# Resistance to antimicrobial agents in *Campylobacter* isolated from chickens raised in intensive and organic farms and its implications for the management of risk to human health

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**Submitted in partial fulfilment of the requirements of Middlesex University for the degree of Doctor of Philosophy**

**May 2006**

# ABSTRACT

The use of antimicrobials in poultry may lead to the emergence of resistant micro-organisms that could cause additional health risk to humans through food consumption. This study aims to investigate the relative health risks from *Campylobacter* and its antimicrobial resistance associated with chicken raised in organic and intensive rearing systems. Three groups of chicken were tested, pre-packaged intensively reared (PIC) and pre-packaged organically reared chickens (POC) both purchased from supermarkets and unwrapped intensively reared (BIC) chickens purchased from butcher' shops in London. Thirty chickens were randomly sampled for each group.

*Campylobacter* was isolated using three culture methods and enumerated using most probable number method (MPN). A modified MPN was also developed for the study. Resistance rates to three antimicrobials were determined using an agar dilution method. Numbers and antimicrobial resistance rates of *campylobacter* were used in consumer risk models to calculate health risks.

The BICs harboured significantly highest numbers of *Campylobacter* ($8.0 \pm 0.81 \log_{10}$MPN/g), followed by the POCs and PICs. All isolates from all groups of chickens were resistant to erythromycin and nalidixic acid. All isolates from the POCs were susceptible to ciprofloxacin, whereas 8.7% of the PICs and 26.7% of the BICs harboured resistant isolates.

The calculated risk of *Campylobacter* associated illness related to the consumption of chicken meals using the dose response relationship model was found to be the highest for the BICs group (33% probability). However, this is the worst case scenario. If elevated internal temperatures ($63^{0}$C-$72^{0}$C) are achieved for a sufficient length of time (1-5 minutes), this risk is reduced to <1%. High resistance rates to antimicrobials may generate additional risk where levels of infection are high.

Potential intervention options for the reduction of *Campylobacter* load in chickens and the control of antimicrobial resistance were considered. The most significant factors found were the initial number of organisms, personal hygiene practices and cooking procedures.

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| | |
|---|---|
| ABR | Antimicrobial resistance |
| ABS | Antimicrobial susceptibility |
| ACMSF | Advisory Committee on the Microbiological safety of food |
| AIDS | Acquired immune deficiency syndrome |
| ALARA | As low as reasonably achievable |
| ALOP | An appropriate level of protection |
| BIC | Unwrapped intensively reared chicken |
| BSAC | The British Society Antimicrobial Chemotherapy |
| BSE | Bovine spongiform encephalopathy |
| CAC | Codex Alimentarius Committee |
| CDC | Centers of Disease Control and Protection |
| cfu | Colony forming unit |
| c-MPN | Conventional Most probable number |
| CR-*Campylobacter* | Ciprofloxacin resistant *Campylobacter* |
| CVM | Center of Veterinary Medicine |
| DEFRA | Department for environment food and rural affair |
| DP | Direct plating |
| ER- *Campylobacter* | Erythromycin resistant *Campylobacter* |
| FISH | Fluorescence In Situ Hybridization |
| FQ- *Campylobacter* | Fluoroquinolone resistant *Campylobacter* |
| FSO | Food safety objective |
| GAP | Good agricultural practice |
| GHP | Good hygiene practice |
| GMP | Good manufacturing practice |
| HACCP | Hazard analysis critical control point |
| JEMRA | The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment |
| MAFF | Ministry of Agriculture, Forestry and Fisheries |
| MIC | The minimal inhibitory concentration |
| MLE | Maximum likelihood estimation |
| MPN | Most probable number |
| MRA | Microbial risk assessment |
| mCCDA | Modified a modified charcoal cefoperazone deoxycholate agar |
| m-MPN | Modified Most probable number method |
| m-QMRA | Modified quantitative microbial risk assessment method |
| NAR- *Campylobacter* | Nalidixic acid resistant *Campylobacter* |
| NCCLS | National Committee of Clinical Laboratory Standards |
| NCTC | National Collection of Type Culture |

| PIC | Pre-package intensively reared chicken |
| POC | Pre-package organically reared chicken |
| QMRA | Quantitative microbial risk assessment |
| TC | Traditional culture method |
| SPS | The application of Sanitary and Phytosanitary Measures |
| UKROFS | United Kingdom Register of Organic Food Standards |
| VNC | Viable but non-culturable |
| WTO | World Trade Organization |

# ACKNOWLEDGEMENT

I am extremely grateful to my Director of Studies Dr Hemda Garelick for all her unlimited patience and great guidance and advice from the first day until now. I am greatly appreciated all her help, moral support good humour and telling me my work was good and interesting, without which the first step to PhD would not be possible.

I would like to thank my supervisors Professor David Ball and Jenny Jacobs for their help and suggestion on my works. I would also like to thank Dr Huw Jones and John Watt for the comments and proof reading and Manika Choudhury for the technical support. I would like to thank many people in the school of Health and Social Sciences, past and present, for valuable support. In particular, I would like to thank Rhona Stephen for making things easier for me. I am grateful to Thammasart University for financial support of my study.

I would also especially like to thank my best friends Charuwan Sriapha and Dr Pensri Watchalayarnn for their help and support throughout this study. Finally, I would like to thank my family for their never-ending love, hope and strength. These all help me to know where the door of the success is and how to find the key to open the door.

# CHAPTER 1

## Introduction

## 1.1 Background of study

Foodborne disease exerts a significant toll on human health (WHO, 2002a). Food and waterborne diarrhoeal diseases are leading causes of illness and death in less developed countries, killing approximately 2.1 million people annually, most of whom are children. Up to one-third of the populations of developed countries are affected by foodborne illnesses each year. Diarrhoea is the most common symptom of foodborne illness; however, other serious symptoms such as organ failure, neural disorder and death can occur. Surveillance and monitoring in a number of countries indicate that foodborne illnesses are on the increase around the world. In the UK, the total number of food poisoning notifications has grown steadily from under 10,000 per annum in the early 1980s to around 90,000 cases in 1998 (WHO, 2001 and Adak *et al.*, 2002).

Although the burden of foodborne diseases is not well documented in many countries, estimation of costs of illness in terms of medical costs and productivity losses has been reported in several countries. These estimations involve the valuation of life and health (Buzby and Roberts,1997). As reported by ERS (2003), it was estimated that in the United States 55-70% of public health costs were attributable to foodborne diseases. In the UK *Campylobacter* infection is estimated to have cost the nation over £ 113 million in 2000 (ACMSF, 2004).

*Campylobacter* infection has been a public health concern for decades. This is because: i) *Campylobacter* has been recognised as the most commonly reported case of acute gastroenteritis in Europe and North America (Barton, 2000; Threfall *et al*, 2000 and Randall *et al*, 2003), ii) the incidence rate of infection as well as total annual costs of illness have been steadily increasing, iii) debilitating long-term complications *(i.e.* Guillain-Berre syndrome) following the infection can occur and cause fatalities, particularly in children, in elderly people, and in immuno-compromised groups.

In addition, concerns have been intensifying after the emergence of fluoroquinolone resistance in *Campylobacter* species (mainly *C.jejuni* and *C.coli*) which has been reported from many countries (Endtz *et al.*, 1991 and Engberg *et al.*, 2001). Antimicrobial resistance in *Campylobacter* is evidently a result of the introduction of antimicrobial agents into animal production (Michael 2001). The uses of antimicrobials in poultry farming are for therapy, prophylaxis and growth promotion. These administrations have been thought to be the crucial factor influencing the development of resistance to antimicrobials. Due to the propensity of bacteria to share genes, resistant genes can be transferred between bacterial

cells, both within species and across species (Collette *et al*, 2001). Some of these bacteria pass to humans through food consumption (mostly of animal origin) and directly cause diseases and act as potential sources of antimicrobial resistance for human pathogens (David and Steven, 2000 and Moore *et al.*, 2001).

To date poultry consumption has been increasing considerably worldwide. This is a result of a shift in consumption patterns due to increasing food prices, greater total food supplies, higher incomes, new information on nutrition and food safety, changes in lifestyles, and technological advances in food production and marketing. So far, to satisfy global demands for poultry and achieve a high yield, most poultry farming for commercial production uses genetically and environmentally manipulated methods in order to accelerate growth. The intensive conditions in which these birds live lead to increased health problems in the flocks (such as bacterial infections). Therefore, antimicrobials are applied to eliminate or prevent the infection. Antimicrobials are also used as growth promoters, diverting nutrition by maintaining a more effective and absorptive gut lining (Aarestrup and Wegener,1999).

Since poultry has been recognised not only as the main animal food product for humans, but also as a significant source of *Campylobacter* infection, the consumption of chicken is highly likely to be a primary route for illnesses related to *Campylobacter* infection. If this organism also carries antimicrobial resistant genes, it would amplify the health risk to consumers, particularly, to vulnerable groups. The extent of this health risk from *Campylobacter* (with and without antimicrobial resistance) is becoming a public health concern (Barton, 2000; and Engberg *et al.*, 2001). As a result, several national public health agencies have developed and established control measures and surveillance programmes for *Campylobacter* infections (Flint, *et al.* 2005).

The issues involved, however, are multifactorial and the working groups established are correspondingly diverse. Although the WHO has attempted to be a link between various agencies, controversies and disagreement continue due to lack of internationally accepted standards on a number of issues (*e.g.* antimicrobial use, surveillance programmes and control measures). Accordingly, strategies and intervention programmes for mitigation of *Campylobacter* have been developed and implemented independently by different groups or agencies and there is little co-ordination (Laisney *et al.*, 1991; FAO, 1997; FSAI, 2001; Kist, 2002; FSA, 2003a and IFT, 2004).

A number of interventions have focused on the reduction of the burden of *Campylobacter* infection carried from farm to table as part of the drive to provide consumers with safe food (Bryan and Doyle,1995 and Kist, 2002). The interventions have been developed and documented following use of microbial risk assessment as a significant scientific tool to assess health risks following the consumption of poultry harbouring *Campylobacter* (Christensen *et al.*, 2001; WHO, 2002b and 2002c; and Nauta *et al*, 2005b). However, other

works have attempted to control the use of antimicrobials on the farms without referring to the numbers or prevalence of *Campylobacter*. This estimation of health risks associated with antimicrobial resistant *Campylobacter* was based on totally different assumptions (Cox and Popken, 2004; Hurd *et al.*, 2004 and Bartholomew *et al.*, 2005). The interventions for the control of antimicrobial resistance are however not based on the risk assessment. Most countries opt to adopt withdrawal of antimicrobial use on the farms despite a notable lack of sufficient data to support the association between antimicrobial use in food-producing animals and antimicrobial resistance in humans (Endtz *et al.*, 1991 and Engberg *et al.*, 2004).

Furthermore, the mitigation of this problem becomes more complicated when it is considered in the context of organic rearing systems. The conflict is that organically reared chickens have been found to harbour high numbers of *Campylobacter*; however, it is thought to be less resistant to antimicrobials due to the rearing practices (EC, 2002 and UK-DEFRA, 2005). These practices involve prudent use of antimicrobials on the farms. It may be assumed that the risk of antimicrobial resistant *Campylobacter* following the consumption of organic chickens should be less than that of intensively reared chickens.

However, there have been no recent studies that consider the comparative health risks from *Campylobacter* (with and without antimicrobial resistance) following the consumption of chicken raised in different rearing systems. Therefore, the assumption stated above may mislead consumers' perception of rearing practices on chicken farms. The perception is that organic chickens are safer or of better quality than standard (intensively reared) chickens. This perception influences their choice of purchase (Hammitt, 1990 and Kist, 2002). In parallel, organically farmed chickens are being increasingly introduced to the market on the presumption that they are safer and free from antimicrobial-resistant bacteria. This has resulted in a price increase despite a notable lack of evidence to support this assumption.

## 1.2 Aims and objectives

### Rationale

This study investigates antimicrobial resistance in *Campylobacter*. *Campylobacter* has been selected for the study because it is recognised as the commonest reported case of gastroenteritis. The consumption of poultry (significant source of *Campylobacter*) has been increasing around the world. In addition, the emergence of resistance to antimicrobials, particularly to drugs of choice used for treatment in campylobacteriosis is becoming a public health concern. As antimicrobials are not permitted to be used for non-therapeutic purpose, organic chickens are unlikely to frequently expose to antimicrobials. As a result, organic chickens may harbour micro-organisms including *Campylobacter* that are less resistant to antimicrobials than those found in intensive chickens.

# Aims

The aims of this study are:i) to investigate and compare the levels of *campylobacter* with and without antimicrobial resistance isolated from chickens reared in different methods, ii) to consider Its implication in terms of risk to human health in the development of risk management options to health risk and iii) to explore intervention options. In order to address these, this study will endeavour to answer the following questions:

1. What are the levels of *Campylobacter* isolated from chickens at retail outlets in which originate from the two different rearing systems (intensive and organic)?
2. What is the resistance rate to three antimicrobial agents of *Campylobacter* isolated from chickens raised in these two different rearing systems?
3. What are the important factors for health (*i.e.* the numbers of *Campylobacter* or the levels of antimicrobial resistance) as far as consumers are concerned?
4. To what extent does *Campylobacter* (with and without antimicrobial-resistance) impose a risk to human health following the consumption of chickens?
5. How useful is microbial risk assessment as a scientific tool for food risk management in this context?

The specific objectives of the study are:

1.  To isolate  and to enumerate numbers of *Campylobacter* in intensively and organically reared chickens at retail outlets, namely, pre-packaged intensively reared chickens (PIC), pre-packaged organically reared chickens (POC) (both purchased from supermarkets), and unwrapped intensively reared chickens (BIC) purchased from butchers'shop.
2.  To test antimicrobial susceptibility in *Campylobacter* isolated from the three types of chickens.
3.  To estimate the exposure of the population to *Campylobacter* with and without antimicrobial-resistance following the consumption of these three types of chicken.
4.  To link the exposure (dose) to *Campylobacter* with and without antimicrobial-resistance with a dose-response relationship model in order to estimate the probability of infection and probability of illness in humans.
5.  To integrate the results and then critically assess the comparative risk associated with the consumption of chickens raised in these two rearing systems.
6.  To evaluate the usefulness of quantitative microbial risk assessment models and whether they can be used as a scientific tool for decision making (risk management).
7.  To further explore potential intervention options currently used by a number of agencies and prioritise in the light of the data collected. Proposals for changes and improvement will be considered.

# 1.3 Conceptual framework

The conceptual framework of the study is as follows: i) microbiological experiments (*i.e.* identification and enumeration of *Campylobacter* and antimicrobial susceptibility to three antimicrobial agents), ii) estimation of health risk using quantitative microbial risk assessment (QMRA) at the consumer level and iii) consideration of risk management options for mitigating the relative risk from *Campylobacter* (with and without antimicrobial resistance) following the consumption of positive- *Campylobacter* chickens raised in different rearing systems. This framework is shown in Figure 1.1.

**Phase 1**

| | |
|---|---|
| Literature reviews | Microbiological experiments<br>-three groups of chickens<br>-three antimicrobial agents |

Detection (isolation and identification)

Enumeration

Antimicrobial susceptibility testing

**Phase 2**

Secondary data/information (model parameters, hygiene parameters)

Risk models from other studies

**Development of modified risk model for estimating ABR-*Campylobacter* (from this study)**

Estimate of health risk of *Campylobacter* with and without antimicrobial resistance (three antimicrobials) :

**Phase 3**

Control measures currently used (literature reviews)

Consideration of Risk management options for
-reduction of *Campylobacter* load in chicken
-reduction of antimicrobial resistant *Campylobacter*

*ABR = antimicrobial resistance

**Figure 1.1** A conceptual framework of the study

## 1.4 Structure of the thesis

The thesis has been divided into ten chapters. Chapters 1-4 cover the general introduction, aims, objectives, conceptual framework and literature reviews. The literature reviews the concept of food safety and provides a context to the study (Chapter 2). It then goes on to review the information and studies specific to *Campylobacter* and antimicrobial resistance (Chapter 3). Chapter 4 reviews the rearing practices in poultry farming including organic farming. Chapters 5 and 6 deal with *Campylobacter* isolation and enumeration and antimicrobial susceptibility testing. Chapters 7 and 8 describe simulation methods for the estimation of health risk from *Campylobacter*. Chapter 9 considers the implications for previousely recommended risk management options based on the findings from Chapters 5-8. Chapter 10 summarises the conclusions, suggestions and future prospects.

## 1.5 Publication

**Soonthornchailku N, Garelick H, Jones H, Jacobs J, Ball D, Choudhury M.** 2006. Resistance to three antimicrobial agents of *Campylobacter* isolated from organically-reared and intensively-reared chickens purchased from retail outlets. Int J Antimicrob Agents 27: 125-130.

## 1.6 Presentations

**1. Soonthornchailkul N.** Antibiotic resistance in *Campylobacter* isolated from chicken. Postgraduate summer conference "Bringing Research to Life" Institute of Social and Health Research. School of Health and Social Sciences. Middlesex University. June 20th 2003.

**2. Soonthornchailkul N.** Incidence and antibiotic resistance level of *Campylobacter* isolated from intensive and organic chicken. Postgraduate summer conference "Relevance of research in the real world" Institute of Social and Health Research. School of Health and Social Sciences. Middlesex University. June 18th 2004.

# CHAPTER 2
## Microbiological Food Safety
## 2.1 Food Safety

This chapter reviews the literature related to microbiological food safety aiming to provide the context in which study of *Campylobacter* in chicken is carried out. The literature review related specifically to *Campylobacter* and antimicrobial resistance is presented in Chapter 3.

## 2.1.1 Background

With the growing global population, improved transportation and communication, the rapid globalisation of food production, processing, distribution and trade, food production has become more industrialised. As the supply chain has lengthened, people are becoming increasingly concerned about the potential for international incidents involving contaminated food and related health risks. The increase in consumers' concerns about health risks from food has been highlighted by well publicised food scares such as BSE and outbreaks and of food poisoning caused by *E.coli*, *Listeria* and *Salmonella*. This has led to regulartory actions over the past twenty years aim at improving the quality of food products and preventing foodborne hazards and illnesses.

WHO asserts that ensuring food safety is an essential function to protect public health from foodborne hazards. Accordingly, food safety must be addressed along the entire food chain with measures based on sound scientific information at both national and international levels. In the last decade food safety has emerged as significant global issue with public health and international trade implications (Buzby and Roberts, 1999 and Satcher, 2000). These shifts have also been motivated by growing public demand for continuous improvement in food safety. As a result, food safety authorities all over the world have acknowledged that food safety must be tackled not only at the national level but also through closer linkages among food safety authorities at the international level. It is important to exchange routine information on food safety issues and to have rapid access to information in case of food hazard emergencies.

There are a number of factors that have led both developing and industrialised countries to establish regulatory systems of food safety. These factors include: the emergence of foodborne hazards and illnesses; the increasing knowledge and awareness of foodborne diseases; the development of advance technologies allowing more accurate detection methods for contaminants in foods; changes in lifestyle, particularly, in food consumption patterns (*i.e.* eating outside the home, buying ready-to-eat meal); the increasing numbers of compromised hosts ( such as AIDS patients, a great number of elderly people in population); expanded food production and international trade; increasing international travel. The

complex linkages between food, technology, pathogens and consumers make it unlikely that the marketplace will be ever entirely free from food hazards. Furthermore, while the cost of foodborne illness estimated by socio-economic methods is so hingh, so is the cost of control, so a compromise has to be turned. The costs of illness and premature death for a number of foodborne illnesses have been used in regulatory cost-benefit and impact analyses in the US (Golan, 2003). Like all cost estimates, the estimations include assumptions about disease incidence, outcome severity, and the level of medical, productivity, and disutility costs. Changes to any of these assumptions could change the cost estimations and, as a result, change the way policymakers rank the risks, prioritise spending, and formulate food safety policies. A sound intervention will induce and further urge manufacturers, regulatory and public health agencies and allied organisations to develop partnerships to improve food safety management.

## 2.1.2 International food safety standards

The growth in world food trade, advance technology, transportation and the increasing mobility of populations are contributing factors to public concern related to food safety, in general and the increasing of emergence of food-borne diseases in particular. Consumers are more aware of food safety and health issues. As a result, consumers are demanding ever greater assurances about the safety and quality of foods they eat. Based on health considerations, the Sixteenth World Health Assembly approved the establishment of the joint Food and Agriculture Organisation of the United Nations (FAO/WHO) Food Standards Programme, with the Codex Alimentarius Commission (CAC) as its principal organ. Its headquater is based in Geneva as part pf the WHO strcture. The CAC has been involved in setting many international standards in food safety (WHO, 2002b) in order to promote food safety and quality and strengthen partnerships with all stakeholders. These include consumers and their representative organisations at the global and national level. The CAC relies on the opinions of scientific expert committees or consultations convened by FAO and WHO on specific issues. The CAC, FAO and WHO have strong interest in promoting national regulatory systems that are based on international principles and guidelines and address all components of the food chain. However, members may maintain or introduce measures which result in higher standards if there is scientific justification or as a consequence of decisions based on an appropriate risk assessment. In 1995, the members of the World Trade Organisation (WTO, 1995) have agreed on Sanitary and Phytosanitary Barriers (the SPS agreement), involving the introduction of international standards, guidelines and recommendations. This Agreement identifies procedures and criteria for the assessment of risk and the determination of appropriate levels of sanitary or phytosanitary protection based on practice in individual countries.

The changes in the methods of food production have induced new risks and increased the risk of infectious agents being disseminated from the original point of production to

consumers. Further challenges arise from the new emergence and re-emergence of food-borne pathogens. Consequently, there is an increased risk to human health as well as implications for international trade in food and ultimately food producers. The need for risk based scientific advice has been increasing (FAO, 2004). The CAC has introduced a food safety system and developed microbial risk analysis as a useful tool to inform actions and decisions aimed at improving food safety and made it equally available to both developing and developed countries (FAO, 2006)

The EU integrated approach to food safety aims to assure a high level of food safety, animal health, animal welfare and plant health through coherent farm-to-table measures and adequate monitoring, while ensuring the effective functioning of the internal market. The European Food Safety Authority (EFSA) is the keystone in the provision of risk assessment and guidelines in the EU regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks. EFSA also ensures that risk management is performed following science-based finding (EFSA, 2006). The Swiss Federal Office of Public Health (SFOPH) is the keystone for foodborne disease surveillance in Switzerland. The SFOPH collects information on foodborne infections and intoxications from regional health authorities. The Swiss Reporting System legally requires the federally registered laboratories to report identifications of all agents listed in the Regulation on Disease Notification of 1999 to the SFOPH. Therefore, only cases that are caused by the agents listed are reported. It is assumed that the numbers of reported cases of foodborne diseases represent a small fraction of their real number (WHO, 2003).

## 2.1.3 The field of food safety

Food safety associated with hazards in the food chain (Figure 2.1) can be classified into three categories. These are based on physical, chemical and biological hazards (Wagstrom, 2004). Each of these hazards needs to be considered separately when assessing food safety risks or when designing a food safety intervention programme. In addition, they must be considered in relation to the periods of time involved in the food chain and production line (Callaway *et al.*, 2003 and Wagstrom, 2004). These periods are related to animal husbandry:

►**Pre-harvest** includes the time in an animal's life from birth (hatching) to loading onto transport to the processing plant.

►**Peri-harvest** accounts for the time after an animal leaves the farm up to the time that it reaches the processing plant.

►**Post-harvest** is the time from processing plant to consumer, including in-plant and distribution processes.
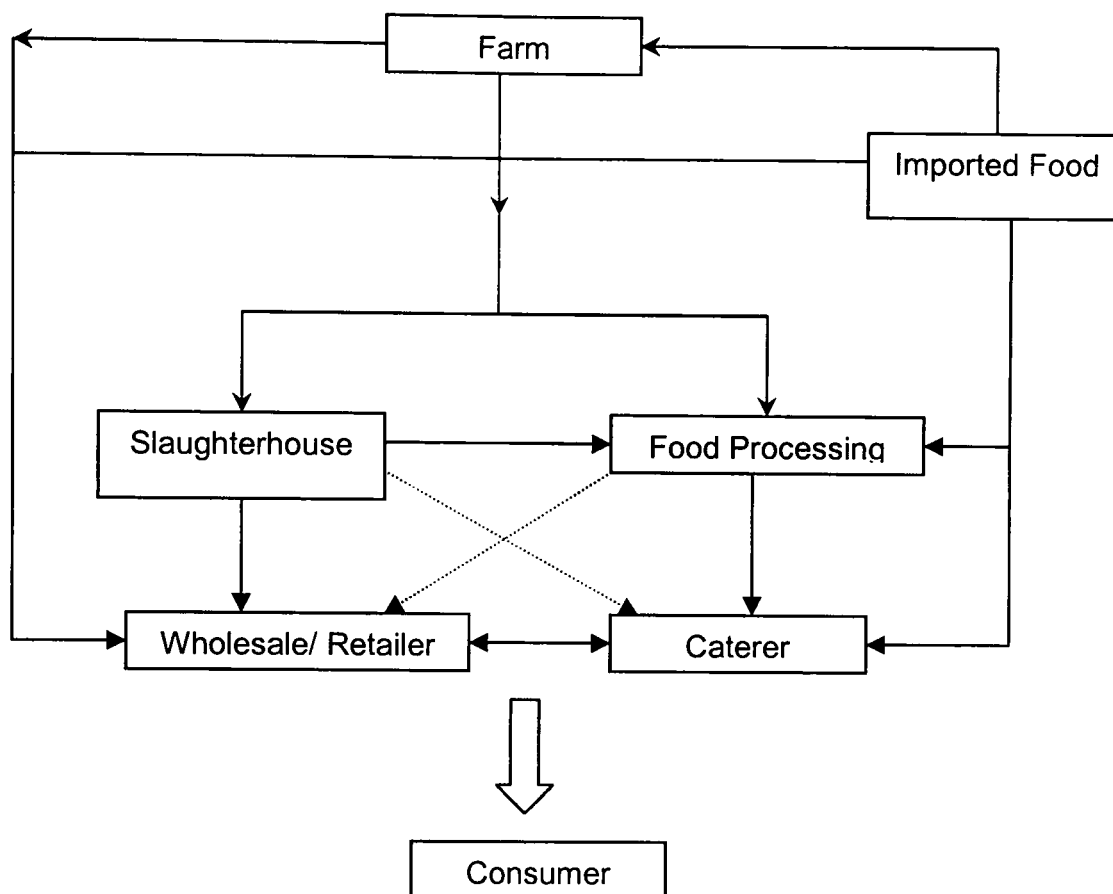
**Figure 2.1** The food chain from production to consumption (adapted from Liu *et al.*, 1999)

## 2.2 Foodborne Disease

Foodborne diseases including intoxication, infection and toxicoinfection (Figure 2.2), are illnesses caused by pathogens and their toxins entering the body through the consumption of contaminated food and drink, or through person-to-person contact (WHO, 2002a). Such contaminations usually arise from improper production, handling, preparation or storage of food. The pathogens and their toxins can damage or destroy host cells, or induce harmful host responses to their presence (IFT, 2002).

When pathogenic micro-organisms are ingested, they colonised the intestine and sometimes invade the mucosa or other tissues. Illness can also be caused by adding pesticides or medicines to food, or by accidentally consuming naturally poisonous substances (*e.g.* poisonous mushrooms, reef fish). In addition, contact between food and pests, especially flies, rodents and cockroaches may cause further contamination of food. Some common gastroenteritis may be occasionally transmitted through the water vector (waterborne pathogens), or other routes, *e.g.* soil, work surfaces, public beaches, irrigation canals, *ect* . These include infections caused by *Shigella*, Hepatitis A, and *Giardia lamblia* and *Cryptosporidium parvum*.

Foodborne diseases are one of the main causes of morbidity and mortality worldwide. It is possible to assume that every person is at risk of foodborne illness, in particular, young children less than 5 years in developing countries (WHO, 2002a).

Foodborne disease

Infections

Intoxications

Toxico-infection

Invasive Infections

Chemical poisoning

Poisonous plant tissues

Poisonous animal tissues

Microbial intoxication

Enterotoxins

Neurotoxins

Other

Intestinal mucosa

Systemic

Other tissues or organs

Mycotoxin

Algal toxins

Bacterial toxins

Diarrhogenic

Emetic

Enterotoxins

Neurotoxins

Other

**Figure 2.2** A classification of the causes of foodborne disease (adapted from Buzby *et al.*,1996)

## 2.2.1 Clinical features

Most foodborne diseases are characterised by symptoms limited to the gastrointestinal tract. After the ingestion of contaminated food or drink, microbes pass through the stomach into the intestine, attaching to the cells lining the intestinal walls and multiplying there. Some types of microbes stay in the intestine. Some produce a toxin that is absorbed into the bloodstream, and some can directly invade the deeper body tissues. The symptoms may appear immediately or may be delayed depending on the pathogenic agents involved (Table 2.1).

The incubation period (the delay between consumption of a contaminated food and appearance of the first symptoms) ranges from hours to days depending on the host, agent and infective dose, which varies according to the agent and the consumer's age and health status.

Most cases of foodborne illnesses are acute as they have a short incubation period and are self-limiting. Acute symptoms including diarrhoea, vomiting, or other gastrointestinal

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manifestations such as dysentery and range from mild to severe and may even result in premature death. However, other pathophysiologic responses may occur independently or accompany acute-phase responses. Chronic sequelae which may impact on the remainder of a patient's life can occur following the acute phase, including ankylosing spondylitis, arthropathies, renal disease, cardiac and neurologic disorders, and nutritional and other malabsorptive disorders (Archer and Young, 1988; Foegeding, 1994; Bunning, 1994 and Bunning *et al.*, 1997). For example, reactive arthritis and Guillain-Barré syndrome may follow campylobacteriosis (Mishu *et al.*, 1993).

There may be one or more symptoms involved. These may be nausea, vomiting, abdominal pain, diarrhoea, fever, headache and tiredness. However, some foodborne pathogens invade deeper tissues or produce toxins that are absorbed and cause systemic symptoms (*e.g.* fever, kidney failure, paralysis). Most cases without underlying illness fully recover without medical treatment or they may require only supportive care. However, some pathogens cause more severe symptoms and take longer time to subside, in particular, in vulnerable groups or immunocompromised hosts (*i.e.* young children, pregnant women, elderly people, and patients with immuno-suppressive administration). These complications can cause permanent health problems (*e.g.* Guillain-Barré syndrome) or even death (Bunning *et al.*, 1997).

**Table 2.1** Major foodborne microbes by major presenting gastrointestinal symptoms (modified from CDC, 2001)

| Probable Microbes | Incubation Period | Probable food sources |
|---|---|---|
| *Staphylococcus aureus* | 1-6 hours | Prepared food (*eg*, salads, dairy, meat) |
| *Bacillus cereus* | 1-6 hours | Rice, meat |
| Noroviruses (Norwalk-like viruses) | 24-48 hours | Shellfish, prepared food, salads, fruit sandwiches |
| *Clostridium perfringens* | 8-16 hours | Meat, poultry, gravy |
| Enteric viruses | 10-72 hours | Faeces-contaminated food or water |
| Enterotoxigenic *Escherichia coli* | 1-3 days | Faeces- contaminated food or water |
| *Cyclospora cayetanensis* | 1-11 days | Imported berries, basil |
| *Clostridium parvum* | 2-28 days | Vegetables, fruit, water, unpasteurised milk |
| *Vibrio parahemolyticus* | 2-48 hours | Raw shellfish |
| Non-typhoidal *Salmonella* species | 1-3 days | Eggs, poultry, meat, unpasteurised milk or juice, fresh produce |
| *Shigella* species | 1-3 days | Faeces-contaminated food and water |
| Shiga toxin-producing *Escherichia coli* | 1-8 days | Ground beef, water unpasteurised milk, juice, raw vegetables, |
| *Campylobacter* species | 2-5 days | Poultry, water unpasteurised milk |

## 2.2.2 Etiological agents

Foodborne diseases can be caused by a variety of pathogenic agents including bacteria, viruses and parasites, toxins and prions. More than 250 different foodborne diseases have been described (CDC, 2001). Other diseases include poisonings caused by harmful toxins or chemicals that have contaminated the food, for example, poisonous mushrooms. The world Health Organisation (WHO, 2002a) indicated that the most virulent foodborne diseases and foodborne pathogens causing illness are Bovine spongiform encephalopathy (BSE), *Campylobacter, Escherichia coli, Salmonella* and *Shigella.*

## Pathogenic bacteria

Bacterial infections are the most common cause of food poisoning. Bean and Griffin (1990) reported that 90% of confirmed foodborne illness cases and death reported to the Centers for Disease Control and Prevention (CDC) are attributed to bacteria. In the United Kingdom during 2000 the following pathogens were identified: *Campylobacter jejuni* 77.3%, *Salmonella* 20.9%, *Escherichia coli* O157:H7 1.4%, with others accounting for less than 0.1% .

Many pathogens, including *Salmonella, Escherichia coli* O157:H7, *Campylobacter,* and *Yersinia enterocolitica,* have reservoirs in healthy food animals, from which they spread to variety of foods. Whilst *Campylobacter* can colonise the intestinal tract of some poultry without any associated clinical consequences, people who consume undercooked chicken from contaminated chicken can get ill.

Farm livestock colonised with foodborne pathogenic bacteria may spread the infection among the herd or flock through water or their manure. After processing at the slaughterhouse, the contaminated meat eventually passes through the food chain to consumers. In the past, the main challenge of foodborne disease prevention lay in interventions to prevent the contamination of human food with sewage or animal manure or in the prevention of spoilage. It is now recognised that this is not sufficient and that the challenge now is to prevent contamination of food throughout production to consumption as contamination may occur at any point in the food chain: on the farm, during slaughter and processing, at the point of sale, or in the home. Thus, the Economic Research Service (ERS) of the US Department of Agriculture (ERS, 2003) measures the costs of foodborne diseases caused by such contamination as well as evaluating the economic consequences of efforts to control contamination by industries and consumers.

In addition, some bacteria, for example, *clostridium botulinum, clostridium perfringens* and *staphylococcus aureus,* excrete exotoxins during bacterial growth. These exotoxins can cause illness even when the microbes that produced them have been killed. Symptoms

typically appear after 1-6 hours depending on the amount of toxin ingested. Table 2.2 shows the common food vehicles for pathogenic bacteria causing illness to humans.

**Table 2.2** Common food vehicles for pathogens

| Pathogen | Food sources |
|---|---|
| *Campylobacter jejuni* or *coli* | Major: poultry.<br>Minor: milk, mushrooms, clams, hamburger, water, cheese, pork, shellfish, eggs, cake icing. |
| *Clostridium perfringens* | Major: meat, meat stews, meat pies, and beef, turkey, and chicken gravies.<br>Minor: beans, seafood. |
| *Escherichia coli O157:H7* | Major: beef particularly ground beef.<br>Minor: poultry, apple cider, raw milk, vegetables, cantaloupe, hot dogs, mayonnaise, salad bar items. |
| *Listeria monocytogenes* | Major: soft cheese, pâté, ground meat.<br>Minor: poultry, dairy products, hot dogs, potato salad, chicken, seafood, vegetables. |
| *Salmonella* (non-typhoid) | Major: poultry, meat, eggs, milk, and their products.<br>Minor: vegetables, fruits, chocolate, peanuts, shellfish. |
| *Staphylococcus aureus* | Major: workers handling foods: meat (especially sliced meat) poultry, fish, canned mushrooms.<br>Minor: dairy products, prepared salad dressing, ham, salami, bakery items, custards, cheese. |
| *Vibrio* spp. | Major: oysters.<br>Minor: other seafood. |

## Other pathogens

### Viruses

Viral infections make up perhaps one third of cases of food poisoning in developed countries. They are usually of intermediate (1-3 days) incubation period and cause illnesses which are self-limited in otherwise healthy individuals. The symptoms are similar to the bacterial forms described above. The common viruses are norovirus (formerly Norwalk virus), rotavirus, hepatitis A and hepatitis E.

### Parasites

Most foodborne parasite infections are Zoonoses including platyhelminthes, *Taenia saginata, Taenia solium, Fasciola hepatica*, tapeworm, nematode and protozoa.

### Natural toxins

Several foods contain naturally occurring toxins such as aflatoxin, alkaloid, ciguatera toxin, mushroom toxin, phytohaemagglutinin, pyrrolizidine alkaloid, shellfish toxin, scombrotoxin and tetrodotoxin.

*Prions*

Prions are a unique type of infectious agent which is an abnormally-structured form of a protein found in the brain. Prions are believed to be the etiological agent for some diseases, for example, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), and kuru in humans, and BSE and scrapie in animals.

## 2.2.3 Incidence

Surveillance and monitoring systems in a number of countries indicate that foodborne diseases are increasing around the world (Sockett, 1993; Henson, 1996 and WHO, 1997). The Centre for Disease Control and Prevention (CDC) estimates that in the United States, approximately 76 million persons contract foodborne illnesses each year, resulting in 325,000 hospitalizations and 5,000 deaths (ERS, 2003). In England and Wales (from 1996 to 2000), an estimated 1,724,315 cases of indigenous foodborne disease occurred per year (3,400 cases for 100,000 inhabitants) resulting in 21,997 hospitalizations and 687 deaths (Adak et al., 2005). There were 750,000 cases in France (1,210 cases per 100,000 inhabitants). In Australia, notification rates for both campylobacteriosis and salmonellosis (common foodborne illnesses) have continued to increase annually.

# 2.3 Microbial Risk Analysis

## 2.3.1 Introduction

Foodborne diseases caused by pathogenic micro-organisms occur when persons consume food(s) contaminated with bacteria, viruses or parasites. Changes in pathogens, food preparation, distribution, consumption, and population immunity have the potential to adversely affect human health and the epidemiology of foodborne diseases. This persistence of the disease burden from food poisoning to public health may lead to a constant threat and new problems emerging, resulting in a major impact on the food industry, government and consumers. Food production and processing, the distribution system and consumption can amplify or attenuate the trend and are a potential source of health hazards. Predicting the impact of a trend in one part of the food continuum requires an understanding of the whole system. As a full understanding of pathogen contamination, infection, and survival is difficult, a systematic approach to assess the impact of the pathogen on human health may improve the quality of public health decisions (WHO, 2002b).

Quantitative risk analysis used in environmental toxicology is a possible approach for managing foodborne diseases. In an attempt to address this, risk analysis has been developed as one of the measures for food safety management. This includes improvement of the understanding of health consequences of foodborne hazards and their associated costs (Schlundt, 2000). However, each country has its own perception, concerns,

acceptance, policy and priorities. Buzby and Roberts (1999) pointed out that consumer perceptions of food safety are influenced by a complex function of factors. These include baseline food safety and risk levels, information on food safety and food risks, public trust in the source of information, and experiences and backgrounds with food safety incidents. In addition, there may be basic differences in how people view and value the consequences of foodborne illness.

Post (2005) observed that conflicts over food safety standards have emerged as one of the most controversial international trade issues in recent years. By consideration of the uptake of the two standards across four very different regulatory environments, the United States, the European Union, Argentina, and the Dominican Republic, the major finding of Post (2005) is that the role of interest groups is of much less importance than theories of political economy. The World Trade Organization (WTO) has encouraged countries to adopt food safety standards facilitated by the Codex Alimentarius Commission (CAC) in order to remove non-tariff barriers to trade (WTO, 1995). As recommended by the Codex, Hazard Analysis and Critical Control Point (HACCP) is currently regarded as the most effective international standard for processing safe food worldwide (CFSAN, 1998; CAC, 2002a and IFT, 2004).

For the World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO), the prevention of foodborne and waterborne diseases remains a major task of these organisations. In this context effective prevention requires a well-balanced set of management strategies. Hence, the CAC has played a central role in developing and standardising the risk analysis for the evaluation of the safety of food and water supplies at an international level.

In addition, in 1993 the Uruguay Round of General Agreement on Tariffs and Trade (GATT) resolved the barriers to the international trade in food. In this agreement attention is particularly focused on public health, and health concerns take precedence over trade issues. The agreement requires that food safety measures taken by individual countries should:

► be applied only to the extent required to protect human health;
► be based on scientific principles;
► not be maintained without scientific evidence;
► be based on the assessment of the risk to health that is appropriate to the circumstance.

Based on the application of the SPS[1], the WTO refers to the term 'an appropriate level of protection' (ALOP)[2] against risks to human life or health, or to animal and plant life (WTO, 1995). The ALOP focuses on public health related to the disease burden associated with a particular hazard/ food combination (WHO, 2002b). This statement indicates that a zero-risk approach is not suitable for a country with a significant trade in agricultural commodities. So far, this concept has been perceived by many WTO members as being about compromised and acceptable risk. Since the complete elimination of risks from food production and consumption is an impossible goal, the reduction of risk to as low as reasonably achievable (ALARA) would be an alternative approach for establishing risk management options (WHO, 2000b).

Microbial risk analysis is an analytical tool for estimating specific risks associated with the microbial contamination of food and food products and indicating how particular production-to-consumption processes or practice contribute to the risks. It also provides risk decision-makers with information which helps them to assess the effectiveness of different interventions from production to consumption (farm-to-fork) chain.

A framework of risk analysis defined by the CAC comprises of: risk assessment; risk management and risk communication (Figure 2.3)
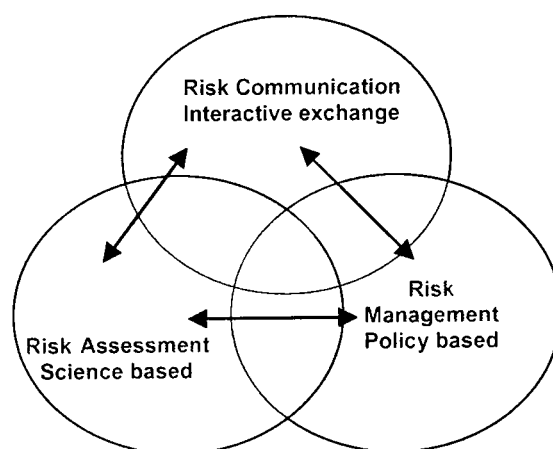


**Figure 2.3** Risk analysis framework (adopted from CAC, 2002b)

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[1] Sanitary and Phytosanitary Measures refer to any measure, procedure, requirement, or regulation, taken by governments to protect human, animal, or plant life or health from the risks arising from the spread of pests, diseases, disease-causing organisms, or from additives, toxins, or contaminants found in food, beverages, or feedstuffs.
[2] An appropriate level of protection (ALOP) is a statement of the degree of public health protection from a particular hazard or food that is to be achieved by the food safety systems implemented within a country

## 2.3.2 Microbial risk assessment (MRA)

This tool is an extension of the principles of chemical risk assessment to estimate the consequences and occurrence of exposures to pathogenic micro-organisms (Hathaway, 2001; Brown and Sringer, 2002 and WHO, 2002b) (Figure 2.4). The performance of MRA will always be limited by the availability of data. In spite of this, risk assessments leading to management options are undertaken based on expert opinions and assumptions which can be validated.

(a) Chemicals                                      (b)Microbiological agents

```
     ┌──────────────────┐                    ┌──────────────────┐
     │   Food safety    │                    │   Food safety    │
     │   objectives     │                    │   objectives     │
     └────────┬─────────┘                    └────────┬─────────┘
              ↓                                        ↓
     ┌──────────────────┐                    ┌──────────────────┐
     │ Food safety policy│                   │  Quantitative risk│
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     ┌──────────────────┐                    ┌──────────────────┐
     │ Appropriate level│                    │ Appropriate level│
     │  of protection   │                    │  of protection   │
     │     (ALOP)       │                    │     (ALOP)       │
     └────────┬─────────┘                    └────────┬─────────┘
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     ┌──────────────────┐                             │
     │ Quantitative risk│                             │
     │    analysis      │                             │
     └────────┬─────────┘                             │
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     │ Incorporation in │                    │ Incorporation in │
     │     HACCP        │                    │     HACCP        │
     └──────────────────┘                    └──────────────────┘
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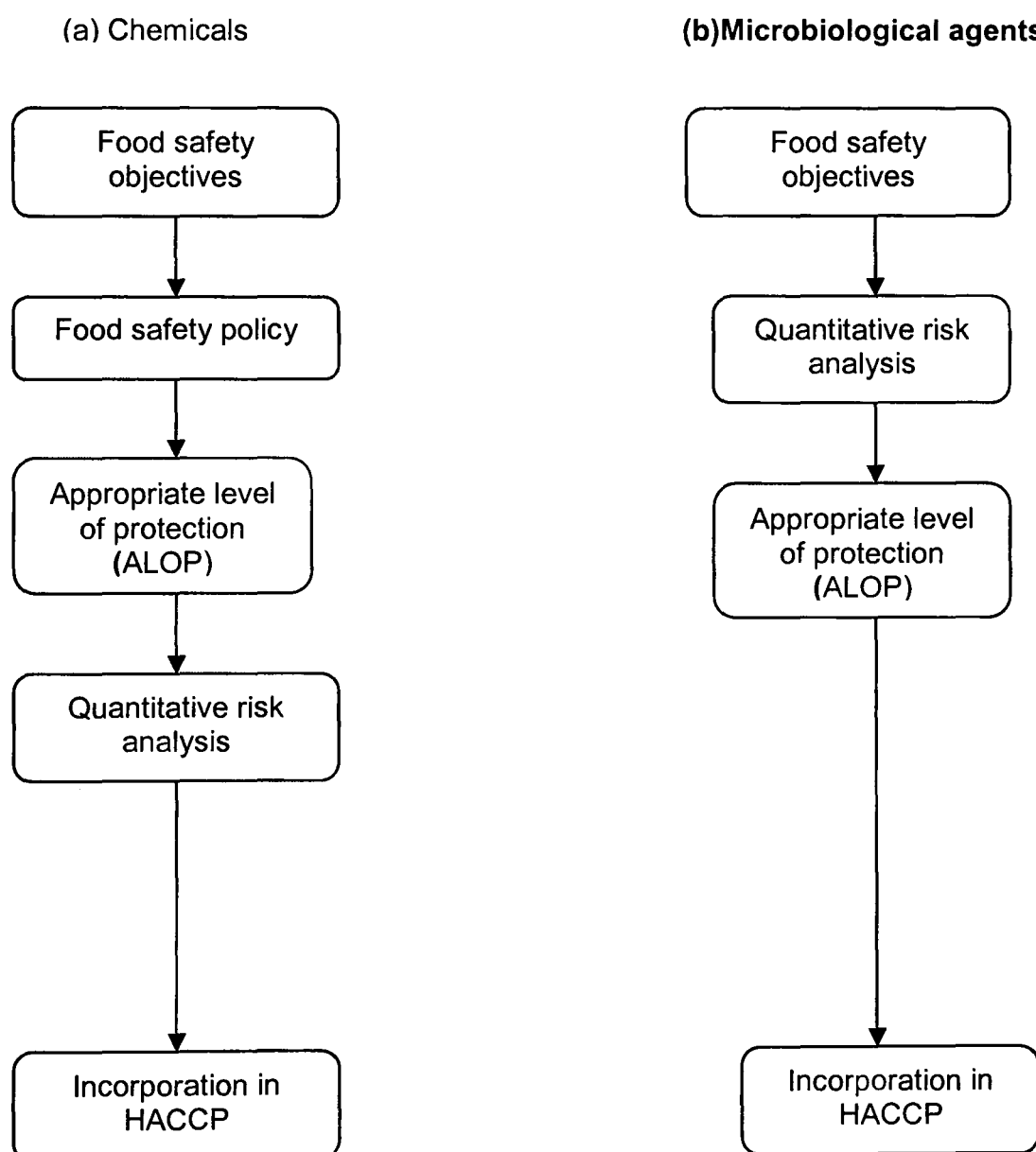
**Figure 2.4** Generic framework of current food safety systems developed for chemicals and microbiological agents (adapted from Hathaway, 2001; Brown and Stringer, 2002 and WHO, 2002b)

The ad hoc Joint FAO/WHO Expert Meetings on Microbial Risk Assessment (JEMRA) have developed and conducted a series of risk assessments for foodborne microbiological hazards at the international level (WHO, 2000). The JEMRA aims to provide a scientific

basis for the relevant risk management deliberations of the CAC, whose purpose is to develop food standards, guidelines and related texts aimed at protecting consumer health and ensuring fair food trade practices.

## Scope of microbial risk assessment

The body of MRA is based on the critical reviews of the best available scientific evidence and consensus, and is derived by integrating information related to adverse health effects. The process defined by the CAC is a scientifically based process consisting of four components (Figure 2.5) (CAC, 2002b).
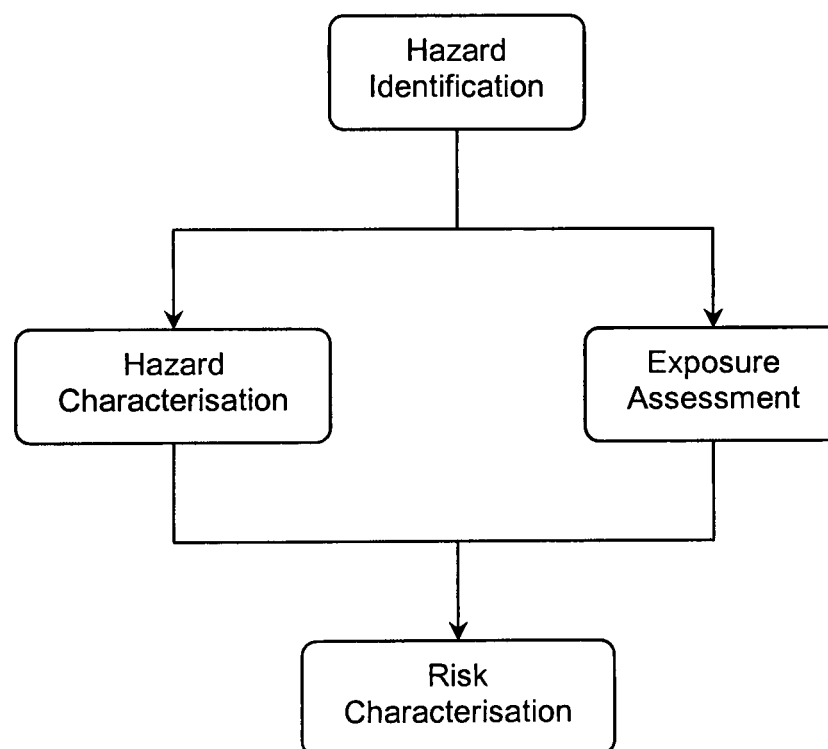


**Figure 2.5** Components of microbial risk assessment (adopted from CAC, 2002b)

The definitions of these four components are:

▶ **Hazard identification** predominantly intends to identify micro-organisms or microbial toxins of concern in food or water. It must include as much information on the hazard of concern as possible.

▶ **Hazard characterisation** provides a description of the nature of adverse health effects that may result from ingestion of a micro-organism. It also focuses on how to quantitatively assess the relationship between the magnitude of foodborne exposure and the likelihood of adverse health effects occurring.

► **Exposure assessment** should provide an estimate, with the associated uncertainty, of the occurrence and level of the pathogen in a specified portion of food at the time of consumption, or in a specified volume of water using a production-to-consumption approach. Whilst a mean value may be used, more accurate estimates will include and estimate the distribution of exposures. This will typically include identification of the annual food and water consumption frequencies and weights or volumes for a given population or subpopulations, and should combine the information to estimate exposure to pathogens in a population through a certain food or water commodity. When data are available, the exposure assessment model should be developed. Finally, the likelihood and magnitude of exposure to the pathogen of concern following the consumption of the food of interests is quantified.

► **Risk characterisation** is performed by the consideration of the results of the hazard identification, hazard characterisation and exposure assessment. This is an integration of the three previous steps in order to obtain a risk estimate (*i.e.*, an estimate of the likelihood and severity of the adverse health effects that would occur in a given population, with the associated uncertainties).

Brown and Stringer (2002) indicated that with the definitions shown above, an MRA is still relatively new and emerging discipline. Although to date some groups of researchers from European countries and New Zealand (Lake, *et al.* 2003) have attempted to perform MRA/QMRA, it appears that few studies for formal MRA have been completed. Additionally, there are many problems related to the availability of relevant data (both quantitative and qualitative) or to issues of handling of variability and uncertainty. Furthermore, each step of MRA undertaken is based on an individual opinion and assumption of each working group and is influenced by the limited availability of trained personnel. Debates have continued over the methodology, for example, how best to model the inputs of the hazard to the supply chain and the resulting outputs with products, and how to express the output of risk assessment in a way that is both accurate and meaningful to food safety managers and consumers.

## Uncertainty and variability in the risk assessment process

Health-risk assessment is a quantitative evaluation and integration of information and relevant scientific data on potential health hazards from exposure to various agents (*e.g.* chemical, biological agents). There are many factors which may be defined as variable or uncertain. The variability refers to real and identifiable differences, which are attributable to the diversity in biological sensitivity and to exposure parameters, between individuals within a population. These differences can be better understood, but are not reduced by further research. On the other hand, uncertainty refers to the lack of knowledge about specific factors, parameters or models. Although in some cases, this may be reduced through further

study or better information, it may not always be possible (USEPA, 1997 and Thompson, 2002).

There are three types of variability defined by US Environmental Protection Agency (1997), which are:

►**Spatial variability** can occur at both macro-scale and micro-scale. For example, the level of contamination of bacteria in animal foods can significantly vary depending on the region or country or farm.

►**Temporal variability** refers to the variations that occur over time and may relate to a short-term or long-term situation, for example, seasonal variation.

►**Inter-receptor variability** can be related to two main factors: i) human characteristics (*i.e.* age, gender, body weight) and ii) human behaviours (*i.e.* consumption pattern, personal hygiene).

The types of uncertainty are categorised as follows:

►**Parameter uncertainty** occurs from the estimation of values from limited data or incomplete information, for example, the errors from measurement, sampling.

►**Modelling uncertainty** arises from the use of inadequate or inappropriate models to perform the assessment and also from the deficiencies of the models in representing reality.

►**Completeness or scenario uncertainty** relates to the inability of the analyst to evaluate all contributions to the risk model. This refers to the problem of assessing what may have been omitted in analysis.

## 2.3.3 Food risk management

Risk management is the process of weighing policy alternatives to accept, minimise or reduce risks associated with food. The goal of management is to protect public health by controlling such risks as effectively as possible through the selection and implementation of appropriate measures (FAO, 1997).

Based on the SPS Agreement, food risk management must consider the measures that protect human, animal or plant life or health within its territory (CAC, 2002a). The SPS Agreement defines a certain level of protection for foodborne microbiological hazard which is to an Appropriate Level of Protection (ALOP). This level of protection is a reflection of a particular country's expressed public health goals for a microbiological hazard(s) associated with a food. It is deemed appropriate by the individual country.

### General principles of food risk management

The implementation of risk management options should be based on principles and strategies that explain the approach taken to risk and safety. Generally, these require

balancing of the risk reduction against other factors such as costs and benefits and (even in same cases) public perception. The World Health Organization (WHO, 2000) proposed same general principles of food safety risk management so as to achieve the goal of food risk management, which are:

▶"Risk management should follow a structured approach including risk evaluation, risk management option assessment, implementation of management decisions and monitoring and review. However, it is not necessary for all of these elements to be included in risk management activities."

▶"Protection of human health should be the primary consideration in risk management decisions. Decisions on acceptable levels of risk should be justified by human health considerations. Considerations of other factors (*i.e.* economics, benefits, technical feasibility and societal preferences) may be taken into account for some appropriate risk management contexts. These considerations should be prioritised and explicit."

▶"Risk management decisions and practices should be transparent. The identification and systematic documentation of all elements of a risk management process should be included so that the rationale is transparent to all interested parties."

▶"Determination of risk assessment policy should be included as a specific component of risk management. Risk assessment policy documents the guidelines for value judgements and policy choices which may need to be applied at specific decision points in the risk assessment process. This should be carried out in advance of risk assessment and in collaboration with risk assessors."

▶"Risk management should ensure the scientific integrity of the risk assessment process by maintaining the functional separation of risk management and risk assessment. This would reduce any conflict of interests between risk assessment and risk management."

▶"Risk management decisions should take into account uncertainty in the output of the risk assessment. The estimates of risk should clearly express not only the variables, but also the uncertainties. The full implications of the range of uncertainty can be taken into account for the risk management decision. If the risk estimation involves high uncertainty, the risk management decision should be more conservative."

▶"Risk management should include clear and interactive communication with consumers and other interested parties concerning all aspects of process. Risk communication is essential for risk management to disseminate the information and opinion to public."

▶"Risk management should be a continuing process that takes into account all newly generated data in the evaluation and review of risk management decisions."

## Risk management framework

The primary goal of the management of risks associated with the consumption of food contaminated with micro-organism is to protect public health by controlling such risks as effectively as possible through the selection and implementation of appropriate measures

(FAO, 1997). As recommended by the CAC (CAC, 2002a), risk management should be conceptualised following the framework illustrated in Figure 2.6.
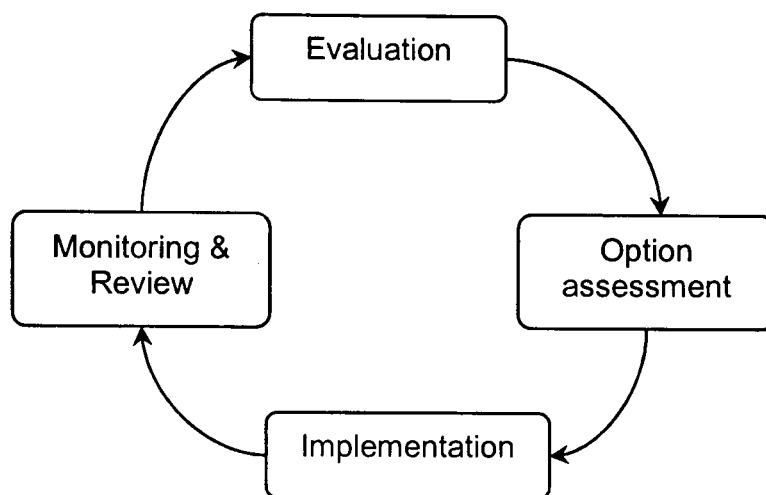


**Figure 2.6** Risk management framework (adopted from WHO, 2000)

The four elements of the framework are:

▶**Preliminary risk assessment activities** include the establishment of a risk profile, facilitating consideration of the issue within a particular context and provide as much information as possible to guide further action (Thompson and Graham, 1996; FAO, 1997 and WHO, 2000). As a result of this process, the risk manager may commit to carry out a risk assessment as an independent scientific process to inform decision-making. The evaluation of risk management options will require information on:

- Identification of a food safety problem

- Establishment of a risk profile

- Ranking of the hazard for risk assessment and risk management

- Establishment of risk assessment policy for conduct of risk assessment

- Commissioning of risk assessment

- Consideration of risk assessment result

▶**Evaluation of risk management options** is the process of weighing available options for managing a food safety issue. It is based on the scientific information on risks and other factors and may include a decision on an appropriate level of consumer protection (Ball and Goats, 1996 and CFSAN, 1998). An important goal is to optimise food control measures, which must be efficient, effective and practical, and technological feasibility at selected points throughout the food-chain. A cost-benefit analysis could be performed at this stage (Cox, 2002). This process will likely be considered by a risk manager in relation to:

- Identification of available management options

- Selection of a preferable management option, including consideration of an appropriate safety standard dependent on the level of acceptable risk

- Final management decision

▶**Implementation of risk management decision:** This will usually involve regulatory food safety measures. Flexibility in the choice of individual measures applied by industry is a desirable element as long as the overall programme can be objectively shown to achieve the stated goals. Ongoing verification of the application of food safety measures is essential.

▶**Monitoring and review**: This final step of risk management considers the assessment of effectiveness of measures taken and, if necessary, reviews risk management decisions and risk assessment. It needs the gathering and analysis of data in order to give an overview of food safety and consumer health. Monitoring of contaminants in food and foodborne disease surveillance should identify new food safety problems as they emerge. Where there is evidence that the goals are not being achieved, redesign of food safety measures is needed.

The outcome, following the application of the framework, should provide the available risk management options in order that risk manager can make a decision on the intervention. In arriving at the decision, human health protection should be the primary consideration over other factors (*e.g.* economic costs, benefits, technical feasibility, risk perceptions, *etc.*). The measures must be effectively able to reduce the prevalence and numbers of pathogens in food, resulting in reducing health risk to humans. A variety of implementations can be selected and set up. However, in practice the factors involved are often complex and unpredictable. It may be necessary to consider which options are appropriate to a specific risk management question (Figure 2.7). Since food safety issues are usually multifactorial, generating unpredictable associations which may lead to unexpected and undesirable problems, flexible responses in risk management may be necessary.

**Figure 2.7** The overall conceptual framework of risk management activities for managing foodborne risks to human health (adopted from WHO, 2002b)
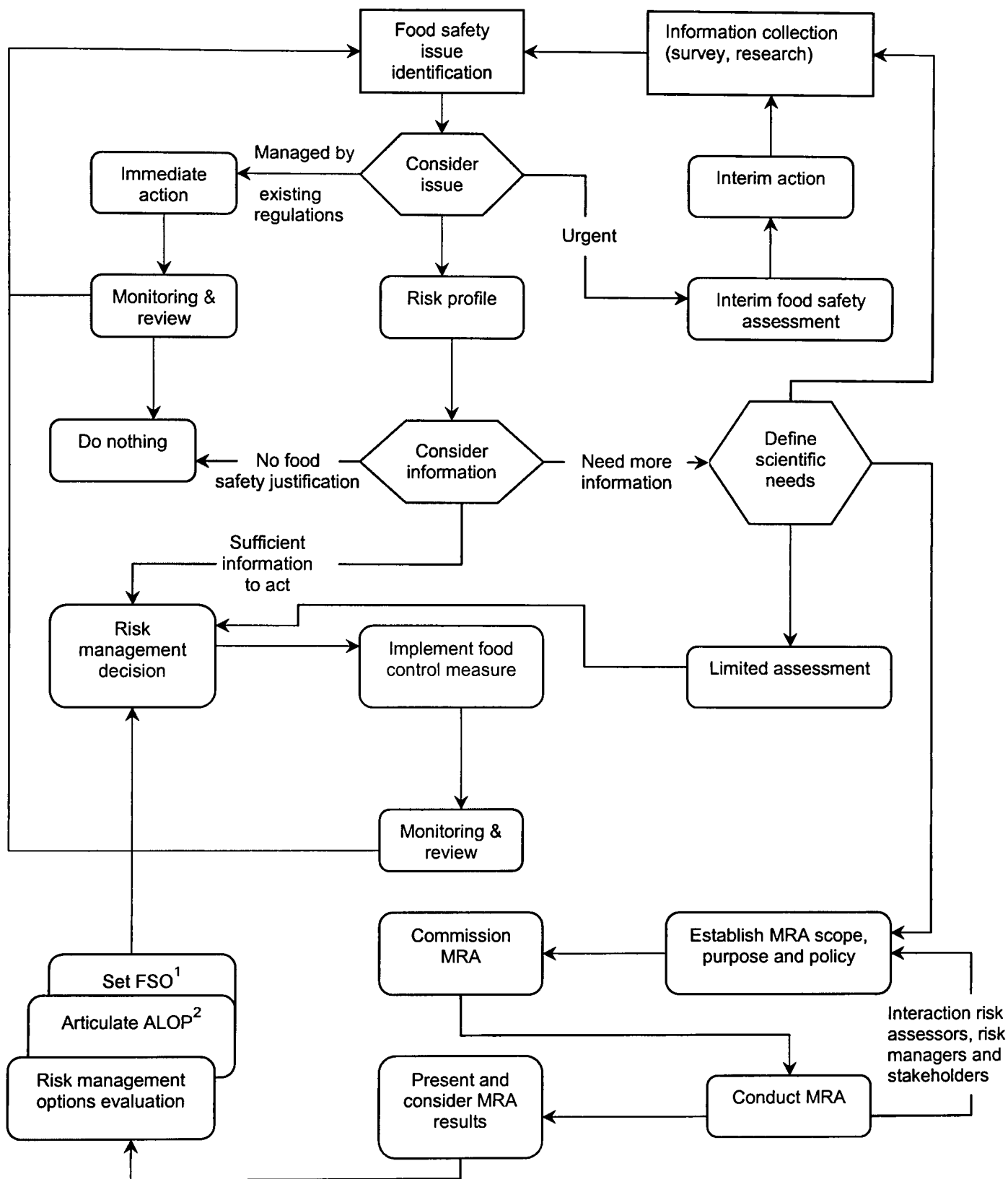
[1]Food Safety Objective (FSO) – the maximum frequency and concentration of a hazard in a food at the time of consumption

[2] Appropriate level of protection (ALOP)

## Evaluation of risk management options

In the assessment of risk management options, all relevant data, knowledge and information pertinent to decisions are often dispersed among various interested parties. Evaluation of risk management options must consider their inherent advantages and disadvantages together with their impact on risks. Relevant considerations include:
- acceptability of the technology or the resulting food product by industry and consumers;
- cost effectiveness;
- technological feasibility;
- expected level of compliance with control measures;
- options for monitoring and review;
- possibility of new risks arising from the options selected

The main point of concern is that who judges an option to be optimal and according to what criteria. For example, steam surface pasteurisation of citrus fruit to remove pathogens may provide the same reduction in risks as washing by hand in an appropriate sanitising solution. For example, following a cost-benefit analysis, where labour costs are high, the former measure may be the most optimal.

## Implementation

Although many food safety measures can be successfully implemented without assessing the risk, in this context (of a more complex issue) implementation of food safety controls should be based on the scientific basis of an MRA. Following the evaluation of risk management options, a wide range of food safety measures, including regulatory standards, guidelines and related issues, may be implemented, either alone or in combination. All parties interested in food safety may be involved in implementation. Implementation of risk management involves complex factors in which competing stakeholders including consumers with imcompatible views and values. Public perceptions should be therefore taken into account. The efficiency of implementation depends on the acceptability of the interventions to stakeholders.

Implementation of possible risk management options would be varied according to the particular circumstances, for example:
- Avoid risk by banning the foods, or limit sales of food that show a history of contamination or toxicity under certain condition.
- Reducing exposure (e.g. not eating specific foods)
- Educating consumers (e.g. labelling products, informing consumers of the risk)
- Controlling initial levels of hazards (e.g. assessing the quality of ingredients by using microbiological criteria)
- Preventing an increase in the levels of hazards (e.g. contamination in the food chain)

- Reducing the level of hazards (*e.g.* disinfection, freezing, pasteurisation, irradiation)
- Removing pathogens (*e.g.* washing, ultra-filtration)

## 2.3.4 Risk communication

Risk communication is currently recognised as involving an interactive discussion and exchange of information about risks associated with foodborne hazards between interested parties, including government, agencies, corporations, media, scientist, organisations and individual citizens (HSE, 1998). A remarkable consideration of risk communication is that the target usually varies between from a variety of audiences. Different audiences are likely to have different interests, values, levels of intelligence, education and understanding. Communicators should recognise and overcome gaps in knowledge as well as obstacles inherent in the uncertainties of scientific risk assessment (Bennett, 1999).

The goal of risk communication is to provide meaningful, relevant and accurate information in clear and understandable terms targeted to a specific audience. It may not resolve all differences between parties. However, it may lead to a better understanding of those differences (FAO, 1998). It may also be a guide to more widely understood and accepted risk management decisions (Edwards *et al.*, 2003).

The expert consultation on risk communication of a joint FAO/WHO (FAO, 1998) proposed that the principles of risk communication must be based on follwing factors:
- know the audience
- involve the scientific experts
- establish expertise in communication
- be a credible source of information
- share responsibility
- differentiate between science and value judgement
- assure transparency
- put the risk in perspective

## 2.4 Consumer perceptions on organically produced foods

Consumer attitudes and perceptions are thought to be significant factors influencing consumer's food choice. The attitudes and perceptions are in fact influenced by geography, history, culture, religion, scientific development, fashion and the media (Williams and Hammitt, 2001). Food related health scares have driven consumer concerns, resulting in demands for safer food. Attitudes and perceptions towards foods related to risk have influenced agricultural policies and practices (Raab and Grobe, 2005). The main hazard of concern has often been pesticides. There are few studies referring to the concerns for other hazards, *e.g.* microbial pathogens. Recently, it was found that consumers tend to

underestimate the probability of foodborne illness from common foodborne pathogens, *i.e.* *Salmonella, Campylobacter* (Zehnder *et al.*, 2003).

It was suggested that most consumers believe their perceived food safety risks are justified (Kraus, *et al.*, 1992 and Graham *et al.*, 1999). The most common belief of consumers about organic foods is that they are safer, healthier and more nutritious, better for the environment and kinder to animals. However, organic foods are more expensive than commercially grown products. In a global survey conducted on the internet across 38 countries, it was found that organic products are purchased mainly for perceived health reasons. Europeans seem to be conscious of other possible benefits of organic products (ACNielsen, 2005). The results of this survey are presented in Figures 2.8 and 2.9
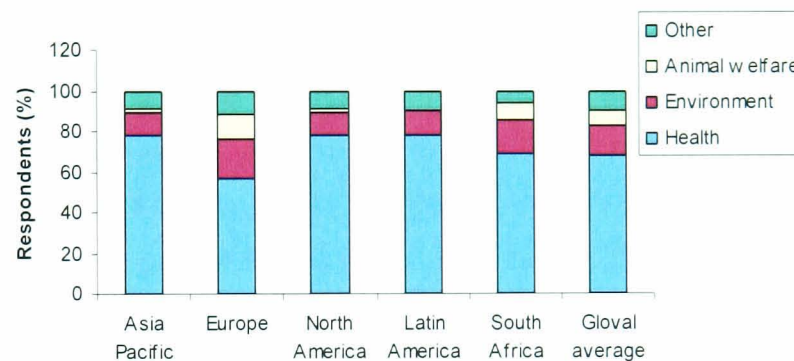


**Figure 2.8** The main reasons for purchasing organic products across the world
(based on data from ACNielsen, 2005)
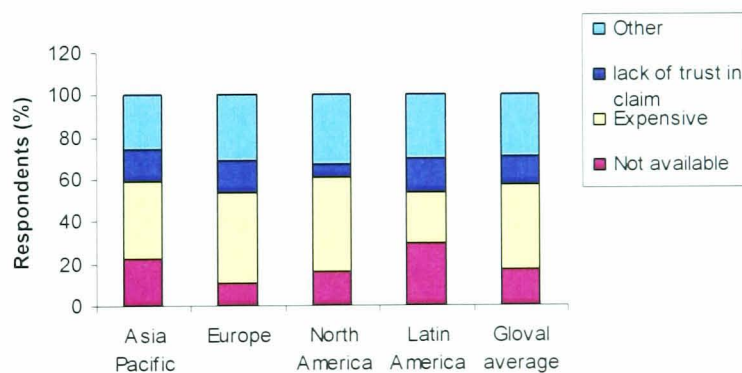


**Figure 2.9** The main reasons for **not** purchasing organic products across the world
(based on data from ACNielsen, 2005)

The choice between organically and conventionally grown products invokes the role of risk perception in decision-making for consumer. Although eating quality becomes more important, the association between diet and health are not well understood, particularly for latent health consequences. Other factors (*e.g.* cost) may play a potential role influencing consumers' food choice as these factors can be demonstrated with hard evidence.

# CHAPTER 3

## *Campylobacter* and Antimicrobial Resistance

## 3.1 Introduction

The genus *Campylobacter* was first identified and named in 1963 by Sebald and Veron (Penner, 1988). The name was derived from the Greek word for a curved rod. Initially, this genus included two species (*C. fetus* and "*C. bubulus*"), which had formally been referred to as *Vibrio* species.

The habitats of *Campylobacter* species vary greatly. Some species appear to be obligated parasites narrowly restricted to one organ or site in the body, whilst others are ubiquitous and are widely distributed in nature (*e.g., C.jejuni*). According to their inability to ferment or oxidise carbohydrate, some isolates would be excluded from this species if the isolates are differentiated following microbiological criteria using general biochemical tests available in the diagnostic laboratory. Therefore, the genetic constitution of the strain has been developed and introduced for the taxonomy of this genus for more than two decades (Penner, 1988)

Following the recognition of the clinical and economic importance of *Campylobacter*, a range of techniques, including molecular techniques such as DNA-rRNA hybridization, 16S ribosomal RNA sequence analysis, and immuno-typing analysis, have been developed for their identification and characterisation. *Campylobacter* species and related taxa belong to the same phylogenetic group named rRNA superfamily VI. The rRNA superfamily VI currently comprises of five genera, which are *Campylobacter, Acrobacter, Helicobacter, Wolinella and Flexispira* (Koneman *et al.,*1997).

At present, the genus contains 16 species and six subspecies of true *Campylobacter* (signified by the partial 16S rRNA sequences). Many species formerly referred to as *Campylobacter* spp. or *Campylobacter*-like organisms have been assigned to other genera, most of which are phylogenetically related.

## 3.2 General characteristics of *Campylobacter*

Member of the genus *Campylobacter* are non-sporeforming, oxidase-positive, gram-negative rods. Actively dividing cells (log-phase cells) are slender, curved or spiral shapes (or S-shaped cells), 0.2 to 0.5 $\mu$m wide and 0.5 to 8.0 $\mu$m long. Some species are predominantly curved or straight rods. Located at one or both ends of the cell are one or more polar or amphitrichous flagella which are highly motile and darting in corkscrew-like fashion, spinning around their long axes and frequently reversing direction (Figure 3.1). This feature may be important in pathogenesis (Adams and Moss, 2000).
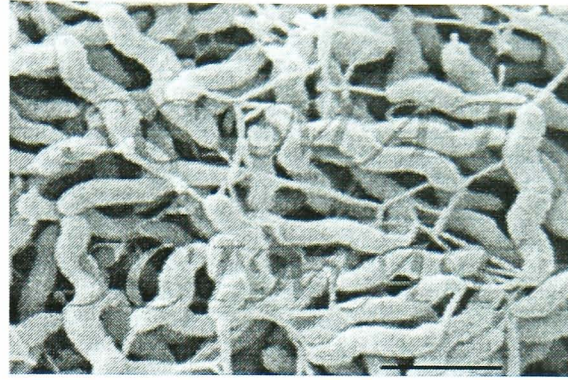
**Figure 3.1** Illustration of *Campylobacter* spp. Bar =1 μm (taken from
http://www.bacteriamuseum.org/species/campylobacter.shtml)

All *Campylobacter* species are unable to ferment or oxidize sugars and usually obtain energy from amino acid or from the tricarboxylic acid cycle (TCA). Typical biochemical reactions include the reduction of fumerate to succinate with indole production reactions. Most species are oxidase positive, however, only *C.jejuni* is hippurate positive. The catalase test is one of few useful tests for differentiating between species. *Campylobacters* are oxygen-sensitive microaerophiles and capnophilic, thus requring atmospheres containing 5-10% oxygen and 3-5% carbon dioxide. The optimal temperature is 37°C, except for *C. jejuni* and *C. coli.* Their optimum growth is at 42°C. They do not grow below 25°C and have poor survival at room temperature. Because of these two conditions, their ability to multiply outside of the animal is severely restricted. Consequently, unlike other foodborne pathogens, they are not normally capable of multiplication in food during processing or storage at temperatures below 25°C (Park, 2002). Although many studies have explored the dormant phase of *Campylobacter* spp., these have focused mainly on *C.jejuni* and *C. coli.* These studies suggested that *Campylobacter* enter a dormant phase under stress conditions. This phase is affected by temperature, oxygenation, moisture, osmolality and microcosm water system (Jones *et al.,* 1991; Lázaro *et al.,* 1999; Hald *et al.,* 2001 and Kam *et al.,* 2001). *C.jejuni* has also shown the ability to tolerate refrigeration and freeze-thawing.

In general, *Campylobacter* species do not grow in conventional aerobic or anaerobic culture systems. Since they are sensitive to hydrogen peroxide and superoxide anions produced in media, lysed animal blood and ferrous sulfate are added to enrichment broths and selective agars in order to neutralise these toxic oxygen products and increase the aerotolerance of the organisms (ICMSF, 1996).

Within the entire genus *Campylobacter, C.jejuni* and *C.coli* are the species most often encountered by medical laboratories responding to gastro-enteritis worldwide (Kothary and Babu, 2001). They jointly account for over 95% of the prevalence of infection in humans, with reported infective doses as low as 500 organisms (Black *et al.,* 1988). Although

infections with *C.upsaliensis, C.lari, C. foetus* and *C.hyointestinalis* may be recognised, they appear to be much less frequent.

It is easy to distinguish *C.jejuni* and *C.coli* from other *Campylobacter* species by their high optimum growth temperature (42°C). *C.jejuni* has two subspecies; subspecies *jejuni* – the familiar cause of enterocolitis in man and subspecies *doylei* – a more fastidious and slower growing organism which does not grow at 43°C. Unlike *C.jejuni*, *C.coli* does not hydrolyse hippurate acid. *C. upsaliensis* is seldom detected by conventional methods used for *C.jejuni* and as for *C.coli*, primarily isolation for this organism usually requires the use of selective filtration, non-selective media and incubation at 37°C. In addition, it requires $H_2$ or formate for the microaerophilic growth (Holt *et al.*, 1994). The differentiation of the most important species of *Campylobacter* is shown in Tables 3.1 and 3.2.

**Table 3.1** Differentiation of *Campylobacter* species related to human disease

| Organism | Biochemical test | | | | | |
|---|---|---|---|---|---|---|
| | Catalase | Nitate | $H_2S_2$ (triple sugar iron) | Urease | Indoxyl acetate | Hippurate hydrolysis |
| *C.coli* | + | + | - | - | + | - |
| *C.concisus* | - | + | + | - | NA | - |
| *C.curvus* | - | + | + | - | + | - |
| *C.fetus* subsp. *fetus* | + | + | - | - | - | - |
| *C.fetus* subsp. *venerealis* | + | + | - | - | - | - |
| *C.gracilis* | - | + | - | - | v | - |
| *C.helveticus* | - | + | - | - | + | - |
| *C.hyoilei* | + | + | + | - | NA | - |
| *C.hyointestinalis* subsp. *hyointestinalis* | + | + | + | - | - | - |
| *C.hyointestinalis* subsp. *lawsonii* | + | + | + | - | - | - |
| *C.jejuni* subsp. *jejuni* | + | + | - | - | + | + |
| *C.jejuni* subsp. *doylei* | v | - | - | - | + | + |
| *C.lari* | + | + | - | - | - | - |
| *C.mucosalis* | - | + | + | - | - | - |
| *C.rectus* | - | + | + | - | + | - |
| *C.showae* | + | + | + | - | + | - |
| *C.upsaliensis* | -(w) | + | - | - | + | - |
| *C.fecalis* | + | + | + | - | - | - |

+, strong reaction; v, 11%-89% of strains are positive ; w, weak reaction; NA, results not available

**Table 3.2** The optimal temperature for growth of *Campylobacter* species

| Organism | Temperature ($^0$C) | | |
|---|---|---|---|
| | 25 | 37 | 42 |
| *C.coli* | - | + | + |
| *C.concisus* | - | + | c |
| *C.curvus* | - | + | + |
| *C.fetus* subsp. *fetus* | + | + | - |
| *C.fetus* subsp. *venerealis* | + | + | - |
| *C.gracilis* | NA | + | NA |
| *C.helveticus* | - | + | + |
| *C.hyoilei* | NA | + | + |
| *C.hyointestinalis* subsp. *hyointestinalis* | v | + | + |
| *C.hyointestinalis* subsp. *lawsonii* | - | + | + |
| *C.jejuni* subsp. *jejuni* | - | + | + |
| *C.jejuni* subsp. *doylei* | - | + | w |
| *C.lari* | c | + | + |
| *C.mucosalis* | - | + | + |
| *C.rectus* | - | + | w |
| *C.showae* | - | + | + |
| *C.upsaliensis* | - | + | + |
| *C.fecalis* | - | + | + |

+, 90% or more of strains are positive; -, 90% or more of strains are negative;v, 11%-89% of strains are positive. w, weak reaction; NA, results not available; c, contradictory reports in literature.

## 3.2.1 Growth condition and survival characteristics

The health risk associated with *Campylobacter* transmitted through the food chain relies on the growth condition and survival of the organism in a food commodity. Usually, *Campylobacter* does not readily grow in a food commodity unless there are the preferable conditions for growth. Despite this, during slaughtering and preparation of raw birds, a high percentage of poultry carcasses may become contaminated with the *Campylobacter*. In addition, Zhao *et al.* (2001) found that rates of cross-contamination were significantly different for each of the store chains of various supermarkets although all of the chains sold the same products. Thereby, pre and post-processing treatments entailing a variety of conditions can affect the survival of *Campylobacter*.

A number of studies have attempted to demonstrate the survival of *Campylobacter*, especially, *C. jejuni* (Storz *et al.*, 1990; Pesci *et al.*, 1994; Wosten *et al.*, 1998 and Day *et al.*, 2000). Black *et al.*, (1988) and Babakhani *et al.* (1993) demonstrated that under the electron microscope organisms reside within epithelial cells lining the gut lumen as well as granulocytes and parenchymal cells located within the lamina propria. These provide the

fastidious, asaccharolytic, slow-growing organism an unoccupied niche where microbial competition is relaxed or nonexistent. Nowadays, the mechanism of how this organism is able to persist in the undesirable conditions and biological and environmental factors that affect pathogen occurrence and survival are still poorly understood (Day et al., 2000). However, the survival rate of Campylobacter is thought to be affected by sensitivity to atmospheric conditions, stress during transport, processing, storage, water activity and competitive organisms (Jones et al., 1991).

## Temperature

The temperature for optimal growth for Campylobacter is $42^{0}C$ (Table 3.3). In general, it appears that Campylobacter is comparatively slow growing (Lake et. al., 2003) and it does not grow under refrigeration conditions. This indicates that Campylobacter does not multiply during slaughtering, post processing, transport and refrigeration. However, at low temperatures (i.e., in refrigerator or freezer) these organisms may be transformed to viable-but non-culturable (VNC) cells which can survive for a prolonged period (Hazeleger et al., 1994). Lake et al.(2003) have found that the survival of Campylobacter in food kept under a condition of refrigeration is better than when kept at room temperature (up to 15 times at $2^{o}C$ longer than at $20^{o}C$) due to oxygen concentration.

Table 3.3. Growth characteristics of Campylobacter (ICMSF, 1996)

| Condition | Minimum | Optimum | Maximum |
|-----------|---------|---------|---------|
| Temperature($^{0}$C) | 32 | 42-43 | 45 |
| pH | 4.9 | 6.5-7.5 | ca.9.0 |
| NaCl(%) | - | 0.5 | 1.5 |
| Water activity($a_w$) | $\geq 0.987$ | 0.997 | - |
| Atmosphere | | $5\%O_2 + 10\%CO_2$ | |

Refrigeration does reduce viable numbers of these organisms on chicken or red meats, but the decrease is not predictable (Kinde et al., 1983 and Koidis and Doyle, 1983). Under these conditions, Campylobacter does not multiply and the numbers decrease depending on the length of storage. Campylobacter is sensitive to freezing with numbers declining faster at the initial reduction phase followed by a slower reduction during long-term storage.

In addition, these bacteria can survive up to an hour on hands and moist surfaces at room temperature. Christensen et al. (2001) summarised the effect of storage temperatures on the number of Campylobacter in chicken products as shown in Table 3.4. C.jejuni and C.coli are sensitive to heat and do not survive cooking or pasteurisation temperatures (D-value are 0.21-2.25 minutes at $55$-$60^{0}C$; ICMFS, 1996). However, Bryan and Doyle (1995) indicated that if the cooking temperature is less than $74^{0}C$, e.g., microwave application, the organisms may survive.

**Table 3.4** Effect of storage temperature on *Campylobacter* in chicken products (adopted from Christensen *et al.*, 2001)

| Chicken product | Storage temp.($^0$C) | Initial Decrease (log cfu/day) | Total Decrease (log cfu/day) | Reference |
|---|---|---|---|---|
| Carcass | -20 | -0.1-1.4/21 | -0.5-2.3/84 | Hänninen, 1981 |
|  | -20 | -0.5/36 | -1.4/64 | Oosterom *et al.*, 1983 |
|  | 4 | -0.6-1/4-7 | - | Oosterom *et al.*, 1983 |
| Drip | -20 | -0.1-1.1/21 | -0.6-2.5/84 | Hänninen, 1981 |
| Drumsticks | -20 | -1.4/7 | -2.7/182 | Yogasundram *et al.*, 1989 |
|  | 4 | -0.7/7 |  |  |
| Breast skin | -20 | -2.4/3 | Ca.-3.7/56 | Lee *et al.*, 1998 |
|  | 4 | 1.4/3 |  | Lee *et al.*, 1998 |
| Breast (meat) | 2 | - | -5-6/24 | Curtis *et al.*, 1995 |
|  | 10 | - | -5-6/13 | Curtis *et al.*, 1995 |

<u>pH</u>:

*Campylobacter* is inactivated at pH below 5.0 and above 9.0. The optimum is 6.5 to 7.5. Bhaduri and Cottrell (2004) found that the pH in ground chicken and chicken skin were 5.96 and 6.41.

## Atmosphere

*Campylobacter* requires a micro-aerobic condition, with oxygen levels less than 5%. However, some isolates can adapte to aerobic conditions, *e.g., C.jejuni*.

## Water activity ($a_w$)

Water activity ($a_w$) is the ratio of the partial vapour pressure of water in equilibrium with the food. This is a physical property that has a direct implication on microbiological safety of food (Beuchat, 1981 and Fontana, 2000). Basically, water activity is controlled by drying, addition of salt (NaCl) or sugar and freezing (Fontana, 2000). The optimal growth conditions for *Campylobacter* is at $a_w$ approximately 0.997 ($\cong$ 0.5% of NaCl). *Campylobacter* does not grow when the $a_w$ is less than 0.98 (salt concentration above 1.5%), thus *Campylobacter* is particularly sensitive to drying conditions. In food processing and packaging (plastic overwraps) methods are generally selected to retain water activity and temperature (water activity is dependent on temperature), thus controlling microbiological growth and the quality of the food product.

## 3.2.2 The role of viable non-culturable stages (VNC)

It is believed that *Campylobacter* can be resistant to four critical stresses, including temperature, oxidative stress, osmotic change and starvation. Although, *Campylobacter* species appear to lack many adaptive responses, some species, *e.g. C.jejuni*, can mount adaptive responses to both acidic and aerobic conditions. The organisms are thought to

transform themselves into a dormant phase, *i.e.*, viable-Non-Culturable state (VNC). In this phase, they are capable of survival without replication in conventional media, whilst maintaining their metabolic activity (Rollins, 1986 and McKay, 1992). The VNC cells cannot be detected by standard culture methods. However, under re-enrichment conditions they may revert to full viability. There is still a controversy over whether to consider the VNC stage as a degeneration form or a dormant phase. Manning *et al.* (2001) reviewed results of genotyping from several studies and proposed that the VNC stage may be a consequence of both natural competence and genomic re-arrangements of *Campylobacter* to overcome the undesirable conditions. This may be the missing explanation of how this organism maintains its diversity and ability to survive in a wide range of habitats. However, the mechanism of this instability is unknown.

A number of studies have demonstrated the viability and VNC phase of *C.jejuni* during low temperature storage. Jones *et al.* (1991) and Solow *et al.* (2003) found that when suspended in microcosm system and incubated at 4°C, various strains of *C.jejuni* enter a VNC phase after 18-28 days. Lázaro *et al.* (1999) detected the changes in cell morphology of *C. jejuni* from spiral to coccoid from. *C. jejuni* cells at late lag phrase were spiral with the length of ca. 1.4859 µm. After starvation for 7 months, the length of the cells decreased to ca. 1.2409 µm at 4°C (Figure 3.2B-E) and ca. 1.2925 µm at 20°C.
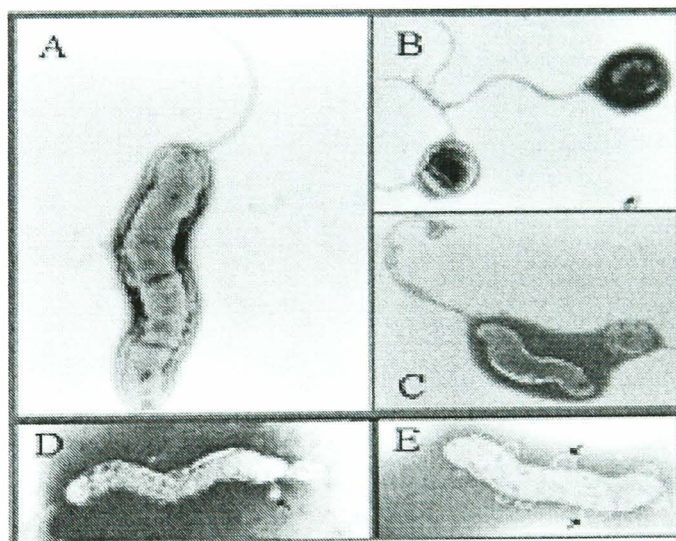


Figure 3.2 Different morphologies of *C.jejuni* cells at late lag phase and after starvation for 7 months: A) Typical spiral cell at late lag phrase; B to E) Transformation of rod shape to coccoid shape after starvation for 7 months (taken from Lázaro *et al.*, 1999)

Tholozan *et al.* (1999) described that ability to enter a VNC stage depends on the strain as well. So far, a number of studies have indicated that a predominantly coccoid morphology, thought to be the beginning of a VNC phase, is associated with exposure to oxygen, change in temperature, and starvation. Hazeleger *et al.*(1998) found that at different storage temperatures, *C.jejuni* strains became non-culturable after about 1 week at 25°C, 3 weeks at 12°C, 6 weeks at 4°C. Reezal *et al.* (1998) postulated that at lower temperature, in particular

at 4°C, the osmolarlity of media has a synergistic effect on the change of morphology of *C. jejuni* from a rod shape to a coccoid shape

The concurrence of culturability and infectivity of VNC was also further discussed by Jones *et al.* (1991) and Sutcliffe *et al.* (1991). Although *C.jejuni* became a coccal form after exposure to water, their antigen remained detectable for several months after they were no longer culturable. In addition, after storing the culture for a minimum of 2 weeks, under the electron microscope most of coccoid forms also showed degenerative change but a small proportion did retain structural integrity and had the appearance associated with viability. After resuscitation of VNC under laboratory conditions, *C.jejuni* strains were found to multiply, but their infectivity weakened and terminated (Hald *et al.*, 2001).

## 3.3 Pathogenicity

Currently, the real mechanism of how the virulence of *Campylobacter* affects the human body is not clear. Prevention and treatment of the infection essentially requires understanding of its nature, regulation and mechanism. Hence, several hypotheses have been proposed on what are the possible determinants of pathogenicity (Tauxe,1987; Black *et al.*, 1988 and Blaser *et al.*, 1997).

Two types of diarrhoea occurring in humans due to *C.jejuni* infection are: i) inflammatory diarrhoea with fever and slimy bloody faeces containing leukocytes and ii) non-inflammatory diarrhoea with watery faeces without blood cells. The indication is that the virulence of *Campylobacter* spp. (*e.g. C.jejuni*) involves both host and pathogenic specific factors. By considering the pathophysiology of *Campylobacter* enteritis, four major virulence factors were presumptive including motility, adherence, invasion and toxin production (Walker *et al.*,1986). Wooldridge and Ketly (1997) also suggested that possible determinants of pathogenicity involved in the induction of campylobacteriosis, diarrhoea in particular, include chemotaxis, motility and flagella, which are required for attachment and colonisation of the gut epithelium. As soon as the colonisation occurs, other possible consequences would go toward iron acquisition, host cell invasion, toxin production, inflammation and active secretion and epithelial disruption with leakage of fluid. However, the mechanisms of host cell invasion remains poorly understood. Flagella mediated motility may be a major influencing factor on the change of cell membrane of host cells or the internal cytoskeletal structure (Wooldridge *et al.*, 1997 and Hanel *et al.*, 1998). It appears that different hosts react differently to invasion, with fluid secretion being dependent on the extent of the host response and degree of epithelial damage.

Wassenaar (1997) summarised the four virulence properties of *Campylobacter* as follows: (i) Motility plays a crucial role in pathogenesis of *C.jejuni*. The flagella characteristic is thought to be the main factor for the attachment sites and penetration into the intestinal cells; (ii)

Adherence of the organisms to the epithelial surface may be important for the colonisation and increase of the local concentration of secreted bacterial products. However, the specific adhesions on the flagella or the body have not been identified; (iii) Following adhesion and penetration, bacteria invade the host cells and multiply. Prevented by the intracellular immune system, invasion levels are however normally low and (iv) Toxins have been considered as a crucial factor for pathogenesis.

The function and role of toxins are not however fully elucidated. It was reported that *C.jejuni* and *C.coli* produce a number of different toxin activities. These include both enterotoxins (defined as secreted proteins able to bind to a cellular receptor, entering the cell and elevating cyclic AMP levels) and cytotoxins (defined as proteins that kill target cells). Gilbert and Slavik (2004) suggested that whilst toxicity may be a primary determinant of *C.jejuni* pathogenicity, *C.jejuni* found in the food chain may not contribute to a toxicity level high enough to cause disease in humans. In an undesirable environment, *C.jejuni* rather produces the proteins enhancing its ability to survive and may play a role of cytotoxicity, many of which are similar to those found in some other bacteria. Only one of these toxins was genetically defined, cytolethal distending toxin (CDT). Moreover, the role of this cytotoxin on foodborne illness has not been fully elucidated (Eyigor *et al.,*1999).

## 3.4 Human campylobacteriosis

### 3.4.1 Acute illness

*Campylobacter* infection displays a broad range of clinical symptoms. These can be mild, self-limiting, non-inflammatory diarrhoea or severe inflammatory bloody and polymorphonuclear leukocytes with severe cramping and fever (Blaser *et al.,* 1979). It is hard to distinguish *Campylobacter* infection from gastro-enteritis caused by other enteric pathogens. In young children, under 5 years old and immunocompromised hosts (*i.e.* elderly people, HIV-positive patients), the illness may exhibit bacteraemia and relapsing or unremitting symptoms.

The incubation period of *Campylobacter* infection may vary from one day to two weeks, typically 1-3 days. The main symptoms are malaise, fever, abdominal pain, abdominal cramp and diarrhoea with or without blood. Commonly, the diarrhoea is self-limiting and may persist for up to a week. However, Allos and Blaser (1995) showed that 20% of symptoms may last from one to three weeks and the organism may be found in faeces for up to 2-3 weeks.

The symptoms of *Campylobacter* infection may also vary in different populations. This may be due to the immune response and the exposure dose. In developed countries, where infection is relatively rare, Its manifestations may be more severe, such as inflammatory diarrhoea containing blood and polymorphonuclear leukocytes with severe cramping and

fever (Blaser *et al.,* 1979). *Campylobacter* infections are seasonal, peaking in late summer and autumn. Sporadic illness associated with food consumption or waterborne exposures has been reported.

By comparison, campylobacteriosis appears to be endemic and non-seasonal in developing countries. The symptoms are typically watery, non-inflammatory, relatively mild diarrhoea. Infection rates are typically highest during the first two years of life and decline with age. There is also attenuation of the symptoms and reduction in convalescent phase of bacterial excretion with increasing age. Convalescent excretion occurred for $14\pm2$ days in Thai children less than 1 year and for $8\pm2$ days in children between 1-5 years (Taylor, 1992).

## 3.4.2 Long term sequelae

Symptoms of campylobacteriosis are usually self-limiting and are usually resolved within a period of 3-10 days. Nonetheless, late complications may occur in a person who is vulnerable, for example, elderly people. These include Guillain-Barré syndrome (an acute peripheral neuropathy), hemolytic uremic syndrome, pancreatitis and reactive arthritis. Among these complications, Guillain-Barré syndrome is the most serious.

Guillain-Barré syndrome (GBS) is an acute, bilateral, ascending paralysis developing typically 1-3 weeks following the onset of diarrhoea. GBS is thought to be associated with *C. jejuni* infection. Several studies determined the prevalence of *C.jejuni* infection leading to GBS, ranging from 15%-66%. Specific *C.jejuni* serotypes, O:19 and O: 41, have been implicated in the development of GBS in certain setting (Kuroki *et al.,* 1993; Mishu *et al,* 1993; Rees *et al,* 1995; Lastovica *et al.,* 1997 and Allos *et al.,* 1998). Post infection arthropathies may occur within two weeks following the onset of diarrhoea. The symptoms appear in more than one joint, commonly knees, ankles, wrists and lower back.

In general, the mortality rate of campylobacteriosis is low and most cases of mortality occur among infants, elderly and immuno-supressive individuals (Tauxe, 1992; Altekruse *et al.,* 1999). Philips (1995) reported that in England and Wales fewer than 10 deaths out of approximately 280,000 cases (<0.0036%) have been reported from 1981 to 1991.

## 3.4.3 Epidemiology

Although *Campylobacter* is a zoonotic organism, colonising the gastrointestinal tract of warm blooded animals, its infection is recognised to date as the most common enteric pathogen caused human illness (Barton, 2000; Threfall *et al.,* 2000; Randall *et al.,* 2003 and Ridley and Newell, 2004). The epidemiology of *Campylobacter* is still however poorly characterised. This is mainly due to the lack of routine microbiological characterisation. In addition, the symptoms of the infection are self-limiting, and thus patients infected with these organisms may not be seen in General Practice. The true population burden must be therefore greater

than that given by national surveillance programmes. Furthermore, surveillance does not exist in some regions or countries.

Oberhelman *et al.*(2000) and Coker, *et al.*(2002) found that despite the lack of national surveillance programmes for campylobacteriosis in most developing countries, *Campylobacter* is the most frequently isolated bacteria from faeces of children, in particular, under 5 years old. Most estimates of the incidence in developing countries are from laboratory-based surveillance of pathogens responsible for diarrhoea. The incidence ranges from 5-20% (Oberhelman *et al.*, 2000). Based on case-control community-based studies, the incidence was 40,000 to 60,000 per 100,000 for children aged less than 5 years and 90/100,000 for the general population. Coker, *et al.*(2002) summarised the isolation rate of *Campylobacter* in faeces which is shown in Table 3.5

Table 3.5 Isolation rate of *Campylobacter* in faeces from diarrhoeal patients, age <5 years, in selected developing countries (taken from Coker, *et al.*2002)

| WHO region and country | Isolation rate (%) |
|---|---|
| **Africa** | |
| - Algeria | 17.7 |
| - Cameroon | 7.7 |
| - Ethiopia | 13.8 |
| - Nigeria | 16.5 |
| - Tanzania | 18.0 |
| - Zimbabwe | 9.3 |
| **Americas** | |
| - Brazil | 9.9 |
| - Guatemala | 12.1 |
| **Eastern Mediterranean** | |
| - Egypt | 9.0 |
| - Jordan | 5.5 |
| **Southeast Asia** | |
| - Bangladesh | 17.4 |
| - Thailand | 13.0 |
| - Laos | 12.1 |

In developed countries, such as in Europe and the United States, epidemiological data have indicated that the incidence of campylobacteriosis has increased steadily. Some European and North America countries have established surveillance programmes, *e.g.,* Euro-surveillance, FoodNet (U.S.A), National Notifiable Diseases Summary program (NNDS, Canada) and Campylobacter Sentinel Surveillance Scheme (UK). These show that the incidence rate varies from country to country. For example, the Netherlands reported 36 per 100,000 between 1997 and 2001 (Van Pelt *et al.*, 2003), with 54.7-65.8/100,000 in Denmark (1997-2003) and 30/100,000 in Sweden. In the United State, it is estimated to be

880/100,000, nearly twice as high as the rate of Salmonella. In several countries it is still unclear whether this observed rise could be attributed to a real increase in incidence, or to an improvement in diagnosis, or to both. Most reported cases of campylobacteriosis occur sporadically, as single cases, or small family outbreaks, and are generally caused by *Campylobacter jejuni*. This may be because there is no international standard isolation method for *Campylobacter*. It is difficult to compare the isolation rate between countries or regions.

In the UK, *Campylobacter* are the most commonly reported cases of infectious intestinal disease (IID) and most cases are sporadic. Ribeiro and Frost (2000) estimated that *Campylobacter* causes IID in 8.7 cases per 1000 person years compared with a rate for all IID of 194 cases/1000 person years.

Adak *et al.*(2002) reported that in 2000 there were 1,388,772 cases of indigenous foodborne disease (IFD) in England and Wales. *Campylobacter* was responsible for 26.85% of IFD cases. Of all cases of campylobacteriosis, 47.62% cases were seen in General Practice (GP), 4.7% cases required hospital admission and 0.0002% were fatal. There has been a steady annual increase in the number of reported infections since surveillance began in 1977 (Figure 3.3). Latest published data for 2004 for laboratory reports of faecal isolates of *Campylobacter* spp. in England and Wales are shown in Figure 3.4.
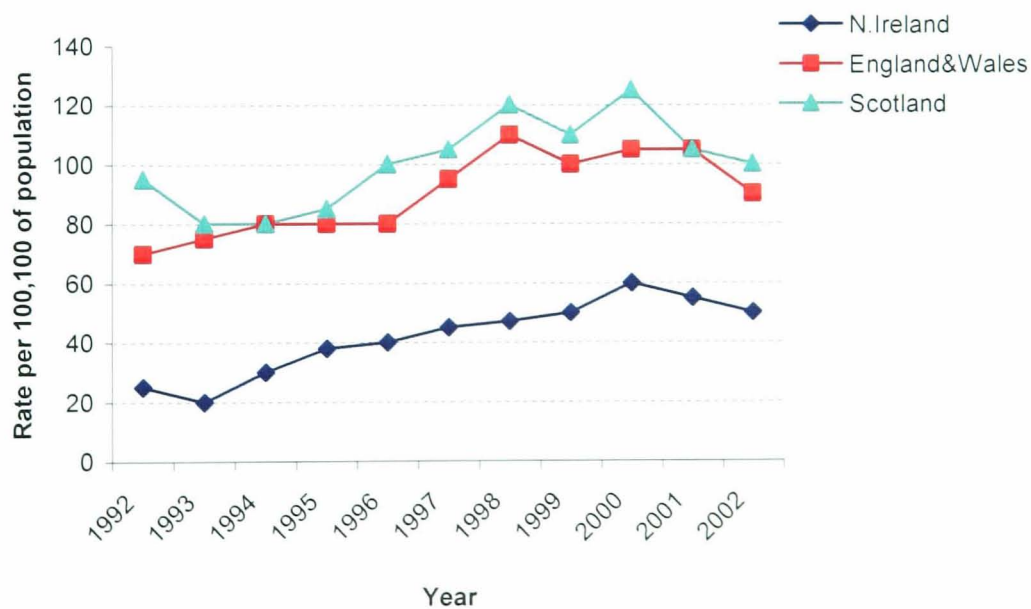


**Figure 3.3** Laboratory reports of *Campylobacter* infection in the UK (source: UK DEFRA, 2002)
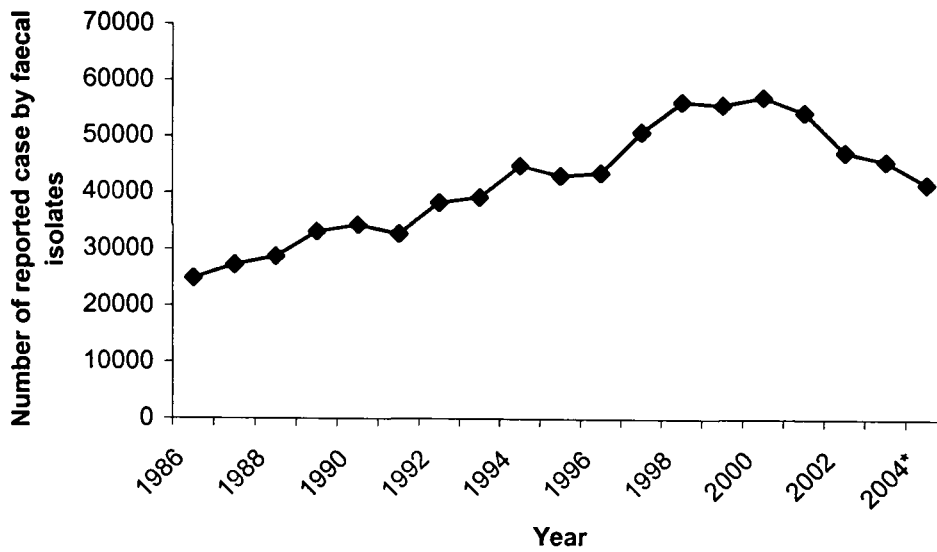
**Figure 3.4** Numbers of reported case of *Campylobacter* spp. by faecal isolates England & Wales, 1986-2004 (source: UK-HPA, 2004)

Buzby and Roberts (1997) estimated that the economic burden due to *Campylobacter* infection in the United States was around US$ 4.3 billion per year. In the UK the average cost of a case of acute *Campylobacter* infection was estimated to be £315. Therefore, *Campylobacter* infections cost the nation over £113 million in 2000 (ACMSF, 2004). As reported by Gillespie *et al.* (2002), the true incidence of campylobacteriosis is however thought to be much higher than that shown in the national report. There may be eight cases unrecognised for every laboratory-confirmed report by national surveillance. Following this assumption, the true annual estimate cost must be higher than £113 million.

The study carried out by ACMSF (2004) found the highest age-specific incidence rate in children under the age of 5 years, with a secondary peak in young adults. In addition, this report also showed that the epidemiology of human *Campylobacter* infection shows a consistent seasonal pattern with the peak in the spring and late summer and a lower rate in the winter.

## 3.5 Sources of *Campylobacter* species

### 3.5.1 Chicken as a major source of *Campylobacter*

Although *Campylobacter* was identified as early as the 1970s, its isolation rate is thought to be underestimated worldwide. This is due to the fastidious nature of these bacteria, especially in food samples, which requires specific media and atmospheric conditions for detection. For the last two decades, following the rise in public concern about infections related to food consumption, several studies have been established to characterise the true reservoirs of these bacteria. Broiler chickens, milk, water and contact with pets and farm animals were identified as sources of infection (Table 3.6). A recent report of a joint

FAO/World Health Organisation (WHO, 2002c) indicated that there is linear relationship between flock prevalence and the probability of human campylobacteriosis.

**Table 3.6** *Campylobacter* species and their host (adapted from Wesley, 1998)

| Species | Host distribution | |
| --- | --- | --- |
| | **Livestock** | **Human** |
| *Campylobacter jejuni\** | Poultry, cattle, pigs and sheep | enteritis |
| *Campylobacter coli\** | Pigs, turkey and cattle | Enteritis |
| *Campylobacter fetus* | Cattle and sheep | Enteritis |
| *Campylobacter concisus* | NA | Periodontal disease |
| *Campylobacter curvus* | NA | Periodontal disease |
| *Campylobacter gracilis* | NA | Head, neck infection |
| *Campylobacter helveticus* | Domestic pets | Enteritis |
| *Campylobacter hyointestinalis* | Pigs, cattle and guinea pigs | Enteritis |
| *Campylobacter lari* | Birds, dogs | Enteritis |
| *Campylobacter mucosalis* | Cattle, pigs | Enteritis |
| *Campylobacter rectus* | NA | Periodontal disease |
| *Campylobacter showae* | NA | Periodontal disease |
| *Campylobacter upsaliensis* | Domestic pets | enteritis |

\*Note *C.jejuni* is found mainly in poultry and *C.coli* is found in pig and turkey
NA = not available

The principal habitat of this foodborne pathogen is, in fact, the alimentary tract of warm-blooded animal. *Campylobacter* is found in cattle, pigs, sheep, dogs and wild animals and birds. Water also plays an important part of the ecology of *Campylobacter*. *Campylobacter* may enter the environment, including drinking water, or be transmitted from host to host through the faecal-oral route. *Campylobacter* has been isolated from surface water, rivers and lakes at prevalences up to 50% (Bolton *et al.*, 1987; Carter *et al.*, 1987; Brennhoved *et al.*, 1992 and Arvanitidou *et al.*, 1995). In addition, 45% of sand samples from bathing beaches contained *Campylobacter* (Bolton *et al.*, 1999a). *Campylobacters* can also be found in dogs and cats. They are therefore considered as zoonoses, infectious agents transmitted to humans from animals.

The prevalence of *Campylobacter* infection in retail chicken is evidently associated with campylobacteriosis in human. The UK recent survey report documented by the Food Standards Agency (2003b) showed that the overall frequency of *Campylobacter* contamination in retail chicken, UK-chicken, was 50%. However, when considering different parts of the UK, England, Wales, Scotland and Northern Ireland, higher isolation rates were found in Northern Ireland (77%) and Scotland (75%). The frequency of contamination of fresh chicken (56%) was higher than for frozen chicken (31%). Whole chickens were more likely to be contaminated (57%) than portions (46%). Although control of *Campylobacter*

contamination in chicken was implemented throughout the whole production chain in the Netherlands, more than 30% of all retail chicken was found to be contaminated with this organism (Katsma et al., 2005).

## 3.5.2 Sources of colonisation in chicken

A number of studies carried out in many countries agree on the importance of a variety routes of infection by *Campylobacter* in poultry production. These routes are contaminated water, vertical transmission from parent flocks, contaminated feed, carry-over from a previous flock, domestic and wild animals, contaminated transport (crates, vehicles), personnel at flock thinning, feed withdrawal and the external environment.

*Flock colonisation and prevalence*

Although very young chicks (one-day and seven-day old) were found negative to *Campylobacter*, broiler chicks can be colonised by *C.jejuni* at 3-4 weeks of age (Berndtson *et al.,* 1992). Unlike *Salmonella* spp., this late colonisation may be a result of early protection from maternal anti-*Campylobacter* antibodies or the presence of bacterial flora antagonistic to *Campylobacter* (Pearson *et al.,* 1993 and Sahin *et al.,* 2001). In addition, several studies support the notion that *Campylobacter* is not transmitted vertically (from parents to chicks). *C.jejuni,* was also found not to contaminate the contents of uncracked eggs (Jones *et al.,*1991; Jacobs-Reitsma *et al,* 2001; and Saleha, 2004).

In poultry, once *Campylobacter* colonises the mucous overlying the epithelial cells in the caeca and small intestine, with the doses as low as 40 cfu, it can rapidly reach high numbers in the caecal content as high as $10^7$-$10^9$ cfu without apparent symptoms (Newell and Fearnley, 2003; and Stern and Robach, 2003). The potential for colonisation is at least 1000-fold in most strains and up to 10,000-fold in some strains. Faecal shedding is therefore presumed to be an important factor in the spreading of organisms around the flocks or the environment.

The proportion of positive-*Campylobacter* broiler flocks varies between countries and regions. In Europe, the prevalence ranges from 18% to >90%, with the northernmost parts having lower rate than southern European countries. In the United States, it appears that nearly 90% of flocks are colonised (Stern and Robach, 2003). Furthermore, there is a seasonal variation in the prevalence of flock colonisation. For example, the rate of infection in summer is higher than in winter. Variation in excretion rates has been also identified. Patrick *et al.* (2004) suggested that limiting bird-to-bird contact or climate changes influences colonisation. Longitudinal studies in the UK have indicated that infection is unpredictable from the previous status of the flocks. The negative flocks often become positive and positive flock can occur even in newly constructed broiler houses (Gregory, *et al.* 1997).

## Feed and litter

Since dryness is unsuitable condition for growth, dry feed or feed additive and fresh litter are not potential sources of infection (Newell and Fearnley. 2003).

## Contaminated water

Pearson *et al.*(1996) demonstrated that *Campylobacter* was isolated from water lines and reservoirs of broiler houses. This suggests that this organism can survive in water. Water contamination usually follows flock colonisation, suggesting that this is a consequence of the tracking up through the water lines of organisms excreted from the birds. In addition, viable non-culturable *Campylobacter* may be responsible for the initial colonisation (ACMSF, 2004).

In broiler houses, drinking water provided by the bell drinkers may also be a vehicle for horizontal transmission from a positive *Campylobacter* bird to others within the houses. A number of studies showed that *Campylobacter* can be part of biofilms found in the water systems of broiler houses (Stern *et al.*,2002; Trachoo and Frank, 2002 and Joshua *et al.*,2006). However, there has been considered debate as to the effect of water-related environmental stresses on the infectivity of *Campylobacter.* Fearnley *et al.*(1998) suggested that the colonisation potential of culturable of *C.jejuni* for chicks is severely compromised by long- term exposure to water. The levels of chlorination in potable water would normally be considered lethal to *C.jejuni.* It appears that water-borne protozoa, for example, *Tetrahymena pyriformis*, may be likely to be reservoirs for *C.jejuni* in water system of broiler houses (Jacobs-Reitsma *et al.*, 2001).

## Domestic and wild animals

As most warm-blooded animals are suitable reservoirs for *Campylobacter*, wild animals are also presumed to be an indirect source of flock colonisation, resulting in contamination through the environment. Evidently, farms with mixed animal species may increase flock infection due to the movement of farm staff. This would transmit the bacteria from wild birds, cattle, sheep or pigs to chickens. Similarly, cats and dogs are also frequently *Campylobacter*-positive. Houseflies have also found to be a source of *C.jejuni* (Shane *et al.*, 1985).

## Contamination during transport at flock thinning

It is suggested that during the thinning process, removing a cohort of birds approximately 5 weeks of age for slaughter, using contaminated crates or vehicles, or gloves and clothing of workers, may introduce *Campylobacter* to negative flocks (ACMSF, 2004). During catching, loading and transport to the processing plant, crate surfaces and lorry decks become contaminated with faeces. Due to budgetary constrains, transport crates are used repeatedly.

In addition, inadequate crate washing results in leaving the washer contaminated with *Campylobacter.*

### Human passage and activities on farm

Farm staff may carry *Campylobacter*-positive faeces around the bird houses to the outside or from the external environment to the houses through boots, external clothes and equipment. The risk of having positive flocks is greater when staff have been tending other food animals prior to entering broiler houses. *C.jejuni* can be recovered from both standing water and soils. Hiett *et al.*(2002) showed that the genotyping of isolates from, particularly, standing water recovered before the flock became positive had the same pattern as those subsequently recovered from the broiler flocks.

# 3.6 Antimicrobial resistance in *Campylobacter*

## 3.6.1 Antimicrobial action on micro-organism

Antimicrobial agents used in the treatment of infectious disease are divided into two groups, which are: i) antibiotics, which are natural substances produced by certain groups of micro-organisms, and ii) chemotherapeutic agents, which are chemically synthesized (McManus, 1997). A hybrid substance is a semi-synthetic antibiotic produced by the microbe that is subsequently modified by the chemist to achieve desired properties.

During infection, bacteria can grow and multiply repeatedly to increase numbers, damaging the host. Antimicrobial action of agents interferes with specific processes of the bacterial cell, which synthesize the essential bio-molecules for growth or division. Antimicrobial agents have various levels of effectiveness against micro-organisms which depends on the main mode of action on the targets in the microbial systems. These modes of action include effects on cell growth/division. These effects are expressed by the following mechanisms: inhibition of cell wall synthesis, inhibition of nucleic acid replication, inhibition of protein synthesis and inhibition of synthesis of essential metabolites (Neu *et al*, 1996).

### Inhibition of bacterial cell wall synthesis

Antimicrobial agents in this group distress the peptidoglycan layer of the cell wall of both gram positive and gram negative organisms. This layer is essential for the survival of bacteria in hypotonic environments. Without this layer, the bacterial cells can be destroyed or damaged, resulting in death.

### Interference of the function of cytoplasmic membrane

As the cytoplasmic membrane composing of lipid, protein and lipoprotein is a diffusion barrier for water, ions, nutrients and transport system, specific antimicrobial agents can cause disorganisation of the membrane, interfering with the function of the bacterial cell.

### Inhibition of nucleic acid synthesis

Antimicrobial agents can inhibit nucleic acid synthesis at different levels. They can: i) inhibit nucleotide synthesis or interconversion, ii) impair the template function of DNA and iii) interfere with the polymerases involved in the replication and transcription of DNA (McManus, 1997).

Quinolones (such as nalidixic acid) interfere with the replication and transcription of DNA by binding to the cleavage DNA gyrase[3] and topoisomerase I. This action causes a detrimental effect on the normal DNA replication process, resulting in death of bacterial cells. Fluoroquinolones is a new derivative group that can interact with DNA gyrase and process a broad spectrum of antimicrobial activity. This new group includes ciprofloxacin, norfloxacin and offloxacin.

### Inhibition of protein synthesis

Macrolides (*e.g.* erythromycin), a large lactone ring compound, can bind the 50S ribosomal subunit and thus impair a peptidyltransferase reaction or translocation or both. These can inhibit protein synthesis.

### Inhibition of synthesis of essential metabolites

Antimicrobial agents in this group interfere with metabolism of essential compounds, *e.g.* tetrahydrofolate, lipids.

## 3.6.2 The development of antimicrobial resistance in pathogenic bacteria

Recent data from European countries suggest that the emergence of antimicrobial resistance in *Campylobacter* is related to antimicrobial use in animal. When an animal is administered with certain antimicrobials, a selective pressure is applied on the bacteria. Some of them have the ability to resist antimicrobials and to express resistance genes. The development of antimicrobial resistant food-borne pathogens would take place in the gut of animals and they may be transferred to other bacteria. Lyons *et al.* (1980) and Marshall *et al.*

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[3] DNA gyrase is essential for relieving torsional strain during replication of circular chromosomes in bacteria.

(1990) stated that intestinal bacterial strains, carrying the resistance genes, are capable of natural transmission to humans. This may affect human health directly, particularly for people who are vulnerable. The United Kingdom set up a Joint Committee on the use of Antimicrobials in Animal Husbandry and Veterinary Medicine. As a result of the Committee's recommendations, the principle of using different antimicrobials for therapy or growth promotion became established in the EU. Recently, the World Health Organization set up *ad hoc* committees to investigate the potential impact on human health from the use of antimicrobials in food-producing animals (WHO, 2004).

The development of antimicrobial resistance amongst pathogenic bacteria has emerged as a public concern. In particular, it is assumed to be associated with foodborne pathogens. Scientists initiated research on the association between the use and overuse of antimicrobials and the development of resistance. It was estimated that approximately 50% of antimicrobial agents released into the biosphere during the last 50 years are used in veterinary practice and agriculture (Mazel and Davies, 1999; Teuber, 2001 and Kümmerer, 2003). Antimicrobial resistant bacteria have been found in farm animals where antimicrobials were heavily used (Smith *et al.*, 2003). Follet (2000) proposed that almost half of total consumption of antimicrobials in the EU is by humans. Collectively, widespread emergence of genes expressing resistance to antimicrobials and selection of new resistant strains occurred only after the agents become widely used in humans and for animals.

## 3.6.3 Antimicrobial use in poultry production

The European Agency for the Evaluation of Medicinal Products (EMEA, 1999) concluded that due to the emergence of multiple antimicrobial resistances there was a need for the establishment of a Joint Committee on the use of Antimicrobials in Animal Husbandry and Veterinary Medicine. The committee stated that these hazards could largely be avoided and recommended that antimicrobials available without prescription in animal feed should be of economic value in livestock production. There should have been, however, little or no application as therapeutic agents in either man or animals and should not be able to impair the efficacy of prescribed therapeutic drugs through the development of resistant strains of the organism.

Antimicrobial agents have been used in livestock and poultry since the early 1950s to treat infections and improve growth (increase rate of weight gain) and feed efficiency. The use of antimicrobials to treat and control disease (using a subtherapeutic concentration) in both farm animals and domestic pets has contributed to improvements in animal health and welfare and to the marked increase in productivity of livestock supplied for human consumption. In general, veterinary surgeons use antimicrobial products to treat one or a number of sick animals in a group resulting in a reduction of symptoms. During enzootic infection, healthy animals may also need to be protected from the spread of disease.

Although antimicrobials are only administered following prescription by veterinary surgeon, farmers believe that in order to increase the rate of weight gain and reduce the amount of feed per unit of gain, certain antimicrobials must be used for growth promotion as well.

Antimicrobial administration in animals is carried out for three main objectives; therapy, prophylaxis and growth promotion. Farmers may treat individual sick animals with a high dose over a short period of time to overcome pathogens by adding antimicrobials into feed or drinking water. Prophylactic treatment, however, would involve moderate to high doses and similarly it is also delivered through feed and water. The three suggested explanations for how antimicrobials can improve growth in animals are: (i) control of subclinical disease; (ii) enhanced nutrient availability; and (iii) minimising activation of the immune response. As they may accelerate growth, antimicrobials tend to be given in feed at sub-therapeutic levels (below 200 g/ton of feed) over extended periods to entire herds and flocks. Although they are given at sub-therapeutic doses, they still exceed the minimal inhibitory concentration of any pathogen (Endtz *et al.,* 1991 and Barton, 2000). Overall the largest quantities are used as a regular supplement for prophylaxis and growth promotion. In Europe a recent report has shown that production of 1 kilogram of animal meat may use approximately 100 milligrams of antimicrobial (EMEA, 1999).

Due to lack of education on the appropriate use of diagnostic services, relevant laboratory support and application of antimicrobial in animal husbandry, the use is apparently careless and uncontrolled (EMEA, 1999). In addition, antimicrobials used as growth promoters may be generally licensed solely as feed additives. However, there is currently little information showing the real situation of how antimicrobial to be used in poultry. Table 3.7 shows the major classes of antimicrobial agents approved for use as prophylaxis and growth promotion in poultry.

**Table 3.7** Major antimicrobial agent classes approved for non-therapeutic use in poultry (Based on Shea, 2004)

| Antimicrobial class | Prophylaxis | Growth promotion |
| --- | --- | --- |
| Aminoglycoside | Yes | No |
| ß-Lactam | | |
| - penicillin | Yes | Yes |
| - cephalosporin | Yes | No |
| Ionophore | Yes | Yes |
| Lincosamide | Yes | Yes |
| Macrolide | Yes | Yes |
| Polypeptide | Yes | Yes |
| Streptogramin | Yes | Yes |
| Sulfonamide | Yes | Yes |
| Tetracycline | Yes | Yes |
| Bambermycins | Yes | Yes |
| Carbadox | Yes | Yes |
| Novobiocin | Yes | No |
| Spectinomycin | Yes | No |

In North America and Europe, it is estimate that approximately 50% (in tonnage) of all antimicrobial production is used in food-producing animals and poultry (FEDESA, 1998). In the UK, approximately tonnes of antimicrobials were used for human therapy, tonnes for animal therapy and prophylaxis and tonnes for growth promoters. Statistics on antimicrobial sales in the European Union and Switzerland for the year 1997 showed that about 52% were used in humans whereas therapeutic animal health antimicrobial use accounted for 33%, and growth promoters for 15% (Figure 3.5).
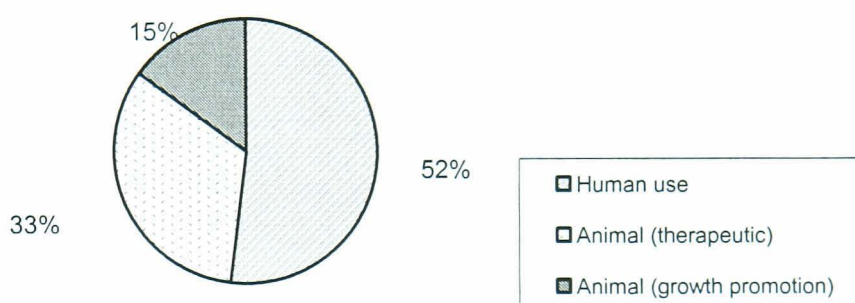


**Figure 3.5** Antimicrobial use in humans and animals in the EU in 1997 (10,500 tonnes of active ingredient at 100% purity) (taken from Follet, 2000).

Sales volumes of various antimicrobials showed in Tables 3.8 and 3.9.

**Table 3.8** Sales volumes of antimicrobial use as therapeutics and growth promoters in different EU member in 1997. (taken from FEDESA, 1998)

| Therapeutic group | Estimated total (% of total) |
|---|---|
| Penicillins | 9 |
| Tetracyclines | 66 |
| Macrolides | 12 |
| Aminoglycosides | 4 |
| Fluoroquinolones | 1 |
| Trimethoprim/Sulphonamides | 2 |
| Other therapeutics | 5 |

**Table 3.9** Sales volumes of antimicrobials in the EU and Switzerland in 1997: Therapeutic groups (tonnes of active ingredients) (Follet, 2000)

| country | Sales of growth promoters (% EU market) | Sales of therapeutics (% EU market) |
|---|---|---|
| Austria | 1 | <1 |
| Belgium+ Luxemburg | 7 | 4 |
| Denmark | 5 | 2 |
| Finland | <1 | <1 |
| France | 21 | 14 |
| Germany | 16 | 14 |
| Greece | 1 | 3 |
| Ireland | 2 | <1 |
| Italy | 6 | 11 |
| Netherlands | 14 | 9 |
| Portugal | 2 | 1 |
| Spain | 12 | 18 |
| Sweden | <1 | <1 |
| UK | 12 | 23 |

## Routes of drug administration

### Feed

Several diet formulas are typically fed to poultry from hatching to market. Pre-starter and starter diets are fed to broilers for up to 19 days after hatching. These diets may contain up to three drugs: (i) a prophylactic coccidiostat; (ii) a growth promoter antimicrobial and (iii) an organic arsenical compound.

### One-day-of-age injection

This route can be applied for drugs that must be approved for usage in poultry. It is for treatment of sick chickens or protection of chicks from injection-site abscesses after vaccination. In addition, mass incubation and hatching techniques create significant bio-aerosols containing various genera of *Enterobacteriaceae*, One-day-of-age injections can be used to improve early viability.

### Water medication

In general, sick birds are medicated through drinking-water systems. Either systemic or intestinal medication can be given by this route. To achieve and maintain therapeutic concentrations, the actual water used for each class of poultry is based on the age of birds, and the environmental temperature is controlled and monitored.

## 3.6.4 Emerging antimicrobial resistance in *Campylobacter*

Many would consider antimicrobial therapy to be unnecessary for campylobacteriosis because of the low mortality and self-limiting symptoms. However, treatment may be indicated for patients whose symptoms develop severely or who are immuno-compromised. In those cases, antimicrobials, at least, can reduce the severity and duration of illness. When

the antimicrobial treatment is indicated, the drugs of choice are either macrolides (*e.g.* erythromycin) or quinolone- fluoroquinolones (*e.g.* nalidixic acid, ciprofloxacin).

*Campylobacter* is found to be resistant to a number of antimicrobial agents, *e.g.*, penicillin, β-lactams, glycopeptides, macrolide and fluoroquinolones (Taylor and Courvalin,1988 and Piddock, 2000). Although the increase in the resistance rate of *Campylobacter* to antimicrobials has been fully recognised for some antimicrobials used in humans and animals, the most alarming increase in resistance is for fluoroquinolones. This is because fluoroquinolones are new, potent, broad-spectrum agents used for a wide range of gram-negative and gram-positive infections as well as being considered as a drug-of-choice for patients whose symptoms are severe. It is also used in adults for therapy and prevention of traveller's diarrhoea (Aarestrup and Wegener, 1999).

The increasing rate of antimicrobial resistant *Campylobacter* has been observed and reported, over the last four decades. Reports from several countries in Europe and North America have shown the rising prevalence of the resistance in humans. Not surprisingly, after introduction of enrofloxacin into animal husbandry in European countries, the increase in fluoroquinolone-resistant *Campylobacter* was up by 35% (EMEA, 1999). In 1993, when enrofloxacin was first licensed for oral use in food producing animals in the UK, a small study expressed the maximum value of the minimal inhibitory concentration (MIC) of enrofloxacin for *C.jejuni* as 1 $\mu$g/ml. Afterwards, the further studies carried out from 1994-1995 showed that 7% of the isolates had a higher MIC, 4 $\mu$g/ml (EMEA, 1999). Endtz *et al.* (1991) presented a long term study of *Campylobacter* resistance to fluoroquinolones. In the Netherlands, from 1982 to 1989, the incidence rate of ciprofloxacin resistance in *Campylobacter* isolated from chickens rose from 0% to 14%, and this increase was in parallel with an increase in humans from 0% to 11%.

MAFF (1998) has carried out an overview of antimicrobial resistance in *Campylobacter*. In the UK less than 1% of *Campylobacter* was found to be resistant to erythromycin. Guant and Piddock (1996) indicated a steady increase in fluoroquinolone resistant *Campylobacter*. Many studies in Spain showed the existence of multi-resistance among ampicillin (69%), erythromycin (1.5%), tetracyclin (43%) and ciprofloxacin (57%). However, in countries that strictly control antimicrobial use like Sweden and Finland, rates of resistance to erythromycin and ciprofloxacin among *Campylobacter* were low. Importantly, resistance to ciprofloxacin was not encountered among human isolates and infrequently among poultry isolates. It is noted that there is no internationally accepted breakpoint for characterising resistance in *Campylobacter* species. Therefore, it is difficult to compare the resistance rate between countries.

## Macrolide resistance

Some studies in the UK and Canada reported that few *Campylobacter* strains from humans (<30%) were resistant to erythromycin (Brunton *et al*, 1978; Piddock *et al.*, 2000 and Moore *et al.* 2001). Aarestrup and Wegener (1999) and Sáenz, *et al.* (2000) reported however that a higher frequency of erythromycin resistance (53-81%) found in *C.coli* isolated from humans and pigs. it is also evident that *C.coli* shows a higher resistance to erythromycin than that found in *C.jejuni.*

## Fluoroquinolones resistance

Several *Campylobacter* species, including *C.jejuni, C.coli, C.fetus* subsp. *fetus* and *C.hyointestinalis*, are usually susceptible to quinolones and fluoroquinolones. In vitro, *C.jejuni* and *C.coli* are highly susceptible to ciprofloxacin, norfloxacin and ofloxacin (Goossens *et al.*, 1985). As the rate of resistance to fluoroquinolones in humans and poultry has been increasing in many countries, this has become a matter of public health concern worldwide. Since then it has been tracked down by a large group of researchers around the world. The resistance varies from country to country as well as from humans to animals. The details were presented in Tables 3.10 and 3.11.

**Table 3.10** Approval date for fluoroquinolones use in humans and food-producing animals (adopted from Anderson *et al*, 2001)

| Country | Human use (year and antimicrobial) | Animal use (year and antimicrobials) |
|---------|-----------------------------------|--------------------------------------|
| Netherlands | 1985, Norfloxacin<br>1988, Ciprofloxacin<br>1989, Pefloxacin<br>1989, Ofloxacin | 1980s, Flumequine<br>1987, Enrofloxacin |
| Spain | 1987, Ciprofloxacin | 1990, Enrofloxacin |
| UK | 1987, Ciprofloxacin | 1993, Enrofloxacin |
| US | 1986, Norfloxacin<br>1987, Ciprofloxacin<br>1990, Ofloxacin<br>1997, Sparfloxacin and Levofloxacin | 1995, Enrofloxacin and Sarafloxacin<br>1998, Enrofloxacin (cattle) |

**Table 3.11** Resistance of *C. jejuni* and *C. coli* to quinolones and fluoroquinolones in human and poultry

| Country and year | Resistance rate (%) | | Reference |
|---|---|---|---|
| | Human | Poultry | |
| Autralia (1997) | 34.1-34.9 | | EMEA( 2001) |
| Belgium (1998) | | 44.2 | Looveren *et al*(2001) |
| Canada (2001) | 47 | | Gaudreau *et al.*(2003) |
| Denmark (2002) | | 5.2 | Engberg *et al.*(2004) |
| - domestic | 9.9 | | |
| - travelers | 50 | | |
| Finland (1997) | 35-37 | | Hakanen *et al.*(2003) |
| Germany (2001) | 45.1 | 45.6 | Luber, *et al.*(2003) |
| Italy (1993) | 25.9 | 47 -56 | EMEA (2001) |
| Japan (1991) | 10.7 | | Engberg *et al.*(2001); Takahasi *et al* (2005) |
| Netherlands (1997) | 29 | 14 | Endtz *et al.*(1991) |
| Singapore | | | Engberg *et al.*(2001) |
| Spain | 88 | 98.7 | Hakanen *et al.*( 2003) |
| Sweden (1993) | 6.1-25.5 | | EMEA(2001) |
| Thailand (1995) | 84 | | Hoge *et al.*(1998); Hakanen *et al.*(2003) |
| United Kingdom (1997) | 11.7-16.2 | 10.8 | Thwaites and Frost(1997) EMEA(2001) |
| United States | 10.8-23.9 | 35-41 | EMEA(2001); Ge *et al.*(2003) |

# 3.6.5 Mechanism of antimicrobial resistance in *Campylobacter*

Antimicrobial resistance is a microbiological phenomenon, which may or may not have clinical implications depending on pharmacokinetic and pharmacodynamic parameters as bacteria develop the resistance to specific antimicrobials. Following survival adaptation, bacteria will develop the potential to inhibit the action of specific antimicrobial agents through cellular mechanisms, for example, genetic mutation, gene transfer or a combination of both. The drug selection pressure depends in part on the concentrations of antimicrobials to which bacteria are exposed and whether a concentration is achieved that can actually assist in the selection for the proliferation of resistant organisms. A tremendous selection pressure has been exerted on bacterial eco-systems in humans and animals. Huovinen (1997) stated that development of bacterial resistance has been expected, rather than beingan unpredicted phenomenon.

Resistance in bacterial populations may or may not be reversible, depending on the antimicrobials used, the bacterial species, selection pressures and other factors. Most likely the adaptation process has a genetic basis. Bacteria store genetic information in genes on

the DNA of their single chromosome. However, they also keep genetic information on accessory pieces of DNA separate from the chromosome, plasmids, or transposons. All resistance genes might either be a fixed part of the bacteria genome or transferable between bacteria. They may be occasionally transferred to the new host by bacteriophages (Neu *et al*, 1996). Thereby, the resistance can be achieved by horizontal acquisition of resistant genes (carried by plasmids or transposons) by recombination of foreign DNA into the chromosome, or by mutations in different chromosomal loci (Martinez and Baquero, 2000).

Resistance can be acquired through chromosomal mutation or acquisition of foreign DNA (plasmids or transposons). Resistance can be an intrinsic property of the bacteria itself, such that every strain in the species is resistant. Bacteria can transfer chromosomal or plasmid DNA containing resistant genes to other bacteria by conjugation, transduction and transformation.

*Campylobacter* species are capable of transformation, *i.e.* facilitation of the uptake of naked DNA. This may become chromosomally integrated by site-specific recombination (Wang *et al.,*1993; and Taylor and Chau 1997). *Campylobacter* also presents the structure of integron-like character which points to the transmission of antimicrobial resistance genes.

Most recent studies of antimicrobial resistance in *Campylobacter* species have been focused on *C.jejuni* and, to a lesser extent, *C.coli*. Both are intrinsically resistant to a number of antimicrobials, including bacitracin, novobiocin, rifampin, streptogramin B, trimethoprim, vancomycin and cephalothin (Taylor and Courvalin, 1988). *C.jejuni* and *C.coli* are usually susceptible to erythromycin and ciprofloxacin. Thereby, they are becoming drugs of choice for campylobacteriosis. As reported by Endtz *et al.* (1991), however, several *Campylobacter* (*i.e.,C.jejuni, C.coli, C.fetus* subsp. *fetus*) are inclined to be resistant to these drugs. Engberg *et al.* (2001) reviewed and proposed mechanisms of fluoroquinolone and macrolide resistance, that are;

## Mechanism of macrolide resistance

Erythromycin resistance in *C.jejuni* and *C.coli* is chromosomally mediated and is due to alteration of the ribosome (Taylor, 1992). The resistance mechanism is not consistent with presence of rRNA methylase, or modification of the antimicrobial or efflux systems (extruding noxious agents through the cytoplasmic membrane). Sequencing of the 23S rRNA genes from erythromycin resistant *Campylobacter* showed the mutation at the sites of 23S rRNA (Figure 3.6).

## Mechanism of fluoroquinolone resistance

Fluoroquinolone resistance in *C.jejuni* appears to be due most often to mutations in the genes encoding subunits of DNA gyrase (*gyrA*) and occasionally to topoisomerase IV (*parC*)

(Figure 3.7). A number of studies suggest that the mutations for quinolone resistance occur primarily in the quinolone resistance-determining region (QRDR) (Wang et al.,1993; Gaunt and Piddock, 1996; Taylor and Chau 1997 and Zirnstein et al.,1999). Fluoroquinolone acts on DNA gyrase and topoisomerase IV. Gyrase is involved in DNA replication, recombination and transcription. It is a tetramer composed of two A and two B subunits which are encoded by the gene gyrA and gyrB. Topoisomerase IV is encoded by the gene parC and parE. The parC plays a role in the partitioning of replicated chromosomes (Luttinger, 1995). In C.jejuni, the region 269-fragment of the gyrA gene encodes for a 41-amino-acid quinolone resistance-determining region (QDRD) develops mutation at amino acid position Thr-86, ACA (threonine). The amino acid Thr-86, ACA (threonine) is substituted to ATA (isoleucine), Asp-90 and Ala-70. Apart from the mutation of Thr-86-to-Ile-gyr A, mutations may be encountered on other proteins, for example, the parC (topoisomerase IV) and parE. However, Cooper et al. (2002) found that the genomes of QRDR in C.jejuni and C.coli do not contain parC and parE.
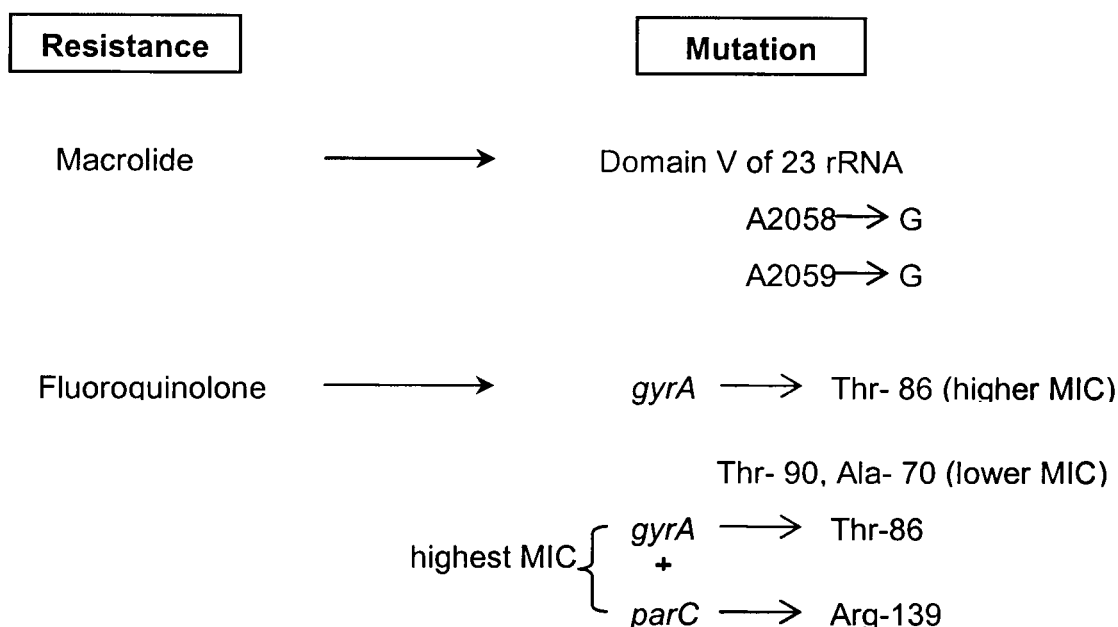


Figure 3.6 Macrolide and fluoroquinolone resistance mechanisms found in Campylobacter (from Engberg et al., 2001)

The resistance mechanisms of Campylobacter developed against the use of these antimicrobials are also summarised in Table 3.12.

Table 3.12 Resistance mechanisms against macrolides and (fluoro)quinolones (modified from EMEA, 1999).

| Class of antimicrobial | Cellular target | Modification | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | | Bacterial cell permeability | | Antimicrobial inactivation | Target | over-production |
| | | Decrease influx | Increase efflux | | | |
| Macrolides | 50S RNA subunit | + | + | + | + | - |
| (Floro)quinolones | DNA gyrase Topoisomerase IV | + | + | - | + | - |

## 3.6.6 Transmission of antimicrobial resistant *Campylobacter* from animals to humans

Over decades the rate of antimicrobial resistance in *Campylobacter* infection has been increasing sharply in many countries. In addition, much of the evidence relating to the potential for transfer of a resistance problem from animals to humans comes to a matter of epidemiology of Zoonoses. Studying the transmission of resistant strains from animals, especially poultry to humans has been difficult because the pathway of transmission is complex (Figure 3.7). However, several studies have attempted to compare not only serotypes but also genotypes of *Campylobacter* isolated from human samples and food samples. It was concluded that food-producing animals could be a substantial source of infection in humans (Orr *et al.,*1995; On *et al.,*1998; Smith, *et al.,*1999 and Owen *et al.,*1999).

Currently, there are no studies that demonstrate a direct causal links between the use of antimicrobial agents in animal husbandry and the rate of antimicrobial resistance in humans. However, three studies have shown an association between strain types of *Campylobacter* in human infection and those found in retail chickens. Other studies have also proposed that the use of antimicrobial agents in poultry husbandry could be one of the factors influencing the development of resistance in *Campylobacter* with the risk that this resistance may pass to humans via food (Endtz *et al.*, 1991; Sarah, 2002; Luber *et al.*, 2003; Randall *et al.*, 2003 and Wagner *et al.*, 2003).

A recent study (Humphrey *et al.*, 2005) has shown that ciprofloxacin-resistant *Campylobacter* strains emerge during fluoroquinolone treatment in commercially reared chickens. Following the increased reporting of antimicrobial resistance in general and fluoroquinolone resistance in particular in *Campylobacter* worldwide, attempts have been made to better control their use in animal husbandry (Sanchez *et al.*, 1994; Piddock *et al.*, 2000; Tollefson, 2002 and Luber *et al.*, 2003).

Moreover, multidrug resistance is also a problem. In particular, multidrug resistance to macrolides and fluoroquinolones must be considered highly undesirable because both of them are drugs-of-choice as well as being first and second-line drugs for treatment of campylobacteriosis. Gaudreau and Giltbert (1998); Sáenz *et al.*(2000) and Randall *et al.* (2003) found cross-resistance between nalidixic acid,ciprofloxacin, erythromycin, tetracycline and ampicillin.

Alternative pathways

Classical pathways

Sewage ← Food animals

Farmer contact

Wild/feral animals/birds → Crops* ← Water

Bacterial contamination of animal food origin

Animal foodstuffs

Wild/feral animals/birds

Crops

Food

Water

Pets* — Contact → Ingestion by human

Ingestion by pets

Sewage

Food handlers

Contact

Survival and proliferation of bacteria in gut

Verternarians, farmers contact

Cross infection

Humans*

* Antimicrobial use, selecting resistance

**Figure 3.7** Mode of the distribution and transmission of antimicrobial susceptible or resistant gastro-intestinally pathogens between animals and humans (taken from Phillips *et al.*, 2004)

## 3.6.7 Definition of susceptibility and resistance to antimicrobials

As defined by the British Society of Antimicrobial Chemotherapy (BSAC), antimicrobial susceptibility and resistance are relative terms and provide an interpretation of the clinical significance of concentrations of an antimicrobial agent.

►**Minimal inhibitory concentration (MIC)** is defined as the lowest concentration of antimicrobial that inhibits the visible growth of a micro-organism after overnight incubation.

►**Break point** is recommended as a measure for the point at which a MIC is sufficiently high to indicate resistance.

►**Laboratory resistance** is the level that is more than a 2-fold increase in the concentration of antimicrobial compared to a normal susceptible strain.

►**Clinical resistance** is the ability of an organism to grow at clinically achievable concentrations of antimicrobial.

# CHAPTER 4

## Organic Chicken Farming

## 4.1 Introduction

With a growing number of consumers switching from red meat to poultry, the chicken and turkey industries have a booming business (Table 4.1). To achieve high productivity at low cost, most poultry farmings use genetically and environmentally manipulated methods to rear chickens. Factory farming methods, especially intensive rearing systems, have been implemented in the poultry industry. In such systems the number of birds typically increases (up to 40,000 birds) without expanding the rearing houses. Birds often have to spend their entire life indoors. The stocking rate for housed broiler chickens is 32 Kg/m$^2$ (EC, 2002). Chickens are bred to develop quickly so they reach slaughter weight at 42 days. Each chicken is given less than half a square foot of space, barely being able to move. They may be crammed into a windowless building, with low levels of artificial light (*e.g.* Figure 4.1). They are not provided with fresh litter and in a fast growing period they are unable to support their bodies. In these conditions their legs and breasts are burnt by the manure and are eventually infected with micro-organisms. For example, millions of chickens in the UK die of heart disease before reaching slaughter weight at 6 weeks of age (*e.g* Figure 4.2). As a result, antimicrobials have been introduced into the system to keep them alive and healthy. This also forces their rate of growth. Many of the antimicrobials used may eventually impact on human health.

**Figure 4.1** Intensively reared farming: inside the house (the example which may not be typical for all chicken farms) (Source: http://www.factoryfarming.com/gallery/broiler01.htm)

**Table 4.1** Poultry meat consumption per capita in selected countries (Kg/capita) (Source: http://www.foodmarketexchange.com/datacenter/product/poultry/chicken)

| Region and country | Amount of consumption (Kg/capita) | | | | |
|---|---|---|---|---|---|
| | 1996 | 1997 | 1998 | 1999 | 2000 |
| **North America** | | | | | |
| Canada | 30.7 | 31.4 | 32.0 | 33.8 | 34.8 |
| Mexico | 18.8 | 18.9 | 19.8 | 21.5 | 22.7 |
| United States | 45.7 | 46.2 | 46.7 | 49.1 | 49.3 |
| **South America** | | | | | |
| Argentina | 19.8 | 22.8 | 25.5 | 25.6 | 24.9 |
| Brazil | 21.5 | 23.2 | 23.4 | 27.3 | 28.2 |
| Colombia | 17.0 | 17.5 | 17.6 | 16.6 | 17.2 |
| Honduras | 7.7 | 10.0 | 10.7 | 10.7 | 10.8 |
| Venezuela | 17.2 | 17.3 | 16.6 | 15.7 | 16.1 |
| **European Union** | | | | | |
| France | 24.8 | 24.7 | 25.1 | 24.4 | 24.9 |
| Germany | 14.1 | 14.7 | 15.0 | 15.2 | 15.2 |
| Italy | 19.2 | 19.5 | 19.5 | 19.1 | 18.7 |
| Netherlands | 21.3 | 21.8 | 22.2 | 21.5 | 21.0 |
| Spain | 26.0 | 24.2 | 24.3 | 26.7 | 26.4 |
| United Kingdom | 26.0 | 25.9 | 27.7 | 28.1 | 28.4 |
| **Eastern European** | | | | | |
| Bulgaria | 11.4 | 12.3 | 14.6 | 15.0 | 15.6 |
| Hungary | 25.0 | 26.1 | 29.4 | 25.4 | 27.4 |
| Poland | 11.1 | 12.7 | 14.0 | 14.2 | 14.3 |
| Russia | 12.2 | 12.7 | 10.6 | 10.6 | 11.3 |
| Ukraine | 5.3 | 5.0 | 4.9 | 5.8 | 5.8 |
| **Middle East** | | | | | |
| Kuwait | 44.5 | 44.7 | 40.8 | 41.7 | 41.1 |
| Saudi Arabia | 32.0 | 35.8 | 34.8 | 35.1 | 34.3 |
| United Arab Emirates | 32.0 | 31.8 | 34.3 | 40.5 | 40.2 |
| **Africa** | | | | | |
| Egypt | 6.0 | 7.0 | 7.6 | 8.9 | 8.5 |
| Republic of South | 21.7 | 21.6 | 24.6 | 26.6 | 26.4 |
| **Asia** | | | | | |
| China (PRC) | 8.2 | 8.8 | 9.0 | 9.3 | 9.6 |
| Hong Kong | 50.3 | 52.5 | 59.0 | 57.0 | 57.4 |
| Indonesia | 4.6 | 4.3 | 2.1 | 2.9 | 3.4 |
| Japan | 14.4 | 14.0 | 13.8 | 13.7 | 13.8 |
| Malaysia | 33.1 | 34.0 | 29.4 | 31.1 | 32.1 |
| Republic of Korea | 10.8 | 10.8 | 9.5 | 11.0 | 11.3 |
| Taiwan | 31.1 | 34.1 | 33.6 | 33.0 | 34.0 |
| Thailand | 12.0 | 12.6 | 11.6 | 12 | 13 |
| **Oceania** | | | | | |
| Australia | 27.0 | 28.3 | 30.5 | 31.4 | 32.4 |

**Figure 4.2** The chicks at the age of 6 weeks

(Source: http://www.factoryfarming.com/gallery/broiler01.htm)

In addition, lighting in the houses is bright so as to encourage maximum feeding and drinking. It may remain on for 23.5 hours a day (*e.g.*Figure 4.3). Prolonged inactivity (rest) is deemd economically undesirable because birds do not eat and then put on weight.

**Figure 4.3** Lighting in the rearing house of intensive chicken farming

(Source: http://www.advocatesforanimals.org.uk/resources/farmed/broilers.html)

Note: All pictures presented in this chapter are taken from an animal welfare resource. These represent typical rearing conditions. There are many worse although there are a few better.

Because of increasing degree of awareness of food safety and health, as well as animal rights, public concerns have increased. Sustainability is a further issue. Alternative farming, for example, organic farming, has been re-established. As reported by DEFRA, poultry meat sales account for 15% of the UK organic meat market (UK-DEFRA 2003). The number of organic poultry slaughtered in the UK expanded from an estimated £1.3 million in 2000/2001 to £ 2.1 million in 2001/2002. Farm gate value increased from £7.2 million to £10.5 million.

## 4.2 Organic chicken farming

### 4.2.1 Definition

According to the proposed Codex definition, organic farming is a form of agriculture that relies on ecosystem management and attempts to reduce or to eliminate external agricultural inputs, especially synthetic ones. It is a holistic production management system that promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. Organic farming emphasises management practices, taking into account that regional conditions require locally adapted systems, using both traditional and scientific knowledge (UKROFS, 2000).

The definition and criteria of what constitutes organic farming is regulated by government through law in a number of countries (e.g. Australia, Norway, Thailand, Taiwan and in the EU). Under such Regulation, each member state is required to establish a national competent authority to ensure adherence to the law, for example, the Department of Environment and Rural Affairs (DEFRA) in the UK. Organic farmers must be registered with a certification body which must be approved by the National Competent Authority and organic food must be produced from a certified farm only. The non-certified farm cannot label products as organically produced food. In Canada, voluntary certification is available, while legislation may be pending (IFOAM, 2005).

There are ten approved certification schemes for organic producers in the UK, of which the five most important in terms of number of licensees are Soil Association (SA), Organic Farmers and Growers (OFG), Scottish Organic Producers Association (SOPA), (Biodynamic Agricultural Association (BDAA) and Organic Food Federation (OFF).

## 4.2.2 General principles of organically produced poultry

The recommendations propose that livestock production must contribute to the equilibrium of systems of agriculture, environment and ecology. It must be done by providing for the nutrient requirement for crops, through the soil's organic matter, and by utilising renewable natural resources. The number of livestock must be closely related to the area available in order to avoid problems of adverse effects, not only on the animal health but also on environmental quality.

Lower stocking rates apply to organic farms. The rate is limited at 21 kg/m$^2$. Organic birds must be reared in open-range conditions and should have free access to open-air runs throughout the day. In addition, chickens have a minimum slaughter age of 81 days, allowing more balanced growth of muscle, bone and vital organs, and virtually doubling their life span compared to intensively farmed chickens (IFOAM, 2005).

The national organic livestock standards (the United Kingdom Register of Organic Food Standards( UKROFS) state that organic poultry must be reared in accordance with standards set for organic livestock and organic livestock products. These standards are described as follows:

## Origin of animals

In the choice of breeds or strains, account must be taken of the capacity of animals to adapt to local conditions. All chicks for broiler production must be less than three days old at the time they leave the production unit where they are produced.

## Feed

Feed is intended to ensure quality of production rather than maximisation of production. It must meet the nutritional requirement of livestock at various stages of their development. However, forced feeding is forbidden. For poultry, the feed formula used in the fattening stage must contain at least 65% cereals. Feed stuff and certain products used in animal nutrition must not have been produced with the use of genetically modified organisms (GMOs) or products derived from GMOs.

## Disease prevention and veterinary treatment

Disease prevention shall be based on the good animal-husbandry practices appropriated to the requirements of poultry, for instance, broiler chicken. Owing to the fact that if animal becomes sick or injured, it is necessary to use veterinary medicinal products, *i.e.*, phytotherapeutic[4] or homeopathic products. The use of either product shall comply with the therapeutic effect for combating illness or injury. In addition those products shall be used in preference to chemically synthesised allopathic[5] veterinary medicinal products or antimicrobials.

The use of chemically synthesised allopathic veterinary medicinal products or antimicrobials for preventive treatments and for promoting growth or production is prohibited. Nonetheless if an identified disease risk occurs and the welfare of animals cannot maintain by management practices alone, it may be permitted. Vaccination is permitted in cases where there is a known disease risk.

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[4] Phytotherapeutic product consists of complex mixtures of one or more active ingredients extracted from plants which are used in most countries for management of various diseases.
[5] Allopathic products usually include registered veterinary pharmaceutical or proprietary medicinal products (*e.g.* antimicrobial agents, painkillers). Some phytotherapeutic treatments could also be viewed as allopathic in their mode of actions.

## Husbandry management practices, transport and identification of livestock products

Poultry must be reared in open-range conditions and cannot be kept in cages. Their houses shall comply with their own grazing, air space, ventilation, feed, water and litter disposal needs.

The size of a group reared together must depend upon the stage of their development and the behavioural needs of the species of concern. During transportation, live animals and birds must be handling with proper care and concern for their welfare, minimising stress and avoiding the likelihood of injury.

# CHAPTER 5
## Detection and Enumeration
## 5.1 Introduction

Since *Campylobacter* was firstly recognised in 1960s, significant detection including isolation and identification methods have been developed in order to understand growth characteristics, genetics, molecular biology and pathogenic mechanisms of this genus as well as host response (Karmali *et al.*, 1986; Walker *et al.*, 1986 and Penner, 1988). In addition, because of its clinical and economic implications, development of detection procedures, pathogenesis, epidemiology and molecular biology have rapidly progressed. Molecular techniques have first been used in clinical laboratories for both direct identification and confirmation (Sails *et al.*, 2001).

### 5.1.1 Detection methods

Although traditional detection methods currently used in most studies are not internationally accepted standard methods, there are some generally agreed procedures. These include pre-enrichment media (with or without blood supplement), selective agar supplemented with antimicrobials, growth conditions (optimal temperature and micro-aerobic atmosphere) and confirmation criteria (Corry *et al.*, 1995; Tran, 1998 and Baylis *et al.*, 2000). Since the first selective medium was successfully developed by Skirrow in 1977, methods for the detection of *Campylobacter* have been developed to recover *C.jejuni* from faecal specimens where the organisms are present in relatively large numbers (Wesley *et al.*, 1983). These methods were based on traditional isolation techniques used for the isolation of pure cultures. However, like other foodborne pathogens, the isolation of *Campylobacter* from foods is often difficult because: i) this organism may be present in lower numbers than numbers of competitor organisms, ii) cells may also be sub-lethally injured by food processing, iii) *Campylobacter* requires specific growth conditions, *e.g.* micro-areobic conditions(5-10% oxygen and 3-5% carbon dioxide), ferric iron and iv) *Campylobacter* is a slow growing bacterium that requires long periods for incubation (Baylis *et al.*, 2000 and Nogva *et al*, 2000). Thus, a number of studies attempted to improve the efficiency of recovery of *Campylobacter* from food (Wesley *et al.*, 1983; Fongsiri *et al.*, 1989; Baylis *et al.*, 2000 and Moore, 2001).

As traditional culture techniques are time-consuming, labour intensive and less efficient methods for detection of *Campylobacter* in food samples, molecular methods (*e.g.* polymerase chain reaction (PCR) based assay, Fluorescence In Situ Hybridisation) have been comparatively developed. They presently are preferable to culture techniques due to improoved speed and accuracy. However, these methods require high performance equipment and unfeasibly high costs. Therefore the two groups of methods currently used

for the detection of *Campylobacter* in food samples are based on: i) traditional culture method involving enrichment and sub-culture on the selective media according to the growth conditions and ii) molecular based methods which, in contrast, detect the organism without enrichment and sub-culture and involve enzymatically replicating DNA. Both types have their advantages and disadvantages.

## 5.1.1.1 A traditional culture technique

A traditional culture method for the detection of *Campylobacter* requires enrichment media and appropriate growth conditions.

Enrichment broths used in recent studies are summarised in Table 5.1[ Bolton broth (BB), Exeter broth, Preston broth and *Campylobacter* Enrichment broth (CEB) supplemented with animal blood (horse or sheep)]. However, the broths are used either micro-aerobically or aerobically. Micro-aerobic incubation has been suggested for growing *Campylobacter*. Tran, 1998 and Baylis *et al.*, 2000 indicated that *Campylobacter* can be recovered under aerobic condition with appropriate media (types of nutrient and supplements) and containing equipment (tightly fitting lid container with head space less than 1-2 cm). These conditions can reduce the oxygen toxic derivatives.

Following pre-enrichment, enriched sample is further sub-cultured onto selective agar and incubated micro-aerobically at 42$^0$C. *Campylobacter* selective agars include nutrient based agar and supplements (antimicrobial agents and animal blood). A variety of *Campylobacter* selective agars have been used in a number of studies. They can be categorised as: i) blood-selective agar (*e.g.* Skirrow, Campy-Cefex, Butzler and modified Butzler, Preston and Exeter) and ii) blood-free selective agar with charcoal as an oxygen quencher (*e.g.* a modified Charcoal Cefoperazone Deoxycholate agar). However, some protocols may combine more than one formula in order to enhance growth rate of *Campylobacter* (Corry *et al.*; 1995; Tran, 1998 and Baylis *et al.*, 2000.

Baylis *et al.* (2000) showed that Bolton broth and *Campylobacter* Enrichment broth were better than Preston broth, and Bolton broth offered the best balance between inhibition of competitor organisms and the growth of *Campylobacter* spp. In addition, a modified charcoal cefoperazone deoxycholate agar (mCCDA) recommended by a number of organisations is most compatible to growth requirements of *Campylobacter* species following pre-enrichment in Bolton, broth (Corry *et al.*, 1995; Donnison, 1998; FDA BAM, 1998; and UK HPA,2002). Pre- enrichment protocols currently used for resuscitation of *Campylobacter* in food samples are shown in Table 5.1.

**Table 5.1** Current pre-enrichment protocols used for resuscitation of *Campylobacter*

| Protocol | Formula | Incubation condition | Reference |
|---|---|---|---|
| Preston broth - original formula | Nutrient broth (not includind yeast extract), 5% lysed horse blood and antimicrobials (polymixin B, rifampin, trimethoprim and cyclohexamide) | Micro-aerobic, 42°C | Bolton and Robertson (1982) |
| - modified formula | Original formula with additional FBP mixture (including sodium pyruvate, sodium metabisulphite and ferrus sulphate) | Aerobic, 37°C for 4hrs following 42°C for 48hrs | Tran (1998) and Baylis *et al.* (2000) |
| Exeter | Modified Preston based formula, FBP mixture, 5% lysed horse blood and antimicrobials (polymixin B, rifampin, trimethoprim, amphotericin and cefoperazone) | Aerobic condition in container fitted with a screw cap with head space < 1 cm. 37°C for 4hrs following 42°C for 24-48hrs | Humphrey (1986) and Martin *et al.*, (2002) |
| Bolton broth | Peptone, yeast extract, alpha- ketoglutaric acid, sodium pyruvate, sodium metabisulphite, sodium carbonate, haemin, lysed horse blood (FDA BAM, 1998) or lake horsed blood (Baylis, *et al,* 2000), antimicrobials (trimethoprim, vancomycin, cefoperazone and cyclohexamide) | Micro-aerobic (FDA BAM, 1998) or aerobic condition in a container fitted with a screw cap with head space < 2 cm (Tran, 1998 and Baylis *et al,* 2000) | Tran (1998), FDA BAM (1998) and Baylis *et al* (2000) |
| *Campylobacter* Enrichment broth (CEB) | Peptone, yeast extract, alpha- ketoglutaric acid, sodium pyruvate, sodium metabisulphite, sodium carbonate, haemin, lake horsed blood, antimicrobials (trimethoprim, vancomycin, cefoperazone and natamycin) | Aerobic condition in a container fitted with a screw cap with head space < 2, 37° C for 4hrs following 42°C for 20-44 hrs | Baylis *et al.* (2000) |
| Park and Sanders broth | Brucella broth, lysed horse blood, antimicrobials (trimethoprim, vancomycin, cefoperazone and cyclohexamide) | Micro-aerobic condition, 32°C for 4 hrs, 37°C for 4 hrs and 42°C for 40-42hrs | Scotter *et al.*(1993) |

## 5.1.1.2 Molecular based methods

Traditional detection methods have been used for the identification of *Campylobacter* in a large number of studies. However, there is great variation in isolation frequencies and different predominant species in certain reservoirs such as animals. This is due to differences in sampling and isolation techniques and characterisation of *Campylobacter* (Jørgensen *et al.*, 2002). Molecular based methods have been considered as superior tests for *Campylobacter* identification and species differentiation. These methods were first used for genotyping of *Campylobacter* in clinical specimens such as faeces. They were applied to identify pure culture isolated from samples using traditional culture methods. The initial objective of genotyping applied to faecal samples obtained from diarrhoeal patients is to differentiate certain species of *Campylobacter* causing enteritis in humans. This information is significantly beneficial for epidemiological studies (Desai *et al.*, 2001). Advantages of molecular methods have led to further applications to directly detect *Campylobacter* spp. in environmental samples such as water and foods (Wegmüller *et al.*, 1993). To date, multiple molecular techniques have been combined in the application of the tests in order to maximise the performance characteristics of the tests, which include accuracy, precision, sensitivity and specificity, *i.e.* polymerase chain reaction/restriction enzyme analysis typing (PCR/REA), amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA and ribotyping (Wassenaar and Newell, 2000; Lehtola *et al.*, 2005 and Schmid *et al.*, 2005).

Applications of molecular methods have been further used for the identification of resistant genes. Several studies have conducted these techniques to identify the antimicrobial resistant region on the *gyrA* gene of *Campylobacter* that expresses evidence of mutation to certain antimicrobials, in particular, (fluoro)quinolones (Zirnstein *et al.*, 1999 and Fluit *et al.*, 2001).

Although these techniques have considerable advantages, they require intricate equipment, skilled staff and are of high consumable cost. Some methods involving the analysis of PCR product have not yet been validated for the use on different viability states of bacteria. Therefore these techniques are not applicable for routine work or in small scale laboratories.

## 5.1.2 Confirmation methods

As traditional culture methods are not species-specific methods, additional indicators are necessary for confirmation of *Campylobacter* spp. Indicators including physical, traditional gram staining, motility, biochemical and genetic characteristics are needed. Specific tests for *Campylobacter* used in this study are outlined below:

## Selective media and growth condition

Selective temperature (42°C) and gas atmospheres or micro-aerobic conditions are required to isolate thermotolerant *Campylobacter*. Table 5.2 expresses the optimal temperature for growth of *Campylobacter* species. Moreover, the agar, mCCDA with its supplement is selective not only by enhancing the growth of *Campylobacter*, but also by suppressing other competitive bacteria (Baylis *et al.*, 2000). As a result, any colonies that are able to grow on this selective media are most likely to be thermotolerant *Campylobacter*.

**Table 5.2** Relationship between temperature and growth for *Campylobacter* species

| Species | Temperature (°C) | | |
| --- | --- | --- | --- |
| | 25 | 37 | 42 |
| *C.coli* | - | + | + |
| *C.concisus* | - | + | c |
| *C.curvus* | - | + | + |
| *C.fetus* subsp. *fetus* | + | + | - |
| *C.fetus* subsp. *venerealis* | + | + | - |
| *C.gracilis* | NA | + | NA |
| *C.helveticus* | - | + | + |
| *C.hyoilei* | NA | + | + |
| *C.hyointestinalis* subsp. *hyointestinalis* | v | + | + |
| *C.hyointestinalis* subsp. *lawsonii* | - | + | + |
| *C.jejuni* subsp. *jejuni* | - | + | + |
| *C.jejuni* subsp. *doylei* | - | + | w |
| *C.lari* | c | + | + |
| *C.mucosalis* | - | + | + |
| *C.rectus* | - | + | w |
| *C.showae* | - | + | + |
| *C.upsaliensis* | - | + | + |
| *C.fecalis* | - | + | + |

+, 90% or more of strains are positive; -, 90% or more of strains are negative; v, 11%-89% of strains are positive; w, weak reaction NA, results not available; c, contradictory reports in literature.

## General typical appearances displayed onto mCCDA

Colonies of *Campylobacter* species are usually odourless and small ranging from pinpoint to 2 mm in diameter. However, colonial morphology is variable and different in several species. *C. jejuni* and *C. lari* show flat, glossy and effuse colonies on selective agar. They have a tendency to spread along the streak lines. Some colonies may resemble droplet fluid. On moist agar a thin and spreading film may be observed. *C.coli* has less effuse and often

convex shape colonies and is overshiny. Figure 5.1 presents typical characteristics of *Campylobacter* spp.
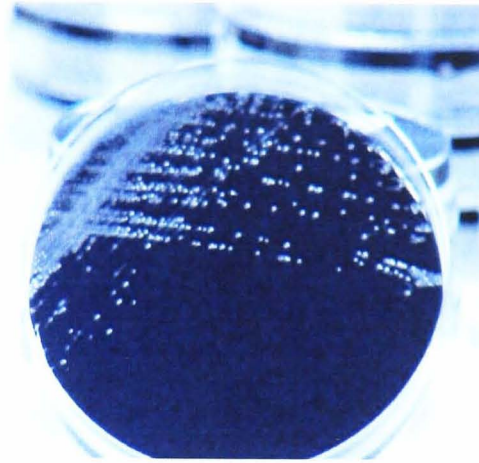


**Figure 5.1** Typical characteristics of *Campylobacter* displayed onto mCCDA

(taken by Soonthornchaikul, 2004)

## Motility

On occasion, other thermophilic bacteria may break through and grow on this selective agar, in particular, *Pseudomonas aeroginosa*. It might be confused with *Campylobacter* species. However, this may be differentiated by the characteristic motility of *Campylobacter* which has darting or corkscrew-like movement.

## Biochemical tests

Additional biochemical tests are necessary for the differentiation *Campylobacter* species. Since the majority of *Campylobacter* species in chickens are *C.jejuni* and *C.coli*, the specific metabolic end-products of *Campylobacter jejuni* were selected for identification. These tests are catalase, oxidase and hippurate hydrolysis test respectively. *C.jejuni* is capable of hydrolyzing hippurate but *C.coli* is not (Penner, 1988). Table 5.3 presents biochemical characteristics of *Campylobacter* spp., which may be used for the initial differentiation amongst species (Engvall *et al.*, 2002).

**Table 5.3** The biochemical characteristics of *Campylobacter* species

| Organism | Biochemical test | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Catalase | Nitate | H$_2$S$_2$ (triple sugar iron) | Urease | Indoxyl acetate | Hippurate hydrolysis |
| *C.coli* | + | + | - | - | + | - |
| *C.concisus* | - | + | + | - | NA | - |
| *C.curvus* | - | + | + | - | + | - |
| *C.fetus* subsp. *fetus* | + | + | - | - | - | - |
| *C.fetus* subsp. *venerealis* | + | + | - | - | - | - |
| *C.gracilis* | - | + | - | - | v | - |
| *C.helveticus* | - | + | - | - | + | - |
| *C.hyoilei* | + | + | + | - | NA | - |
| *C.hyointestinalis* subsp. *hyointestinalis* | + | + | + | - | - | - |
| *C.hyointestinalis* subsp. *lawsonii* | + | + | + | - | - | - |
| *C.jejuni* subsp. *jejuni* | + | + | - | - | + | + |
| *C.jejuni* subsp. *doylei* | v | - | - | - | + | + |
| *C.lari* | + | + | - | - | - | - |
| *C.mucosalis* | - | + | + | - | - | - |
| *C.rectus* | - | + | + | - | + | - |
| *C.showae* | + | + | + | - | + | - |
| *C.upsaliensis* | -(w) | + | - | - | + | - |
| *C.fecalis* | + | + | + | - | - | - |

+, strong reaction; W, weak reaction; NA, results not available

**Note:** All *Campylobacter* species are oxidase postive

*Hippurate test*

*C.jejuni* is able to hydrolyse hippurate acid (N-benzoylglycine; C$_9$H$_9$NO$_3$). This reaction can be detected by a colour change following the reaction with the reagent colour, ninhydrin, demonstrated in Figure 5.2.

Hippuricase *(from C.jejuni)*

$H_2O_2$, heat

$C_9H_9NO_3$ $\longrightarrow$ Lactic acid + glycine

Ninhydrin

**Dark purple**
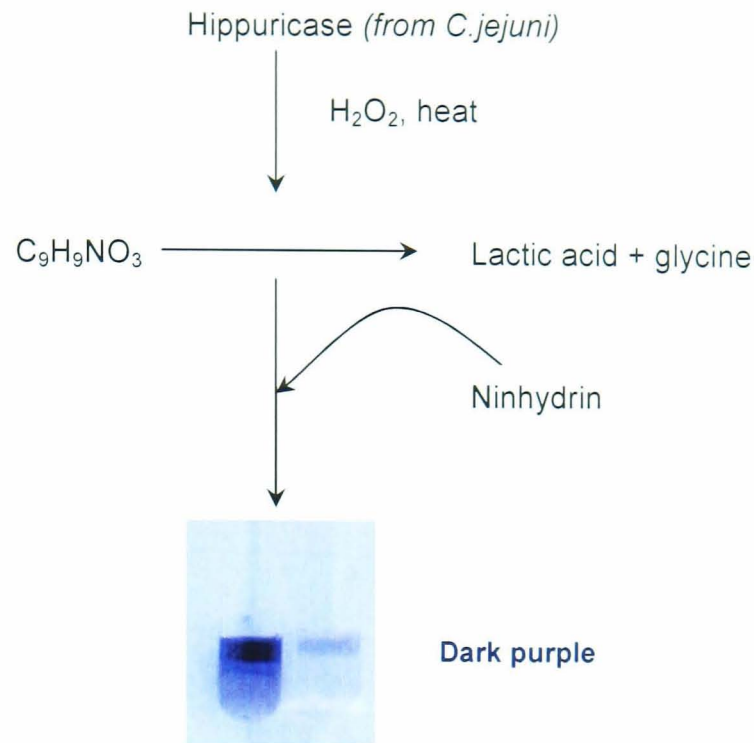
**Figure 5.2** Hippurate test

<u>**Fluorescence In Situ Hybridisation (FISH)**</u>

Fluorescence In Situ Hybridisation (FISH) is a molecular cytogenetic method. It allows DNA or RNA sequences to be detected through the use of fluorescent labelled oligonucleotide probes complementary to the appropriate regions (*e.g.*16s rRNA).The bound fluorescent probe can then be visualised using a fluorescent microscope. This method combines the precision of molecular genetics with visual information from microscopy (Amann *et al.*, 1995).

## 5.1.3 Enumeration method

Since microbial risk analysis has been adopted for the development of food safety management in order to control *Campylobacter* in the food chain, the prevalence and numbers of *Campylobacter* are needed for the estimation of health risk. Several studies have attempted to enumerate *Campylobacter* species in poultry as a significant source of *Campylobacter* (Beuchat, 1985; Wang 2002; Donnison, 2003 and El-Shibiny *et al.*, 2005). There are a number of methods available for enumeration of *Campylobacter* in food samples, including culture and non-culture methods. Various methods based on culture techniques have been used including direct plating (before enrichment) or the conventional Most Probable Number (c-MPN). However, under the environmental stresses which these bacteria are exposed to during food production and subsequent storage, *Campylobacter* may change to a viable-but-non-culturable (VNC) form. As such conventional culture methods would be less sensitive in its detection. These methods are tedious and time-consuming. Over the last decade, several studies have developed rapid methods to directly detect *Campylobacter*. These are based on polymerase chain reaction (PCR), DNA hybridization, enzyme immunoassays (EIAs) and Fluorescence In Situ Hybridisation (FISH)

(On, 1996; Waage, 1999; Moreno *et al*, 2000 and Waller *et al*, 2000). Although these non-culturing approaches are fast and specific, they require intricate operations which may not be available in general laboratories. Microbiologists see advantages and disadvantages of these methods. Selection of appropriate methods must be considered on the basis for the recovery of *Campylobacter*, performances of the tests (*e.g.* sensitivity, specificity, false positive and false negative), confirmation rates and practical aspects (availability, readability and friendliness). Comparison of advantages and disadvantages of two different based methods are presented in Table 5.4.

**Table 5.4** Comparison of enumeration methods used for *Campylobacter* in food samples

| Methods | Advantages | Disadvantages |
|---------|------------|---------------|
| Culture-based method (*i.e.* MPN, DP) | -less complicated procedures<br>-do not require skilled operator<br>-can be performed in any laboratory<br>-detect viable cell only | -time consuming<br>-less sensitive for non-viable cells<br>-require a large number of materials |
| Non-culturing based method (*e.g.* Hybridisation) | -fast<br>-high sensitivity and specificity | - high cost<br>-require skilled operator<br>-may produce other hazards, *e.g.* radio-isotopes<br>-cannot distinguish between viable and dead cells |

In this study, the selected methods used for determination of positive *Campylobacter* chicken samples were i) direct plating, ii) MPN and iii) traditional culture isolation. In addition, the numbers of *Campylobacter* isolated from chickens were calculated using the MPN technique.

## 5.1.3.1 The direct plating method

A number of studies reported the success of using direct plating (before enrichment) to determine the numbers of *Campylobacter* in faecal samples from humans or animals. These types of sample usually contain very high numbers of viable *Campylobacter* (Stern and Robach, 2003; and Oyarzabal *et al.*, 2005). Most food samples however harbour low numbers of *Campylobacter* and the organisms may be partially damaged during food processing and subsequent storage. Enumeration of *Campylobacter* in food samples prior to recovery damaged cells appears unsuitable and less sensitive to obtain the cells (FDA BAM, 1998).

## 5.1.3.2 The most probable number (MPN) method

A most probable number (MPN) method is particularly efficient for enumeration of low concentrations of bacteria, especially in milk and water, and for those foods whose particulate matter may interfere with accurate colony counts (FAD BAM 1998). The assumption of the MPN is that bacteria in tested samples are distributed randomly and not clustered together. The growth medium and conditions of incubation are chosen so that every inoculum containing at least one viable organism will produce detectable growth. It is therefore sensitive only for viable organisms.

The principle of an MPN technique is that the detection of organism at each dilution of the sample from first dilution to such a degree that inocula will sometimes but not always contain viable organisms. The numbers of inocula producing growth at each dilution will imply an estimate of the original, undiluted concentration of bacteria in the sample (FDA BAM, 1998).

Finally, the estimate of MPN level of *Campylobacter* can be calculated from the MPN index based upon the application of probability to the numbers of observed positive growth responses after incubation (Mageau, 1998 and FDA, 2001). The most probable number of bacteria is therefore scored from the MPN table.

A conventional MPN has been used for enumeration of *Campylobacter* in several types of samples including faeces, coacal content, water and food (Stern and Robach, 2003). This technique is simple and sensitive especially for samples harbouring low concentrations of bacteria as it includes a pre-enrichment stage. However, it requires two media, a number of test tubes, confirmation criteria and time.

## 5.2 Materials and Methods

In this chapter detection and enumeration of *Campylobacter* were carried out with three groups of chickens, PICs, POCs and BICs (see section 5.2.1). All samples were processed for isolation and identification (see section 5.2.1 to 5.2.5). The numbers of *Campylobacter* were enumerated (see section 5.2.4) and the framework of experiment regimes in this chapter is presented in Figure 5.3.
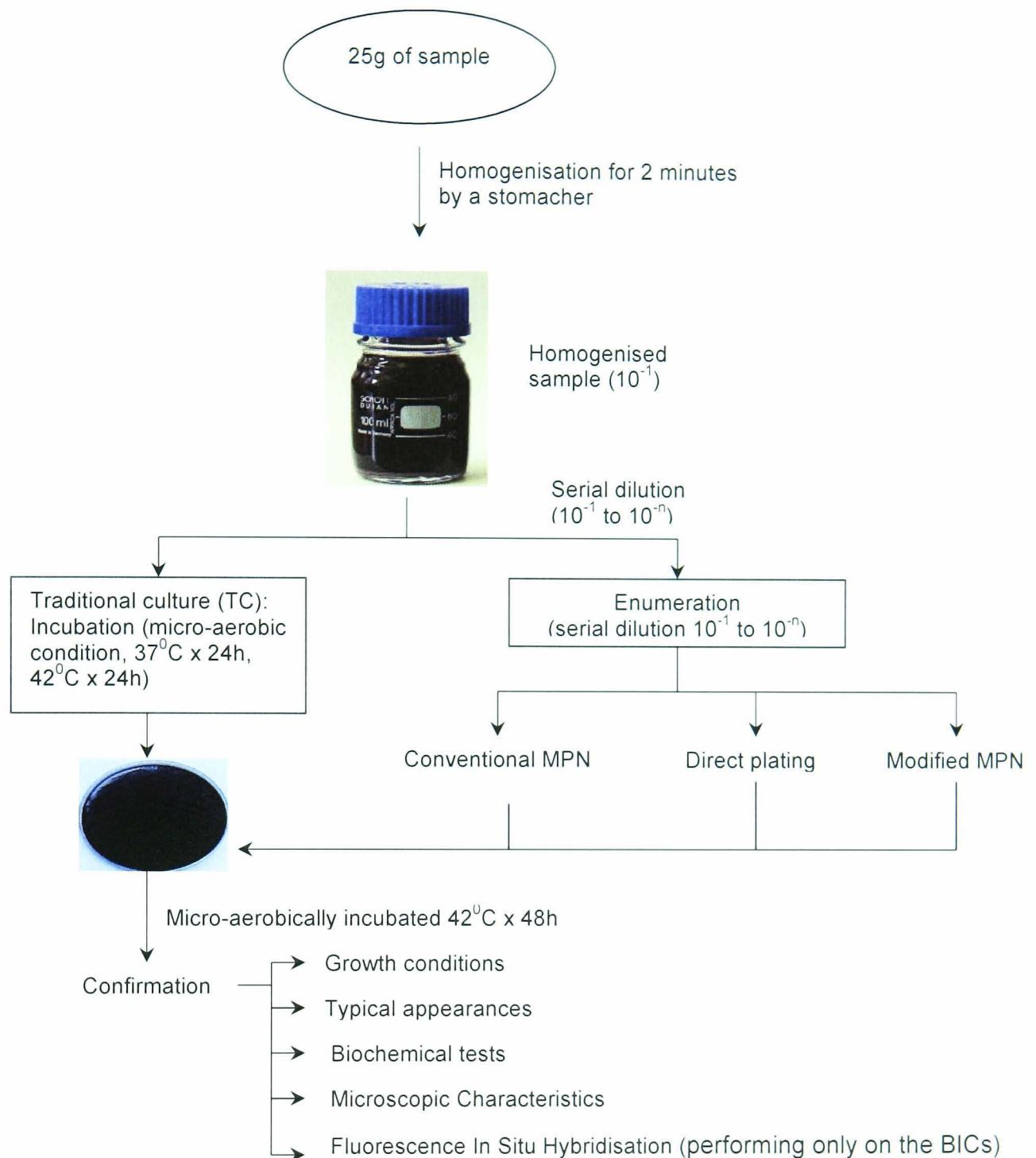
**Figure 5.3** A framework of experiment regimes (detection and enumeration)

## 5.2.1 Sampling Regime

### Type and number of samples

The samples in this study were whole carcasses of fresh broiler chickens. A broiler chicken is a bird in which the tip of the sternum is flexible (not ossified) and it is raised specifically for meat. A fresh chicken carcass is defined as chicken meat not stiffened by the cooling process, which is to be kept at a temperature not below $-2^0C$ and not higher than $4^0C$ at any time (UK-DEFRA 2004b). The whole carcass includes the whole body of the bird. Removal

of the heart, liver, lungs, gizzard, crop, kidneys and legs at the tarsus or the head are optional. However, carcasses sampled in this study were without giblets.

Three groups of fresh whole chickens were selected. These were; 91) a pre-packaged intensively reared chicken (PIC), (2) a pre-packaged organically reared chicken (POC), grown in accordance with the standards certified by the UKROF (the definition and conditions of these are found in chapter 4, and (3) an unwrapped intensively reared chicken (BIC). All raw chickens purchased from supermarkets (PICs and POCs) were pre-packaged whilst the raw chicken purchased from local butchers' shops (BICs) were unwrapped. Both PIC and POC group were labelled as reared and processed in accordance with the EEC Poultry Meat Marketing Standards Regulations 1999 (UK-DEFRA 2004a). In contrast, no labeling or information was available on the origin and processing of the BIC group.

30 fresh pre-packaged intensively reared chickens (PICs) and 30 fresh pre-packaged organically reared chickens (POCs) were randomly purchased from supermarkets. 30 fresh unwrapped intensively reared chickens (BICs) were purchased from butcher's shops. The chickens purchased from butcher's shops (no label) were assumed to be the intensively reared chicken based on the price, which is cheaper than that of an organic or a free-range chicken. Since there is no evidence to assure that the unwrapped organic chickens sold at retail shops comply to the UKROFS criteria, unwrapped organic chickens were not included in this study.

**Sampling programme**

*Selection of chicken outlets*

In order to reduce farm-specific effects and to sample as representatively as possible, the PIC and POC groups were randomly purchased from five separate major supermarkets chains. These chains represent over 70% of total UK retailer's volume sales, which are TESCO (30%), Sainsbury (21%), Safeway or Morrison (12% or 7%), Marks & Spencer and Waitrose (1%) (Oligopoly Watch, 2003). Although, Marks & Spencer and Waitrose share a small volume, these shops regularly supply organic chicken. Tesco, Sainsbury and Safeway provide organic chicken occasionally in selected branches. Ten separate butcher's shops were selected for the BIC purchase. All supermarkets and butcher's shops were located in North East London, the London Borough of Haringey, Barnet and Enfield (Figure 5.4).

In order to reduce possible temporal variation effects, timing of purchases from all supermarkets and butcher's shops was undertaken using 40 separate randomly allocated purchasing days (Appendix 2). In each purchase the name of supermarket or of butcher's shops, type of chicken and numbers of whole chickens were randomly drawn with no prior knowledge of their *Campylobacter* status.

**Figure 5.4** London borough map showing location of Enfield, Barnet and Haringey.

*Transport and storage of sample*

Each pre-packaged chicken was kept in a separate plastic bag. And all of them were transported and exposed to conditions that reflect the condition that may be experienced by the average consumer. On arrival at the lab, each chicken's type, source, weight, expiry date and price was recorded. Each chicken was then given a code on it as 'I1' (to 30) or 'O1 (to 30)' or `B1 (to 30)'. The letter **'I'** represents the PIC group, the letter **O** represents the POC group and the letter **B** represents the BIC group. The number reflects the order of purchase for each chicken. Following the coding all chickens were stored in the fridge ($5^{\circ}$-$10^{\circ}$C) until the next day when they were processed for *Campylobacter* isolation.

## 5.2. 2 Media components

### Enrichment broth

*Campylobacter* requires specific nutrient to recover the cell prior to be sub-cultured (Skirrow, 1974). The media must contain peptones as a nutrient source, laked horse blood for growth and antibiotics to prevent other competitive bacteria. Based on the recommendation of the U.S. Food and Drug Administration (FDA BAM, 1998) and using method proposed by Baylis *et al.* (2000), this study used Bolton selective enrichment broth (CM0983, Oxoid Ltd, Co., Basingstoke, Hants, UK) for *Campylobacter* isolation. The compositions of Bolton broth are shown in Appendix 1-1.

In order to quench toxic oxygen, *e.g.* hydrogen peroxide, which may form on the media exposure to light, 5% laked horse blood (SR0048, Oxoid Ltd, Co., Basingstoke, Hants, UK) was added to the broth.

A vial of Bolton broth selective supplement (SR0183 Oxoid Ltd, Co., Basingstoke, Hants, UK), which consists of three antibiotics, was added to 500 ml of broth to suppress the growth of other micro-organisms which may complete with *Campylobacter*. The components of selective supplement are presented in Appendix 1-2.

### *Campylobacter* blood-free selective agar and the selective supplement

The use of *Campylobacter* Blood-Free Selective Agar is specified by the UK Ministry of Agriculture, Fisheries and Food (MAFF) in a validated method for isolation of *Campylobacter* from foods (MAFF, 1993). A vial of mCCDA selective supplement (SR 0155: Oxoid Ltd, Co., Basingstoke, Hants, UK) was added to the 500 ml medium for suppressing other micro-organisms. The composition of Modified Charcoal Cefoperazone Deoxycholate agar (mCCDA: CM0739 Oxoid Ltd, Co., Basingstoke, Hants, UK) and CCDA selective supplement are described Appendix1-3.

## 5.2.3 Sample preparation

Three samples were taken from three different parts of each whole broiler carcass. These were: a) meat and skin, b) a whole tail and c) internal cavity (Figure 5.5).
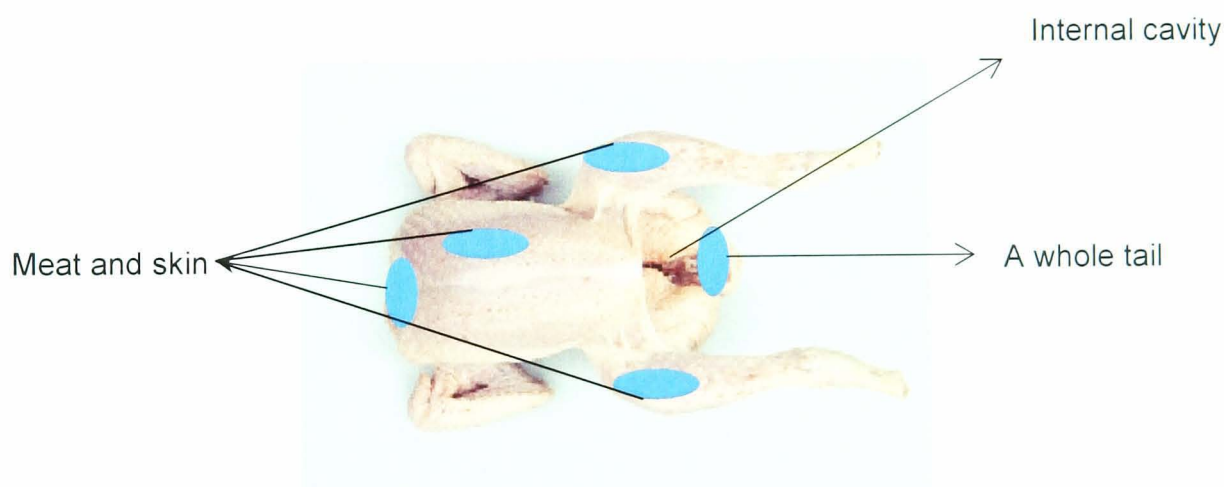


**Figure 5.5** Three sampling parts of a chicken carcass

A total of 100 g of meat and skin were cut from the neck, the breast and both thighs, and put into a sterile bag. These were cut into very small pieces and mixed thoroughly. 25 g was put into a stomacher bag containing 100 ml of Bolton broth. The sample was homogenised in a stomacher at a frequency of 230 rpm for 2 minutes. The homogenate solution was then poured into a 300-ml glass bottle with a screwcap. 125 ml Bolton broth was added. The final dilution of homogenised sample was $10^{-1}$.

The whole tail of each broiler was cut off and then weighed, and put into a sterilised plastic bag containing 100 ml of Bolton broth. It was homogenised by a stomacher for 2 minutes and poured into a 300-ml glass bottle with screwcap. The final volume was determined based on the original weight of the tail making a dilution to $10^{-1}$.

The liquid in the internal cavity was swabbed with 10 moistened specified 100 cm$^2$ (Duffy *et al.*, 2001) and pre-weighed, sterile cotton swabs. After liquid collection, all swabs were weighed again in order to obtain the approximate volume of liquid. Then the swabs were put into a 100-ml glass bottle with screwcap containing the appropriate volume giving the final dilution equal to $10^{-1}$ dilution. The sample was mixed thoroughly by using vortex (Vortex Genie2) for 2 minutes.

## 5.2.4 Enumeration

Two significant approaches long used for bacterial enumeration are the conventional most probable number (MPN) and direct plating (DP) method. Each method has advantage and disadvantage for enumeration of *Campylobacter*. Both methods are used in this study. However, a conventional MPN (c-MPN) requires a large quantity of materials (for enrichment, sub-culture and confirmation), incubator space and time consuming, the new modified MPN (m-MPN) was then developed for replacement of a c-MPN in order to reduce some of these disadvantages (*i.e.* material use and incubator space).

### 5.2.4.1 MPN and Direct plating methods

#### The conventional MPN

A conventional MPN was performed by using a 5-tube set for three consecutive volumes, 10, 1 and 0.1 ml (Oblinger and Koburger, 1975). The homogenate samples (see section 5.2.3) were diluted by 10-fold serial dilutions. Aliquots of 10 ml of each dilution were transferred into a test tube containing 10 ml of Bolton broth one by one for 5 test tubes. Similarly, aliquots of 1 and 0.1 ml were also transferred into other set of 5 test tubes containing 5 ml of Bolton broth. All tubes were incubated micro-aerobically at 37° C for 24 hours and 48°C for 24 hours. The micro-aerobic atmosphere was generated by using commercial gas-generating kits (BBL™ GasPack™) in an airtight container. Presumptive positive results were identified from the change in media turbidity. 30 μl aliquots of presumptive positive samples were streaked onto a mCCDA and micro-aerobically incubated at 42°C for 48 hours. Presumptive colonies of *Campylobacter* were identified by the specific appearance of colony on the agar. These were then inspected under a light microscope for motility and gram staining and were tested for hippurate hydrolysis.

## The modified MPN (m-MPN)

A modified MPN (m-MPN) was developed based on the principle of a c-MPN. The modifications were carried out on three aspects: i) a 48-well plate was used instead of sets of 5 tubes for the volumes of 1 ml and 0.1 ml and ii) the new volumes of 10 ml and 1 ml volume were used for the first sample dilution and iii) for each subsequent sample dilution these volumes were represent 0.1 ml of the appropriate previous volume (e.g. volume of original 1 and 0.1 ml of the original concentration ($10^{-1}$) are considered equivalent to 10 ml and 1 ml of the concentration of $10^{-2}$. The m-MPN is then interpreted through the use of the c-MPN table. Tests comparing the c-MPN and the m-MPN were carried out in parallel. The reliability of the m-MPN was statistically analysed before the m-MPN was used throughout the study. These are illustrated in Figure 5.6.
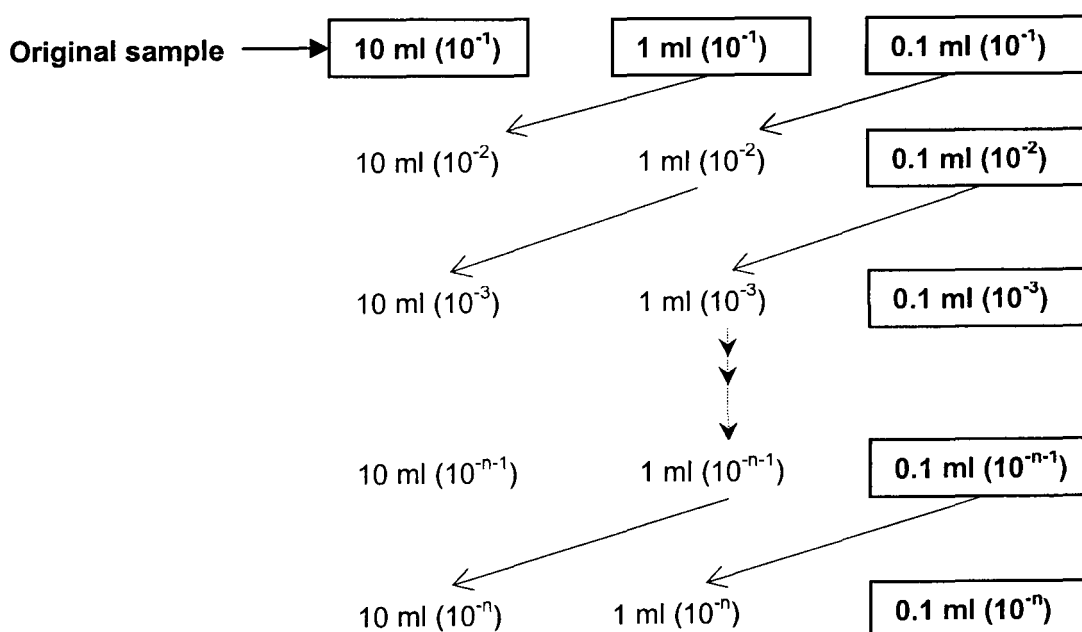


**Figure 5.6** Algorithm of modified volumes of serial dilutions used in the m-MPN

Aliquots of 10ml of the original homogenate samples ($10^{-1}$ dilution) were processed in the same way in the c-MPN. Using the new modified volumes, 1 ml aliquots of $10^{-1}$ were transferred into a well plate for 5 wells, following with consecutive volumes, 0.1 ml aliquots $10^{-1}$ to $10^{-n}$ (PIC and POC $10^{-1}$-$10^{-7}$ and BIC $10^{-1}$-$10^{-12}$). These volumes were transferred into a well plate for 5 wells in each dilution (Figure 5.7a). To make the final volume of each well to 1.4 ml (maximum volume of each well is approximately 1.5 ml), Bolton broth was added into all wells.

All 48-well plates were micro-aerobically incubated at 37oC for 24 hours and then at 42oC for 24 hours. The presumptive positive results were identified from the change of media turbidity like the conventional method stated above. Aliquots of each presumptive positive sample (30 µl) were dropped onto a mCCDA (Figure 5.7b). They were micro-aerobically

incubated at $42^{\circ}C$ for 48 hours. Presumptive colonies of *Campylobacter* were identified by the specific appearance of a colony on the agar. These were then inspected under the light microscope for motility and gram staining and were tested for the hippurate hydrolysis.

The positive control organism was *Campylobacter jejuni* (NCTC 11322). The negative control organisms were *Escherichia coli* (NCTC 10418), *Pseudomonas aeroginosa* (NCTC 10662) and *Staphylococcus aureus* (NCTC 6571). The negative control sample was cod fish and the blank was the media (Bolton broth supplemented with lake horse blood and SR0183) sterilised. These were processed in the same way as chicken samples.
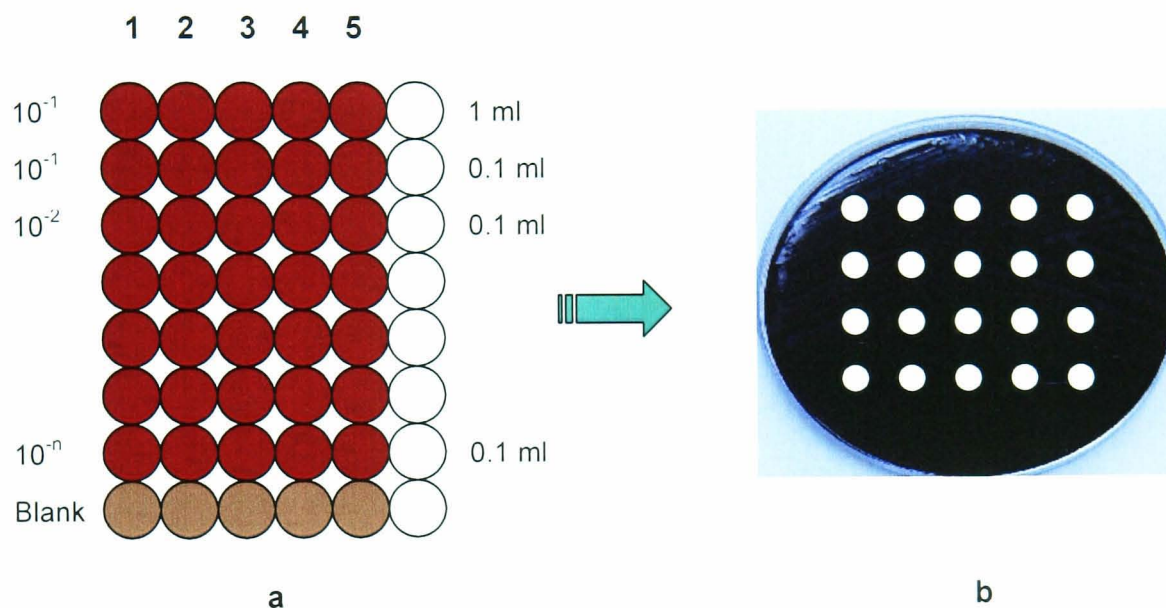


**Figure 5.7** The m-MPN method using 6 x 8 well plate

## The Direct plating method

Aliquots of pre-enrichment homogenate (100 µl ) were directly dropped onto a mCCDA plate, and then streaked out on the surface of the agar in duplicate plate. All inoculated plates were micro-aerobically incubated at $42^{\circ}C$ for 48 hours.

## 5.2.4.2 Protocol of enumeration

Prior to application of the new m-MPN method was tested in parallel with a conventional MPN in order to evaluate its reliability. The supplementary tests were then carried out for 20 samples of chicken parts. The results were statistically compared. After supplementary tests showed no significant difference between a c-MPN and m-MPN, the new modified technique was used for enumeration instead of a c-MPN throughout this study.

The enumerations and isolations of *Campylobacter* from three groups of chickens were carried out using the m-MPN and DP methods. A scheme of detection including enumeration applied to three groups of chickens is presented in Figure 5.8.
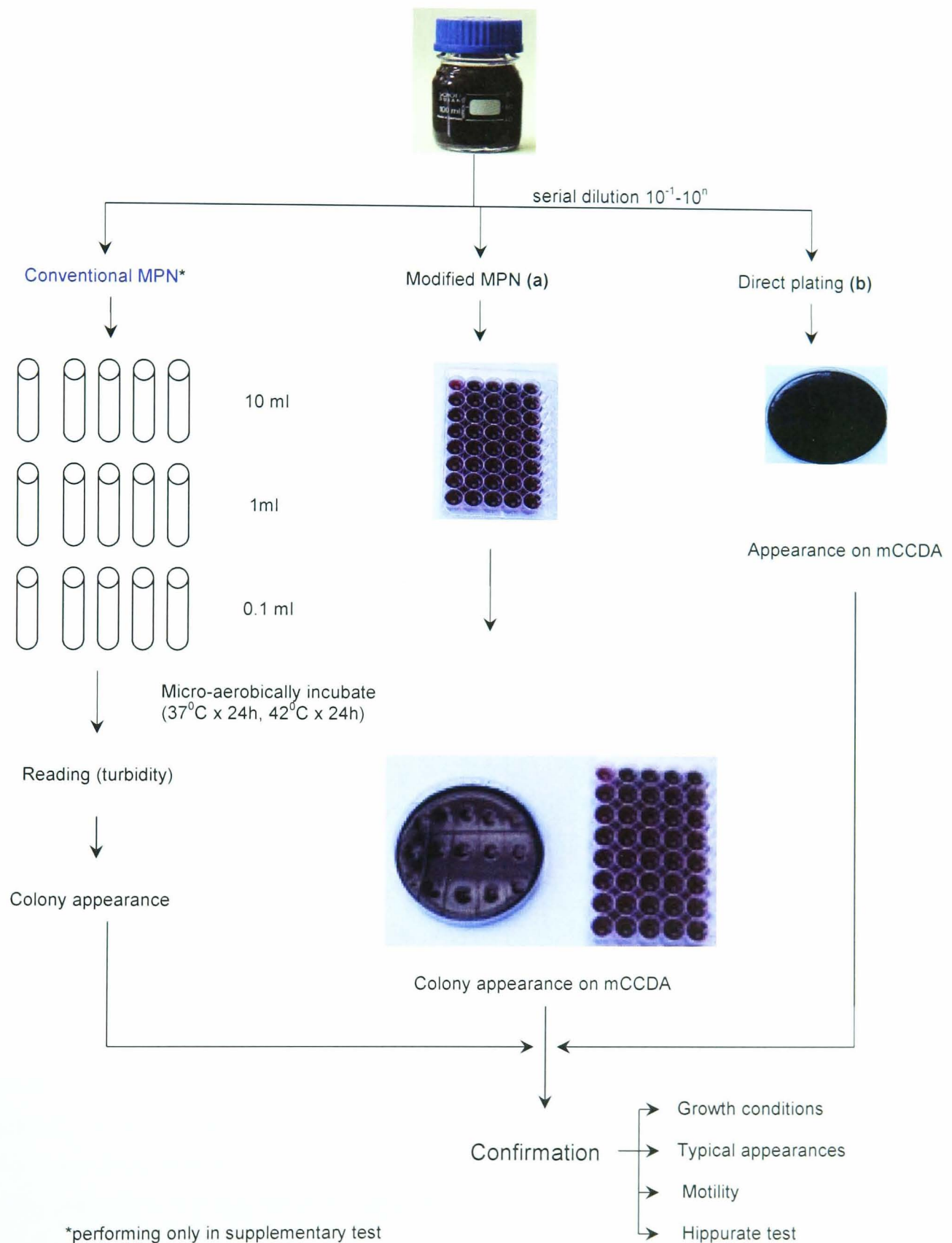


**Figure 5.8** Scheme of *Campylobacter* enumeration methods

## 5.2.5 Isolation method

The isolation and identification methods used in this study were based on the standard methods recommended by the US Food and Drug Authority (FDA BAM,1998) and the UK Health Protection Agency (UK HPA, 2002) which include enrichment, sub-culture and confirmation methods.

*Campylobacter* in food samples deteriorates with time. They therefore require an enrichment stage for optimal isolation and cultivation. Each sample was pre-enriched in Bolton broth (section 5.2.3), and stored in a glass bottle fitted with a screw cap leaving a head-space of less than 2 cm (Tran, 1998 and Baylis *et al.*, 2000). The inoculated broth was aerobically incubated at 37°C for 24 hours followed with 42°C for another 24 hours. After enrichment, 30μl of the inoculated broth were streaked onto 2 duplicate plates of mCCDA. The plates were then incubated under a micro-aerobic condition, using a commercial gas-generating kits (BBL™ GasPack™) in an airtight container (an aerobic gas jar), at 42°C for 48 hours.

## 5.2.6 Identification methods

Following cultivation on mCCDA (section 5.2.5), all plates were examined for the presence of typical and atypical colonies. The presumptive colonies (one or more typical colonies) were selected from each mCCDA plate for further confirmation using characteristics explained in section 5.1.2.

Presumptive colonies were confirmed by: i) selective media and growth condition, ii) general typical appearances, iii) traditional gram staining, iv) cell morphology and motility, v) biochemical tests (including catalase test, oxidase test and hippurate test) and vi) FISH. The principles of confirmation methods for *Campylobacter*, mainly *C.jejuni and C.coli* are explained in section 5.1.2.

**Procedures of confirmation methods**

Motility

Motility was determined by a wet mount method (Koneman *et al.*, 1997). A very small drop of suspect *Campylobacter* suspension was applied onto a slide and covered with a coverslip. The preparation was examined immediately, using light microscopy with the magnification 1000. *Campylobacter* species were highly motile slender rods with spiral morphology.

Catalase test

Suspect colonies were spread out on the slide. A drop of 3% hydrogen peroxide was dropped over the colonies. Positive result was detected by gas production by visual observation of bubble formation.

## Oxidase test

2 or 3 drops of 1%NNNN-tetramethyl-p-phenylenedianine dihydrochloride were placed on the centre of filter paper (Whatman No.1) and left to allow for a few seconds for absorption. A loopful of presumptive colony was smeared onto the paper by using a plastic loop. The reaction of the enzyme with oxygen produces a dark purple colour in positive samples within 5-10 seconds.

## Hippurate hydrolysis test

A loopful or more of suspect colonies was inoculated into 0.4 ml of 1% aqueous sodium hippurate and incubated in a water bath at 37°C for 4 hours. 0.2 ml of Ninhydrin solution was slowly added to form an overlay, taking care not to shake the tubes after Ninhydrin addition. The tubes were re-incubated at 37°C for 20 minutes. Hydrolysis of hippurate was indicated by a colour change to dark purple due to the release of glycine. A purple colour is a positive result for *C.jejuni* (Figure 5.2). No colour or a faint trace of purple is a negative result.

It is interesting to observe that the number of loopfuls is important for the reaction. This appears to be dependent on the characteristic of colonies isolated from chicken sample. For example, if the colonies are thick, creamy and loose, a loopful would be sufficient for the reaction. In contrast, more than a loopful would be required if the colonies showed thin, flat and sticky characteristics.

## FISH

This study performed the FISH method by using a partial 16S rRNA gene sequence (DNA probe) as a probe to detect *Campylobacter* species (Amann *et al.*, 1995, Christensen *et al.*, 1999 and Moreno *et al.*, 2001).

*The probe type*

There were two probes are used in this study, EUB 338 and CAM 1. The former is a universal probe complementary to the domain bacteria, which are common and live almost anywhere. It was used as a positive control to select all bacteria in samples. The sequence of EUB 338 is 5'-FGCTGCCTCCCGTAGGAGT (prepared by Sigma Genosys, Cambridge, U.K.).

The latter is complementary to a portion of the 16s rRNA gene conserved in the domain of *Campylobacter*. The CAM 1 probe is specific for *C.jejuni* and *C.coli*. This probe is prepared and labelled by Sigma Genosys (Cambridge, U.K.) with 5(6)-carboxy-fluorecein-N-hydroxysuccinimide ester (FLUOS) and tetramethylrhodomine-5-isothyocyanate (TRITC), of

which the maximum absorbance is 555 nm and the maximum emission 580 nm. The sequence of the oligonucleotide is 5'OCTGCCTCTCCCTCACTCT.

*Probe preparation*

The oligonucleotide, CAM 1 or EUB 338 was normalised in 1XTris-EDTA buffer (TE buffer; 10mM TrisHCl and 0.1mM EDTA). To prepare a stock solution of 1 µg/µl, the probe was dissolved in TE buffer in proportion to the amount of mass (based on the preparation of the company), which is 1:1. This stock solution was maintained at $-40^{\circ}$C until required. For a working solution (100 ng/µl), an aliquot of stock solution (1µl) was dissolved in 9 µl of 1X TE buffer.

Procedure

*Fixation*: aliquot of each homogenate sample (1 ml) (see section 5.2.3) was put into a 2-ml micro-centrifuged tube. The cells were harvested by centrifuging at 940-990 rpm for 5 minutes and the supernatant discarded. The pellet was washed twice with 1 ml of 1x PBS. The cells were fixed in 1 ml of paraformaldehyde (4%) at $4^{\circ}$C for 10 - 20 minutes.

*Sample preparation*: Following fixation, the cells were washed once again with 1 ml of 1xPBS and re-suspended it with 1xPBS and ice-cold absolute ethanol at a 1:1 ratio. A 5 µl aliquot of fixed sample was applied onto a gelatine-coated well slide (Figure 5.9), air-dried and dehydrated with serial concentrations of ethanol (50%, 80%, 98-100%).
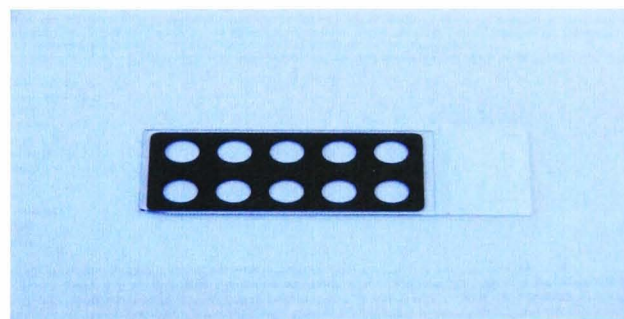


**Figure 5.9** A 10-well slide

*Hybridization*: The cells were hybridized with the CAM1 and EUB 338 probes (volumes applied are presented in Table 5.5). The cells were incubated to enable hybridisation at $46^{\circ}$C for 2 hr.

**Table 5.5** Proportion of CAM 1 and EUB 338 used for hybridization

| Numbers of probes | Volume (µl) | | |
| --- | --- | --- | --- |
| | CAM 1 | EUB 338 | HB* |
| 1 probe | 100 | - | - |
| 2 probes | 50 | 50 | - |

\* Hybridization buffer (HB) was not combined with the probes in this study

*Washing*: The remaining probes were washed out with a warm washing buffer (the buffer was pre-heated at temperature $60^0$C in a water-bath). The slide was then rinsed with sterile water.

*Mounting and visualisation:* a slide was mounted (wet) with the antifade agent (1,4-phenylene diamine:1,4 phenylene diamine, 86% glycerol, 1xPBS, sodium bicarbonate and sodium carbonate) and visualized using a fluorescence microscope at 1000 magnification (Olympus® BX51/BX52).

The model illustrated in Figure 5.10 displays the flow chart of the general procedures of FISH.



Fixation

Sample preparation

Hybridization

Washing

Mounting

Visualisation
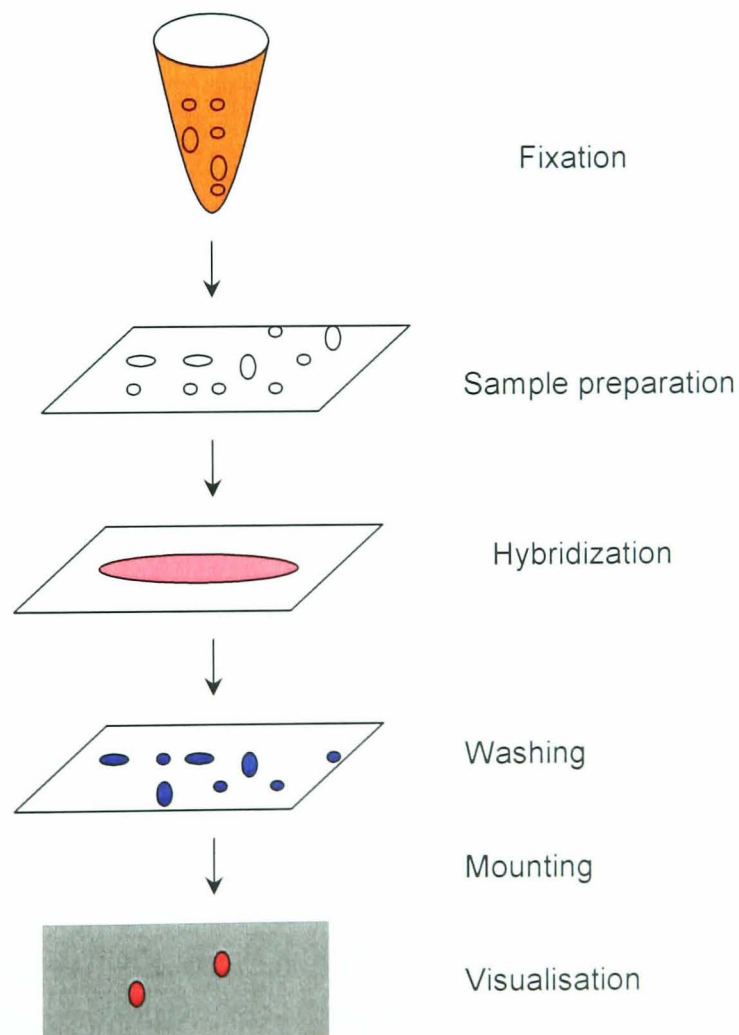
**Figure 5.10** Flow chart of a FISH method (adopted from Moter and Göbel, 2000)

## 5.3 Data analysis and statistical approaches

### 5.3.1 Data transformation

From literature reviews, MPN values usually show skewed distribution pattern. The MPN values in this study were transformed to be logarithmIt for the calculation. The transformed values become symmetric in shape at the natural log level (Limpert *et al.*, 2001, Bohannan and Hughes, 2003) and are therefore log-normally distributed. The lognormal distribution is the function of x given a mean ($\mu$) and a standard deviation ($\sigma$);

$$f(x \setminus \mu, \sigma) = \frac{1}{x\sigma\sqrt{2\pi}} e^{-(\ln x - \mu)^2 / 2\sigma^2}$$

The geometric mean, standard deviation and variance were used as representive parameters for statistical analysis. These parameters are determined by the formulas as follows;

Mean $\quad = \exp(\mu + \sigma/2)\quad$ [or using @ risk, v 4.5*, RiskLognormal2 ($\mu,\sigma$)],

Variance $\quad = [\exp(\sigma^2) - 1]\exp(2\mu + \sigma^2)$

Standard deviation = $\sqrt{[\exp(\sigma^2) - 1]\exp(2\mu + \sigma^2)}$

\* @ Risk (Risk analysis and simulation add-in for Microsoft Excel release 4.5, Palisade Corporation, Newfield, NY, USA)

### 5.3.2 Statistical approach

#### Comparison of two MPN methods

To ascertain whether the difference between the two methods of MPN, conventional MPN and modified MPN, is significantly different, the data were analysed statistically using the Mann-Whitney.

#### Comparison of the levels of MPN between three groups of chicken

Under conditions explained in section 5.2.1, all samples are considered to be randomly selected and independent. The differences of the levels of MPN of *Campylobacter* isolated from samples obtained from three groups of chicken were examined using the Analysis of Variance (ANOVA) test (using Minitab release 14). Prior to ANOVA approach, the equality of variance of the data was tested. Multiple comparisons were performed after ANOVA in order to determine where a difference in the samples' means exists. The means of all samples were compared by Tukey's Honestly Significant Difference (HSD) Test.

All statistical analyses were performed using Minitab, release 14, 2005.

## Comparison of the isolation rate

The difference in isolation rates between two groups was analysed using a 2 proportions test, computing a confidence interval and testing the hypothesis test using Fisher's exact test. A chi-square test was performed to compute the difference of isolation rates of *Campylobacter*-positive chicken between three groups of chicken, PIC, POC and BIC.

## 5.3.3 Estimating sensitivity and specificity of detection method with an imperfect gold standard

The qualitative measurement of detection for micro-organisms in a sample is expressed by a positive or a negative reading. As there are several detection methods usually used for identifying the status of tested samples, index tests must be evaluated by comparison with a perfect gold standard, which assesses the real status of sample with certainty (Joseph *et al.*, 1995). The performance of such a test is usually expressed by its sensitivity and specificity. Sensitivity and specificity of index tests can be estimated using the 2 X 2 table under a binomial sampling model. Ideally, if a gold standard exists, the observed performance is the true performance. There is no bias from measurement error, where parameters a, b, c and d are equal to zero. Table 5.6 demonstrates the performance of an index test when a perfect gold standard is available.

**Table 5.6** Observed status of index test when a perfect gold standard is known (parameters a, b, c and d are bias due to measurement error)

| Index test (I) | True status | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Positive | | | Negative | | | |
| | Reference test (R) | | | Reference test | | | |
| | Positive | False negative | Sub-total | False positive | Negative | Sub-total | Total |
| Positive | $x_{11}$-a | a (=0) | $x_{11}$ | c (=0) | $x_{12}$-c | $x_{21}$ | $n_{I+}$ |
| Negative | $x_{21}$-b | b (=0) | $x_{21}$ | d (=0) | $x_{22}$-d | $x_{22}$ | $n_{I-}$ |
| Total | $n_{R+}$+(a+b) | a+b | $n_{R+}$ | c+d | $n_{R-}$-(c+d) | $n_{R-}$ | N |

**Where:** $x_{11}$-a is that truth = +, reference = +, index = +; a is that truth = +, reference = -, index = +

$x_{12}$-c is that truth = -, reference = -, index = +; c is that truth = -, reference = +, index = +

$x_{21}$-b is that truth = +, reference = +, index = -; b is that truth = +, reference = -, index = -

$x_{22}$-d is that truth = -, reference = -, index = -; d is that truth = -, reference = +, index = -

$$Sensitivity = x_{11} / n_{R+} \qquad [5\text{-}1]$$

$$Specificity = x_{22} / n_{R-} \qquad [5\text{-}2]$$

$$False_{Pos} = x_{12} / n_{I+} \qquad [5\text{-}3]$$

$$False_{Neg} = x_{21} / n_{I-} \qquad [5\text{-}4]$$

However, it has been recognised that a perfect gold standard may be unavailable, particularly for index tests used in diagnosis of microbiological infection. This is due to measurement error or possible high costs, (Joseph *et al.*, 1995 and Hadgu, 1999). More frequently available but imperfect reference tests are used as a gold standard. Misclassification by imperfect gold standard may introduce bias and result in error of sensitivity, specificity and predictive prevalence (Hadgu, 1999). These errors can be resolved using two different approaches: i) improving an imperfect gold standard (*i.e.* using technologic advanced methods, using more than one reference test) and ii) applying mathematical and statistical resolutions (Joseph, 1995; Hadgu, 1999 and Dendukuri *et al.*, 2004).

The three culture methods for detecting *Campylobacter* used in this study were the m-MPN, the TC and the DP. Limitations of these methods have been recognised as *Campylobacter* in food samples may change to the viable-non-culturable form (VNC) that cannot be detected by conventional culture methods. Although most recent studies used direct plating to enumerate as well as to detect *Campylobacter* (Mead *et al.*,1995; Stern *et al.*1995; Bashors *et al.*, 2004 and Keener *et al.* 2004), most samples were obtained from live birds rather than food samples (carcasses). *Campylobacter* can grow and multiply easily in the gut of live birds. Thus, it is easier to detect *Campylobacter* from caecal content without enrichment as the bacteria are likely to be in good condition for growth. Chen and Stern (2001) postulated that the good colonisers were isolated from caeca or caecal droppings, whereas most poor colonisers were found from broiler wash. The test performances (*i.e.* sensitivity, specificity, false positive and false negative) of these methods are uncertain for food samples. Relative comparison for the performances of an index test requires a gold standard (Joseph *et al.*, 1995). However, none of the methods used here (the TC, m-MPN and DP) can be a gold standard. The performances of these three methods may include uncertainties resulting from using an imperfect test as a gold standard. Evaluation of test performances is therefore necessary.

In this study, evaluation of test performances was considered following two criteria: i) within pre-enrichment methods (the TC and the m-MPN) and ii) between enrichment (the m-MPN) and before enrichment methods (the DP). For the former, sensitivity, specificity, false positive and false negative of the TC and the m-MPN were calculated twice with different gold standards by switching between the TC or the m-MPN as a gold standard. Similarly, for the latter, performances were evaluated using either the m-MPN or the DP as a gold standard for each other.

As neither of them can be a gold standard, errors occur. This may result in decrease or increase of the performances, affecting the true prevalence. Although recently, several studies suggested that molecular methods show higher performances than conventional culture methods (Wassenaar and Newell, 2000), these methods are expensive and also

detect both viable and non-viable cells. Threfore, whilst the molecular based methods overestimate the prevalence, the culture based methods underestimate the prevalence. The application of mathematics is therefore considered as an alternative approach in revising the bias from measurement error. It has been suggested that this problem can be resolved using mathematical functions, *e.g.* Bayesian estimation, discrepant analysis (Joseph, 1995; Hadgu, 1999 and Dendukuri *et al.*, 2004). The performances of parameters have to be revised following new assumption as presented in Table 5.7. The parameters a, b, c and d are biased due to measurement errors.

**Table 5.7** Assumption of observed status of index test when using an imperfect gold standard: parameter $x_{ij}$ as in table 5.6 and parameter a, b, c and d as measurement error

| Index test | True status of sample | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Positive | | | Negative | | | |
| | Reference test | | | Reference test | | | |
| | Positive | False negative | Sub-total | False positive | Negative | Sub-total | |
| Positive | $x_{11}$-a | a | $x_{11}$ | c | $x_{12}$-c | $x_{12}$ | $n_{I+}$ |
| Negative | $x_{21}$-b | b | $x_{21}$ | d | $x_{22}$-d | $x_{22}$ | $n_{I-}$ |
| Total | $n_{R+}$+(a+b) | a+b | $n_{R+}$ | c+d | $n_{R-}$-(c+d) | $n_{R-}$ | N |

As the reference test is thought to be imperfect, the new cross-classification of events can be further developed as demonstrated in Table 5.8.

**Table 5.8** Observed status of index test when using an imperfect gold standard

| Index test | Imperfect reference | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Positive | | | Negative | | | |
| | True status | | | True status | | | |
| | Positive | False pos. | Sub-total of observed pos. | False neg. | Negative | Sub-total of observed neg. | |
| Positive | $x_{11}$-a | c | $x_{11}$-a+c | a | $x_{12}$-c | $x_{12}$+a-c | $n_{I+}$ |
| Negative | $x_{21}$-b | d | $x_{21}$-b+d | b | $x_{22}$+b-d | $x_{22}$+b-d | $n_{I-}$ |
| Total | $n_{R+}$-(a+b) | c+d | $n_{R+}$-(a+b)+(c+d) | a+b | $n_{R}$-(c+d) | $n_{R}$-(c+d)+ (a+b) | N |

Following Table 5.8, the new observed data can be re-arranged and presented in Table 5.9.

**Table 5.9** New observed status of index test after adjustment

| Index test | Imperfect reference | | Total |
| --- | --- | --- | --- |
| | Positive | Negative | |
| Positive | $x_{11}$-a+c | $x_{12}$+a-c | $n_{I+}$ |
| Negative | $x_{21}$-b+d | $x_{22}$+b-d | $n_{I-}$ |
| Total | $n_{R+}$-(a+b)+c+d | $n_{R-}$-(c+d)+ a+b | N |

Therefore, new sensitivity, specificity, false positive and false negative of index performance can now be re-expressed as follows:

$$Sensitivity = x_{11} - (a + c)/\{n_{R+} - (a + b) + (c + d)\} \qquad [5\text{-}5]$$

$$Specificity = x_{22} + (b - d)/\{n_{R-} - (c + d) + (a + b)\} \qquad [5\text{-}6]$$

$$False_{Pos} = x_{12} + (a - c)/n_{I+} \qquad [5\text{-}7]$$

$$false_{Neg} = x_{21} + (b - d)/n_{I-} \qquad [5\text{-}8]$$

When comparing with the relative performance of index test (using a perfect gold standard), the new expressions as presented in equations 5-5 to 5-8 can be developed to show the errors of measurement due to the use of an imperfect reference as a gold standard (Table 5.10).

**Table 5.10** Comparison of performance expressions between using a perfect gold standard and an imperfect gold standard

| Performance parameters | A perfect gold standard | An imperfect gold standard |
| --- | --- | --- |
| Sensitivity | $x_{11}/n_{R+}$ | $x_{11}$-(a+c)/{$n_{R+}$-(a+b)+(c+d)} |
| Specificity | $x_{22}/n_{R-}$ | $x_{22}$+(b-d)/{$n_{R-}$(c+d)+(a+b)} |
| False positive | $x_{12}/n_{I+}$ | $X_{12}$+(a-c)/$n_{I+}$ |
| False negative | $x_{21}/n_{I-}$ | $x_{21}$+(b-d)/$n_{I-}$ |

Tables 5.7 to 5.10 present the eight parameters that cannot be determined through measurement. These parameters are taken as uncertainties, affecting the true status of performance characteristics of an index test. This problem arises from misclassification. Joseph et al.(1995) proposed a solution to improve the performances of the index test using Bayesian estimation. The principle is that: 1) prior distributions of unknown parameters are firstly constructed over all unknown values, 2) The likelihood functions of missing information

are analysed using latent class analysis (LCA) (Kaldor and Clayton, 1985; Tanner and Wong, 1987; Water and Irwig, 1988; and Joseph *et al.*, 1995). It is therefore likely that the posterior distributions are a proportional product of prior distributions time likelihood functions using Bayes theorem.

### *Posterior distribution ∝ prior distributions x likelihood functions*

A prior distribution over all model parameters can be drawn from previous data, expert opinions or a combination of both. Joseph *et al.*(1995 and 2000) also demonstrated likelihood functions when using two diagnostic tests. These functions were derived from prevalence of positive results ( $\pi$ ), sensitivity of test 1 ($S_1$) and test 2 ($S_2$) and specificity ($C_1$ and $C_2$). These likelihood contributors are illustrated in Table 5.11

**Table 5.11** Possible likelihood contributors of observed and latent data for two detection tests (adopted from Joseph, *et al.*, 1995).

| Product | Truth | test 1 result | Test 2 result | Likelihood |
|---------|-------|---------------|---------------|------------|
| $y_1$ | + | + | + | $\pi S_1 S_2$ |
| $y_2$ | + | + | - | $\pi S_1(1-S_2)$ |
| $y_3$ | + | - | + | $\pi(1-S_1)S_2$ |
| $y_4$ | + | - | - | $\pi(1-S_1)(1-S_2)$ |
| $u - y_1$ | - | + | + | $(1-\pi)(1-C_1)(1-C_2)$ |
| $v - y_2$ | - | + | - | $(1-\pi)(1-C_1)C_2$ |
| $w - y_3$ | - | - | + | $(1-\pi)C_1(1-C_2)$ |
| $x - y_4$ | - | - | - | $(1-\pi)C_1C_2$ |

$\pi$ denotes prevalence of positive sample in population

$S_1$ denotes sensitivity of index test 1 using index test 2 as reference standard

$S_2$ denotes sensitivity of index test 2 using index test 1 as reference standard

$C_1$ denotes specificity of index test 1 using index test 2 as reference standard

$C_2$ denotes specificity of index test 2 using index test 1 as reference standard

Information presented in Table 5.11 can be re-written by 2 x 2 table as shown in Table 5.12 .

**Table 5.12** Observed and latent data for the case of two detection methods when using imperfect reference as a gold standard.

| Test 2 | True status | | | | | | Total |
|--------|-------------|---|---|---|---|---|-------|
| | Positive | | | Negative | | | |
| | Test 1 | | | Test 1 | | | |
| | + | - | Sub-total | + | - | Sub-total | |
| + | $y_1$ | $y_3$ | $y_1+y_3$ | $u-y_1$ | $w-y_3$ | $(u-y_1)+(w-y_3)$ | **a** |
| - | $y_2$ | $y_4$ | $y_2+y_4$ | $v-y_2$ | $z-y_4$ | $(v-y_2)+(z-y_4)$ | **b** |
| Total | $y_1+y_2$ | $y_3+y_4$ | $(y_1+y_3)+(y_2+y_4)$ | $(u+v)-(y_1+y_2)$ | $(w+z)-(y_3+y_4)$ | $(u+v+w+z)-(y_1+y_2+y_3+y_4)$ | **N** |

The new cross-classification can now be re-expressed as the real situation of using two diagnostic methods for calculating performance status. Table 5.13 shows the observed parameters following the new classification.

**Table 5.13** Observed data of two detection methods in the absence of a gold standard following table (adapted from Joseph et al., 1995)

| Test 1 (Index test) | Test 2 (Reference) | | Total |
| --- | --- | --- | --- |
| | Positive | Negative | |
| **Positive** | $y_1+(u-y_1)=u$ | $y_3+(w-y_3)=w$ | **u+w** |
| **Negative** | $y_2+(v-y_2)=v$ | $y_4+(x-y_4)=z$ | **v+z** |
| | u+v | w+z | **N** |

Parameters u, v, w and z are the actual values determined and these values include uncertainties due to measurement error. These parameters can be re-adjusted using Bayes' theorem to estimate the posterior distribution of performance characteristics. Given assumption propsed by Joseph et al. (1995) (shown in Tables 5.11-5.13), the joint posterior distribution is proportional to the product of the likelihood function and the prior distribution. If $(\alpha_\pi, \beta_\pi)$, $(\alpha_S, \beta_S)$ and $(\alpha_C, \beta_C)$ represent the prior beta distribution of the parameters for $\pi$, S and C, respectively, the joint posterior distribution formulated by Joseph et al. (1995) is given by

$$\pi^{u+v+\alpha_\pi}(1-\pi)^{N-u-v-\beta_\pi}S^{u+\beta_S}(1-S)^{v+\beta_S}C^{z+\beta_C}(1-C)^{\alpha-u+\beta_C} \qquad [5-9]$$

The joint posterior distribution (equation 5-9) can be constructed using the Gibbs sampler which is iterative with Markov-chain Monte Carlo (MCMC) technique (Appendix 3-2). This technique was carried out using Winbugs software (release 1.4). (Winbugs software is available at http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml.)

## 5.4 Results

### 5.4.1. Comparison of MPN levels estimated by two methods

A change in turbidity of the media and colony formation on the mCCDA were used to describe a presumptive positive or negative result in the MPN test (Figure 5.11).



**Turbidity**

**Colony formation**
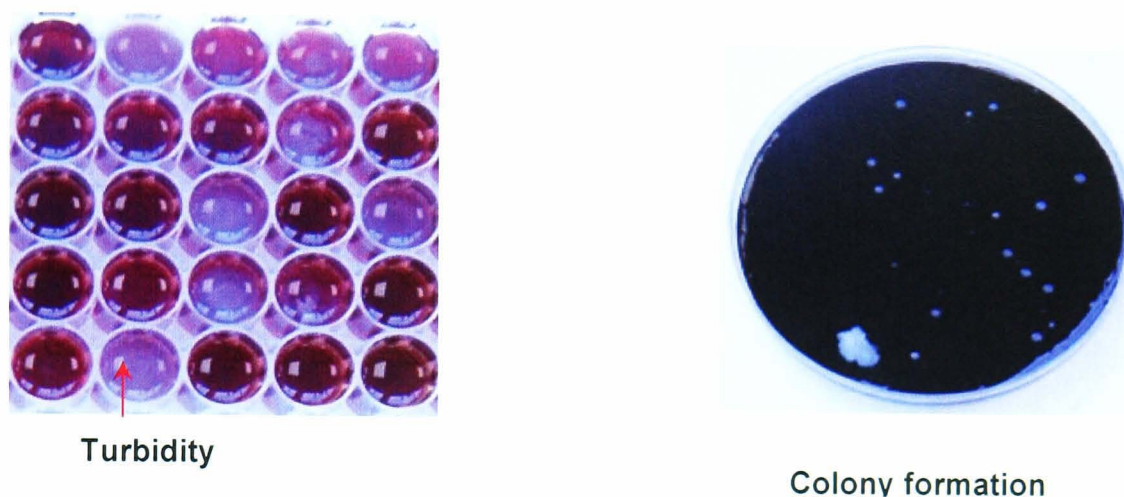
**Figure 5.11** Characteristics indicating the positive results for enumeration of *Campylobacter*

These presumptive results were analysed using the Mann-Whitney test. Although levels of MPN determined from the change of turbidity in the c-MPN method were lower than those expressed by the colony formation, this difference was not significant. Results from the m-MPN method in contrast showed a significant difference between the values interpreted by the two indicators at *p<0.001*. When comparing the two methods using the turbidity characteristics, the values estimated from the c-MPN were significantly higher than those from the m-MPN (*p= 0.002*). *However, there was no significant difference between the two methods when using colony formation as the indicator (p>0.05)*. Table 5.14 shows the results of this section.

**Table 5.14** *Campylobacter* levels determined by the c-MPN and the m-MPN method.

| Method | *Campylobacter* level ($log_{10}$MPN/g): | | | | *P-value* |
| | Turbidity | | Colony appearance | | |
| | Mean±SE* | 95%CI** | Mean±SE | 95%CI | |
|---|---|---|---|---|---|
| Conventional MPN (c-MPN) | 3.87±1.34 | 1.24, 6.5 | 4.23±1.44 | 1.40,7.06 | NS |
| Modified MPN (m-MPN) | 1.18±0.36 | 0.47,1.88 | 4.68±1.54 | 1.67, 7.69 | < 0.001 |
| *P-value* | 0.002 | | NS | | |

\* Geometric mean and geometric SE , ** 95% confidence interval of the mean

NS = No significant difference

## 5.4.2 Determination of test performances for three detection methods in the absence of a gold standard

### 5.4.2.1 Pre-adjustment performances of three detection methods

Tables 5.15 and 5.16 show the performance characteristics of three detection methods using different gold standards. When considering the difference between with and without enrichment methods for detecting *Campylobacter*, using the TC method as a gold standard , the m-MPN method showed higher sensitivity (91.8%) than that found for the DP (54.4%). However, it also gave a higher rate of false positive (26.2%). Although the DP was more specific than the m-MPN (66.7%), it contained very high rate of false negative (85.7%).

When using the m-MPN as a gold standard, the TC was found to have a lower sensitivity, but higher specificity than those shown in the m-MPN.

**Table 5.15** Performance of index test when using different reference tests as a gold standard: a) m-MPN, b) traditional culture and c) direct plating

| Index test | Gold standard: m-MPN | | G + | G- | Performance characteristics | Percentage (%) |
|---|---|---|---|---|---|---|
| TC | n = 180 | I + | 112 | 10 | Sensitivity | 71.8 |
| | | | | | Specificity | 58.3 |
| | | I - | 44 | 14 | False positive | 8.2 |
| | | | | | False negative | 75.9 |
| | **Gold standard: TC** | | G + | G- | **Performance characteristics** | **Percentage (%)** |
| m-MPN | n = 180 | I + | 112 | 44 | Sensitivity | 91.8 |
| | | | | | Specificity | 24.1 |
| | | I - | 10 | 14 | False positive | 26.2 |
| | | | | | False negative | 46.7 |
| | **Gold standard: m-MPN** | | G + | G- | **Performance characteristics** | **Percentage (%)** |
| DP | n = 88 | I + | 43 | 3 | Sensitivity | 54.4 |
| | | | | | Specificity | 66.7 |
| | | I - | 36 | 6 | False positive | 6.5 |
| | | | | | False negative | 85.7 |
| | **Gold standard: DP** | | G + | G- | **Performance characteristics** | **Percentage (%)** |
| m-MPN | n = 88 | I + | 43 | 36 | Sensitivity | 93.5 |
| | | | | | Specificity | 14.3 |
| | | I - | 3 | 6 | False positive | 45.6 |
| | | | | | False negative | 33.3 |

G+ = a gold standard shows positive results; G- = a gold standard shows negative results

I+ = an index test shows positive results;    I- = an index test shows negative results

**Table 5.16** Summary of comparison of performance characteristics of three index tests using different reference tests as a gold standard

| Gold standard / Index test | Performance parameters | | | | |
|---|---|---|---|---|---|
| | Predictive prevalence (%) | Sensitivity (%) | Specificity (%) | False positive(%) | False negative(%) |
| Traditional culture: m-MPN | 62.2 | 91.8 | 24.1 | 28.2 | 41.7 |
| m-MPN: Traditional culture | 62.2 | 71.8 | 58.3 | 6.5 | 75.9 |
| m-MPN : Direct plating | 48.9 | 54.4 | 66.7 | 6.5 | 85.7 |
| Direct plating : m-MPN | 48.9 | 93.5 | 14.28 | 45.6 | 33.3 |

## 5.4.2.2 Post-adjustment performances of the three detection methods using Bayesian estimation

Using an imperfect gold standard, previous values of the performances of the index tests were thought to include uncertainties occurring from measurement errors. Therefore, Bayesian estimation was applied to determine the joint posterior distribution following the studies of Joseph, *et al.* (1995 and 2001). As there is currently no previous information on the performance characteristics of these three methods, the prior distribution cannot be performed. Therefore, the true joint distribution (equation 5-9) cannot be calculated due to no information of beta-coefficient represented with $\alpha$, $\beta$. However, in order to demonstrate the approach to resolve the evaluation of performance characteristics of detection methods for *Campylobacter* in the absence of a gold standard, the beta prior densities (espressed with Beta coefficient $\alpha$, $\beta$) were adapted from the study of Joseph *et al.*, (1995), which performed the beta prior densities for the test parameters in the diagnosis of *Strongyloides* infection. Following this (Table 5.17), the prior coefficients selected were based on the fact that the TC and the m-MPN are performed after the samples were enriched. Under these conditions, *Campylobacter* cells are thought to be recovered from stressed environment during food processing. Thus, these cells are easier to detect (Moore, 2001). In contrast, the DP was performed without enrichment. Therefore, it is assumed that the TC and the m-MPN have high sensitivity and the DP has high specificity. The coefficients of the beta prior densities used for the demonstration are shown in Table 5.18

**Table 5.17** Coefficient of the beta prior densities for the test parameters in the diagnosis of *Strongyloides* infection (taken from Joseph *et al.*,1995)

| Test parameters | Stool examination | | | Serology | | |
|---|---|---|---|---|---|---|
| | Range (%) | Beta coefficients | | Range (%) | Beta coefficients | |
| | | α | β | | α | β |
| Sensitivity | 5-45 | 4.44 | 13.31 | 65-95 | 21.96 | 5.49 |
| Specificity | 90-100 | 71.25 | 3.75 | 35-100 | 4.1 | 1.76 |

**Table 5.18** Ranges and coefficients of the beta prior densities of three detection methods for *Campylobacter* (adapted from Joseph *et al.*, 1995)

| Performance parameters | TC | | | m-MPN | | | DP | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range (%) | α | β | Range (%) | α | β | Range (%) | α | β |
| Sensitivity | 35-100 | 4.1 | 1.76 | 65-95 | 21.96 | 5.49 | 5-45 | 4.4 | 13.31 |
| Specificity | 5-45 | 4.4 | 13.31 | 5-45 | 4.4 | 13.31 | 65-95 | 21.96 | 5.49 |

## Comparison of posterior performance parameters of three methods for the combination of the two tests

Assuming the prior distributions in Table 5.18 are correct, the posterior values confirmed the highest sensitivity, but lowest specificity of the m-MPN method. The TC does not demonstrate a good performance for both sensitivity and specificity. However, it is still higher than the DP. Although the DP showed very low sensitivity, it is very specific to negative samples. Table 5.19 shows the posterior sensitivity and specificity when using the two tests.

Figure 5.12 shows the densities of posterior distribution of sensitivity and specificity as calculated through simulation using Winbug software released 1.4.1. The densities of both sensitivity and specificity from the m-MPN method are found to be highly skewed, whereas the densities from the TC are symmetrical.

**Table 5.19** Posterior median and 95% confidence interval (CI) for the combination of the two tests: TC and m-MPN; m-MPN and DP

| Test | Parameter | Posterior values | | | | | |
|---|---|---|---|---|---|---|---|
| | | TC vs m-MPN | | TC vs DP | | m-MPN vs DP | |
| | | Median | 95% CI | Median | 95% CI | Median | 95% CI |
| -TC : | Sensitivity | 0.61 | 0.33-0.90 | NA | | NA | |
| | Specificity | 0.30 | 0.11-0.47 | | | | |
| -m-MPN: | Sensitivity | 0.81 | 0.64-0.92 | NA | | NA | |
| | Specificity | 0.24 | 0.08-0.46 | | | | |
| -m-MPN : | Sensitivity | | | | | 0.93 | 0.73-0.98 |
| | Specificity | | | | | 0.10 | 0.02-0.48 |
| -DP : | Sensitivity | | | | | 0.24 | 0.08-0.47 |
| | Specificity | | | | | 0.81 | 0.64-0.93 |

**Sensitivity**          **Specificity**
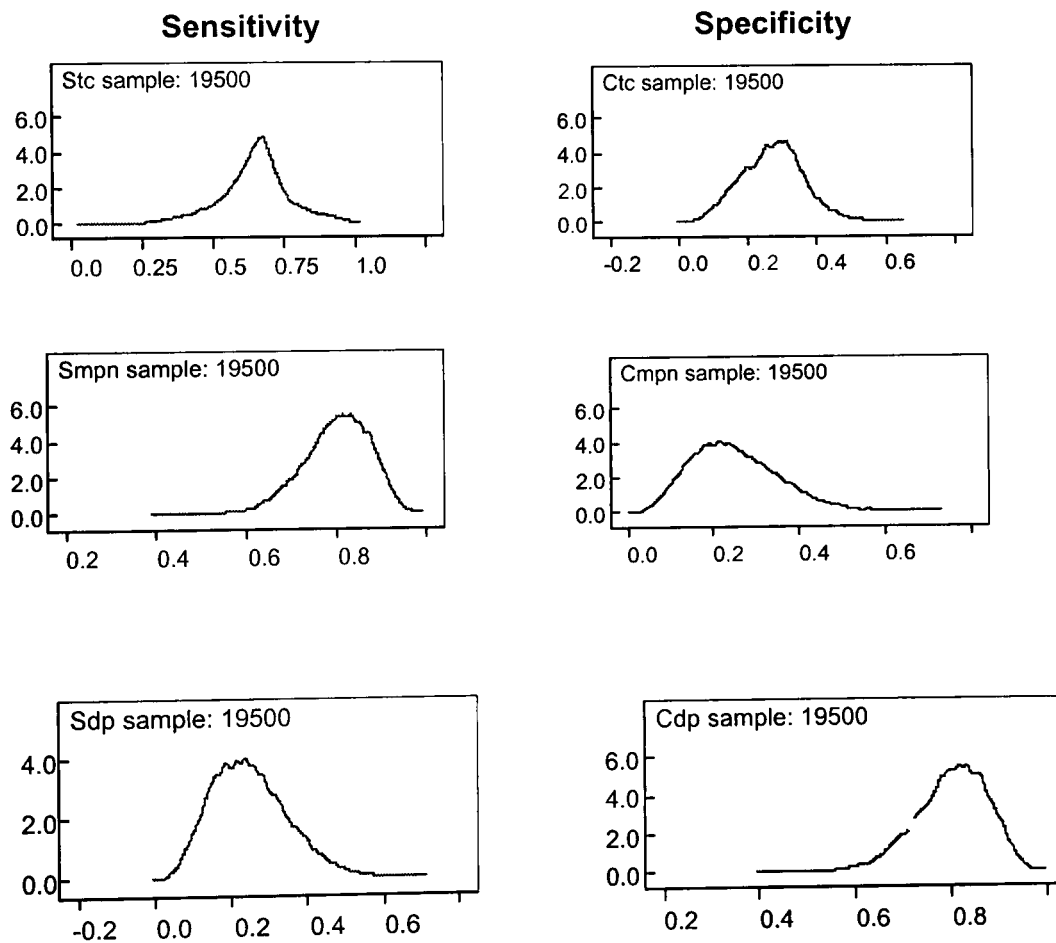


**Figure 5.12** Posterior distributions of sensitivity and specificity of three detection methods (tc = traditional method, mpn = modified MPN, dp = direct plating, S =sensitivity and C = specificity)

## 5.4.3 Isolation rates of *Campylobacter* isolated from three groups of chicken

### Rates of isolation from whole carcasses

Table 5.20 shows the isolation rate of *Campylobacter*. The isolation rates of positive-*Campylobacter* carcasses determined by the m-MPN method demonstrated a significantly higher rate than those determined by the TC and the DP in all groups of chicken ($p=0.03$ within the PICs, $p<0.001$ within the POCs and $p=0.007$ within the BICs). The differences of the isolation rate between the different groups range from 7 to 13%.

The isolation rates found in the BIC group showed the highest values whether it was determined by the TC (93%) or the m-MPN (100%) or the DP (90%). The rates found in the PIC group, in contrast, showed the lowest values (80% for the TC, 90% for the m-MPN and 77.8% for the DP). The isolation rates between these three groups are significantly different at $p=0.04$, except for that determined by the m-MPN. In addition, when using the FISH method for identification of *Campylobacter* in the BIC group, which was performed before the samples were enriched, both the m-MPN and the FISH were capable of detecting the organism at the same rate (100%).The image of *Campylobacter* cell hybridised with the CAM 1 probe visualised under the fluorescence microscope is shown in Figure 5.13

**Table 5.20** Isolation rate (per carcass) of *Campylobacter* obtained from three types of chickens

| Type of chicken | Isolation rate/ carcass (%) | | | | |
|---|---|---|---|---|---|
| | Detection procedure | | | | |
| | TC | m-MPN | DP | FISH* | P-value** |
| PIC | 80.00 | 90.00 | 77.78 | * | 0.03 |
| POC | 83.33 | 96.67 | 77.78 | * | <0.001 |
| BIC | 93 | 100 | 90 | 100 | 0.007 |
| *P-value* | 0.04 | NS | 0.04 | NA | |

\* Fluorescence In Situ Hybridization (FISH) performing on the BICs only

\** Significant difference between TC, m-MPN and DP, FISH was not included

NA =not applicable, NS = no significant difference
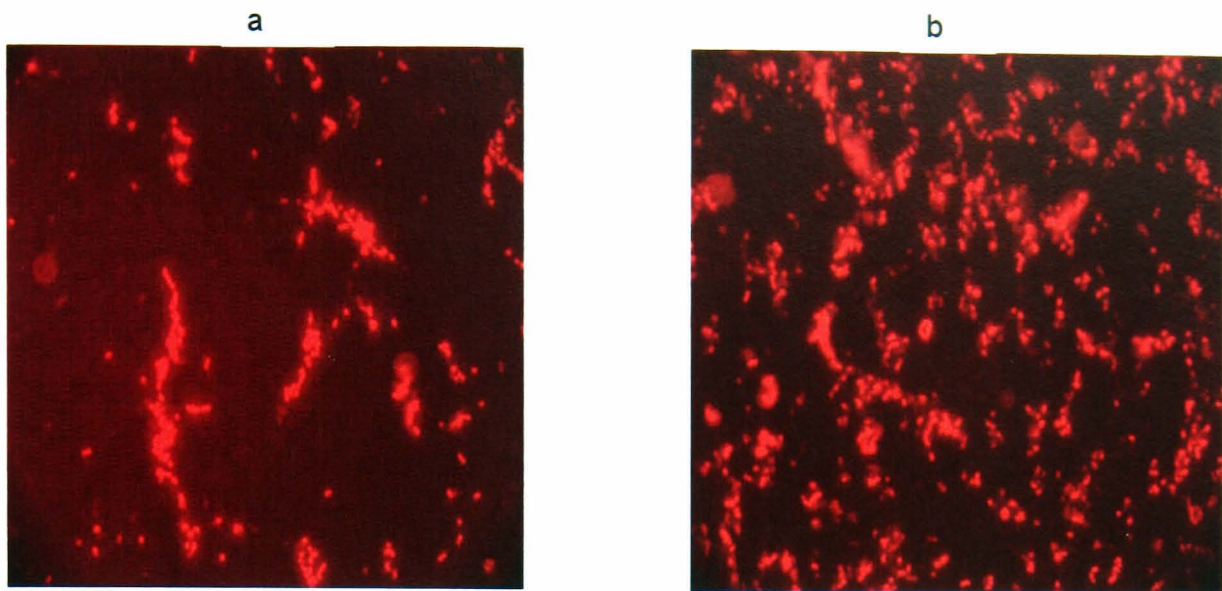
**Figure 5.13** The image of *Campylobacter* cell hybridised with the CAM 1 probe and visualised by fluorescence microscope (x 1000): a) *C.jejuni* NCTC 11322 (reference strain) and b) *Campylobacter* isolates from the BIC

## Rate per part of chicken

Using the TC method, the rate of isolation of *Campylobacter* obtained from meat or cavity or tail part of the BIC group showed a significantly higher level (~93%) than those found in the PIC and the POC samples ($p=0.04$) (Table 5.21).

However, when indicating by the m-MPN, only the outcomes from cavity samples showed significant difference ($p=0.002$) whereas the PIC-cavity samples showed the lowest level at 70%. Although the rates found in meat and tail samples differ between these three groups, they were no statistically significant differences.

When using the DP method for all groups, the isolation rates from different parts of the chicken were found to be significantly different ($p= 0.002$), with the lowest value in cavity samples (22% for the PICs, 33% for the POCs and 46% for the BICs).

The rates estimated using the direct plating provided the significant lowest value compared to the other two methods. These significant findings were similarly noted in all three groups of the chicken. The m-MPN gives the highest rate of detection in all cases.

In relation to the four detection methods used for the BIC group, the m-MPN and the FISH gave the highest rate (100%) of detection, following by the TC (93%). The direct plating method contributed the lowest rate of isolation (46 – 80%).

**Table 5.21** Isolation rate (per part) of *Campylobacter* obtained from three types of chickens determined by the TC, the m-MPN, the DP and the FISH methods

| Type of chicken | Isolation rate/ part of chicken (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TC | | | mMPN | | | Direct plating | | | FISH* | | |
| | Meat | Cavity | Tail | Meat | Cavity | Tail | Meat | Cavity | Tail | Meat | Cavity | Tail |
| PIC[1] | 86.67 | 60.00 | 70.00 | 83.33 | 70.00 | 83.33 | 55.56 | 22.22 | 44.44 | * | * | * |
| POC[2] | 76.67 | 73.33 | 76.67 | 90.00 | 90.00 | 90.00 | 77.78 | 33.33 | 72.22 | * | * | * |
| BIC[3] | 93.33 | 92.86 | 93.33 | 100 | 100 | 100 | 80.00 | 46.43 | 80.00 | 100 | 100 | 100 |
| *P-value* | *0.04* | *0.04* | *0.04* | NS | *0.002* | NS | *0.002* | *0.002* | *0.002* | NA | NA | NA |

[1]significant at $p<0.001$ for the rates on meat or cavity or tail determined by TC, m-MPN and DP sample within PICs
[2]significant at $p=0.03$ for the rates on meat or cavity or tail determined by TC, m-MPN and DP sample within POCs
[3]significant at $p<0.001$ for the rates on meat or cavity or tail determined by TC, m-MPN and DP sample within BICs
*NA = not applicable, NS = no significant difference

## Prevalence of positive-hippurate *Campylobacter*

The proportion of positive-hippurate *Campylobacter* slightly varied from part to part or group of chicken (Table 5.22). 65- 83 %, 63-82 % and 70-73 % of isolates from the three parts of the PICs, the POCs and the BICs, respectively, that were positive for the hippurate hydrolysis test. The frequencies found in meat and tail samples of all groups of chickens were not significantly different, whilst those occurring in cavity samples indicated a significant difference at $p= 0.01$. Within the same group of chicken, the cavity samples of the PIC group exhibited the highest frequency of positive hippurate (83%) and was significantly different ($p=0.013$) between the three parts. In contrast, the cavity samples of the POC group displayed the lowest positive-hippurate *Campylobacter* (64%) ($p=0.016$) compared to the other two parts. The levels of positive results for hippurate test of the three parts of the BIC group did not demonstrate a significant difference.

**Table 5.22** The outcomes of hippurate hydrolysis test of *Campylobacter* isolated from three parts of chickens

| Type of chicken | Rate of positive-hippurate test (%) | | | *p-value* |
|---|---|---|---|---|
| | Meat | Cavity | Tail | |
| PIC | 65.39 | 83.33 | 76.19 | **0.013** |
| POC | 73.91 | 63.64 | 82.61 | **0.016** |
| BIC | 73.33 | 73.33 | 70.00 | **NS** |
| *p-value* | NS | 0.01 | NS | |

NS = no significant difference

## Level of *Campylobacter* in three groups of chicken

The levels of *Campylobacter* isolated from three groups of chicken in this topic were taken into account as the pooled samples and samples from individual parts (meat or cavity or tail). The data were all normally distributed after logarithm transformation (Figures 5.14: a, b and c)
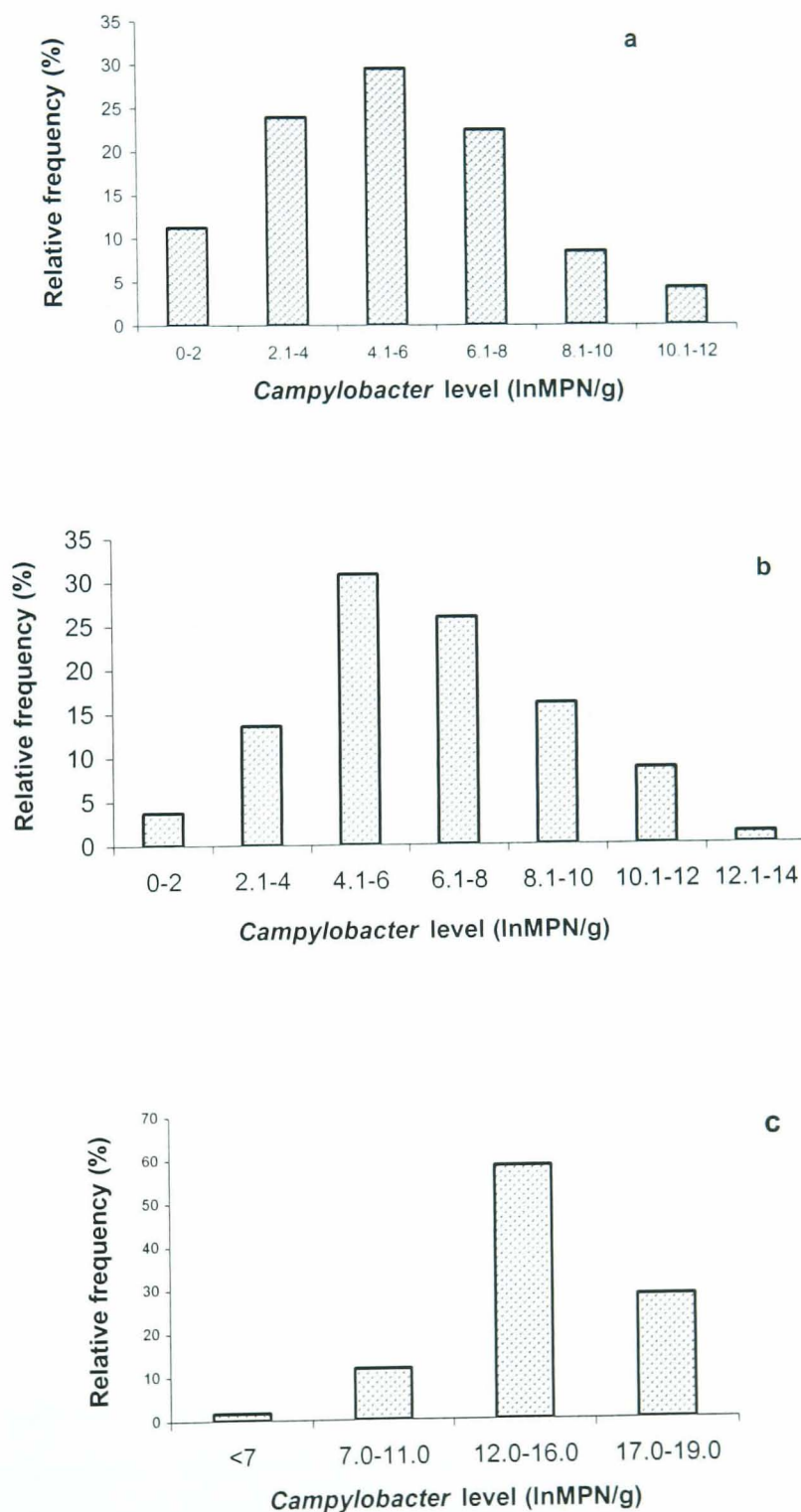


Figure 5.14 Distribution of the levels of *Campylobacter* per carcass in the three types of chickens: a) PIC, b) POC and c) BIC

Table 5.23 shows results calculated as pooled samples accumulating values from three parts of the chicken. Significantly higher levels were found in the BICs, with the lowest levels in the PICs at $p < 0.001$ with multiple statistic differences between three means (using HSD test). Although the level was slightly different between the PICs and the POCs, it still remained statistically different. These findings are highlighted in Figure 5.15.

**Table 5.23** Levels of *Campylobacter* isolated from three types of chickens (per carcass)

| Type of chicken | Level of *Campylobacter* ($\log_{10}$MPN/g) | |
| --- | --- | --- |
| | Mean±SE | 95% (CI)* |
| PIC | 3.73±0.59 | 2.98, 4.48 |
| POC | 4.44±0.64 | 3.70, 5.22 |
| BIC | 8.10±0.81 | 6.57, 9.23 |
| **P-value** | <0.001 | |

* 95%Confidence interval of the mean
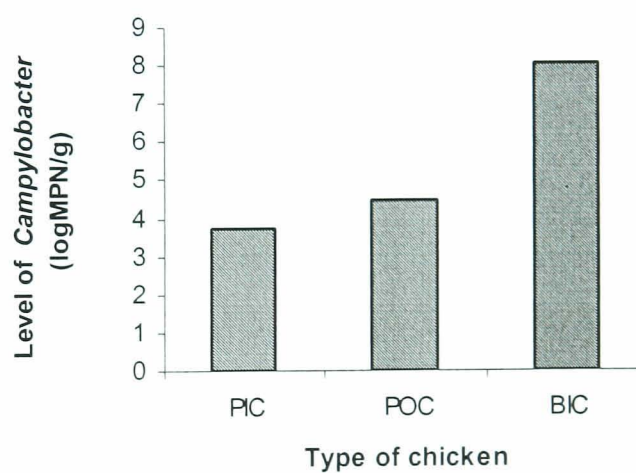


**Figure 5.15** Level of *Campylobacter* per carcass comparing PIC, POC and BIC

A high significant difference ($p < 0.001$) was also noted for the levels estimated from individual parts, meat or cavity or tail samples (Table 5.24 and Figure 5.16). Regarding the difference within the group, representing by meat, cavity and tail, no significant difference was found for the PICs. In contrast, in the POCs and the BICs, the highest level was noted from the samples obtained from tail and followed by meat and cavity samples. These findings also showed a significant difference at $p = 0.003$ for the POCs and at $p < 0.001$ for the BIC group.

**Table 5.24** Levels of *Campylobacter* isolated from three types of chickens grouping at the level per individual part of chicken

| Type of chicken | Type of sample | Level of *Campylobacter* ($\log_{10}$ MPN/g) | | |
| --- | --- | --- | --- | --- |
| | | Mean + SE | 95% CI[*] | P-value |
| PIC | Meat[a] | 3.72 ± 0.64 | 2.53, 5.02 | |
| | Cavity[b] | 3.64 ± 0.72 | 2.23, 5.04 | NS |
| | Tail[c] | 3.63 ± 0.62 | 2.43, 4.84 | |
| POC | Meat[a] | 4.00 ± 0.60 | 2.82, 5.18 | |
| | Cavity[b] | 3.63 ± 0.62 | 2.42, 4.85 | 0.003 |
| | Tail[c] | 4.80 ± 0.68 | 3.46, 6.14 | |
| BIC | Meat[a] | 7.28 ± 0.88 | 5.54, 9.00 | |
| | Cavity[b] | 6.35 ± 0.89 | 4.61, 8.09 | <0.001 |
| | Tail[c] | 7.49 ± 0.90 | 5.74, 9.24 | |

[*] 95%Confidence interval of the mean

[a] Significant at *p-value* <0.001 for MPN levels of meat samples between PIC, POC and BIC group

[b] Significant at *p-value* <0.001 for MPN levels of cavity samples between PIC, POC and BIC group

[c] Significant at *p-value* <0.001 for MPN levels of tail samples between PIC, POC and BIC group
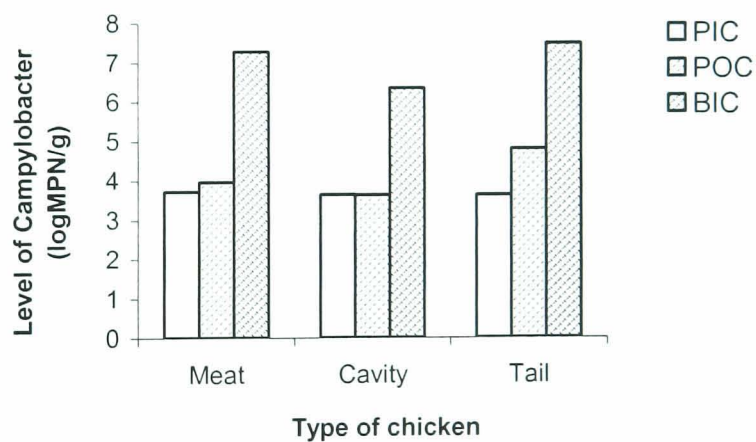


**Figure 5.16** Level of *Campylobacter* per part of chicken (meat or cavity or tail) and between PIC, POC and BIC

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# 5.5 Discussion

## 5.5.1 Reliability of the new modified MPN

To date, there is no internationally accepted standard method for the enumeration of *Campylobacter* in food samples. This is because of its fastidious requirements for growth. Both conventional methods (*i.e.* MPN, direct plate count) and molecular-based methods (*e.g.* PCR/REA, PFGE) have their advantages and disadvantages. Consequently, the results of the enumerations of *Campylobacter* carried out over decades are difficult to compare as there were major differences in the methodologies used (*i.e.* type of media, concentrations, sampling regime, and type of samples). These differences are presented in Table 5.25

**Table 5.25** *Campylobacter* prevalence in chicken (post processing) in selected countries

| Country | Type of sample | Method | Prevalence (%) | Reference |
|---|---|---|---|---|
| Belgium | carcass | convention | 28.5 | Uyttendaele *et al.*, |
| | breast | isolation | 21.7 | 1999 |
| | skin | | 23.5 | |
| Denmark | carcass | no information | 37.7 | EC (2003) |
| Finland | carcass | no information | 10.6 | EC (2003) |
| Ireland | a carcass wash | no information | 58 | EC (2003) |
| New Zealands | carcass | conventional isolation | 63 | Bongkot, 1997 |
| Sweden | carcass | no information | 9.3 | EC (2003) |
| UK | chicken portion | conventional isolation | 83.3 | Kramer *et al.* , 2000 |
| USA | pre-packaged broiler (grocery) | conventional isolation | 98 | Stern and Line, 1992 |

Since the environments during the slaughter and processing as well as storage at retail points are unfavourable for the growth of *Campylobacter* (affecting the viability of the cells) (Beuchart, 1985; Chaveerach *et al.*, 2003; Stern *et al*, 2003 and Vashin and Stoyanchev, 2004), the enumeration prior to enrichment of these organisms may be less efficient in determining actual levels. It is evident that the direct plating method is performed before the homogenate is enriched. In contrast, the principle of the conventional MPN is that the enumeration occurs alongside the enrichment. Several studies have used these methods to enumerate *Campylobacter* in chickens as presented in Table 5.26.

**Table 5.26** Overview of the enumeration studies of *Campylobacter* in chicken

| Type of sample | Level | Unit | Method | Reference |
|---|---|---|---|---|
| Caecal content (Farm) | 6-10 | $\log_{10}$ cfu/g | Direct plating | Beery *et al.*(1988), Stern *et al.* (1988) |
| Caecal content (Farm) | 6.83 | $\log_{10}$ cfu/g | Direct plating | Stern *et al.* (1995) |
| Caecal content (Farm) | 6.4 | $\log_{10}$ cfu/g | Direct plating | Mead *et al.* (1995) |
| Feathers | 5.4 | $\log_{10}$ cfu/g | Direct plating | Berrang *et al*, 1999 |
| Skin | 3.8 | | | |
| Caeca | 7.3 | | | |
| Parts of chicken | 2-3 | $\log_{10}$ cfu/g | Direct plating | Berrang *et al*, 2001 |
| Crate wash water | >4 | $\log_{10}$ MPN/ml | MPN | Slader *et al.* (2002) |
| Carcass rinse + neck skin (slaughterhouse) | 9 | $\log_{10}$ cfu/g | Direct plating | Bull *et al.*, (2003) |
| -at entrance | 8 | | | |
| -post bleed | 7-8 | | | |
| -post defeathering | 6-7 | | | |
| -post evisceration | 5-7 | | | |
| -post chill | 5 | | | |
| -post packaging | | | | |
| Faeces | 3-6 | $\log_{10}$ cfu/g | Direct plating | Stern and Robach (2003) |
| Carcass rinse | 3-5 | | | |
| Feather | 8 | $\log_{10}$ cfu/g | Direct plating | Keener *et al.*(2004) |
| Carcass wash | 4.75 | $\log_{10}$ cfu/g | Direct plating | Bashors *et al.*(2004) |
| Packaging (retail) | 4.64 | $\log_{10}$ cfu/g | Direct plating | Keener *et al.*(2004) |
| Caecal content (Farm) | 4.95-6.00 | $\log_{10}$ cfu/g | Direct plating | Nauta *et al.* (2005b) |
| Caecal content (Farm) | | $\log_{10}$ cfu/g | Direct plating | El-Shibiny *et al.*(2005) |
| -organic chicken | 4.5-9.7 | | | |
| -free range chicken | 6.7-9.2 | | | |
| Caecal content (Farm) | 7.15-9.20 | $\log_{10}$ cfu/g | Direct plating | Nauta *et al.* (2005b) |

The conventional MPN, using a set of test tubes (either 3 or 5 tubes), is laborious and consumes time, space and materials. For this reason, the conventional MPN (c-MPN) was modified by using a 48-well plate instead of a set of test tubes. As there was no significant difference in the results of the enumeration between the c-MPN and the m-MPN (section 5.4.1), this modified method is comparable to the conventional MPN. Nevertheless, because of interference from lake horse blood added in the broth (Figure 5.17a), the turbidity occurring in the wells is not easy to be noticed by the naked eye, except for samples harbouring high concentrations of organisms (Figure 5.17b).



a                                                    b
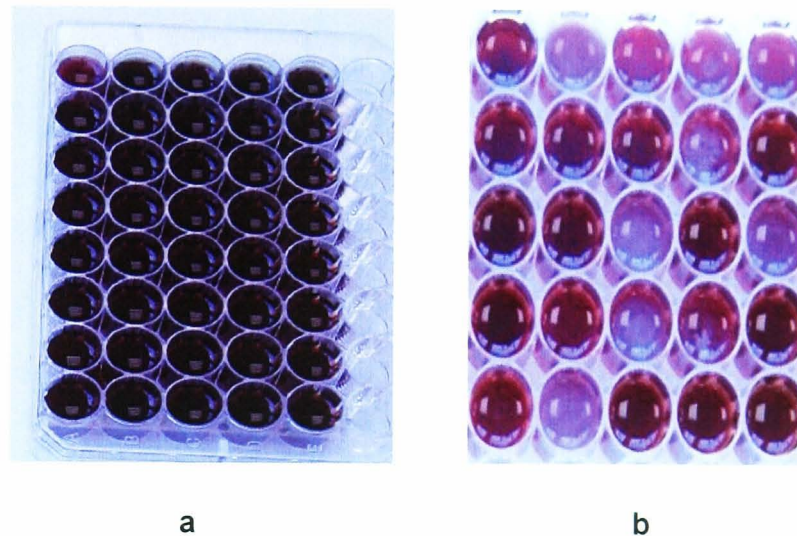
**Figure 5.17** Samples in Bolton broth after enrichment: a) sample harbouring a low concentration of organisms and b) sample harbouring a high concentration of organisms

The presumptive positive result of the m-MPN must be indicated by the colony formation onto the mCCDA. However, the c-MPN, interpreting by the turbidity is comparable to that determined by colony formation. Thus, the results of c-MPN may be directly readable from the change of the turbidity. It is interesting to further consider whether the turbidity can be detected by the naked eyes in a sample harbouring low concentration of organisms. If not, there is still a need to use colony formation on the agar and confirmation methods as a positive indicator. Comparing between turbidity and colony formation, it is suggested that colony formation is a more precise indicator for positive samples. The whole processes using both methods needed at least five days to gain sufficiently precise detection. This is one of the disadvantages of the MPN method. Nonetheless, the new modified method (m-MPN) can reduce the use of materials, specifically, the media.

Although the enumeration of *Campylobacter* by both the c-MPN and the m-MPN is performed under enrichment conditions or the enrichment processes do not change the

status of the original samples (positive or negative). Enrichment is used for recovering *Campylobacter* in the samples. If the original sample is negative, after enrichment, it still remains negative. In addition, by the assumption of MPN determination, the sample showing a positive tube/well represents that there is at least one colony of organism in the original tested sample (Swaroop, 1951; Oblinger and Koburger, 1975 and US-FDA/CFSAN, 2001). It is therefore suggested that enrichment-enumeration method does not increase the levels of MPN in the tested samples.

## 5.5.2 Comparison of test performances for the three detection methods in the absence of a gold standard

Two groups of conventional culture methods have been used for detecting *Campylobacter* in a number of studies (Beuchat, 1985, Wang, 2002 and Stern and Robach, 2003). These are: i) with enrichment method (*i.e.* Traditional culture (TC) and MPN methods) and ii) without enrichment method (*i.e.* direct plating method, DP).

None of these three tests in the current study gave perfect performance status. For example, the m-MPN showed high sensitivity but low specificity. Amongst these tests, the TC and the m-MPN were performed with the enrichment stage. With the enrichment stage, *Campylobacter* is in better condition than that in the sample without enrichment. Therefore, the TC and the m-MPN have high sensitivity.

As only the viable *Campylobacter* can cause infection, the three methods used in this study are appropriate to provide the association between the prevalence of positive chicken at retail point and the consequence to human health. However, the performance characteristics of the tests are important for determining the true prevalence. Amongst these three methods, each of them shows advantages and disadvantages regarding sensitivity and specificity. The bias due to measurement error can be resolved using the application of Bayesian estimation. The demonstration of using Bayesian application to adjust the performance of these tests supports that the m-MPN and the TC have high sensitivity but low specificity. This suggests that detection of *Campylobacter* in chicken samples should be performed using at least two methods.

## 5.5.3 Isolation rates and levels of *Campylobacter* in three groups of chicken

The varying results in isolation rates highlight the importance of considering the type and condition of the samples in relation to the selection of enumeration and detection methods. The direct plating did not perform well in terms of both isolation and detection for *Campylobacter* in chicken carcasses, suggesting that the direct plating is less sensitive for

the detection of *Campylobacter* in food samples. The findings from the individual parts of chicken clearly indicate that the DP provided the significant lowest rate of detection, in particular, of cavity sample in three groups (22-46%). Berrang *et al.*(2001) also reported significant difference in the levels of *Campylobacter* from different chicken parts (*e.g.* breast, thighs and drumsticks) with and without skin.

These differences in isolation rate between different parts of the same chicken may be a result of variation in: i) levels of *Campylobacter* colonising in the gut prior to slaughter process, ii) rates of cross-contamination and iii) pathways of cross-contamination. The results clearly indicate that the BIC group showed higher rate of not only colonisation but also cross-contamination. This is supported by the different results found between pre-packaged and unwrapped chicken. Since the BIC group were all unwrapped, the carcasses must be openly exposed to each other. This increases the chance of a negative-*Campylobacter* chicken being contaminated with a positive one. This cross-contamination after slaughter processes plays a significant role of spreading and consequent increased positive-*Campylobacter* chickens exposure to consumers. The similar isolation rates in three parts of chicken in the BIC group indicate that BIC-chickens unwrapped and of unknown origin, exhibited high colonisation and cross-contamination. However, further studies are needed in order to evaluate whether the significant cross-contamination takes place pre- or post slaughtering.

Recent studies reported variable prevalence of *Campylobacter* in chicken (Stern *et al.*,1988; Mead *et al.*,1995 and Berrang *et al*, 1999). These differences indicate considerable variation in isolation method, source of sample, type of sample (whole or part of chicken, fresh or frozen, with or without giblets), sampling methods or country as well as actual variation in prevalence of *Campylobacter*. It was proposed by Jones *et al.* (1991) that the faecal isolation rate of *Campylobacter* in normal flock can range from 0 to 100%. During the slaughter process, faecal content from birds can easily be spread to the surface of chicken carcasses or equipment (Izat *et al.*, 1988; Khalafalla, 1990 and van de Giessen *et al.*, 1992). The follicles of the chicken skin create the micro-aerophilic environment, which is a preferable habitat of *Campylobacter* spp. This is coupled with the cold humid processing environment, promoting the survival of these organisms on carcasses (Cloak *et al.*, 2001 and Solow *et al.*, 2003). It is plausible that the slaughter process is the major factor in the spreading of the organisms from positive to negative carcasses.

The levels of *Campylobacter* in each group of chicken support the assumption of colonisation and cross-contamination. Very high concentrations were found in the BIC group (6-7 $\log_{10}$MPN/g of sample) compared with the PIC and the POC groups. The differences between these groups of chicken are found to be highly significant ($p<0.001$), with the significant lowest in the PIC group ($3.72\pm0.59$ $\log_{10}$MPN/g).

Several studies showed the concentration of *Campylobacter* in the caecal content of live birds on the farms. The concentrations range from 6 $log_{10}$ cfu/g to 9 $log_{10}$ cfu/g. However, most of them, except for one study of El-Shibiny *et al* (2005), did not describe the specific type of chicken rearing (*i.e.* intensive or organic method). El-Shibiny *et al.* (2005) showed a very high concentration of *Campylobacter* obtained from the caecal content of both organic and free range chicken (5-10 $log_{10}$ cfu/g). As caecal content is thought to be main source of *Campylobacter*, the transport crates were then prone to be contaminated with *Campylobacter* through the shedding from birds. Slader *et al.* (2002) and Hansson *et al* (2005) presented that most transport crates were contaminated with *Campylobacter* (85%) and the levels were higher than $10^4$ MPN/ml.

Other studies investigated the concentration of *Campylobacter* during slaughter processes (Mead *et al*, 1995; Bull *et al.*, 2003; Stern and Robach, 2003 and Bashors *et al.*2004). The concentrations varied in different steps of the processing. However, they were still high being between 5 $log_{10}$ cfu/g to 9 $log_{10}$ cfu/g throughout the processes. Presumably, by the end of the slaughter process the negative-*Campylobacter* chicken would become positive. A number of studies indicated that during slaughter and processing, *Campylobacter* may be transmitted through the skin of the birds as the feather follicles were thought to be the entry for *Campylobacter* into the subcutaneous layer (Berndtson *et al*, 1992; and Berrang and Dickens, 2000). This assumption was supported by the study of Uyttendaele *et al.* (1999), which showed that skinless parts of chicken were less likely to be contaminated with *Campylobacter*.

The results from the BIC group indicate that cross-contamination may be responsible for the high level of *Campylobacter*. It is possible that at high levels *Campylobacter* may have high probability of spreading to be contaminated to other carcasses. This is supported by results obtained from the individual parts of the chicken. Significantly lower levels were found in cavity samples. The results from the POC groups indicating significant higher levels than found in the PIC group supports the assumption that organic chickens allowed to be outside most of the day tend to be exposed to *Campylobacter* from the environment (UK-DEFRA, 2005). By the time of processing in a slaughterhouse, the carcass harbouring high numbers of organisms can be a high contributor to other carcasses.

Considering information collected from other studies related to the concentration and the prevalence of *Campylobacter* in chicken from farms to points of sale, it is unlikely to find the negative-*Campylobacter* chicken at the time of purchase of consumers. However, there is a big gap of the isolation rates per carcass at the point of sale amongst several studies, ranging from less than 10% to 98% (Table 5.25). This may be due to lack of internationally accepted methodology using from country to country. In addition, the imperative point is that most studies were not conducted consecutively from farm to slaughterhouse and to retail outlet. It is therefore difficult to analyse, compare and conclude the critical point of the real

situation of *Campylobacter* in the food chain. As a result, managing further reductions in *Campylobacter* contamination in poultry in many countries cannot be efficiently achieved. An achievement in the reduction of *Campylobacter* contamination could depend upon stringent controls of infection and contamination from farm to consumption.

Finally, specified by the hippurate hydrolysis test, *C. jejuni* was the most commonly isolated species from all groups of chicken in this study (approximately 70-80%). However, further confirmation of *Campylobacter* requires more specific indicators, *e.g.* genotyping. Solow *et al.*, 2003 have highlighted such confirmation difficulty in suggesting that the incidence of *C.coli* may be underestimated because *C.coli* is more sensitive than *C.jejuni* to antimicrobials used in the selective media.

## 5.6 Summary

This chapter describes the development of methods for enumeration of *Campylobacter* in food samples. Three methods were compared: i) traditional culture (TC), ii) modified MPN (m-MPN) and iii) direct plating (DP). The findings show that:

1. The levels of *Campylobacter* in chicken samplesdetermined by the new modified MPN $(4.68 \pm 1.54 \log_{10}MPN/g)^*$ were found to be comparable to that by the conventional MPN $(4.23 \pm 1.44 \log_{10}MPN/g)$.

2. All three methods exhibit imperfect performances in detection of *Campylobacter* carried in chicken samples (section 5.4.2.1). The efficiency of detection can be improved using the combination of at least two methods such as the MPN and the direct plating.

3. Based on three detection methods, isolation rates of *Campylobacter* per carcass were 78-90% for the PICs, 78-97% for the POCs and 90-100% for the BICs.

4. Comparison of the three detection methods shows that direct plating had the lowest rate of detection for *Campylobacter* (77-90%). The TC and the m-MPN had a detection rate of 80-100%.

5. When comparing samples from three parts of the chicken, the samples obtained from the cavity gave the lowest prevalence for *Campylobacter*, particularly when using the DP (60-90% for the TC, 70-100% for the m-MPN and 22-46% for the DP).

6. The BIC group was found to harbour high levels of *Campylobacter* $(8.10 \pm 0.81 \log_{10}MPN/g)$, followed by the POC $(4.44 \pm 0.64 \log_{10}MPN/g)$ and the PIC $(3.73 \pm 0.59 \log_{10}MPN/g)$.

7. The levels of MPN found in the three different parts of chickens indicate that contamination with *Campylobacter* in chickens can occur during pre- and post slaughtering processes (Tables 5.21 and 5.24).

*Note: $\log_{10}N \pm SE$

# CHAPTER 6

## Antimicrobial Susceptibility Testing

## 6.1 Introduction

Antimicrobial susceptibility testing (*In-vitro*) is used for predicting the success or failure of antimicrobial therapy, in which micro-organisms are divided into treatable and non-treatable group on the basis of the breakpoint. The susceptibility of micro-organisms to antimicrobial can be determined in a quantitative or qualitative way. Quantitative methods will result in the data that can be related to the actual concentrations of antimicrobial agents inhibiting the visible growth of micro-organisms, *e.g.*, the minimal inhibitory concentration (MIC). Qualitative methods categorise micro-organisms as susceptible, intermediate or resistant. However, the breakpoint (see 6.1.2) may differ between different susceptibility testing methods or may be set at different levels to reflect differences in the pharmacokinetics or pharmacodynamics of an antimicrobial in different animal species or ages.

## 6.1.1 Susceptibility testing methods

Several methods have been used for both a quantitative or qualitative analysis of antimicrobial susceptibility. These include agar/broth dilution method, agar diffusion method, Epsilon test (E-test) and molecular methods. However, molecular methods are not routinely used in laboratory.

### Agar/broth dilution method

Different dilutions methods designed to determine the MIC are widely used the comparative testing of antimicrobial agents, in particular, new agents. These methods are reliable than other methods such as disc diffusion method (EUCAST, 2000). Procedures for the standardisation of dilution methods are described by a number of organisations, including the BSAC, the US NCCLS.

These methods are applied to determine the lowest concentration of an antimicrobial that inhibits visible *in vitro* growth of micro-organisms (MIC). The MIC is considered as a gold standard for determining the susceptibility of organisms to antimicrobial agents. Thus, dilution methods are internationally accepted as the reference standard when other methods are tested. A disadvantage of the dilution methods is that they can be costly and time consuming. Due to lack of the internationally accepted breakpoints, the interpretative criteria for the MIC must be agreed before the tests are performed in order to provide a meaningful comparison of resistance rates (Kahlmeter *et al.*, 2003).

## Agar diffusion method (Kirby-Bauer diffusion method)

Agar or disc diffusion susceptibility testing is amongst the most widely adopted alternative approaches. Disc diffusion method is based on an approximation of the effect of antimicrobial on bacterial growth on solid medium. The principle of this testing is based on the inoculation of the surface of an agar plate with a suspension of test organism followed by application of a paper disc containing a defined quantity of antimicrobial agent (BSAC, 2002). The interaction of the antimicrobial agent diffusing through the medium with the organism on the surface of the agar plate results in inhibition of bacterial growth for a variable distance around the paper disc, expressing by the zone diameter (Figure 6.1). A zone of growth inhibition around the antimicrobial disc will occur if the organism is susceptible to the antibiotic. Standardized regression curves have been developed that correlate inhibition zone size to the minimal inhibitory concentration of the antimicrobial. However, even though it is the most common test for antimicrobial effectiveness, not all bacteria can be tested using disc diffusion.

The concentration of the antibiotic at different distances from the disc will depend on its original concentration on the disc and its diffusion rate through the agar. There is a relationship between the diameter of the zone of inhibition of bacterial growth around the disc and the MIC of the test organism for the particular antimicrobial agent. The antimicrobial content of the paper disc is a key determinant of the diameter of the zone of inhibition of growth. The relationship between MIC (as determined under defined conditions) and the diameter of the zone of inhibition is complex and affected by many variables. In general terms, the lower the MIC for a particular antimicrobial agent, the larger the diameter of the zone of inhibition of growth (Cormican et al., 2005).
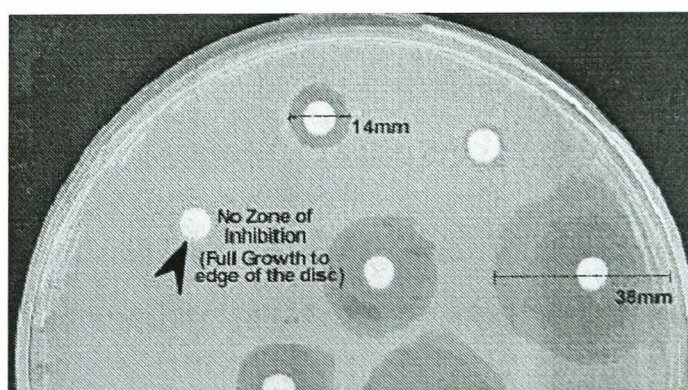


**Figure 6.1** Disc Diffusion Test

(Source: http://www.poultry.baytril.com/119/SusceptibilityTesting.htm)

## Epsilon test (E-test)

The Epsilon test combines the principles of dilution method with agar diffusion method. This gradient diffusion method uses commercial strips instead of disc and allows determining the

MIC values. In this test fixed concentration of antimicrobial agents, representing the cut-off points of susceptibility or resistance, are incorporated with into agar or broth dilution method. Figure 6.2 shows an elliptical zone of inhibition results.

The Epsilon Test



Epsilon Test (schematic). The resolution zone of inhibition (with area) crosses the antibiotic gradient (scale on strip) at the MIC (here, ca. 0.125 µg/ml)
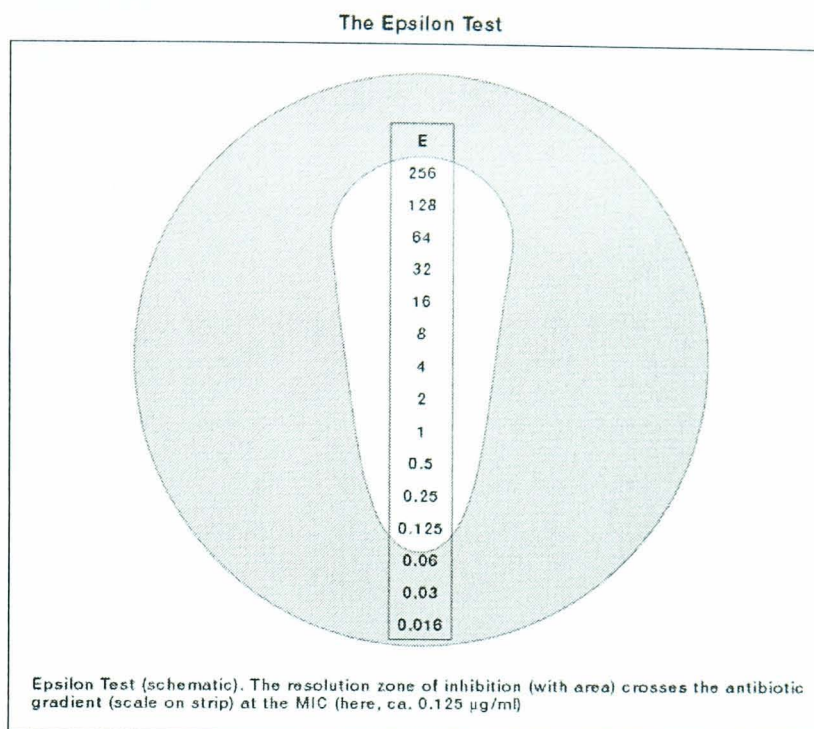
**Figure 6.2** An elliptical zone of inhibition from the Epsilon test

(Source: http://www.poultry.baytril.com/119/SusceptibilityTesting.htm)

## Molecular methods

These methods are mainly based on the polymerase chain reaction and DNA-DNA hybridisation method. These molecular methods are usually used for identification of bacterial strains, *e.g. Campylobacter jejuni*. They can be applied to a lesser extent to directly detect resistance gene in clinical samples as well. However, the procedures involve complex processes, in particular, in gram-negative bacteria which have the diversity of resistance mechanisms. Molecular methods are then not routinely used in clinical laboratories (Fluit *et al.*, 2001).

## 6.1.2 Breakpoint consideration

The setting of breakpoints to a certain antimicrobial agent resulting from the MIC values or zone of inhibition diameters is a contentious subject and has been the focus of much debate among microbiologists, regulators and industry. There are many aspects to the determination of breakpoint interpretive criteria. These include microbiological (the pattern of distribution of MIC measurements for a group of isolates of the same species), pharmacological (achievable blood and tissue levels of the antimicrobial agent), animal models and clinical trial (Kahlmeter *et al.*, 2003). The issue is further complicated by differences in the interaction between an antimicrobial agent and different species or groups

of bacteria. Therefore, a single antimicrobial agent (*e.g.* ampicillin) may have different breakpoint interpretive criteria.

The European Committee for Antimicrobial Susceptibility Testing (EUCAST) defines separate breakpoints for epidemiological cut-off values and clinical breakpoint. The epidemiological cut-off values are used for the detection of bacteria with resistance mechanisms and the monitoring of resistance development. The clinical breakpoint is used for guidance of therapy (Kahlmeter *et al.*, 2003).

Clinical breakpoints are defined based on three criteria, which are: i) "A micro-organism is defined as clinically susceptible (S) by a level of antimicrobial activity associated with a high likelihood of therapeutic success, or by applying the appropriate breakpoint in a defined phenotypic test system", ii) "A micro-organism is defined as clinically intermediate (I) by a level of antimicrobial activity associated with indeterminate therapeutic effect, or by applying the appropriate breakpoints in a defined phenotypic test system" and iii) "A micro-organism is defined as clinically resistant (R) by a level of antimicrobial activity associated with a high likelihood of therapeutic failure, or by applying the appropriate breakpoint in a defined phenotypic test system".

These breakpoints may be altered with legitimate changes in circumstances. Clinical breakpoints are presented as $S \leq x$ mg/L; $I > x$, $\leq y$ mg/L; $R > y$ mg/L.

Microbiological resistance and epidemiological cut-off values are defined based on the basis of the following: i) "A micro-organism is defined as wild type (WT) for a species by the absence of acquired and mutational resistance mechanisms to the drug in question, or by applying the appropriate cut-off value in a defined phenotypic test system" and ii) "A micro-organism is defined as microbiological resistance - Non-Wild Type (NWT) (NWT) for a species by the presence of an acquired or mutational resistance mechanism to the drug in question, or by applying the appropriate cut-off value in a defined phenotypic test system".
In addition, this cut-off value will not be altered by changing circumstances as well as wild type micro-organisms may or may not respond clinically to antimicrobial treatment. The wild type is presented as $WT \leq z$ mg/L and non-wild type as $NWT > z$ mg/L (z values is obtained from reference database of EUCAST)

It is becoming clear that there are no standard methods for antimicrobial susceptibility testing for *Campylobacter* spp. However, the approved method described by the National Committee for Clinical Laboratory Standards (NCCLS, 2001), an agar dilution method, has been widely used to measure the susceptibility. This method will provide the quantitative values in interpreting the rate of resistance. In addition, it is also internationally accepted as a reference method for the comparative testing.

For this reason, the *in vitro* agar dilution method to determine the susceptibility of *Campylobacter* to three antimicrobials was selected for this study.

## 6.2 An agar dilution method

### 6.2.1 Principle

The determination of the minimal inhibitory concentration (MIC) of antimicrobials was performed by using twofold serial dilutions of the antimicrobial agent in the agar growth medium. The results were interpreted by the identification of the lowest concentration (MIC) of an antimicrobial agent that inhibits visible growth of *Campylobacter* isolates on the agar (NCCLS, 2001).

### 6.2.2 Materials and methods

#### Sample and Isolate

The details of the sampling regime (30 fresh PICs, 30 fresh POCs and 30 fresh BICs), isolation and identification were described in Chapter 5, section 5.21. All isolates from all positive-*Campylobacter* samples were tested for the MIC of three antimicrobials, ciprofloxacin, erythromycin and nalidixic acid. These three antimicrobials selected are drugs of choice in the treatment of campylobacteriosis.

#### Media and inoculum preparation

As recommended by NCCLS, Mueller-Hinton broth was used for the preparation of equivalent inoculum (Oxoid: CM 0337;1 litre of this medium contained 300g dehydrated beef infusion, 17.5g casein hydrolysate and 1.5g starch at pH $7.3\pm0.2$). Two groups of antimicrobials were used in this study: i) macrolide (erythromycin) and ii) (fluoro)quinolone (ciprofloxacin and nalidixic acid). Twofold serial dilutions of these antimicrobials were added into a molten Mueller-Hinton agar. Stock solution and twofold agar plate are described in appendix 1-4.

#### Procedures

The minimal inhibitory concentration (MIC) of each antimicrobial agent was determined by the agar dilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2001). Following identification, five purified isolates grown on an mCCDA from each positive sample (meat and skin, cavity and tail) underwent susceptibility tests with three antimicrobial agents, ciprofloxacin (Bayer Ltd. Co., Newbury, Berkshire,UK),

erythromycin (Sigma Ltd. Co., Poole, Dorset, UK) and nalidixic acid (Sigma Ltd. Co., Poole, Dorset, UK).

Each isolate was inoculated into Mueller-Hinton broth and incubated micro-aerobically at 37°C overnight. The bacterial suspension was adjusted to ca. 0.5 McFarland turbidity standard, with the aim of producing broth culture that would yield similar viable counts of approximately $10^8$ CFU/ml (0.5 McFarland standard preparation is described in Appendix 1-5). It was diluted 1:10. An aliquot (10 μL) of the final diluted inoculum was applied onto twofold antimicrobial Mueller–Hinton agar (ciprofloxacin at a range from 0.125-8 mg/L or erythromycin at a ranged from 8.0- 512 mg/L or nalidixic acid at range from 8.0-512 mg/L) supplemented with 5% defibrinated horse blood. The agar plates were incubated under micro-aerobic condition produced by using gas-generating kits (BBL™ GasPack™) and kept in an airtight container at 42 °C for 48 h (Figure 6.3).

The standard strains for control for antimicrobial action are *C.jejuni* (NCTC 11322) *Eacherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662) and *Staphylococcus aureus* (NCTC 6571). The reference strains were obtained from the National Collection of Type Cultures (NCTC), Health Protection Agency (UK). All strains were tested in parallel with the samples. Agar without antimicrobial was used as a positive control for growth.



5 pure isolates

Muller-Hinton broth

micro-aerobically incubated overnight

Muller-Hinton agar
(doubling concentration)

Micro-aerobically incubate 42°C, 48h

MIC
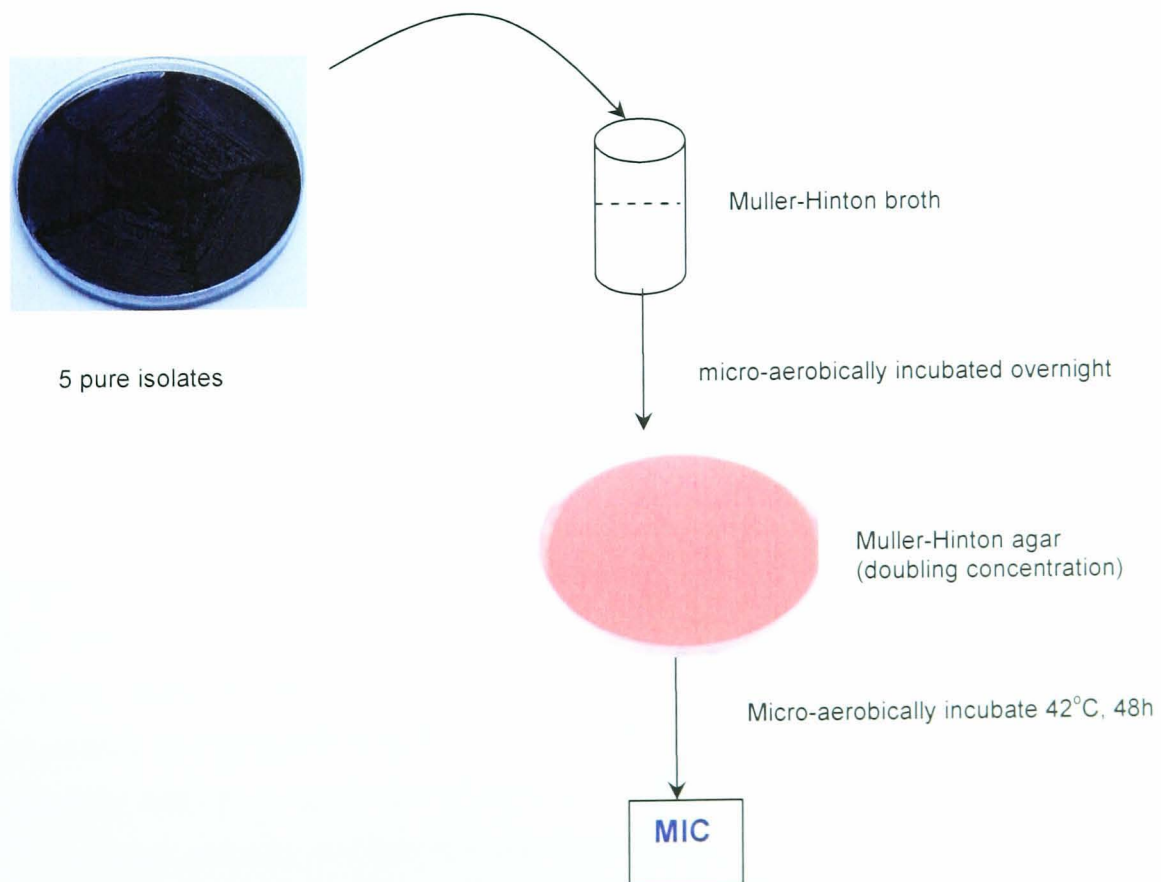
**Figure 6.3** Antimicrobial susceptibility testing: an agar dilution method

## The MIC determination

After 48-hour incubation, the MIC endpoint was determined at the concentration where a marked reduction took place in the appearance of growth on the test plate, which was compared with the growth on the blank control plate (without antimicrobials). Examples of how to interpret the MIC are shown in Figure 6.4.



**Figure 6.4** Examples of MIC determination: Lane A as MIC= 16mg/L, Lane B as MIC= 4mg/L, Lane C as MIC= 8mg/L, Lane D as MIC= 8mg/L and Lane E as MIC= 4mg/L.

## Interpretative breakpoint

The MIC is defined as the lowest concentration of antimicrobial agent that inhibits visible growth of organisms. The $MIC_{50}$ is equivalent to the concentration of antimicrobial agent that inhibits growth of 50% of *Campylobacter* samples.

Breakpoint concentrations published by the British Society for Antimicrobial Chemotherapy (BSAC) and NCCLS (Thwaites and Frost, 1999; Sarah, 2002; Tollefson and Flynn, 2002; Luber *et al.*, 2003 and Randall *et al.*, 2003) were used for the definition and determination of the resistance of *Campylobacter* isolates. The following cut-off concentrations were used: for ciprofloxacin MIC $\geq$ 4 mg/L, for erythromycin MIC $\geq$ 8 mg/L and for nalidixic acid MIC $\geq$16mg/L. Each chicken carcass was considered positive for resistance if it was found to harbour one or more resistant isolates. In addition, the resistance rate of isolates was also

considered by assuming that each isolate in each sample is independent and selected randomly.

## 6.3 Data analysis

MIC data analysis: Analysis of difference of MICs for the three chicken sources (PIC, POC, BIC) was undertaken by ANOVA (Ryan and Joiner, 2003). Additionally, analysis of the difference between the three chicken parts (body, cavity and tail) within each group was carried out in the same way.

Resistant rates data analysis: The chi-square test was used to test for association of resistance rates with the three groups of chickens and for association of resistance rates with the different chicken parts (body, cavity and tail).

# 6.4 Results

## 6.4.1 MIC determination

The results of MICs of three antimicrobial agents, ciprofloxacin, erythromycin and nalidixic acid for *Campylobacter* isolated from three groups of chicken are summarised in Table 6.1. These results show significant difference ($p=0.009$) for the MICs of nalidixic acid for the three groups of chickens. No significant difference of MICs of ciprofloxacin and of erythromycin was found for the same groups. The $MIC_{50}$ of ciprofloxacin and erythromycin for *Campylobacter* isolated from pre-packaged organic chicken (POC), pre-packaged intensive chicken (PIC) and butcher-intensive chicken (BIC) were the same (1mg/L for ciprofloxacin 128 mg/L for erythromycin). However, the highest values of MIC of each antimicrobial agent for isolates from intensive chickens were found to be higher than those found in isolates from organic chickens, with the highest values found in isolates from butcher's unpackaged samples (ciprofloxacin: 4-8 mg/L for intensive and 2 mg/L for organic, erythromycin: 512 mg/L for intensive and 256 mg/L for organic, and nalidixic acid: 256-512 mg/L for intensive and 128 mg/L for organic). $MIC_{50}$ of nalidixic acid for samples from the PIC and BIC groups showed the same level (64 mg/L), and were higher than that found in isolates from the POC group (32 mg/L).

Analysis of the difference in the MICs was carried out for the three parts of the chicken carcasses (body [meat and skin], cavity and whole tail). These analyses showed a significant difference ($p = 0.026$) for resistance to erythromycin in *Campylobacter* isolated from the POC group, with the lowest MIC found in the cavity samples, compared to the other two parts. No significant differences could be demonstrated for the two other antimicrobial agents.

**Table 6.1** The MIC value of three antimicrobial agents for *Campylobacter* isolates obtained from three types of chickens

| Antimicrobial | Retail source of chicken | MIC (mg/L) | | |
| --- | --- | --- | --- | --- |
| | | Range | $MIC_{50}$[a] | $MIC_{90}$[b] |
| Ciprofloxacin | PIC | 0.125-4 | 1 | 2 |
| | POC | 0.125-2 | 1 | 2 |
| | BIC | 0.25-8. | 1 | 4 |
| Erythromycin | PIC | 32-512 | 128 | 256 |
| | POC | 16-256 | 128 | 256 |
| | BIC | 8-512 | 128 | 256 |
| Nalidixic acid | PIC | 16-512 | 64 | 128 |
| | POC | 8-128 | 32 | 64 |
| | BIC | 8-512 | 64 | 256 |

[a] $MIC_{50}$ is equivalent to the concentration of antimicrobial agent that inhibits growth of 50% of *Campylobacter* samples
[b] $MIC_{90}$ is equivalent to the concentration of antimicrobial agent that inhibits growth of 90% of *Campylobacter* samples

## 6.4.2 Resistance rates

Using the pre-set breakpoint, 100% of chickens in all three groups were found to harbour *Campylobacter* isolates resistant to erythromycin and nalidixic acid. Chickens from the BIC group exhibited the highest resistance rate to ciprofloxacin (26.7% of chickens with resistant isolates and 11.6% of all isolates) followed by a lower rate for PIC group (8.7% chicken with resistant isolates and 2.9% of all isolates) whereas no isolates from the POC group were resistant to ciprofloxacin (Table 6.2).

Resistance rates were calculated for samples from different parts of the chicken carcasses (body [meat and skin], cavity and tail). These show that rates of resistance to ciprofloxacin in isolates from meat and skin of the PIC group were higher than those found on the cavity and tail samples. In contrast, isolates from the cavity fluid obtained from the BIC group showed the highest resistance rate to ciprofloxacin. The resistance rates to erythromycin and nalidixic acid found among the different carcass parts in the POC, PIC and BIC groups were all in the high range of 82-100% (Table 6.2).

The distributions of percentage of samples determined for the median MIC of three antimicrobial agents from the POC, PIC and BIC groups are illustrated in Figures 6.5 to 6.7.

**Table 6.2** resistance rates of three antimicrobial agents for *Campylobacter* isolates obtained from three types of chickens

| Antibiotic | Retail source of chicken | Chickens with resistant isolates[a] (%) | Resistant isolates[b] (%) | Chicken parts with resistant isolate (%) | | |
|---|---|---|---|---|---|---|
| | | | | Body | Cavity | Tail |
| Ciprofloxacin[c,f] | PIC | 8.7 | 2.9 | 5.6 | 0 | 0 |
| | POC | 0 | 0 | 0 | 0 | 0 |
| | BIC | 26.7 | 11.6 | 10.7 | 20.8 | 9.0 |
| Erythromycin[d,g] | PIC | 100 | 100 | 100 | 100 | 100 |
| | POC | 100 | 100 | 100 | 100 | 100 |
| | BIC | 100 | 100 | 100 | 100 | 100 |
| Nalidixic acid[e,h] | PIC | 100 | 100 | 100 | 100 | 100 |
| | POC | 100 | 95 | 93.8 | 94 | 100 |
| | BIC | 100 | 81.4 | 82.1 | 83.3 | 90.9 |

[a] percentage of chicken carcases harbouring one or more resistant isolates

[b] percentage of isolates found to be resistant to a specific antimicrobial agent.

[c,d] no significant difference for the MICs of ciprofloxacin and erythromycin amongst POC, PIC and BIC

[e] significant at $p = 0.009$ for the MICs of nalidixic acid amongst POC, PIC and BIC

[f] significant at $p=0.012$ for the percentage of chickens harbouring ciprofloxacin-resistant isolates amongst POC, PIC and BIC

[g,h] no significant difference for the percentage of chickens harbouring erythromycin-resistant and nalidixic acid-resistant isolates among POC, PIC and BIC
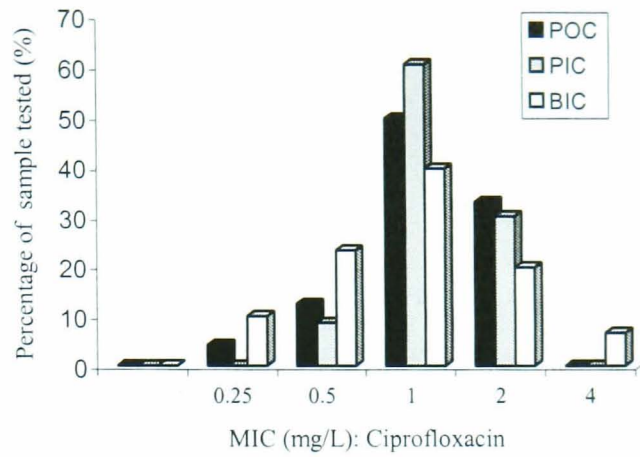
**Figure 6.5** Comparison of median MIC of ciprofloxacin in *Campylobacter* isolated from PIC, POC and BIC
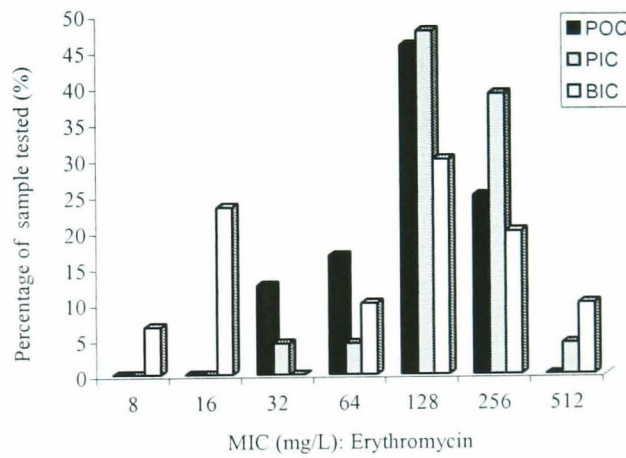


**Figure 6.6** Comparison of median MIC of erythromycin in *Campylobacter* isolated from PIC, POC and BIC
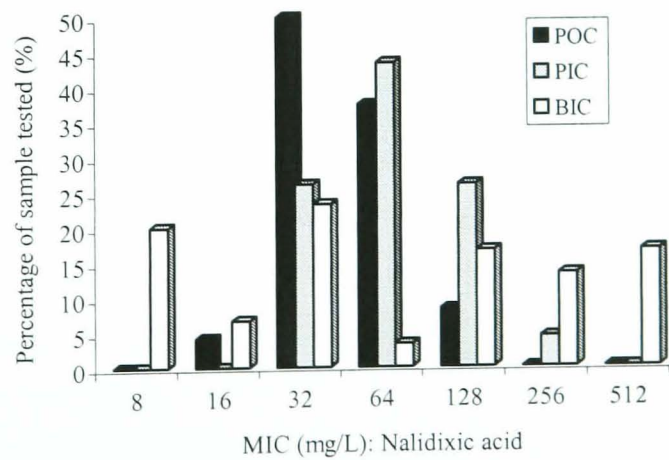


**Figure 6.7** Comparison of median MIC of nalidixic acid in *Campylobacter* isolated from PIC, POC and BIC

# 6.5 Discussion

The results in the study show a significant difference of MICs of nalidixic acid for *Campylobacter* isolated from the three groups of chicken tested. The MICs of ciprofloxacin and of erythromycin did not demonstrate a significant difference between these three groups. However, the highest MIC values of all three antimicrobial agents for isolates from intensively farmed chicken were higher than those found in organically reared chicken. This is further highlighted in Figures 6.5-6.7, showing the distribution of percentage of samples with median MIC values. This is particularly prominent in Figure 6.7 with relation to nalidixic acid MICs in chickens from butcher's shops. Rates of resistance to ciprofloxacin (as determined by the application of the pre-set break point) also demonstrate the highest rate of resistance in the BIC group (26.7%) compared with the PIC and POC groups (at 8.7% and 0% respectively). These findings indicate a potentially worrying trend in chickens from unlabelled and therefore presumed less controlled sources.

The resistance rates to erythromycin and nalidixic acid per carcass were found to be the same among the POC, PIC and BIC groups, being 100% in each. Other studies (Loovern, *et al.*, 2001; Ge, *et al.*,2003 and Luber *et al.*, 2003) found varying rates of resistance to the three antimicrobial agents tested. These found comparatively higher levels of resistance to ciprofolaxacin (30-40%) and lower levels of resistance to erythromycin (30-60%) and nalidixic acid (40%). Although reported results vary, the rates of antimicrobial resistance are high and are a cause of concern as is the continuing reporting of increasing rates of resistance to antimicrobial agents in general and to fluoroquinolones in particular, following their use in poultry rearing (Piddock, 1995; Lucey, *et al.*, 2000b; McDemott, *et al.*, 2002; Ge *et al.*, 2003 and Humphrey, *et al.*, 2005).

Interesting findings of resistance rates in the different parts of the chickens have emerged. Ciprofloxacin-resistance rates in the PIC group were highest in the meat and skin. Although no statistical significance could be demonstrated, this result would be consistent with a post-slaughtering contamination of these chickens, indicating that this process may play a role in the spread of resistant strains.

The equivalent data for ciprofloxacin-resistance rates from the BIC samples indicated higher levels in the cavity compared with the other parts, suggesting that treatment on the farm may be play a role in the development of resistance in these chickens. Since these chickens were purchased from sources that do not provide information on their origin, the findings suggest that the chickens in the BIC group may have been subjected to less stringent regulatory controls than those in the other groups.

The possibility that the development of antimicrobial resistance in *Campylobacter* may be associated with the use of antimicrobial agents on the farm makes it imperative to have

more reliable information about the level of their use. A recent study (Humphrey, *et al.*, 2005) has shown that ciprofloxacin-resistant *Campylobacter* strains emerge during fluoroquinolone treatment in commercially reared chickens. Following the increased reporting of antimicrobial resistance in general, and fluoroquinolone resistance in particular, in *Campylobacter* worldwide, attempts have been made to better control their use in animal husbandry (Sanchez *et al.*, 1994; Piddock *et al.*, 2000; Tollefson *et al.*, 2002 and Ge *et al.*, 2003). The continued presence of very high rates of resistance to erythromycin and nalidixic acid in *Campylobacter* from both intensive and organic chickens found in this study (with particularly high levels of multi-resistance in the BIC group), indicate that these antimicrobial agents may have been used on the farm.

Several reports have indicated that antimicrobial agents used as growth promoters may have a critical role in influencing the development of antimicrobial resistance in bacteria including *Campylobacter* (Sanchez *et al.*, 1994; WHO, 1997; McDermott *et al.*, 2002; Tollefson and Flynn, 2002; Luber *et al.*, 2003 and Randall *et al.*, 2003). Despite the complete ban on this type of use in chicken husbandry, *Campylobacter* still shows resistance to some antimicrobial agents. These findings indicate that substituting antimicrobial agents used as growth promoters for therapeutic purposes may contribute to the maintenance of antimicrobial resistant strains. Evidence from Denmark shows an increase in their use for therapeutic purposes following the banning of the use of antimicrobial agents as growth promoters (Arnold *et al.*, 2004). In addition, the slaughtering process, as well as pre and post slaughtering processes (*e.g.* thinning, transportation and packaging), may play a further role in the spreading of resistant strains.

Currently, there are no studies that demonstrate a direct correlation between the use of antimicrobial agents in animal husbandry and the rate of antimicrobial resistance in humans. However, three studies have shown an association between strain types of *Campylobacter* in human infection and those found in retail chickens (Smith *et al.*, 1999; Kramer *et al.*, 2000 and FSA, 2003) and other studies have proposed that the use of antimicrobial agents in poultry husbandry could be one of the factors influencing the development of resistance in *Campylobacter* with the risk that this resistance may pass to humans via food (Endtz *et al.*, 1991; Loovern *et al.*, 2001; Sarah, 2002; Luber *et al.*, 2003; Randall *et al.*, 2003 and Wagner *et al.*, 2003).

This study has found that all *Campylobacter* strains isolated from both intensively and organically reared chicken have a high resistance rate to erythromycin and nalidixic acid. This indicates that the antimicrobial agents currently administered to patients for the treatment of *Campylobacter* infection may not be the most effective. This is further emphasised by the occurrence of some co-resistance to ciprofloxacin and erythromycin in intensively reared chickens.

The lack of internationally accepted breakpoints for *Campylobacter* makes it difficult to clearly interpret and compare the results from different studies (Salazar-Lindo *et al.,* 1986; Loovern *et al.,* 2001; Sarah 2002 and Luber *et al.,* 2003). There is therefore an urgent need to harmonise and standardise, both the methods for antimicrobial susceptibility testing and the use of breakpoint values for resistance determination for *Campylobacter*. This will make surveillance programmes more effective in monitoring the development of antimicrobial resistance in *Campylobacter* worldwide.

## 6.6 Summarry

Resistance to the three antimicrobials in *Campylobacter* isolated from the PIC, POC and BIC groups were determined using an agar dilution method.

1. The $MIC_{50}$ values of ciprofloxacin and erythromycin for *Campylobacter* isolated from these three types of chickens were the same (1 mg/L for ciprofloxacin and 128 mg/L for erythromycin). The $MIC_{50}$ of nalidixic acid for samples from the PIC (64 mg/L) and BIC (64 mg/L) groups were higher than that found in isolates from the POC group (32 mg/L).

2. Significant differences in the MICs of nalidixic acid were found ($p=0.009$), whereas no significant differences in the MICs of ciprofloxacin and erythromycin were found for the same groups ($p>0.01$).

3. Using pre-set breakpoints, 100% of chickens in these three types were found to harbour isolates identified as resistant to erythromycin and nalidixic acid. All isolates from the POCs were susceptible to ciprofloxacin. The BICs exhibited the highest resistance rate to ciprofloxacin (26.7% of chicken with resistant isolates and 11.6% of all isolates resistant) followed by a lower rate for the PICs (8.7% of chicken with resistant isolates and 2.9% of all isolates resistant).

4. The rates of resistance to ciprofloxacin in isolates from meat and skin of the PICs were higher than those found on the cavity and tails samples. In contrast, the isolates from the cavity fluid obtained fron the BICs showed the highest resistance to ciprofloxacin (Table 6.2). The resistance rates to erythromycin and nalidixic acid found amongst the different carcass's parts in these three types were all in high range (82-100%).

# CHAPTER 7

# Assessment of consumer exposure to *Campylobacter*

## Background

This study has three phases (section 1.4). This chapter is one part of the work of phase 2 which deals with the estimation of health risk from *Campylobacter* following the consumption of three groups of chickens. This chapter is about the estimation of the exposure (dose) to *Campylobacter* of a person consuming chicken meals or salad. It also compares the additional risks from antimicrobial resistance associated with three different sources of chicken (PIC, POC and BIC group).

Estimation of exposure to *Campylobacter* was carried out using the results from Chapter 5 (the numbers and prevalence of *Campylobacter* isolated from three groups of chickens) and Chapter 6 (levels of antimicrobial resistance to three antimicrobials, ciprofloxacin, erythromycin and nalidixic acid). Secondary data and relevant information obtained from the literature were also selected and then incorporated with the primary data to estimate the doses.

The exposure to *Campylobacter* was calculated using two different criteria based on: 1) all *Campylobacter* including antimicrobial susceptible and antimicrobial resistant isolates and 2) antimicrobial resistant *Campylobacter* isolates.

The work in this chapter is in three parts (Figure7.1). The first part **(part A)** describes the complete risk exposure model for *Campylobacter* from production (farm level) to consumption (consumer level). Although this study focuses on exposure to *Campylobacter* at the consumer level, it is necessary to consider the whole process from farm to consumption. This shows how consumers can be exposed to *Campylobacter* through the food chain. This helps when considering risk management options (see Chapter 9). It also presents what, and how, many data input required for the calculations from farm to consumption. This is an explanation of why this study considered the exposure dose at the consumer end only. The selected models are also described and presented. The second part **(part B)** identifies the origins of the data input to the selected models at a consumer level. The results from part B are presented and discussed in **part C**. The limitations arising from the exposure assessment modelling are also described.

A fully quantitative microbial risk assessment (QMRA) model involves a high level of complexity of data, which is almost impossible without considerably more data than is currently available. The main deficiencies are the availability of data related to the farm-to-table pathway of the pathogen. The approache used in this chapter is to estimate exposure

to *Campylobacter* following the consumption of chicken. This provides an assessment of the comparative doses resulting from the consumption of chicken raised by different rearing practices from the consumer perspective.

The calculations of exposure following the consumption of chicken harbouring *Campylobacter* are carried out as a comparative estimation between three groups of chicken. The exact numeric result(s) is/are not as important as the relative effects on management options.

The modelling approaches take into account the growth and inactivation of *Campylobacter* following cross-contamination, heat treatment and refrigeration in private kitchens. Human exposure to *Campylobacter* from chicken meals and salad is also considered in relation to age and gender. The age and gender of consumers are taken into account into the models to introduce variable hygiene levels during food preparation and variable sizes of meals. Finally, the outputs of the exposure assessment are used for the estimation of health risk (see Chapter8).These approaches are:

**Modelling approach 1** covers the transfer of *Campylobacter* (with and without antimicrobial resistance) from a positive chicken to salad during food handling in private kitchens, including the effect of refrigeration.

**Modelling approach 2** addresses the changes in prevalence and numbers of *Campylobacter* (with and without antimicrobial resistance) in chicken carcasses throughout the cooking steps, including the effect of refrigeration.
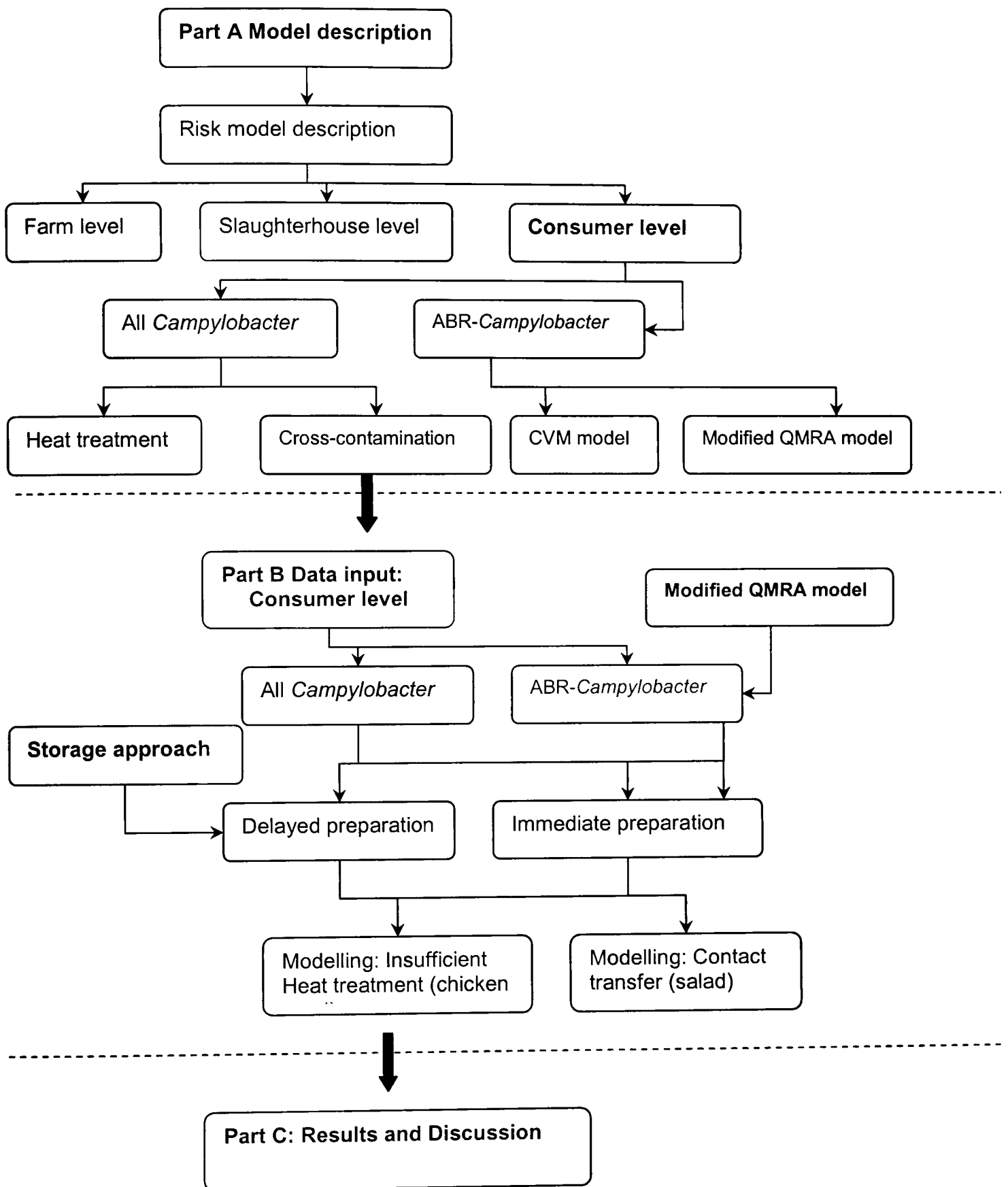
**Figure 7.1** A conceptual framework of Chapter 7

# PART A: The exposure model description

## 7.1 Introduction

Illness resulting from *Campylobacter* infection is affected by a complex multi-factorial process associated with bacterial virulence, types of foods, size of meal consumed and the individual's immune system (Anderson *et al.*, 2001). The magnitude of infection and the degree of illness are affected by dose of **viable** *Campylobacter* ingested, the dose-response relationship, and related factors, *i.e.*, size of meal, age, gender, and consumption pattern (Teunis *et al.*, 1996 and Hass *et al.*,1999).

Exposure assessment involves the systematic modelling of the transmission of *Campylobacter* through the food chain from production to consumption. These models relate to the growth and inactivation, which are performed as a function of probability given prevalence, numbers of *Campylobacter* and the amount of chicken consumed along the consecutive processes of food pathways, taking into account both variability and uncertainty (Nauta *et al.*, 2000, WHO, 2001 and Nauta *et al.*, 2005a). By the end of the modelling, the outcomes show the estimate of the amount of viable *campylobacter* ingested and surviving the immune system of humans and the likely consequences for human health (Nauta, 2000; Christensen *et al.*, 2001; WHO, 2001 and Nauta *et al.*, 2005b).

## The predictive growth and inactivation model

Food products actually show a low viable pathogenic contamination level. However, it has been found that the microbial dynamics at low numbers are important. This is because the viability of an individual cell has a much more pronounced effect on the probability of infection. The consumption of foods harbouring low numbers of organisms can be likely to cause illness if these organisms are in good condition or can be resuscitated (Kozak *et al*, 1996; Uyttendaele *et al*, 1999; Rørvik, 2000 and Standaert *et al*, 2005).

An estimate of microbial risk to human health following the consumption of foods involves the prevalence and the numbers of organisms in foods. The changes in both microbial prevalence and numbers may occur throughout the food chain. Hence, the risk model for the exposure assessment has been developed based on these dynamic changes of micro-organism from production to consumption, involving growth and inactivation following the change in temperature and time.

Predictive growth models have been developed since the 1980s. They are based on the relationship between bacterial growth and the change in the number of micro-organisms over time. This is influenced by a set of intrinsic and extrinsic parameters (or conditions), governing growth, survival and control of desirable and undesirable micro-organisms in food

systems. Three degrees of modelling: primary, secondary and tertiary are available. Whilst the primary models describe the growth and inactivation in the simplest way, using first order kinetics, the secondary and tertiary models attempt to account for all relevant parameters as well as to integrate data for all aspects in response to environmental factors influencing the micro-organism. Van Gerwan and Zwietering (1998) summarised and proposed some possible functions for use in predictive modelling. These are presented in Table 7.1.

**Table 7.1** Models described the growth curve of microbial cells under stationary conditions (taken from van Gerwan and Zwietering, 1998)

| Growth curve | Equation |
| --- | --- |
| Exponential[1] | $\ln(n_0) = \ln(n_0) + \mu t$ |
| Lag-exponential[2] | $\ln(n_o) = \ln(n_0), \ t < \lambda$ |
| | $\ln(n_0) = \ln(n_0) + \mu(t - \lambda), \ t \geq \lambda$ |
| Logistic[3] | $\ln(n_0) = \ln(n_0) + \dfrac{a}{[1 + \exp(b - cx)]}$ |
| Reparameterised Gompertz[4] | $\ln(n_0) = \ln(n_0) + A\exp\{-\exp[\dfrac{\mu_{max} \times \exp(1)}{A}(\lambda - t) + 1]\}$ |
| Baranyi[5] | $\ln(n_0) = \ln(n_0) + \mu_{max}A_n(t) - \ln[1 + \dfrac{\exp(\mu_{max}A_n(t) - 1}{\exp(A)}]$ |
| Jones[6] | $\ln(n) = \ln(n_0 - \ln(2)\{\exp[\frac{t-d}{c}] - \exp[\frac{-(t-d)}{c}] - \exp[\frac{-d}{c}] + \exp[\frac{d}{c}]\}$ |
| | $+ \ln(2)a(1 - (1 + \frac{t}{b} + \frac{1}{2}.(\frac{t}{b})^2 + \frac{1}{6}.(\frac{t}{b})^3)\exp(\frac{-t}{b}))$ |
| Probability[7] | $P(t) = \dfrac{P_{max}}{(1 + \exp[k(\theta - t)])}$ |

[1] $n_0$ is the initial number of organisms, $\mu$ is specific growth rate, t is time

[2] $\lambda$ is the lag time

[3] a,b and c are fit parameters for logistic equation

[4] $\mu_{max}$ is the maximum specific growth rate (h⁻¹), A is the maximum level of increase: ln $(n_x/n_0)$.

[4,5] $A_n$ as defined by Barayi, et al. (1994)

[6] d is a fit parameter

[7] P(t), probability of growth at time t; $P_{max}$, maximum probability; k, rate constant; $\theta$ time to the midpoint of the function

# 7.2 Exposure modelling

Estimation of exposure to *Campylobacter* through the consumption of chicken involves complex and inter-related processes, describing the probability and likelihood of the

exposure from the initial to the final stage in selected food pathways. Given this complexity, it is necessary to separate the overall chain into a number of distinct parts, each representing a specific stage in the production to consumption line (WHO, 2001).

The joint FAO/WHO working group (WHO, 2001, Christensen *et al.* (2001) and Nauta *et al.* (2005a) described the main stages of exposure modelling which include primary production (on farm), processing at slaughterhouses, retail outlets and food handling and preparation in the household with likely transport and storage between various stages. Figure 7.2 illustrates the most likely pathway by which *Campylobacter* may be transmitted to humans through the consumption of chicken. Three levels of risk model for *Campylobacter* have currently been developed due to availability of data which are i) a farm model, ii) a slaughterhouse model and iii) a consumer model.
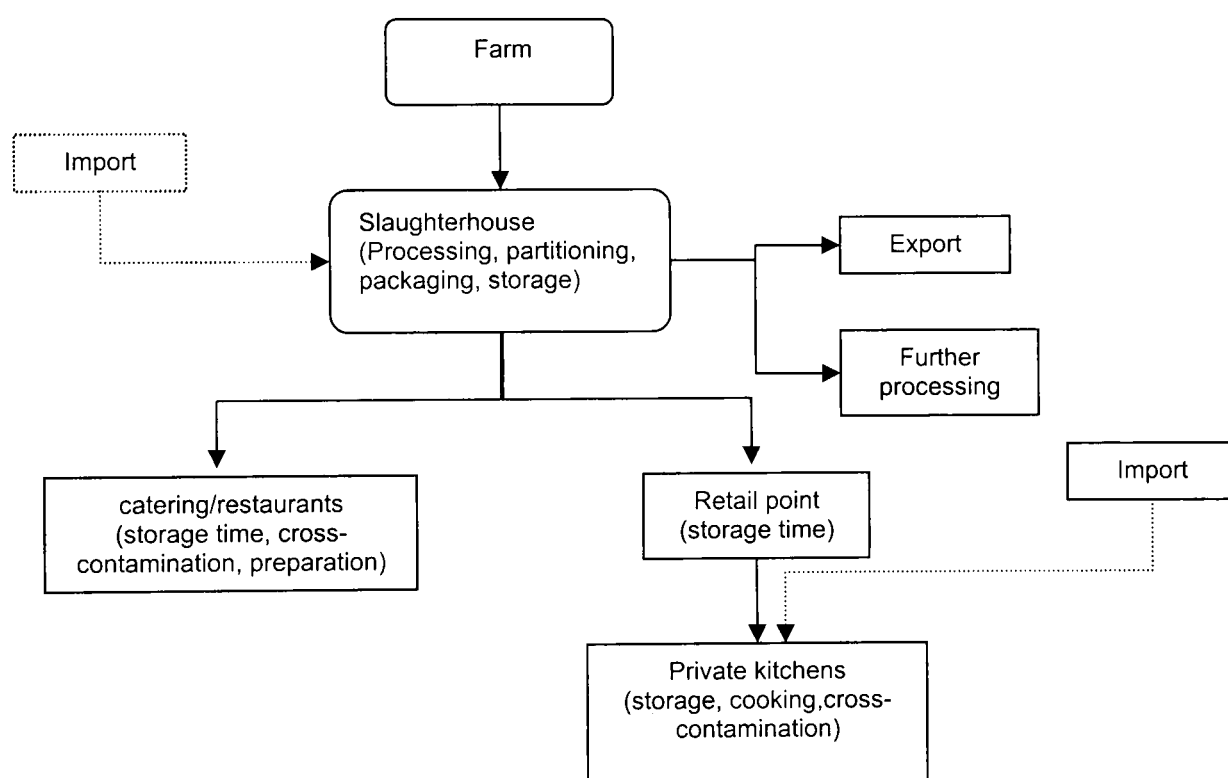


**Figure 7.2** An overview of a pathway of chicken products from production to consumers (Christensen *et al.*, 2001)

## 7.2.1 A farm model

Risk modelling at the farm level aims to estimate the rate of colonisation, transmission and contamination within and between flocks and prevalence of *Campylobacter* (WHO, 2001 and Katsma *et al.*, 2005). It also aims to quantify the levels of *Campylobacter* likely to be present in the gut of a colonised chicken before entering the slaughterhouse and processing.

The estimation of exposure to *Campylobacter* on the farm involves the colonisation in the gut and the contamination of the exterior of the birds. Thus, the modelling approach considers the probability of a flock being colonised with *Campylobacter* and contamination on farm and during transport.

The probability of flocks being positive for *Campylobacter* ($P_{pb}$) was described by a joint FAO/WHO working group (WHO, 2001) as a function of the flock prevalence and the within-flock prevalence. This function is expressed by equation 7-1.

$$P_{pb} = P_{fp} \times P_{wfp} \qquad \text{[7-1]}$$

Where;

$P_{fp}$ is the flock prevalence, which is the proportion of the national flock that is positive. A positive flock is defined as a flock that shows at one bird colonised with *Campylobacter*.

$P_{wfp}$ is the within-flock prevalence of a positive flock at the time of slaughter, which is directly related to the rate of transmission and is therefore a time dependent event for a positive flock. The transmission of *Campylobacter* within a flock was rapid. A number of studies demonstrated that once one chick in a flock is infected with *Campylobacter*, the whole flock becomes positive within 4-7 days (Shanker *et al.*, 1990; Shreeve *et al.*, 2000 and Jacobs-Reitsma *et al.*, 2001).

The estimate for $P_{wfp}$ is $\qquad P_{wfp}(t) = \dfrac{I(t)}{N} \qquad \text{[7-2]}$

Where;

*I(t)* is the number of colonised birds within a flock at time *t*

*t* is the time since the introduction of the infection

*N* is total numbers of bird within a flock (a flock size).

Katsma *et al.* (2005) described and proposed the additional model for the transmission of *Campylobacter* within a flock, *I(t)*, by fitting to a logistic[9] growth curve for population (see Table 7.1). The addition model is shown in equation 7-3

$$I(t) = \dfrac{KNI_0}{I_0 + (KN - I_0)e^{-rt}} \qquad \text{[7-3]}$$

Where,
N is the flock size
K is the carrying capacity (the upper limit of population growth)
I is the number of colonised chickens in the population,
r is the growth rate of the colonised chicken in the population (in the absence of intra-specific competition)
t represents the time since the introduction of the infection.

The calculation of contamination on the farm and during transport is based on: i) *Campylobacter* status of the flock, ii) faeces shedding from chickens, iii) transmission time

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[9] A logistic model assumes that a population N (t) of individuals, cells or inanimate objects grows of diffuses at an exponential rate $\alpha$ until the approach of a limit or capacity *K* slows the growth.

since the introduction of the infection, iv) number of newly colonised birds and v) the distance between negative and positive groups in the transport vehicle (WHO, 2001).

## 7.2.2 Slaughterhouse model

The poultry processing at a slaughterhouse is a dynamic process consisting of a series of stages, which starts with slaughter and moves through processing to the transport of the final sale product. The different technological stages of slaughter processes may decrease or increase contamination (Nauta *et al.*, 2005a). During slaughter and processing, it is expected that *Campylobacter* from positive chickens could be transmitted to the exterior of other carcass or the environment.

In order to calculate the level of cross-contamination of *Campylobacter* between chicken products, it is necessary to understand each stage of processing and the relative consequences of one stage to others. A number of models consider the probability that a random chicken product will be contaminated with these micro-organisms and the likely number of *Campylobacter* in contaminated products. In addition, the models also assess the stages of processing most likely to impact on the levels of contamination (WHO, 2001; Christensen *et al.*, 2001 and Nauta *et al.*, 2005b).

## Stages of chicken processing in the slaughterhouse

In general, the processing of chicken consists of seven or eight main stages as illustrated in Figure 7.3. These are:

### Hanging area- shackling, killing, bleeding

On entry, birds are removed from crates, hung upside down, stunned and bled for up to two minutes. Although birds are hung in close proximity and come in to contact with each other and the machines, the effect of this stage on cross-contamination is assumed to be negligible.

```
┌─────────────────┐                    ┌─────────────────┐
│ Live chicken-   │                    │ Hanging area-   │
│ unloading area* │───────────────────>│ shackled, killed│
└─────────────────┘                    └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Scalding/Picking/│
                                       │ washing         │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Defeathering    │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Evisceration    │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Washing/Chilled │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Portioning      │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Grading/Packing │
                                       └─────────────────┘
                                                │
                                                ▼
                                       ┌─────────────────┐
                                       │ Distribution    │
                                       └─────────────────┘
```
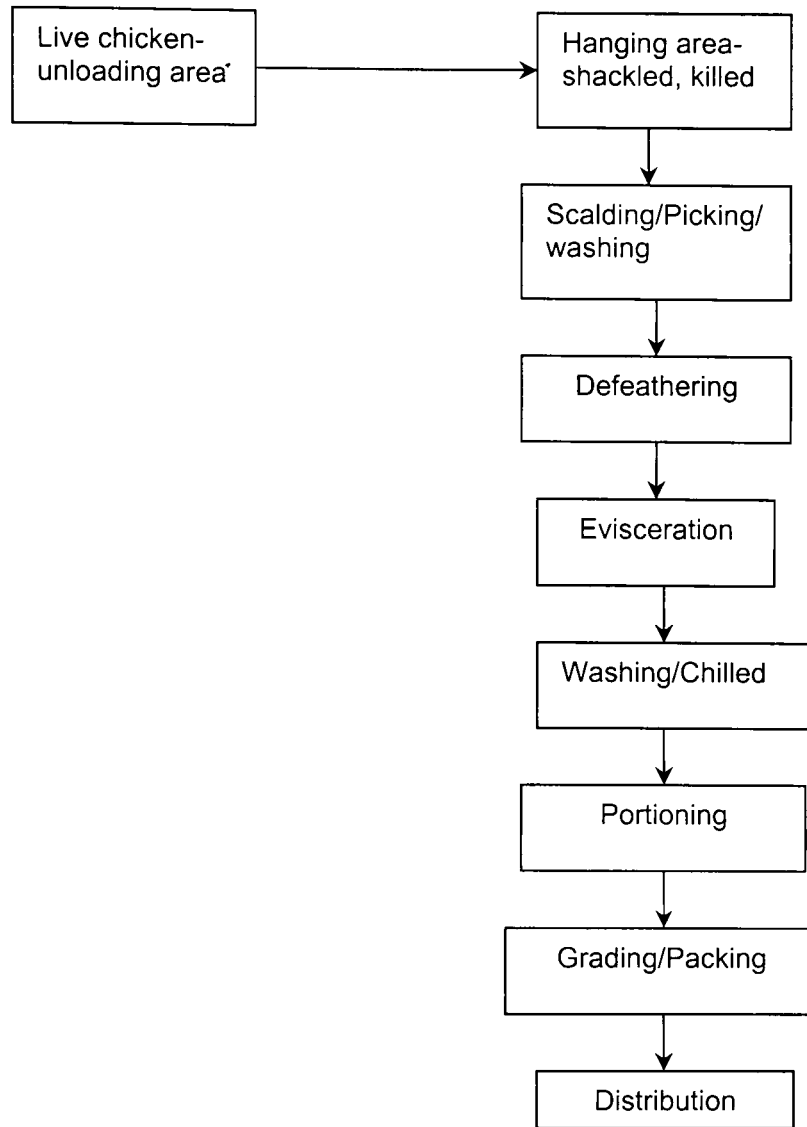
**Figure 7.3** The main stages of poultry processing at a slaughterhouse (adapted from WHO, 2001)

## Scalding

After slaughter, birds are immersed in a scald tank. This loosens the feathers and facilitates plucking. At this point, birds may involuntarily defecate, leading to accumulation of faecal matter in the tank. If the birds are colonised with organisms, this results in contamination of the scald water and may lead to further contamination to the exterior of the carcasses.

## De-feathering

This process is a mechanical process comprising a series of machines that are the major sites of potential cross contamination. Oosterom *et al.*(1983) and Izat *et al.*(1988) showed that de-feathering increases the prevalence of contaminated carcasses.

## Evisceration

Following the removal of head and feet, chickens are eviscerated, removing the internal organs. In this process the birds are either mechanically or manually eviscerated and the intestines usually remain intact. If the intestines are damaged, this may lead to a risk of gross contamination. However, Oosterom *et al.*(1983) and Izat *et al.*(1988) demonstrated that even if the viscera remain intact, the levels of enteric bacteria on the exterior of the carcass can still increase.

## Washing

In compliance with EU regulation, following the evisceration, the carcass is washed internally and externally. Even though the inside-outside wash is capable of removing visible faecal contamination, it does not eliminate bacteria attached to the surface. However, attachment is a time-dependent process. Washing the carcass at different stages may loosen the attachment. Cudjoe *et al.*(1991) showed that the washing procedure typically reduced the numbers of *Campylobacter* on a carcass by approximately 90%.

## Chilling

Within the EU, the chilling processes used to maintain the temperature at $4^0$C or less may involve air-chillers, water chillers and spray chillers (cryogenic nitrogen chillers). Technically, the choice of chilling procedures is based on whether the carcass is intended to be a fresh or frozen product. For example, air-chillers are generally used for a carcass sold as a fresh product. The methods of applying water are used for frozen products. Laisney *et al.*(1991) reported that water chilling reduces the levels of contamination of *Campylobacter* on carcasses since they move through a counter-flow current. In addition, addition of chlorine to chill water prevents the cross-contamination of organisms washed off into the water. Nonetheless, air chilling does not change the levels of *Campylobacter* due to its ability to survive under these conditions (Cudjoe *et al.*, 1991). Spray chillers cause less cross-contamination

## Portioning

Currently the preferred method for the jointing of the carcass is by mechanical or semi-mechanical methods. These methods allow the fast line speeds and higher throughout required by the industry. The contact between carcasses and a machine or operator's hands increases, resulting in higher rates of cross-contamination. This results from the re-distribution of the contamination of the processed load or carries over contamination from the previous day. Gill (1999) showed that the automatic portioning equipment is likely to be a potential source of contamination.

## Grading and packaging

Grading and packaging is governed by the scald and chill procedure. Water chilled carcasses are packed in polythene bags with or without giblets. Air chilled products are usually packed without giblets on polystyrene trays and wrapped in cling film. Since there is no further information to affirm whether there is potential for cross-contamination at this stage, it is assumed that no cross-contamination occurs on the carcasses (WHO, 2001).

## Model description

A risk model describing the poultry processing has been formulated by the joint working group of FAO/WHO (WHO, 2001). The model considers the stages of processing which may impact upon the level of *Campylobacter* contaminating a carcass. However, recently Nauta *et al.*(2005b) also developed and simplified the model of poultry processing for the *Campylobacter* Risk Management and Assessment (CARMA) project. This model considers growth or inactivation and removal as factors changing the level and prevalence of *Campylobacter*. As temperatures in the processing plant should never reach the minimum growth temperature for these micro-organisms, **it is assumed that *Campylobacter* will not grow in this environment.** Thus, the model considers only inactivation and removal of *Campylobacter* during the consecutive processing stages. This modelling process involves a number of different factors affecting individual stages at each point of time. Nauta *et al.*(2005a) generated a slaughterhouse model (Figure 7.4) as a stochastic process  and developed a scenario of a model based on the principle of an input-output model (West, 1986). The principle of the model is that the input of stage "S" is the product (output) from the previous stage (stage "S-1").
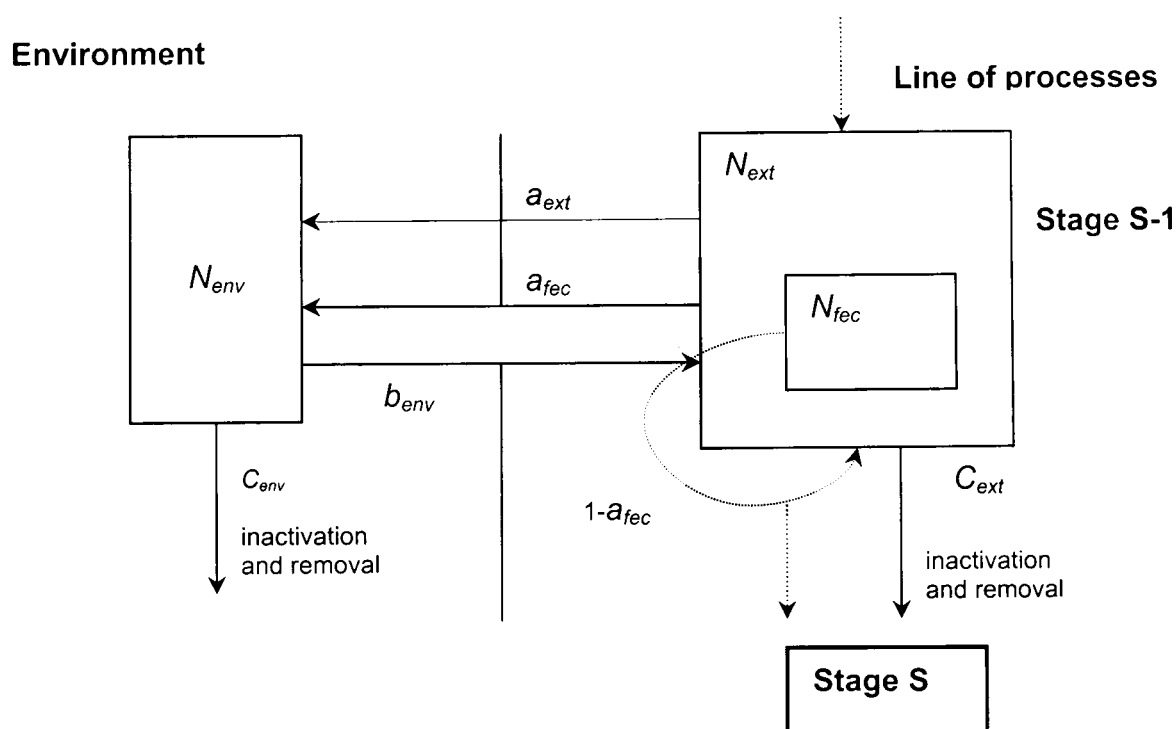


**Figure 7.4** Diagram of the basic poultry processing model (adopted from Nauta *et al.*,2005a) (Definition of parameters is given in Tables 7.2 and 7.3)

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**Table 7.2** Model parameters of model equations per stage $S$ and carcass $i$ (adopted from Nauta *et al*,2005a)

| Parameter | Description |
| --- | --- |
| $a_{ext,S}$ | probability per cfu *Campylobacter* on the exterior (skin and feathers) to move from the carcasses exterior to the environment. |
| $b_{env,S}$ | probability per cfu *Campylobacter* in the environment to move from the environment to the carcass exterior. |
| $a_{fec,S}$ | probability per cfu *Campylobacter* in the leaking faeces to move to the environment, corresponding probability 1- $a_{fec,S}$ per cfu to move from the interior to the exterior of the carcass directly). |
| $c_{env,S}$ | probability of inactivation or removal per cfu *Campylobacter* in the environment, which is not transferred to the carcass exterior. |
| $c_{ext,S}$ | probability of inactivation or removal per cfu *Campylobacter* on the carcass exterior, which is not transferred to the environment. |
| $P_{fec,S}$ | probability of faecal leakage per carcass. |
| $P_{anim}$ | fraction of the animals in a positive colonised flock. |

**Table 7.3** Model variables of model equations per stage $S$ and carcass $i$ (after Nauta *et al*,2005a)

| Parameter | Description |
| --- | --- |
| $m_{S(i)}$ | amount of faeces (g) that leaks from carcass $i$, given faecal leakage; sampled from a lognormal distribution with mean $\mu_{m,S}$ and standard deviation $\sigma_{m,S}$. |
| $w_{fec,S}(i)$ | amount of faeces (g) that leaks from carcass $i$: with probability $P_{fec,S}$ $w_{fec,S}(i)=0$, where $w_{fec,S}(i) = m_S(i)$ |
| $C_{fec,}(i)$ | *Campylobacter* concentration in the faeces of carcass $i$, identical per carcass $i$ for all stages (cfu/g faeces), taken the value from a lognormal distribution ($10^\text{Normal}$ ($\mu_C,\sigma_C$) |
| $N_{fec,S}(i)$ | numbers of *Campylobacter* in the leakage from carcass $i$. $N_{fec,S}(i) = w_{fec,S}(i) \times C_{fec}(i)$. |
| $N_{ext,S}(i)$ | numbers of *Campylobacter* on the exterior after processing of carcass $i$. $N_{ext,input}(i)$ is model input, taken from a lognormal distribution ($10^\text{Normal}$ ($\mu_N,\sigma_N$) |
| $N_{env,S}$ | numbers of *Campylobacter* in the environment after processing of carcass $i$. |

The model considers inactivation and removal of *Campylobacter* at each stage, given a carcass, $i$, entering the process stage $S$. A carcass is contaminated with $N_{ext,S-1}(i)$ colony-forming units (cfu) of *Campylobacter* on the exterior. If $S$ is the de-feathering stage, the previous stage $S-1$ refers to the product after scalding, *etc*. At stage $S$, $N_{fec,S}(i)$ (cfu) obtained

from the numbers of *Campylobacter* in faeces dripping out from carcass *i*, thus, $N_{fec}$ is the product of amount of faeces ($W_{fec,S}(i)$) and *Campylobacter* concentration in faeces ($C_{fec}(i)$).

In this system, *Campylobacter* from the exterior or faeces occasionally contaminates the environment, *i.e.* faeces or fluid leak from the positive carcass and remains on a machine or worker's hands. This contamination may be transferred back to other carcasses through the water, equipment, hands, *etc.* At this stage, the numbers of *Campylobacter* in the environment {($N_{env, S}(i)$) can be counted. Given the condition at each stage, the model equations per stage *S* and carcass *i* are;

$$N_{ext,S}(i) = (1 - a_{ext,S})(1 - c_{ext,S})N_{ext,S-1}(i) + b_{env,S}N_{env,S}(i-1) + (1 - a_{fec,S})N_{fec,S}(i) \qquad [7\text{-}4]$$

$$N_{env,S}(i) = a_{ext,S}N_{ext,S}(i) + (1 - b_{env,S})(1 - c_{env,S})N_{env,S}(i-1) + a_{fec,S}N_{fec,S}(i) \qquad [7\text{-}5]$$

## Model input

Nauta *et al.*(2005a) described and analysed the model equations 7-4 and 7-5 further. It is assumed that at any stage *S* the number of *Campylobacter* added and removed per passing carcass is the same, thus resulting in $\Delta N_{env,S} = 0$. Although this equilibrium state is not possible, it is evident that this situation is likely to be approached after the passage of a few carcasses. Therefore, the value of $\Delta N_{env,S}$ is equal to zero if;

$$N_{env,S} = \frac{a_{ext}N_{ext,S-1} + a_{fec}N_{fec}}{b_{env} + c_{env} - b_{env}c_{env}} \qquad [7\text{-}6]$$

Where, $N_{fec}$ is a product of $w_{fec}$ and $C_{fec}$. If log($N_{ext,S-1}$) and log ($C_{fec}$) is a normal distribution with means $\mu_N$ and $\mu_C$ and variances $\sigma^2_N$ and $\sigma^2_C$ , respectively, the expected value of $E(N_{env,S})$ is given by;

$$E(N_{env,S}) = \frac{a_{ext}E(N_{ext,S-1}) + a_{fec}E(w_{fec})E(C_{fec})}{b_{env} + c_{env} - b_{env}c_{env}} \qquad [7\text{-}7]$$

Then, $$E(N_{env,S}) = \frac{a_{ext}10^{\mu_N + \ln(10)\frac{\sigma^2_N}{2}} + a_{fec}P_{fec}\mu_m P_{anim}10^{\mu_C + \ln(10)\frac{\sigma^2_C}{2}}}{b_{env} + c_{env} - b_{env}c_{env}} \qquad [7\text{-}8]$$

In addition, the expected of value $E(N_{ext,S})$ can be determined from equations 7-4 to 7-8 as follows:

$$E(N_{ext,S})$$

$$= (1 - a_{ext})(1 - c_{ext})E(N_{ext,S-1}) + b_{env}E(N_{env,S}) + (1 - a_{fec})(E(w_{fec})E(C_{fec}))$$

$$= (1 - a_{ext})(1 - c_{ext})10^{\mu_N + \ln(10)\frac{\sigma^2_N}{2}} + b_{env}E(N_{env,S}) + (1 - a_{fec})P_{fec}\mu_m P_{anim}10^{\mu_C + \ln(10)\frac{\sigma^2_C}{2}}$$

$$............ \qquad [7\text{-}9]$$

Based on an input-output model, the numbers of Campylobacter in carcass *i* at the end of each stage *S* is the output which will be the input to the next consecutive stage. Applying the equations 7-8 and 7-9 the expected numbers of Campylobacter in carcass *i* at each stage *S* can be calculated consecutively (Figure 7.5).

output from stage **S-1**

input of stage **S**

Initial level at the beginning of process.......$N_0$

$N_0$

Scalding.......$N_{ext}$

$N_{ext, scalding}$

De-feathering.......$N_{ext,}$

$N_{ext, defeathering}$

Evisceration.......$N_{ext,}$

$N_{ext, eviscerationg}$

Washing.......$N_{ext,}$

$N_{ext, washingg}$

Chilling.......$N_{ext}$

$N_{env,S}(i)$
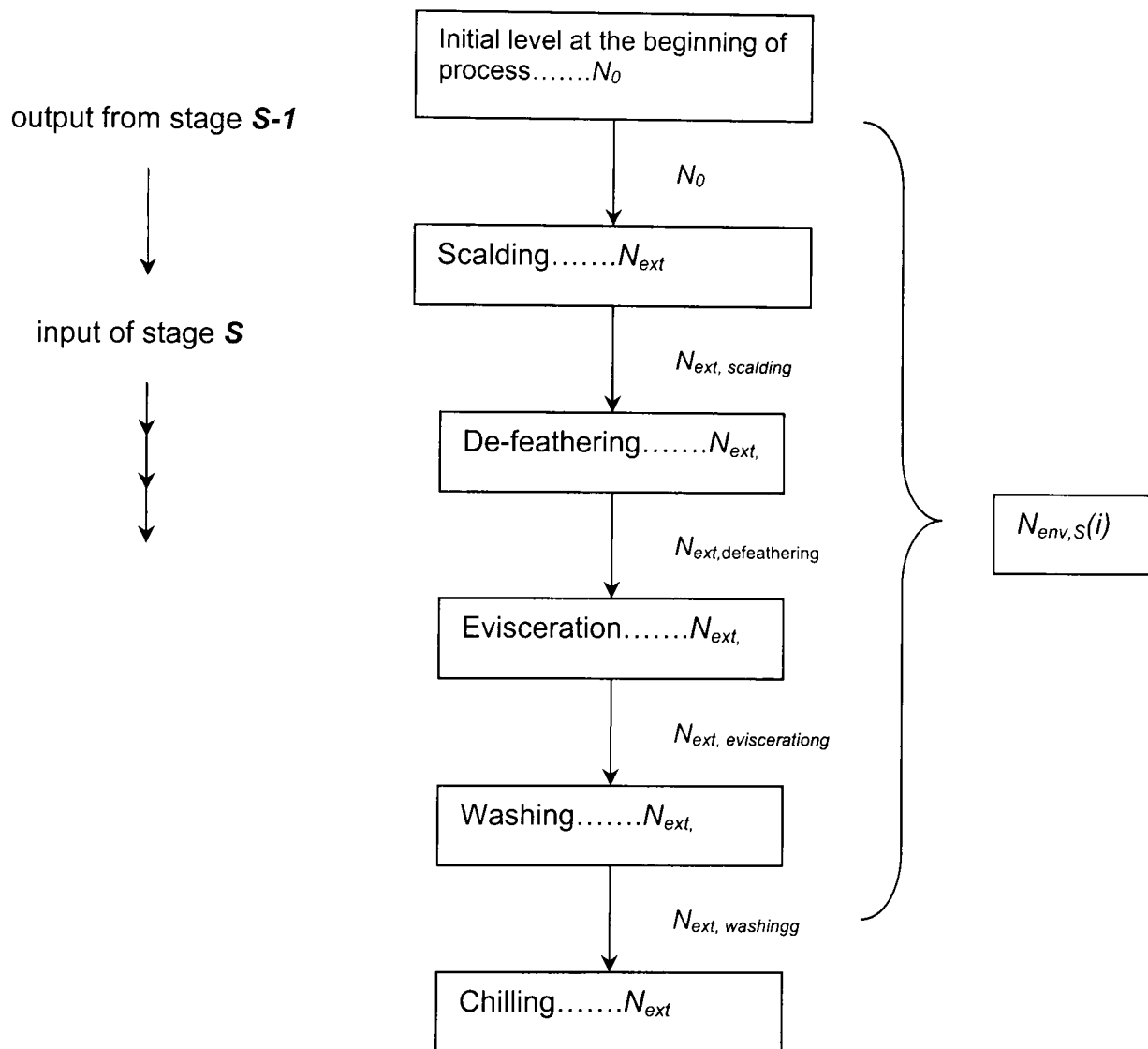
**Figure 7.5** Algorithm of input-output process used for poultry slaughter and processing (based on the model presented by Nauta *et al.*, 2005a).

## 7.2.3 Modelling consumer exposure to *Campylobacter*

Two criteria considered for health risk related to Campylobacter following the consumption of three groups of chickens in this study are: 1) health risk related to all Campylobacter (with

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and without antimicrobial resistant isolates) and 2) health risk related to antimicrobial resistant *Campylobacter* only. Therefore, exposure assessment was independently performed, firstly for all *Campylobacter* and secondly for antimicrobial resistant *Campylobacter*.

Exposure assessment for all *Campylobacter* is first presented in this section. From this point the word "*Campylobacter*" means all *Campylobacter* including susceptible and resistant isolates. Antimicrobial resistant *Campylobacter* (ABR- *Campylobacter*) refers to the isolates found to be resistant to three antimicrobial agents (ciprofloxacin, erythromycin and nalidixic acid).

*Campylobacter* is the most common pathogen found in raw fresh chickens brought into the home. During meal preparation, these micro-organisms are obviously transferred to hands, utensils, preparation surfaces or other food items (Christensen *et al.*, 2001). Thus, individuals can be exposed to *Campylobacter* from fresh chicken through these pathways. These include direct contamination from the chicken to any food stuffs or indirect contamination of preparation surfaces through hands and subsequent ingestion. The exposure level is therefore associated with the detail of the events during preparation.

The FAO/WHO risk assessment model assumes that liquid from fresh carcasses containing micro-organisms (included *Campylobacter*) may drip onto the hands, surfaces, utensils or cutting boards. These events would facilitate the organisms' movement throughout the whole pathway from preparation to consumption.

It is possible that the levels of hygiene practice by a person handling foods may have the greatest effect on the level of *Campylobacter* in any private kitchen. In England and Wales, it was estimated that 35% of outbreaks of foodborne illnesses were related to cooking with insufficient heat treatment and 28% were related to cross-contamination during preparation (Ryan *et al*, 1996). Other reports from the USA and Sweden showed similar results (Worsford and Griffith, 1997 and Zhao *et al*, 1998 ).

This study estimates the exposure to *Campylobacter* following the consumption of three groups of chickens using the combination of risk models developed by a joint FAO/WHO committee (WHO, 2001; Christensen *et al.*, 2001 and Nauta *et al.*, 2005b). The risk models of how consumers handle and prepare meals in private kitchens were developed according two scenarios. These are:

▶ insufficient heat treatment of the chicken resulting in the survival of *Campylobacter*

▶ cross contamination of *Campylobacter* due to improper handling procedures (personal hygiene).

The pathways of these two scenarios following the blue blocks are demonstrated in Figure 7.6.

Levels of *Campylobacter* in raw chicken at Retail outlet

Household

Storage → Preparation/ Cooking ← Personal hygiene

Storage → Cross-contamination to other meals

Preparation/ Cooking → Insufficient Heat treatment

Preparation/ Cooking → Cross-contamination (utensil, cutting board, hand) to other meals

Numbers of *Campylobacter* in contaminated meals ← Size of meal

Consumption pattern

Age, sex group of person preparing

Age, sex group of person ingesting meals

Hygiene level

→ Probability of ingesting a risk meal / a chicken risk meal at home

Exposure dose of *Campylobacter* following consumption of contaminated meal (per serving)

Infective dose

Probability of infection from *Campylobacter* contaminated

Probability of illness from *Campylobacter* contaminated

**Figure 7.6** A stochastic pathway of a consumer model of risk assessment (developed by Soonthornchaikul, 2005.)

## Scenario 1: Insufficient heat treatment

*Campylobacter*, in general, is sensitive to the effect of thermal processing. The joint FAO/WHO meeting group (WHO, 2001) developed a model of heat treatment specifically for *Campylobacter*. It is evident that *Campylobacter* tends to remain attached to the surface of carcasses. Sustained high surface temperatures ($>70^0$C) will generate enough heat to eliminate the organism. However, under some conditions some *Campylobacter* may survive

to cause infection. Given insufficient intensity of heat treatment, the estimation of the exposure dose is based on the survival rate of *Campylobacter*.

Due to the scarcity of data, the modelling of insufficient heat treatment is performed using an exponential growth model (see Table 7.1, section 7.1). The model provides a mechanistic explanation based on whole cell inactivation kinetics (including cell injury) associated with cooking. Also described are the alternative explanations of the means by which *Campylobacter* in the thermally protected areas (such as cracks, air pockets or deep within the carcass mass) might survive an otherwise thorough heat treatment.

The joint of FAO/WHO working group proposes three approaches to the modelling of heat treatment, which depend on **the assumptions described below** (WHO, 2001). These approaches are: 1) Internal temperature approach, 2) Protected area approach and 3) The heat transfer approach.

## 1) Internal temperature approach

The assumption of this approach is that a representative point in the chicken mass (*e.g.* a drumstick portion) **can be expected to receive the mildest heat treatment.** The selection of this point is partially based on the existence of a cooking temperature survey which measured the internal temperature of heat treatment in chicken portions (*e.g.*drumsticks). The reduction in cell numbers at this point is therefore calculated by using a step-wise time-temperature profile and applying a primary kinetic function at each time-step, (Van Gerwen and Zwietering, 1998; and van Gerwen *et al.*, 2000). This also includes the possibility of an increase in cell numbers in the period where the chicken is being heated through the range of temperatures. The variability of consumer practices is taken into account in this approach by varying the stopping point of the final temperature of cooking in the simulated time-temperature profile. The variation in the stopping point is based on survey data measuring the final temperature (Doyle & Roman, 1981 and Gill & Harris 1982).

## 2) Protected area approach

This approach makes four assumptions: i) that only a fraction of the carcasses will experience some type of undercooking; ii) that the only cells which have any possibility of survival are within an area which is relatively protected (or insulated) from the heat of the oven; iii) that some fraction of the cells will be located in the protected areas and iv) that a reduction is calculated based on the internal temperature at this protected area and the time for which this maximum temperature is applied.

## 3) Heat transfer treatment

This approach is for calculating the internal time-temperature profile at a number of different depths in the meat of the carcass. This requires transient heat transfer models and parameters of thermal properties applied on the chickens which are generally available in food engineering texts. The approach is based on; i) the proportion of bacterial load found at the surface and at various depths into the carcass, ii) a simplified characterisation of the roasting of a carcass regarding specific heat transfer assumptions and iii) the oven temperature and the time at which the chicken is removed from the oven. Therefore, the reduction in cell numbers can be characterised at each depth into the chicken meat by considering the reduction in each simulated time-step.

## Scenario 2: Cross-contamination

Recently, several studies investigated the pathways of cross-contamination of bacteria during food preparation in either a private kitchen or other catering establishments (Worsefold and Griffith, 1997). These noted that either personal hygiene practices or unsafe food handling in a private kitchen is associated with a large number of factors. Thus, estimating the risk of infection through cross-contamination requires extensive data. Since, to date, the availability of essential data is limited, risk assessment of cross-contamination involves a large degree of uncertainty.

To assess the potential of cross-contamination in spreading organisms during meal preparation, the prevalence and numbers of *Campylobacter* transferred to meals during preparation should be taken into account. This study focuses on cross-contamination by hands and contacted surfaces and cutting boards, due to data availability.

## 1) Cross contamination as a result of hygiene practices by a food preparer (by hands)

Several studies have investigated consumers' habits regarding hand washing. Washing hands after handling raw food animals is thought to be a crucial factor in minimising cross-contamination. Brown *et al.* (1988) found that washing hands could totally eliminate *Campylobacter* remaining on hands, whereas, handling raw chicken without hand washing results in other food items becaming contaminated with *Campylobacter*. This is supported by the study of De Boer and Hanne (1990) which investigated the contamination with *Campylobacter* during handling of *Campylobacter*-positive chickens. 73% of trials showed positive findings for detection of *Campylobacter* on hands. After 3 minutes *Campylobacter* spp. could still be detected in 55% of trials. In addition, Coates *et al.* (1987) showed that *Campylobacter* from the liquid of raw meat stained on fingers could survive up to an hour. It also supported the assertion that hand washing (with water or water with soap) combined

with drying can effectively eliminate *Campylobacter*. However, washing without drying cannot remove the organisms from fingers.

Christensen *et al.*(2005) indicated that hygiene levels during food preparation vary significantly with age and gender of food preparers. There may be an association between the hygiene practices of food preparers and likelihood of *Campylobacter* infection within different age and gender groups. The results of most studies related to unsafe food handling are summarised in Tables 7.4

**Table 7.4** Consumer behaviours related to hand-washing after handling raw meat and poultry (adopted from the WHO, 2001).

| Statement & Observation | Respondent agreeing with the statement (%) | Reference |
|---|---|---|
| Washing hands not performed after handling | 34 | Altekruse *et al.*, 1999 (USA) |
| | 55.8 | Jay *et al.*, 1999 (Australia) |
| | 36 | Worsford & Griffith, 1997 (UK) |
| | 58 | |
| Washing hands not important in relation food hygiene | 18.4 | Jay *et al.*, 1999 (Australia) |
| Personal hygiene not important for prevention of foodborne disease | 62 | Christensen *et al.*, 2005 |
| Drying of hands performed after hand wash | 70 | Christensen *et al.*, 2005 |

## 2) Cross contamination by contacted surfaces

The information from the outcomes of interviews and observations showed the likelihood of cross-contamination during food preparation in a private kitchen (Tables 7.5 and 7.6). Christensen *et al.*(2001) reported that the prevalence of *Campylobacter* spp. on cutting boards was 50%. It was found on 9% of vegetable samples handled on a cutting board previously used for a raw chicken. Although a chicken was already cooked (heat treatment), *Campylobacter* was detected on the cooked chicken handled on a cutting board previously used for a raw chicken (De Boer and Hahne, 1990). Martin *et al.*(1999) stated that it is possible to isolate *Campylobacter* spp from contaminated domestic kitchen surfaces 50 minutes after the area was observably dry. Furthermore, *Campylobacter* spp were isolated from the outer layer of packaging of chicken and offal products sold at retail points (Bolton *et al.*,1999b and Burgess *et al.*,2005).

**Table 7.5** Food handling procedures related to age and gender (adopted from WHO, 2001).

| Statement & Observation | Respondent agreeing with the statement (%) | | Related to age group (%) | Reference |
|---|---|---|---|---|
| | Male | Female | | |
| Washing hands not performed after handling raw meat and poultry | 47 | 25 | 18-29: 42<br>30-64: 32<br>>65: 29 | Altekruse *et al.*, 1995 |
| Cutting board not changed or washed after handling raw meat and poultry | 47 | 28 | 18-29: 47<br>30-64: 29<br>>65: 24 | Altekruse *et al.*, 1995 |
| Cutting board not sufficiently washed | | | 17-35: 45<br>36-45: 38<br>>46: 33 | Jay *et al.*, 1999 |
| Utensil not sufficiently washed | | | 17-35: 32<br>36-45: 28<br>>46: 27 | Jay *et al.*, 1999 |
| Clean utensils and change of cutting boards are not important issues in preventing foodborne disease | 51 | 46 | <24: 63<br>25-34: 47<br>35-54: 41 | Christensen *et al.*, 2001 |
| Sufficient heat treatment not recognised as a preventive option to food borne disease | 51 | 57 | <24: 55<br>25-34: 52<br>35-54: 50 | Christensen *et al.*, 2001 |

**Table 7.6** Consumer behaviours related to cross-contamination by contacted surfaces (adopted from WHO, 2001).

| Statement & Observation | Respondents agreeing with the statement (%) | Reference |
|---|---|---|
| Knives and cutting boards not cleaned in warm water + soap after handling raw meat and poultry and before cutting vegetables and salads | 46 | Williamson *et al.*, 1992 (USA) |
| Cutting board not washed after handling raw meat and poultry | 33 19.5 | Altekruse *et al.*, 1995 (USA) |
| The kitchen facilities not sufficiently cleaned to avoid cross-contamination | 11.6 | Jay *et al.*, 1999 (Australia) |
| Food items handled on not sufficiently cleaned cutting boards | 25 | Worsford & Griffith, 1997 (UK) |
| Meat and poultry packaging materials stored in the food handling area | 18 | Worsford & Griffith, 1997 (UK) |
| Food items handled in a way that could lead to cross-contamination | 76 | Daniels, 1998 (USA & Canada) |

## 7.2.4 The development of consumer model as applied to be used in this study for *Campylobacter*

Based on "insufficient heat treatment approach" (WHO, 2001 and Christensen *et al.*, 2001) and "cross-contamination approach (Nauta *et al.*, 2005) (sections 7.2.3), the models of heat treatment and cross-contamination were selected for use in the estimation of the number of *Campylobacter* on a carcass which may be transferred to person consuming that meal. Additionally, the effect of refrigeration (storage) on the number of *Campylobacter* was also considered for delayed preparation.

### 1) Modelling heat treatment

The modelling was developed using those three options of insufficient heat treatment (section 7.2.3) to estimate numbers of *Campylobacter* in a chicken after thermal processing. As stated previously, under heat treatment, the number of *Campylobacter* may increase through growth, or decrease by inactivation. Modelling is then carried out by using a predictive growth model associated with temperature and time during cooking. The

exponential models selected for the representation of both growth and inactivation are as follows:

Table 51. Given a population of bacteria where growth is unrestricted, the rate of increase in the population is proportional to the size of the population itself,

$$\frac{dN}{dt} = \mu N$$

Where N = number of bacteria, t= time, μ= growth rate at which the population changes over time, t

$$N = N_0 e^{ut}$$

$$\ln N = \ln N_0 + ut \text{ , when } T_{stop} < T_c \qquad [7\text{-}10]$$

2) The reduction of bacteria at given time and temperature follows first order kinetics (described by the The Arrhenius equation[10]), and therefore is;

$$\log_{10}(\frac{N}{N_0}) = -kt$$

$$\log N = \log N_0 - kt \text{ , where } k = 2.303/D$$

Thus;
$$\ln N = \ln N_0 - (\frac{t}{D}), \text{ when } T_{stop} > T_c \qquad [7\text{-}11]$$

**Where:**

$N_0$     is the population size at the beginning ($t_0$), prior to heat treatment for time $t$

$\mu$     is the specific growth rate constant

$D$     is the D-value which is the time required for a 1 log reduction[11] in the size of the bacterial population at a given temperature.

$T_{stop}$     is the internal temperature of cooking reaching the protected area

$t$     is a period of time that the protected area exposes to $T_{stop}$

$T_c$     is the temperature above which growth of *Campylobacter* does not occur and a number of organisms begin declining, assuming $T_c$ is $46^0C$ (Doyle and Roman,1981).

## The value of μ, *D*, T$_{stop}$ and *t*

The growth rate constant and the D-value can be determined as a function of temperature. The FAO/WHO report (WHO, 2001) quantifies the relationship between the value of μ and D for *Campylobacter* with least squares regression models against temperature. The regression models are;

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[10] The Arrhenius equation is a simple, but remarkably accurate, formula for the temperature dependence of a chemical reaction rate.

[11] Each log reduction is a reduction of 90%. So, a 1 log reduction is a 90% reduction, a 2 log reduction is 99%, and a 5 log reduction is 99.999%.

$$\mu = 1.4943\ln(T_{stop})-5.0885 \qquad \text{[7-12]}$$
$$D = 1426e^{0.169T} \qquad \text{[7-13]}$$

During cooking the protected areas were thought to be exposed to $60^0$ to $65^0$C for a period of time ($t$) (WHO, 2001). The final temperature ($T_{stop}$) at the internal part of a chicken (thermally protected area) can be estimated by the mean value of the temperature between $60^0$C and $65^0$C. This mean value of $T_{stop}$ is estimated from the probability distribution. However, due to a scarcity of data, a triangular distribution was selected for calculating the mean value of $T_{stop}$. The triangular distribution is a function of the minimum, most likely and maximum values. These values are 60,64 and 65, respectively. The distribution equation is then calculated by;

$$T_{stop} = \text{RiskPERT (60,64,65)} \text{ (using @Risk*, standard release 4.5 software)}$$

(*@ Risk is the risk analysis and simulation software produced by Palisade corporation.)

Similarly, a period of time (t) over which the protected area is exposed to the final temperature was taken from the literature (Bryan and Doyle, 1995). The values ranged from 0.5 to 1.5 minutes and the mean value is calculated by a triangular distribution. The equation is;

$$t = \text{RiskPERT (0.5,1.0,1.5) (using @Risk, standard v 4.5*)}$$

## 2) Modelling cross contamination: A contact transfer model

Due to the scarcity of data, the estimation of health risk from cross contamination must take into account a large degree of uncertainty and variability associated with food handling procedures in private kitchens (WHO, 2001; Christensen *et al.*, 2001 and Nauta *et al.*, 2005b). A contact transfer model assumes that some organisms are transferred from a raw chicken to preparation surfaces (cutting board, utensil, *etc.*) or hands. These organisms are then transferred from the preparation surfaces to prepared meals and may be directly ingested. The transfer of cross-contamination during food preparation is considered as a single event in which *Campylobacter* is transferred from the chicken to utensils or cutting board, hands or other preparation surfaces and then to prepared food (*e.g.* a cooked chicken, salad) (Figure 7.7).
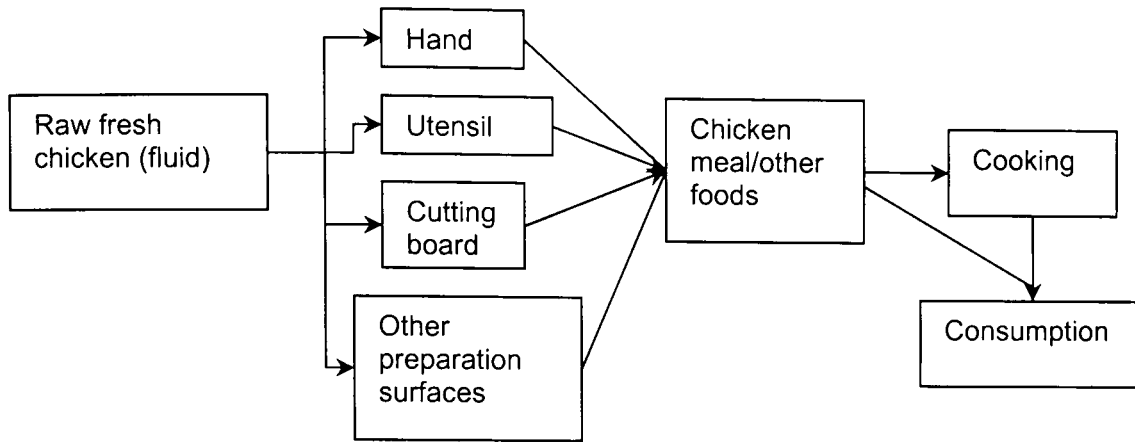
**Figure 7.7** The pathway of cross-contamination as a single event (adopted from WHO,2001)

Zhao *et al.*(1998); a joint FAO/WHO working group (WHO, 2001) and Christensen (2001) developed mathematical functions describing the transfer of *Campylobacter* from a raw chicken to contacted surfaces. The function consists of: i) the fraction of cells transferred from chicken to preparation surfaces and hands ($F_{chic\_prep}$); and ii) the fraction of cells from preparation surfaces and hands to meals ($F_{prep\_prep}$).

$$F_{transfer} = F_{chic\_prep} \cdot F_{prep\_prep} \qquad\qquad [7\text{-}14]$$

The transfer model is illustrated in Figure 7.8.
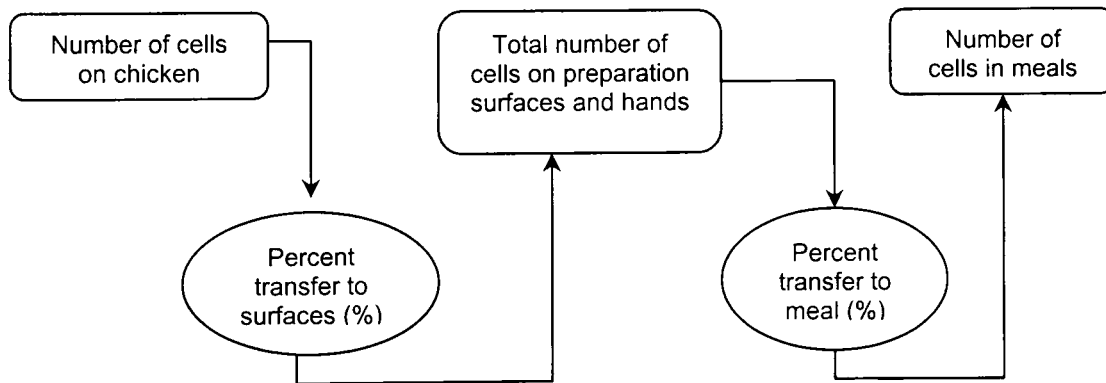


**Figure 7.8** Steps in the contact transfer model (adopted from WHO, 2001).

Using the assumptions stated above, Nauta *et al* (2005b) developed a new model for contact transfer, which expresses the probability that each colony forming unit (cfu) of *Campylobacter* in chicken meat is transferred to hands, cutting board and eventually salad. The transfer coefficients convey the probability of *Campylobacter* that can survive during meal preparation, and contaminated salad, which is subsequently ingested. Given this assumption, the final number of *Campylobacter* transferred from chicken *I* to the salad is;

$$N_{salad}(i) = [(t_{C,H} \times t_{H,H} \times t_{H,S}) + (t_{C,B} \times t_{B,B} \times t_{B,S})]t_{S,S}N_0(i)$$

[7-15]

**Where:**

$N_{salad}$ (i)   = numbers of *Campylobacter* in salad which are transferred from chicken (i)—

$t_{CH}$       = a coefficient for the transfer of *Campylobacter* from chicken to hands.

$t_{HH}$       = a coefficient for the transfer of *Campylobacter* from hands with or without washing

$t_{HS}$       = a coefficient for the transfer of *Campylobacter* from hands to salad.

$t_{CB}$       = a coefficient for the transfer of *Campylobacter* from chicken to cutting boards.

$t_{BB}$       = a coefficient for the transfer of *Campylobacter* from cutting boards with/ without washing

## 3) Modelling storage (Refrigeration)

It is likely that a chicken purchased from a retail outlet may not be cooked immediately, being kept in somewhere between 1-7$^0$C (Nauta *et al.*, 2005b). The storage time may vary, especially due to the variability of consumer behaviour. The variety of time-temperature profiles encountered is presumably large and unknown. In addition, the survival dynamics of *Campylobacter* during storage are not well established.

Nauta *et al.*(2005b) developed a modelling approach for the effect of storage on the number of *Campylobacter*. The model is based on the data of the Campylobacter Risk Management and Assessment (CARMA) project. By using Maximum Likelihood Estimation (MLE), the mean and variance of the numbers of *Campylobacter* in chicken products stored in plastic bags for a week at 4$^0$C were estimated by using the LogNormal distribution. According to this, there is a decrease of the numbers between 0.1 and 1.9 logs, with a mean value of 0.9. Finally, the risk model applied to a primary inactivation model is;

$$\log N_{storage} = \log N_0 - r_{storage}$$

[7-16]

**Where:**

$N_{storage}$   is the numbers of *Campylobacter* after storage

$r_{storage}$   is the variable of which is associated with the survival dynamics of *Campylobacter* during storage. This variable is incorporated with a BetaPert Distribution with the minimum, most likely and maximum values, that is; $r_{storage}$ ~BetaPert (0.1,0.9,2.1)

LogNormal distribution is defined with reference to the normal distribution. A random variable *X* is lognormal if its natural logarithm, y= *log(x)*, is normal.

**Note**: Nauta *et al.* (2005b) explained that the value of 0.1 and 0.9 are obtained from the MLE stated above. The maximum value is otherwise taken to be larger than that obtained from MLE regarding the small sample size.

## 7.2.5 The consumer model as applied to antimicrobial resistant *Campylobacter*

Cox and Popken (2004) summarised two different approaches for risk assessment of antimicrobial resistant bacteria.

1) The first method tracks the antimicrobial resistant microbial load from multi-sources, including chicken, through people. Finally, it estimates the number of patients infected and treatment failures, which will occur with and without a ban on antimicrobial use. This approach is close to the QMRA currently used for "farm-to-fork" modelling. It also requires extensive data to satisfy the model.

2) The Second method takes the opposite starting point. It starts from patients infected with antimicrobial resistant bacteria and determines the maximum proportion of cases that could have been caused by antimicrobial use in chicken. This approach therefore requires data on cases, trends and genotypes of cases. This approach was developed by the FDA-Center for Veterinary Medicine (Bartholomew *et al.*, 2005), which will be referred to the CVM model.

Currently, only the second of these, the CVM model developed by the U.S. Food and Drug Administration Centre for Veterinary Medicine, was available to be used for estimation of the exposure to antimicrobial resistant *Campylobacter* (Bartholomew *et al.*, 2005). However, the model does not possess all the features seen in a current quantitative microbial risk assessment (QMRA) for *Campylobacter*. The outcome of the CVM model, then, does not show the level of exposure. It expresses the proportion of illness related to antimicrobial resistant isolates. Therefore, in this study a new modified model was developed to quantify the exposure level. This modified model is based on the principles of exposure assessment of a QMRA (see section 7.2.3). Thus, there are two models used for antimicrobial resistant *Campylobacter* in this study. They are: i) the CVM-model and ii) a modified QMRA model (from this point "a modified model" refers to a modified QMRA model for antimicrobial resistant *Campylobacter*).

## The CVM model

The U.S. Food and Drug Administration-Centre for Veterinary Medicine (FDA-CVM) developed a relative risk model to estimate the health impact of luoroquinolonese-resistant *Campylobacter* (Bartholomew *et al.*, 2005). Due to some limitations in the model, Bartholomew *et al.*(2005) re-described and also extended the implication of the CVM risk model to a current QMRA model. The CVM model assumed that susceptible and resistant *Campylobacter* are equally likely to survive and to cause illness. Given this assumption, this model is based on a linear relationship between the weight (in pounds) of chicken consumed containing luoroquinolonese-resistant *Campylobacter* and the annual number of cases of

fluoroquinolonese-resistant campylobacteriosis in the U.S. The relationship is expressed in Figure 7.9.
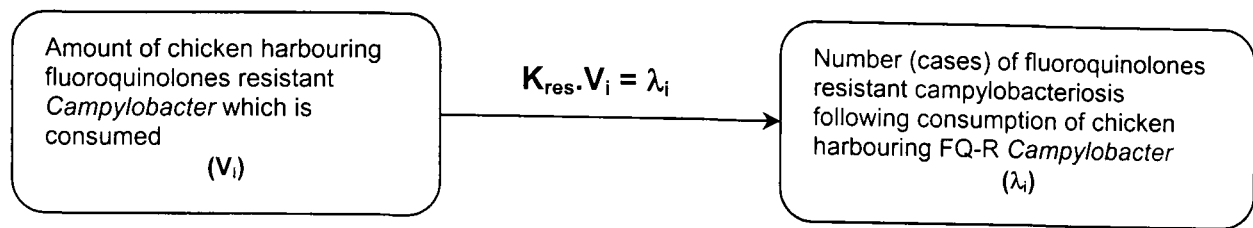


Figure 7.9 The CVM risk model (adopted from Bartholomew et al,2005).

Herein, the model equation can be written as

$$\lambda_i = K_{res} V_i \qquad [7\text{-}17]$$

**Where**:

$V_i$ is the exposure in year $i$ which is estimated by the product between the annual weight of chicken consumed (pounds per capita), the population size, the proportion of carcasses contaminated with *Campylobacter* and the proportion of antimicrobial resistant *Campylobacter* (ABR-*Campylobacter*).

$\lambda_i$ is the proportion of human campylobacteriosis with antimicrobial resistance associated with the consumption of chicken.

$K_{res}$ is the population-based dose-response parameter associated with $V_i$ and $\lambda_i$

That is; $\qquad\qquad K_{res} = \dfrac{\lambda_i}{V_i} \qquad\qquad [7\text{-}18]$

Given the linear relationship between $\lambda$ and $V$ (Bartholomew et al., 2005), the value of $K_{res}$ is therefore a constant.

As seen in the model equation, the model describes the risk related to a proportion of chicken harbouring antimicrobial-resistance which is consumed. It does not consider the numbers of all *Campylobacter* carried in the chickens consumed. This implies that the risk is associated only with the amount of **raw chicken** harbouring antimicrobial resistant *Campylobacter* which is consumed. The CVM model does not speculate on the exposure to antimicrobial-resistant *Campylobacter* (ABR-*Campylobacter*) following the consumption of **cooked chicken**.

## The modified QMRA model

An estimation of the exposure to ABR-*Campylobacter* is determined using two scenarios of the current QMRA models for all *Campylobacter* (see section 7.2.3). However, the number of ABR-*Campylobacter* ($N_{0,ABR}$) input to the models is obtained from the multiplication of the

initial numbers of *Campylobacter*, $N_0$, (see section 5.4.3, Chapter 5) and the fraction of antimicrobial resistant isolates (see section 6.4.2, Chapter 6). It is assumed that the distribution of antimicrobial resistant *Campylobacter* (ABR-*Campylobacter*) in chicken is random and fits a Poisson distribution. Each ABR-*Campylobacter* is independent and can survive the immune system and initiate the infection.

First, given the resistance rate of isolates populated in a chicken carcass, the number of ABR- *Campylobacter* in chickens is determined by;

$$N_{0,ABR} = N_0 \times P_{ABR(i)} \qquad \text{[7-19]}$$

**Where:**

$N_{0,ABR}$    is the number of ABR-*Campylobacter* (MPN/g)

$N_0$    is the initial number of *Campylobacter* (MPN/g)

$P_{ABR}(i)$    is the rate of resistance to three antimicrobials of *Campylobacter*, where *i* names to ciprofloxacin or erythromycin or nalidixic acid.

Next, the value $N_{0,ABR}$ is processed by the similar methods as described in section 7.2.4.

# PART B: Data input for the consumer model

Following the model development, estimations of the exposure to *Campylobacter* were considered for: 1) exposure to all *Campylobacter* and 2) ABR- *Campylobacter*.

1) The first section (7.3) is for all *Campylobacter*. The raw data of the MPN values of *Campylobacter* (section 5.4.3) and other model parameters (secondary data) were inputted to three models dealing with heat treatment, cross-contamination and storage.

2) The second section deals with antimicrobial resistant *Campylobacter*. The approach requires both the numbers of *Campylobacter* and the rate of resistance to three antimicrobial agents (section 6.4.2). The data were input to the modified QMRA model. As the CVM is not for estimating exposure dose, data input for the CVM model and results are presented and discussed in Chapter 8 dealing with health risk characterisation.

## 7.3 All *Campylobacter* isolates

## 7.3.1 The number of in the chicken meal or salad at the time consumption

In this section, the exposure to *Campylobacter* associated with the consumption of three groups of chickens was calculated following two scenarios. These are: 1) immediate preparation and 2) delayed preparation. Two modelling approaches (heat treatment and cross-contamination) were applied in each scenario.

The three scenarios are illustrated in Figure 7.10
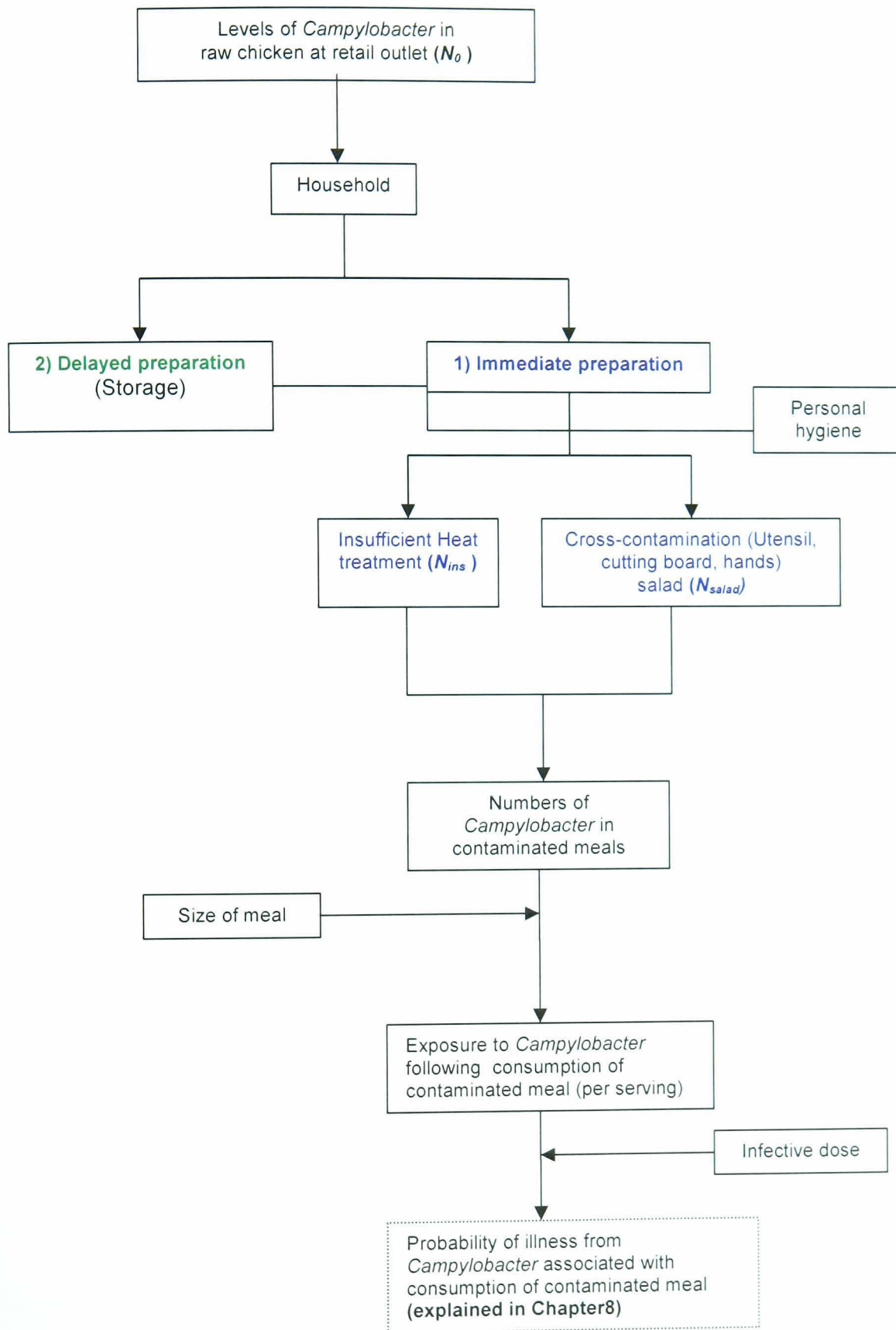
**Figure 7.10** Scenarios and data inputted of a consumer modelling approaches

(developed by Soonthornchaikul, 2005)

# Scenario 1: Immediate preparation (heat treatment)

## 1) Modelling approach: Insufficient heat treat meant

1. It is assumed that the fresh whole *Campylobacter*-positive chicken purchased from the retail point is immediately prepared and cooked via heat treatment (*e.g.*roast, grill). During preparation the liquid containing these organisms touches and stains a cutting board, utensils and hands of a preparer. The numbers of *Campylobacter* remaining in the carcass after transfering to the contacted surfaces and hands can be calculated from equation 7-20 (Christensen *et al.*, 2001).

$$N_{0/transfer} = N_0 - R_{CCB} - R_{CBS} \qquad [7\text{-}20]$$

**Where:**

$N_0$ = initial numbers of *Campylobacter* in chicken

$N_{0/Transfer}$ = final numbers of *Campylobacter* in chicken after being transferred to preparation surfaces

$R_{CCB}$ = Log-reduction of *Campylobacter* from raw chicken to cutting board

$R_{CBS}$ = Log-reduction of *Campylobacter* from cutting board to other foods

2. If the chicken is well cooked, these organisms on the outer layer are likely to be inactivated. Bryan and Doyle (1995) stated that during roasting, frying and grilling, surfaces of chicken usually reach temperature of $74^0$C. However, Daniels (1998) reported approximately 24% of individuals did not cook properly and did not ensure that the heat reaching protected areas is sufficient to inactivate *Campylobacter*. Some cells (ca. 10-20%) in these protected areas may thus survive.

The numbers of *Campylobacter* in the chicken insufficiently cooked ($N_{ins}$) can be determined following two conditions: i) Growth when $T<T_C$ and ii) Inactivation when $T>T_C$. The calculations are based on the equations described below

♦ Given $T<T_C$, $\qquad\qquad\qquad\qquad \ln N_{ins} = \ln N_{0/transfer} + ut$

♦ Given $T>T_C$, $\qquad\qquad\qquad\qquad \ln N_{ins} = \ln N_{0/transfer} - (\dfrac{t}{D})$

The model parameters are described in Table 7.7.

**Table 7.7** The model parameters

| Parameter | Description | Function | Unit |
|---|---|---|---|
| [1]$N_0$ | numbers of *Campylobacter* in chicken at the beginning | Taken from section 5.4.3 , Chapter 5 | |
| [2]$R_{CCB}$ | Log-reduction of *Campylobacter* from raw chicken to cutting board | RiskPert (1,2,6) | |
| $R_{CBS}$ | Log-reduction of *Campylobacter* from raw cutting board to other foods | RiskPert (1,2,6) | |
| [3]t-exposure | A time that a protected area is exposed to the maximum heat | Risk Pert (0.5,1.0,1.5) | minute |
| $T_c$ | A temperature at which the growth of *Campylobacter* does not occur (46$^0$C) | Taken from Roman and Doyle(1984) | $^0$C |
| $T_{stop,prot}$;< $T_c$ | The maximum internal temperature in protected area, but lower than $T_c$ | [4]Risk Pert (37,42,46) | $^0$C |
| $T_{stop,prot}$;> $T_c$ | The maximum internal temperature in protected area and higher than $T_c$ | [5]Risk Pert (60,64,65) | $^0$C |
| $\mu$ | A specific growth rate constant | [6]$\mu = 1.4943\ln(T_{stop})-5.0885$ | |
| D-value | A time for 1 log reduction of the size population | [7]$D = 14926e^{0.169T_{stop}}$ | minute |

**Note:**

1. All raw data of $N_0$ were input to the model equations for the calculation of exposure dose using @Risk software.
2. $R_{CCB}$ and $R_{CBS}$ following Christensen *et al.*(2001)
3. time of exposure adopted from a joint FAO/WHO report (WHO, 2001)
4. $T_{min}$ and $T_{most\ likely}$ of $T_{stop,prot}$;< $T_c$ were considered from the optimal temperature (37$^0$C or 42$^0$C) and $T_{max}$ was equal to $T_c$ ($T_c$ = 46$^0$C).
5. Parameters for $T_{stop,prot}$;> $T_c$ were taken from a joint FAO/WHO report (WHO, 2001).
6 and 7 Parameter for $\mu$ and D were taken from a joint FAO/WHO report (WHO, 2001).

## 2) Modelling approach: Cross-contamination

In relation to food handling in a private kitchen, it is more likely that the liquid from or direct contact with the *Campylobacter*-positive chicken may contaminate other foods, contacted surfaces or persons preparing a meal. Due to availability of secondary data, this study focuses on salad contaminated with *Campylobacter* through cross-contamination. The

numbers of *Campylobacter* in salad are dependent on the transfer fraction from chicken to a cutting board and then to salad. It is important to note that the probability of illness from the consumption of salad is associated with the number of **viable *Campylobacter* at the time of consumption.**

Given these assumptions, the final number of *Campylobacter* in salad can be obtained from the modelling approach described by Nauta *et al.*(2005b). The model equation is;

$$N_{salas}(i) = [(t_{C,H} \times t_{H,H} \times t_{H,S}) + (t_{C,B} \times t_{B,B} \times t_{B,S})]t_{S,S}N_0(i)$$

Based on the data collected from the literature, Nauta *et al.* (2005a) applied probability distributions to calculate the mean values of the transfer coefficients. These values are expressed in Table 7.8.

**Table 7.8** The transfer coefficients per cfu of *Campylobacter* associated with food handling procedures (adopted from Nauta *et al.*, 2005b).

| Source of transfer | Mean value of coefficient | Probability distribution | Reference |
|---|---|---|---|
| Chicken to hand ($t_{C,H}$) | 0.0415 | Beta (1.78,41.1) | Montville *et al.*, 2001 |
| Chicken to cutting board ($t_{C,B}$) | 0.0125 | 10^Normal(0.098,0.61)% | Kusumaningrum *et al*, 2004 |
| Hand to salad ($t_{H,S}$) | 0.207 | Beta (0.6,2.3) | Montville *et al.*, 2001 |
| Cutting board to salad ($t_{B,S}$) | 0.343 | 10^Normal(1.535,0.32)% | Kusumaningrum *et al*, 2004 |
| Hand washing ($t_{H,H}$) | 0.0347 | Beta (0.24,6.67) | Chen *et al.*, 2001 |
| Cutting board washing ($t_{B,B}$) | 0.0000464 | BetaPert (0,0.4,1)log reduction | Smith *et al.*,2003 |

## Scenario 2: Delayed preparation

Table 51.┌ If the chicken is not cooked immediately, it must be stored at a temperature between 1-7⁰C for a period of time. It is then taken out, raised to room temperature and cooked following the scenario 1. The modelling approach is performed by applying the model parameters from the CARMA project (Nauta *et al*, 2005b). The model is;

$$\log N_{storage} = \log N_0 - r_{storage}$$

The parameters are shown in Table 7.9.

**Table 7.9** The model parameters for the storage model

| Parameter | Description | Function/Expression | Unit |
|-----------|-------------|---------------------|------|
| $N_0$ | The numbers of *Campylobacter* in chicken at the beginning (before storage) | Taken from chapter 5 | $\log_{10}MPN/g$ |
| $N_{0/s}$ | The numbers of *Campylobacter* after storage | $\log N_0 - r_{storage}$ | $\log_{10}MPN/g$ |
| $*r_{storage}$ | the decrease of *Campylobacter* after storage | BetaPert (0.1,0.9,2.1) | $\log_{10}MPN/g$ |

* adopted from the study of Nauta *et al.*, 2005b

2. Following the storage modelling, the new value, $N_{0,S}$, is fed into the models presented in scenario 1, insufficient heat treatment (a chicken meal) and cross-contamination (salad) (see section 7.3.1: scenario 1).

## 7.3.2 The dose of *Campylobacter* ingested per serving by age and gender

The risk associated with the consumption of *Campylobacter*-positive chickens depends on the number of organisms ingested and this is related to the size of the meal. The exposure dose to *Campylobacter* ingested is the number of organisms per gram of food (*e.g.* chicken meal) multiplied by the amount of food consumed. It is possible that the size of the meal consumed may depend on the age and gender of the consumer.

Although national dietary surveys carried out in most countries provide food consumption, the data on foods consumed are usually in terms of food groups (*e.g.* red meat, poultry, daily product) rather than specific food items (*e.g.* chicken). Specific dietary surveys for the consumption of chicken and related meals in populations are uncommon. One example of such a survey is a Danish study (Christensen *et al.*,2001) which estimates the size of the chicken meal and salad consumed by person given age and gender. These are shown in Tables 7.10 and 7.11, respectively.

**Table 7.10** Distribution of the sizes of chicken meals consumed given age and gender (adapted from Christensen *et al.*, 2001).

| Age and sex group | Mean (gram) | Standard deviation | Distribution |
|---|---|---|---|
| Female < 18 years | 128.8 | 81.8 | LogNormal($\alpha,\beta$) |
| Female 18-29 years | 151.1 | 92.9 | LogNormal($\alpha,\beta$) |
| Female 30-65 years | 154.1 | 98.5 | LogNormal($\alpha,\beta$) |
| Female >65 years | 159.2 | 100.2 | LogNormal($\alpha,\beta$) |
| **Average (all age group)** | **148.3** | | |
| male < 18 years | 128.8 | 81.8 | LogNormal($\alpha,\beta$) |
| male 18-29 years | 256.4 | 197.4 | LogNormal($\alpha,\beta$) |
| male 30-65 years | 189.0 | 126.9 | LogNormal($\alpha,\beta$) |
| male >65 years | 178.4 | 129.0 | LogNormal($\alpha,\beta$) |
| **Average (all age group)** | **188.15** | | |

**Table 7.11** Distribution of the sizes of salad portions consumed given age and gender (adapted from Christensen *et al.*, 2001).

| Age and sex group | Mean (gram) | Standard deviation | Distribution |
|---|---|---|---|
| Female < 18 years | 54.6 | 36.7 | LogNormal($\alpha,\beta$) |
| Female 18-29 years | 66.5 | 30.5 | LogNormal($\alpha,\beta$) |
| Female 30-65 years | 67.6 | 47.5 | LogNormal($\alpha,\beta$) |
| Female >65 years | 67.1 | 46.3 | LogNormal($\alpha,\beta$) |
| **Average (all age group)** | **63.95** | | |
| male < 18 years | 54.6 | 36.7 | LogNormal($\alpha,\beta$) |
| male 18-29 years | 106.5 | 88.4 | LogNormal($\alpha,\beta$) |
| male 30-65 years | 94.1 | 65.6 | LogNormal($\alpha,\beta$) |
| male >65 years | 87.9 | 51.8 | LogNormal($\alpha,\beta$) |
| **Average (all age group)** | **85.78** | | |

As *Campylobacter* in food items behaves as Poisson distributed, the exposure dose must be calculated by the distribution function. Estimation of the exposure to *Campylobacter* per serving following the consumption of chicken or salad given gender can be calculated using a probabilistic function proposed by Christensen *et al.* (2001). These functions are;

♦ Number of *Campylobacter* in a chicken meal consumed given age and gender ($N_{C/AGCS}$) is:

$$N_{C/AGCS} = Poisson(S_{C/AGCS} \times 10^{N_0 - R_{CCB} - R_{CBC}})$$  [7-21]

◆ Number of *Campylobacter* in salad consumed given age and gender ($N_{S/AGCS}$) is:

$$N_{S/AGCS} = Poisson(S_{S/AGCS} \times 10^{N_0 - R_{CCB} - R_{CBS}})$$  [7-22]

**Where:**

$N_0$ = initial number of *Campylobacter* in chicken

$R_{CCB}$ = *Campylobacter* log reduction from chicken to cutting board

$R_{CBC}$ = *Campylobacter* log reduction from cutting board to chicken

$R_{CBS}$ = *Campylobacter* log reduction from cutting board to salad

$S_{C/AGCS}$ = size of chicken serving given age and gender

$S_{S/AGCS}$ = size of salad serving given age and gender

# 7.4 Antimicrobial-resistant *Campylobacter*

## The initial number of antimicrobial-resistant *Campylobacter* in chickens

It is assumed that the number of *Campylobacter* isolated from chicken includes both antimicrobial-susceptible *Campylobacter* (ABS-*Campylobacter*) and antimicrobial -resistant *Campylobacter* (ABR-*Campylobacter*). Given this assumption, the number of ABR-*Campylobacter* per carcass is the product of the number of all *Campylobacter* multiplied by the rate of antimicrobial resistance per carcass (see section 6.4.2, Chapter 6). Similarly, the number of ABR-*Campylobacter* per isolate is the product of the number of all *Campylobacter* multiplied by the rate of antibiotic resistance per isolate (see section 6.4.2, Chapter 6). The fractions of antimicrobial resistant *Campylobacter* are presented in Table 7.12.

**Table 7.12** Fraction of antimicrobial resistant *Campylobacter* isolated from three groups of chicken

| Type of chicken[1] | Fraction/carcass[2] | | | Fraction/isolate[3] | | |
|---|---|---|---|---|---|---|
| | Cp[4] | Eryth[5] | NA[6] | Cp | Eryth | NA |
| PIC | 0.087 | 1 | 1 | 0.029 | 1 | 1 |
| POC | 0 | 1 | 1 | 0 | 1 | 0.95 |
| BIC | 0.267 | 1 | 1 | 0.116 | 1 | 0.814 |

[1]Type of chicken: pre-packaged intensively reared (PIC), pre-packaged organically reared (POC) and unwrapped intensively reared chicken (BIC)

[2] percentage of chicken carcases harbouring one or more resistant isolates

[3] percentage of isolates found to be resistant to a specific antimicrobial agent.

[4] Cp = ciprofloxacin, [5] Eryth = erythromycin, [6] NA = Nalidixic acid

The initial numbers of ABR-*Campylobacter* were modelled for estimation of exposure to ABR-*Campylobacter* following the consumption of three groups of chicken harbouring antimicrobial resistant isolates. The estimations were performed using similar modelling approaches used for all *Campylobacter* (immediate preparation and delayed preparation) as shown in sections 7.3.1 and 7.3.2.

# 7.5 An overview of modelling approaches applied for exposure assessment

All selected probabilistic models used for estimation of the exposure to *Campylobacter* (with and without antimicrobial resistance) in this chapter are described in Tables 7.13 and 7.14.

**Table 7.13** The modelling approaches used for estimation of the exposure doses to **all** *Campylobacter* **isolates** following the consumption of three groups of chickens

| Modellling Approach | Model description and equations | Remark |
|---|---|---|
| **1) Heat treatment** (a chicken meal) | 1. $N_{0/transfer} = N_0 - R_{CCB} - R_{CBS}$ | see eq. [7-20] |
| | 2. $\ln N_{ins} = \ln N_{0/transfer} + ut$ ; when T$_{stop}$< T$_C$ | see eq. [7-10] |
| | or $\ln N_{ins} = \ln N_{0/transfer} - (\dfrac{t}{D})$ ;when T$_{stop}$>T$_C$ | see eq. [7-11] |
| **2)Cross-contamination** (salad) | $N_{salas}(i) = [(t_{C,H} \times t_{H,H} \times t_{H,S}) + (t_{C,B} \times t_{B,B} \times t_{B,S})] t_{S,S} N_0(i)$ | see eq. [7-15] |
| **3) Storage** - Numbers after refrigeration - Heat treatment - cross-contamination to salad | $\log N_{storage} = \log N_0 - r_{storage}$ <br><br> - see scenario 1 <br> - see scenario 1 | see eq. [7-16] |

**Table 7.14** The modelling approaches used for estimation of the exposure doses to antimicrobial resistant *Campylobacter* isolates following the consumption of three groups of chickens

| Modellling Approach | Modelling description and equations | Remark |
|---|---|---|
| **Number of ABR-** *Campylobacter* | $N_{0,ABR} = N_0 \times P_{ABR(i)}$ | |
| **1) Heat treatment** (a chicken meal) | 1. $N_{0,ABR/transfer} = N_{0,ABR} - R_{CCB} - R_{CBS}$ | see eq. [7-20] |
| | 2. $\ln N_{ins} = \ln N_{0,ABRtransfer} + ut$; when $T_{stop} < T_C$ | see eq.[7-10] |
| | or $\ln N_{ins} = \ln N_{0,ABRtransfer} - (\dfrac{t}{D})$; when $T_{stop} > T_C$ | see eq. [7-11] |
| **2)Cross-contamination** (salad) | $N_{salas}(i) = [(t_{C,H} \times t_{H,H} \times t_{H,S}) + (t_{C,B} \times t_{B,B} \times t_{B,S})] t_{S,S} N_{0,ABR}(i)$ | see eq. [7-15] |
| **3) Storage** - Numbers after refrigeration - Heat treatment - cross-contamination to salad | $\log N_{storage} = \log N_0 - r_{storage}$ <br> - see scenario 1 <br> - see scenario 1 | see eq. [7-16] |

**Note:** All raw data of $N_0$ were used for the calculation of the exposure doses. The calculations were performed using @Risk software, which the values of parameters are taken into account as probabilistic distribution (*e.g.* a lognormal distribution for the mean values of MPN, a Poisson distribution for the probability of getting ill given exposure to one organism).

# PART C: Results and Discussion

The difference in exposure to *Campylobacter* between two groups of chicken were analysed using a Mann-Whitney test. Analysis of Variance (ANOVA) was performed to test the difference of the doses between three groups of chickens.

## 7.6 Results

These results consider the exposure to *Campylobacter* ingested by person consuming chicken meals and salad. These meals are assumed to be associated with three types of chickens, (PIC, POC and BIC).

The exposure doses are calculated using the numbers of *Campylobacter* and amount of chicken or salad consumed. Two conditions of meal preparation taken into account were: 1) the chicken was cooked immediately and 2) the chicken was kept in the refrigerator for a short period of time before being cooked. The results described below were outlined following three modelling approaches (heat treatment, cross-contamination and pre-cooking storage).

### 7.6.1 The effect of heat treatment on the number of *Campylobacter* in chicken immediately cooked

Following the assumptions in each modelling approach, the survival of *Campylobacter* in any chicken meal is dependent on the internal temperature and exposure time (Table 7.7) in the protected area during heat treatment. These two parameters were measured as an indicator for the prediction of the change of the numbers of *Campylobacter* in a chicken meal after being cooked.

The numbers of *Campylobacter* in a cooked chicken meal given the internal temperature higher than $46^0C$ ($T>T_C$) are significantly lower than that of $T<T_C$ ($p<0.001$). 2-4 log reduction can be achieved when using the model given $T<T_C$ compared with 3-6 log reduction using the model given $T>T_C$. After heat treatment, the numbers of *Campylobacter* in meals associated with the BIC group still show the highest level significantly ($p<0.001$). These results are presented in Table 7.15 and Figure 7.11.

**Table 7.15** Comparison of the final numbers of *Campylobacter* after thermal processing between three groups of chicken: Immediate preparation

| Type of chicken[1] | Number of *Campylobacter* ($\log_{10}$MPN/g):mean$\pm$SE[2] | | | p-value |
|---|---|---|---|---|
| | $N_0$[3] | $N_{ins}$[4]; T<$T_c$ | $N_{ins}$[5]; T>$T_c$ | |
| PIC | 3.73$\pm$0.59 | -1.08$\pm$0. 06 | -2.51$\pm$-0.20 | *<0.001* |
| POC | 4.44$\pm$0.64 | -0.44$\pm$0.19 | -1.88$\pm$0.07 | *<0.001* |
| BIC | 8.10$\pm$0.81 | 3.31$\pm$0.65 | 1.88$\pm$0.70 | *<0.001* |
| *p-value* | *<0.001* | *<0.001* | *<0.001* | |

[1] Type of chicken: Pre-packaged intensively reared (PIC), Pre-packaged organically reared (POC) and Butcher intensively reared chicken (BIC)

[2] mean and SE are expressed as geometric values

[3] Number of *Campylobacter* at $t_0$ (the initial number after some cells were transferred)

[4] Number of *Campylobacter* after heat treatment; when the internal temperature is lower than $46^0$C.

[5] Number of *Campylobacter* after heat treatment; when the internal temperature is higher than $46^0$C.
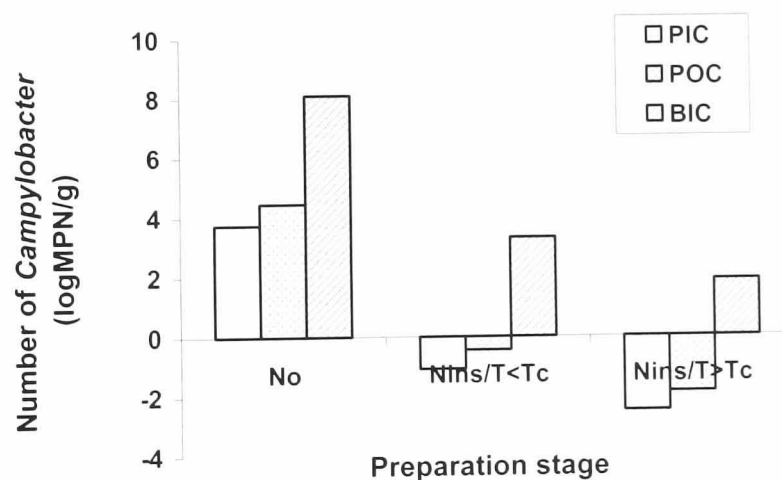


**Figure 7.11** The number of *Campylobacter* in the immediately cooked chicken associated with PIC, POC and BIC given T<$T_C$ or T>$T_C$

## 7.7.2 The number of *Campylobacter* transferred to salad

The numbers of *Campylobacter* transferred to a cutting board, utensils and hands are dependent on the initial numbers in the carcasses. The lowest level is found in salad contaminated with *Campylobacter* from the PIC group (-0.24$\pm$0.28 $\log_{10}$MPN/g), whereas the highest level (4.14$\pm$0.64 $\log_{10}$MPN/g) is found in the BIC group, for which the initial level of *Campylobacter* is also very high. Table 7.16 and Figure 7.12 show the details of the findings. There is a significant difference for the numbers of *Campylobacter* transferred to salad from those found in PIC, POC and BIC (*p<0.001*).

**Table 7.16** The numbers of *Campylobacter* transferred from three types of chickens to salad

| Type of chicken | Number of *Campylobacter* ($\log_{10}$MPN/g): mean$\pm$SE | |
| --- | --- | --- |
| | $N_0{}^1$ | $N_{salad}{}^2$ |
| PIC | 3.73$\pm$0.59 | -0.24$\pm$0.28 |
| POC | 4.44$\pm$0.44 | 0.39$\pm$0.36 |
| BIC | 8.10$\pm$0.81 | 4.14$\pm$0.64 |
| *p-value* | *<0.001* | *<0.001* |
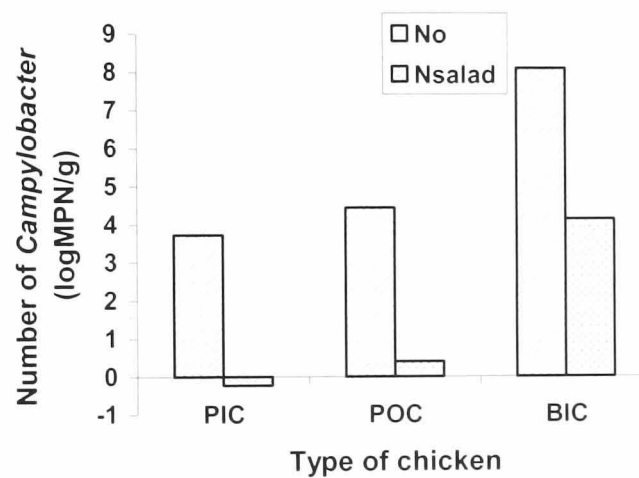


**Figure 7.12** The number of *Campylobacter* transferred from PIC, POC and BIC to salad

## 7.7.3 The number of *Campylobacter* in chicken and salad after storage and cooking

Table 7.17 and Figure 7.13 demonstrate the effect of storage on the numbers of *Campylobacter*. It is evident that after storage the numbers of *Campylobacter* in chicken meals related to these three types of chicken decrease significantly ($p<0.001$). In addition, the numbers in chicken using the model given $T>T_C$ are significantly lower than those given $T<T_C$ ($p<0.001$). Furthermore, the chicken meal associated with the BIC group contained the significantly highest numbers of *Campylobacter* ($p<0.001$).

**Table 7.17** The numbers of *Campylobacter* in a chicken meal and salad associated with three types of chickens: after storage

| Type of chicken | Number of *Campylobacter* ($\log_{10}$MPN/g) (mean$\pm$SE) | | | | |
| | | | | $N_{ins/s}$[3,4] | |
| | $N_{0/transfer}$[1] | $N_s$[2] | $N_{salad}$ | $T<T_c$ | $T>T_c$ |
|---|---|---|---|---|---|
| PIC[4] | 3.73$\pm$0.59 | 2.75$\pm$0.53 | -1.21$\pm$0.04 | -2.03$\pm$-0.11 | -3.46$\pm$-0.37 |
| POC[5] | 4.44$\pm$0.44 | 3.48$\pm$0.58 | -0.48$\pm$0.20 | -1.31$\pm$0.07 | -2.75$\pm$0.33 |
| BIC[6] | 8.10$\pm$0.81 | 7.13$\pm$0.78 | 3.17$\pm$0.58 | 2.34$\pm$ 0.53 | 0.91$\pm$0.43 |
| *p-value* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* |

[1] Number of *Campylobacter* in chicken before storage (at $t_0$)

[2] Number of *Campylobacter* in chicken after storage

[3] Number of *Campylobacter* in chicken after storage and cooking given $T<T_c$ and $T>T_c$

[4] significant at $p=0.00005$ for the numbers of *Campylobacter* after cooking between $T<T_c$ and $T>T_c$ within a group of PIC or POC or BIC
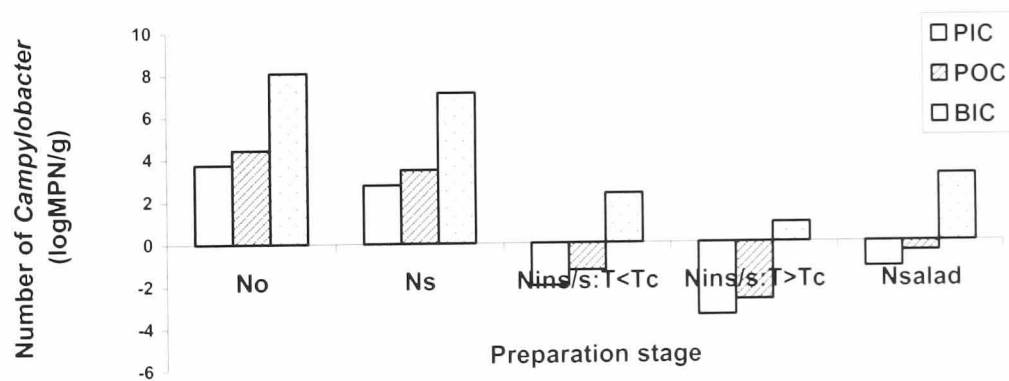


**Figure 7.13** The numbers of *Campylobacter* in chicken meal associated with PIC, POCand BIC after storage and heat treatment, given $T<T_c$ and $T>T_c$ and the numbers in contaminated salad

## 7.6.4 The exposure to (all) *Campylobacter*

Initially, the exposure to *Campylobacter* related to the consumption of three types of chickens was calculated following the modelling approaches (Tables 7.13 and 7.14). These doses were also considered in respect of the size of the meal at different age and gender groups. The results showed no significant difference of exposure doses to *Campylobacter* between different serving sizes given age groups. The whole results are presented in Appendix 4.

Therefore, these following results of the exposure to *Campylobacter* are calculated using the average serving size of a chicken meal or salad for all age groups within the same sex (Tables 7.10 and 7.11). The average serving size of chicken meals consumed by female groups (age from <18 to > 65 years) was 148.3 g. and by male groups (same range of age groups) was 188.5 g. Similarly, the average of the size of salad consumed by females was 63.9 g. and by males was 85.8 g.

## Immediate preparation: a chicken meal and salad

Using the average serving size of a chicken meal or salad consumed by female and male groups (stated above), the doses of *Campylobacter* ingested following the consumption of a chicken meal given gender and internal temperature reaching the protected area were found to be very high in relation to the BIC group (Table 7.18), particularly, when $T<T_C$ (about 5 logMPN/serving for both sexes). Although when $T>T_C$, the dose is still higher than those associated with the PIC and the POC group. After heat treatment, the level of exposure associated with the PIC and the POC group was very low (less than 1 logMPN/serving). The exposure dose associated with the consumption of salad exhibited higher values than those associated with the chicken meal. The difference is about 1-2 logMPN/serving. This is become clearer when using the model given $T>T_C$.

**Table 7.18** The numbers of *Campylobacter* ingested per serving of a chicken meal or salad immediately prepared, given sex and internal temperature at the protected area

| Type of chicken | Dose of *Campylobacter* ingested (MPN/serving): Immediate preparation | | | | | |
| | Salad | | Chicken meal: $T<T_c$ | | Chicken meal: $T>T_c$ | |
| | Female | Male | Female | Male | Female | Male |
| PIC | 38 | 51 | 12 | 16 | 0 | 1 |
| POC | 157 | 211 | 54 | 68 | 2 | 2 |
| BIC | $8.82 \times 10^5$ | $1.18 \times 10^6$ | $3.03 \times 10^5$ | $3.85 \times 10^5$ | $1.12 \times 10^4$ | $1.41 \times 10^4$ |

## Delayed preparation

As shown in Table 7.19, the results are in parallel with those found for immediate preparation. The consumption of a chicken meal or salad contaminated with *Campylobacter*-positive BIC is responsible for the highest level of exposure (3-4 logMPN/serving for a chicken meal and 4-5 logMPN/serving for salad). This also shows that the levels of exposure following the consumption of salad are higher than those from a chicken meal. It is noted that after cooking the levels of exposure to *Campylobacter* associated with the consumption of the PIC-meals PIC and the POC–meals were considerably lower (< 1 logMPN/serving).

**Table 7.19** The numbers of *Campylobacter* ingested per serving of chicken meal and salad associated with three types of chicken after storage, given sex and internal temperature at the protected area

| Type of chicken | Dose of *Campylobacter* ingested (MPN/serving): Delayed preparation | | | | | |
|---|---|---|---|---|---|---|
| | Salad | | Chicken meal: $T<T_c$ | | Chicken meal: $T>T_c$ | |
| | Female | Male | Female | Male | Female | Male |
| PIC | 4 | 5 | 1 | 2 | 0 | 0 |
| POC | 21 | 28 | 7 | 9 | 0 | 0 |
| BIC | $9.52 \times 10^4$ | $1.28 \times 10^5$ | $3.27 \times 10^4$ | $4.15 \times 10^4$ | $1.20 \times 10^3$ | $1.52 \times 10^3$ |

## 7.6.5 Exposure to ABR- *Campylobacter*

As shown in Table 7.20, the numbers of ciprofloxacin-resistant *Campylobacter* (CR-*Campylobacter*) ingested per serving of a chicken meal or salad associated with the BIC group exhibited the highest value, particularly from salad. It is evident that both types of meal associated with the POC group were completely free from CR-*Campylobacter*. In the PIC group, the numbers were down to zero when the internal temperature was higher than $46^0$C. The exposure to CR-*Campylobacter* per carcass or per isolate was found to be very similar in all groups.

**Table 7.20** The numbers of CR- *Campylobacter* ingested per serving of a chicken meal and salad immediately prepared from three types of chicken, given sex and internal temperature at the protected area: a) rate of resistance per carcass and b) rate of resistance per isolate

| Type of chicken | Dose of CR-*Campylobacter* ingested (MPN/serving): Immediate preparation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a: given rate of resistance per carcass | | | | | | b: given rate of resistance per isolate | | | | | |
| | Salad | | Chicken: $T<T_c$ | | Chicken:$T>T_c$ | | Salad | | Chicken:$T<T_c$ | | Chicken:$T>T_c$ | |
| | F* | M* | F | M | F | M | F | M | F | M | F | M |
| PIC | 3 | 4 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| POC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BIC | $10^5$ | $10^5$ | $10^5$ | $10^5$ | $10^3$ | $10^3$ | $10^5$ | $10^5$ | $10^4$ | $10^4$ | $10^3$ | $10^3$ |

* Sex group: F = female and M = male

The numbers of erythromycin-resistant *Campylobacter* (ER- *Campylobacter*) or nalidixic acid-resistant *Campylobacter* (NAR- *Campylobacter*) associated with the consumption of chicken meals or salads were not greatly different (Tables 7.21 and 7.22). However, the findings associated with the BIC group (4-6 logMPN/serving) were still considerably higher than those found in the PIC (1 logMPN/serving) and POC groups (1-2 logMPN/serving). The exposure dose whether taken into account as per carcass or per isolate was the same.

**Table 7.21** The numbers of ER- *Campylobacter* ingested per serving of chicken meal and salad immediately prepared from three types of chicken given sex and internal temperature at the protected area: a) rate of resistance per carcass and b) rate of resistance per isolate

| Type of chicken | Dose of ER-*Campylobacter* ingested (MPN/serving): Immediate preparation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | per carcass | | | | | | per isolate | | | | | |
| | Salad | | Chicken:T<$T_c$ | | Chicken:T>$T_c$ | | Salad | | Chicken:T<$T_c$ | | Chicken:T>$T_c$ | |
| | F | M | F | M | F | M | F | M | F | M | F | M |
| PIC | 36 | 49 | 13 | 16 | 0 | 1 | 36 | 49 | 13 | 16 | 0 | 1 |
| POC | $10^2$ | $10^2$ | 54 | 68 | 2 | 3 | $10^2$ | $10^2$ | 54 | 68 | 2 | 3 |
| BIC | $10^6$ | $10^6$ | $10^5$ | $10^5$ | $10^4$ | $10^4$ | $10^6$ | $10^6$ | $10^5$ | $10^5$ | $10^4$ | $10^4$ |

**Table 7.22** The numbers of NAR- *Campylobacter* ingested per serving of chicken meal and salad immediately prepared from three types of chicken given sex and internal temperature at the protected area: a) rate of resistance per carcass and b) rate of resistance per isolate

| Type of chicken | Dose of NAR-*Campylobacter* ingested (MPN/serving): Immediate preparation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | per carcass | | | | | | per isolate | | | | | |
| | Salad | | Chicken:T<$T_c$ | | Chicken:T>$T_c$ | | Salad | | Chicken:T<$T_c$ | | Chicken:T>$T_c$ | |
| | F | M | F | M | F | M | F | M | F | M | F | M |
| PIC | 36 | 49 | 13 | 16 | 0 | 1 | 36 | 49 | 13 | 16 | 0 | 1 |
| POC | $10^2$ | $10^2$ | 54 | 68 | 2 | 3 | $10^2$ | $10^2$ | 51 | 65 | 2 | 2 |
| BIC | $10^6$ | $10^6$ | $10^5$ | $10^5$ | $10^4$ | $10^4$ | $10^6$ | $10^6$ | $10^5$ | $10^5$ | $10^4$ | $10^4$ |

# 7.7 Discussion

It is clear that cooking parameters, such as temperature, are dependent on the behaviour of individual consumers. There were few studies that carried out personal hygiene practice during food handling in the household kitchen. (Montville, *et al*, 2001; Chen *et al*, 2001 and Christensen *et al.*, 2005). The estimation of the dose at the time of consumption always involves both variability and uncertainty. Hence, probabilistic approaches have been used to estimate the dose from the best-case to the worst-case scenarios. On one hand, the best-case is considered to be where an individual consumes a small chicken portion containing few organisms that are inactivated during cooking. On the other hand, the worst-case is considered to be where an individual consumes a large portion of highly contaminated and improperly cooked chicken.

## 7.7.1 The exposure dose after the heat treatment

Although the internal temperature of cooking can reduce the number of *Campylobacter to* undetectable levels, the numbers in the BIC group after passing the heat treatment even given $T>T_C$ were found still considerable. Based on the assumption of insufficient heat treatment (described in section 7.3.1) and both data collected in this study and secondary data, after the heat treatment, the exposure to *Campylobacter* related to the BIC-chicken meal remains very high. This could suggest that: i) there would be significant numbers of *Campylobacter* remaining in the thermally protected areas of the carcass, ii) the models used are not sensitive to the model parameters and iii) the values of the parameters (internal tempersture and time) used may not be reasonably representing values. The outcomes of a sensitivity anaylysis indicate that the models used are sensitive to the changes in the values of parameters and thus increasing of either the internal temperature (from $63^0$C to $72^0$C) at the protected area or the time (from 1 to 5 minutes) can reduce the dose of *Campylobacter* from $10^8$ MPN/g to < 1 MPN/g. The results and further discussion of a sensitivity analysis are presented in section 8.4.3. For this reason, highly concentrated chickens should be cooked at very high temperatures raising internal temperature at the protected areas. Alternatively, cooking time may be increased in parallel. This however, would lead to the question arising of whether there can be in practice temperature that is completely sufficient in reducing the number of high number of *Campylobacter* effecting the meal, *i.e.* taste, appearance, nutritive values. As the numbers of *Campylobacter* in the PICs and the POCs are not very high ($\leq 5$ log MPN $g^{-1}$meat), given $T>T_C$ the organisms were almost completely eliminated. Koidis and Doyle (1983) recognised that cooking meat at 58-60$^0$C for 2 min or more will inactivate $10^6$*C.jejuni* $g^{-1}$meat. Therefore, this suggests that the dose of *Campylobacter* from the consumption of a chicken meal involves two factors, the initial numbers of *Campylobacter* and cooking procedure parameters, mainly temperature and time.

## 7.7.2 The exposure dose related to the transfer from the positive carcass to salad

The dose of *Campylobacter* related to the consumption of salad contaminated through contact with uncooked chicken was found to be higher than that from the consumption of an adequately cooked chicken meal. A sufficient cooking temperature for a whole chicken is $82^0C$ or is that in practical the centre of meat should not be pink (USDA, 1999). This may be a consequence of the direct transfer of *Campylobacter* from the uncooked chicken to salad which is usually consumed without heat treatment. This finding is further emphasised by the outcomes found in the BICs. Since the BICs are contaminated with very high concentrations of *Campylobacter*, the numbers transferred to salad are considerably higher than those from PICs and POCs. Several factors may influence the number of *Campylobacter* transferred from raw chicken to a cutting board and further to a prepared meal, *i.e.* the amount of drip fluid, the contact area, the time lag between placing the raw chicken and the prepared chicken on the cutting board (Rosenquist *et al.*,2003). Nauta *et al.*(2005b) considered the transfer coefficients for the cross-contamination from a raw chicken to salad which is related to the initial numbers of *Campylobacter* in the carcass (section 7.2.4). Therefore, the initial number of the organisms is the significant factor influencing the level of the exposure as well as personal hygiene practices in the private kitchen. This supports the notion that proper handling of food can decrease the transfer coefficient of the cross-contamination, resulting in a reduction in the dose of *Campylobacter* transferred to salad. Anderson *et al.*(2001) found similar results when estimating the health risk following the consumption of hamburgers.

## 7.7.3 The exposure dose to *Campylobacter* after pre-cooking storage

The exposure doses to *Campylobacter* per serving of meal following the consumption of a chicken meal or salad associated with chicken stored in a refrigerator before being cooked were lower than those found in the immediate preparation. This is highlighted by the finding from the PIC and POC groups, in particular the dose of *Campylobacter* after heat treatment given $T>T_C$ (the dose equals 0). Although typical food storage conditions are not associated with the growth of *Campylobacter*, the length of time at which cells can survive at ambient and refrigeration temperatures are significant when considering the health risk, as the infective dose is as low as 500 cells (Robinson, 1981; Black *et al.*, 1988 and Solow *et al.*, 2003).

## 7.7.4 The exposure dose to ABR-*Campylobacter*

The dose of ABR-*Campylobacter* is dependent both on the initial numbers of *Campylobacter* and on the rate of resistance to tested antimicrobials. Similarly, the heat treatment can reduce the dose of ABR-*Campylobacter* if the initial number is not too high. As the initial

number of *Campylobacter* in the PICs was lower than that found in the POCs, the dose of CR- *Campylobacter* isolates associated with the PICs was gradually declined to zero after heat treatment. This makes the final outcome similar to that found in the POCs, which were free from CR- *Campylobacter*. The difference of the dose of ER-*Campylobacter* or NAR-*Campylobacter*, in a chicken meal after being cooked, between the PICs, the POCs and the BICs emphasises that the initial number of *Campylobacter* in the carcass is the most important factor influencing the level of the exposure to ABR-*Campylobacter*. The dose of antimicrobial resistant *Campylobacter* could therefore be reduced by lowering the total numbers of *Campylobacter*. This can be achieved by lowering the *Campylobacter* load at various production points and at the kitchen through the good hygiene practices. This is supported with the outcomes of a sensitivity analysis.

Since the serving sizes of meal at different age or gender groups were only slightly different, individuals in all age and gender groups have a similar chance of being infected and getting ill following the consumption of chicken harbouring *Campylobacter*. It can therefore be assumed that this estimate is for the general population, except for young children or other vulnerable group.

The severity of *Campylobacter* infection involves health status, immune system, infective dose and individual susceptibility to pathogens. *Campylobacter* infection in vulnerable groups such as young children can develop to severe conditions (*e.g.* high fever, septicaemia). These conditions then require intensive treatments including antimicrobial administration. Therefore, development of antimicrobial resistance in *Campylobacter* species can cause the failure for antimicrobial treatment in these groups.

## 7.7.5 Sensitivity analysis

It is important to note that the estimation of exposure to *Campylobacter* was determined based on a number of prior assumptions and included the model parameters. The mean values of the internal temperature and exposure time were adopted from the literature. Given these, the numbers of *Campylobacter* associated with the BIC as calculated by the model as worringly high. It is important to consider whether the risk is truly high or whether the model is providing an inaccurate estimate. It is interesting to investigate the sensitivity of model parameters used. A sensitivity analysis was therefore performed in order to identify the sensitivity of the model to the two main model parameters (internal temperature and exposure time). These two parameters are thought to have the greatest impact on the numbers of *Campylobacter*. The results of a sensitivity analysis which are presented and discussed in details in sections 8.4.3 and 8.5.3 demonstrate that.

# 7.8 Limitations of a consumer exposure model

The estimation of the exposure to *Campylobacter* relies on the information and data (*e.g.* scientific data) and the assumptions taken throughout the entire risk model. The models of exposure dose used in this chapter consider two significant factors influencing on the numbers of *Campylobacter* in a chicken meal or salad. These two factors are the cooking practice (heat treatment) and kitchen hygiene (cross-contamination). These models require considerable data. However, at present, the data available are not complete. Thus, there are gaps between the data used, which can be further considered as follows:

## 7.8.1 The information on cooking practice

This study combines two modelling approaches recommended by the joint FAO/WHO working group (WHO, 2001) for the estimation of exposure to *Campylobacter* after heat treatment. These models, based on two approaches, are: 1) the internal temperature approach and 2) the protected areas approach. The modelling approaches are based on observations of the time-temperature profiles in the centre of the drumstick portions of a whole roasted chicken as well as the area in the carcass where the least heat treatment is achieved (WHO, 2001). Thereby, the expected dose of viable *Campylobacter* after cooking is affected by temperature and time. It is becoming clear that there is currently little information on time-temperature profiles for growth and inactivation of *Campylobacter* during the cooking process. The final temperature at the inner part of the carcass (which is assumed to be an insulated area for *Campylobacter* during roasting) is also not reported completely. The ranges of time or temperature used in the models had to be taken from different studies due to incomplete data. These studies were unfortunately performed with different designs and criteria, in particular, the observational definition of undercooking and the actual heat treatment applied. Thus, uncertainties can occur alongside the pathways of the assumption (On *et al*, 1996; Teunis *et al.*,1997 and Kang *et al.*, 2000).

In addition, it is assumed that the organisms are evenly distributed not only on the surface but also in other locations on the carcass including the inner part of the meat. In fact, there has been no evidence to indicate the distribution of *Campylobacter* inside the meat in a chicken carcass. Although some studies implied that a significant habitat of *Campylobacter* may be on the skin of a carcass (Burgess *et al.*, 2005), it has not been demonstrated by direct measurements.

## 7.8.2 Cross-contamination regarding the kitchen hygiene

This model assumed that there is transfer of *Campylobacter* from a contaminated raw chicken to preparation surfaces and subsequently from these surfaces to uncooked foods such as salad. There are some limitations which need to be considered, when referring to this:

1) There are little survey data and direct observational data on consumer practices in the domestic kitchen (preparation and handling of chicken, for example). The few available studies come from the UK and some countries in North America. However, these studies did not establish the relationship between consumer practices in the kitchen and the transfer coefficient of the organisms, *e.g.* the numbers of organisms remain on the cutting board (whether washed or unwashed). The data fed in to the model in this study were taken from different studies and sources. Different pathways of contamination may contribute the different quantities of *Campylobacter* to the final meal. There might be a number of plausible dose-response models whose fits are consistent with the data. In other word, the dose-response model depends on the assumptions and data available. Frequently, the assumptions of a model are developed from emergent problems, subject to interests and data available in parallel with the principle and theory (Cox, 2005).

2) Except for the report from a joint FAO/WHO (WHO, 2001), there is no data on the concentration of *Campylobacter* in the fluid attached to the chicken and how many organisms there are in the fluid dripping onto the preparation surfaces.

3) Although the dietary survey in the UK is carried out annually, data on the consumption and consumption patterns of chicken or related food items are scarce. There is currently no specific data which estimate different types (*e.g.* intensively reared or organically reared chicken) and amounts of a certain chicken consumed. Hence, most data used in this chapter were adopted from several studies and different sources, including the consumption data of a chicken meal and salad.

## 7.9 Summary

Estimations of the *Campylobacter* doses that consumers may expose t, following the consumption of chicken meals and salads, were calculated through risk models developed from the literature. The risk models include insufficient heat treatment and cross-contamination. The modelling approaches were performed for two conditions: i) immediate preparation and ii) delayed preparation. The results in this chapter indicate that the most significant factors affecting the risk from exposure to *Campylobacter* following the consumption of a *Campylobacter*-positive chicken are the numbers of organisms in the raw chicken, personal hygiene practices and cooking procedures (mainly temperature and time).

1. Given the assumptions and data used, the highest dose of *Campylobacter* was calculated to be associated with the consumption of the BICs ($10^2$ -$10^3$ MPN/g), followed by the POCs and the PICs (less than 10 MPN/g). The exposure to *Campylobacter* related to the BIC-chicken meals remains very high, indicating that: i) there may be significant numbers of *Campylobacter* remaining in the thermally protected areas of the carcass, ii) the models used are not sensitive to the model parameters and/or iii) the values of the parameters (internal tempersture and time) used may not be resonably sufficient. The outcomes of a sensitivity anaylysis

indicate that the models used are sensitive to the changes in the values of the parameters and thus increasing of either the internal temperature (from $63^0$C to $72^0$C) at the protected area or the time (from 1 to 5 minutes) can reduce the dose to < 1MPN/g. The results and further discussion of the sensitivity analysis were presented in section 8.4.3. This indicates that if elevated internal temperatures ($63^0$C-$72^0$C) are achieved for a sufficient length of time (1-5 minutes), the risk can be reduced to a very low level.

2. The above findings indicate that the models are appropriate for use. However, the values of model parameters taken from the literature may not be sufficient and lead to an inaccurate estimate. As currently the information related to cooking practices in kitchens is insufficient, further studies are needed.

3. The exposure dose to *Campylobacter* related to salad was found to be higher (1.7 logMPN/serving, 2 logMPN/serving and 6 logMPN/serving for the PICs, POCs and BICs, respectively) than that related to an adequately cooked chicken meal (<1 logMPN/serving, <1 logMPN/serving and 4 logMPN/serving for the PICs, POCs and BICs, respectively). It is important to note that this contamination is related to the uncooked chicken which directly transmits the organisms to the salad during food preparation in the kitchen. Therefore, the reduction of the exposure dose to *Campylobacter* can be achieved by good personal hygiene practices in the kitchen. (Table 7.18).

4. Storage in a refrigerator can reduce the number of *Campylobacter* to < 10 MPN/g for the PICs, to < 20 MPN/g for the POCs and to $10^3$ -$10^5$ MPN/g for the BICs.

5. The exposure dose to antimicrobial resistant *Campylobacter* depends not only on the rate of resistance but also on the initial number of *Campylobacter*. This is clearly supported by the results. The PICs harbour higher numbers of CR-*Campylobacter*, although lower total numbers than that found in the POCs. The BIC group harbour both high total numbers and resistance rate to ciprofloxacin (section7.6.5).

6. As this study aims to compare the relative health risk from *Campylobacter* with and without antimicrobial resistance following the consumption of three types of chicken (PICs, POCs and BICs), it assumes that any uncertainty in the exposure dose of the host arising from the limitations of the model may affect all these three types equally. In addition, as long as the underlying mathematical assumptions are true, good estimates on relative changes can be calculated and the model can be used to compare the relative exposure to *Campylobacter* following the consumption of different chickens. The results of the sensitivity analysis support this assertion.

# CHAPTER 8

## Health Risk from *Campylobacter*

## 8.1 Introduction

Infection with *Campylobacter* following the consumption of contaminated foods, chickens in this context, involves the interaction between host, pathogen and food matrix (Figure 8.1).
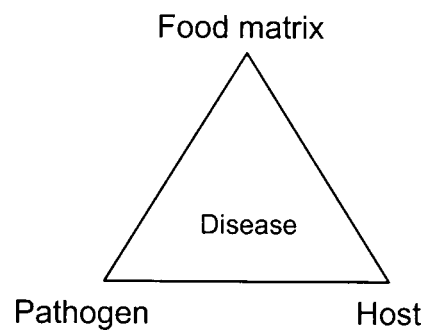


**Figure 8.1** Epidemiology triangle for foodborne disease (adopted from Coleman and Marks, 1998)

The infection is not necessarily accompanied by symptoms. Usually symptoms of campylobacteriosis in humans are self-limiting and do not require intensive treatments (Coker *et al.*, 2002). During the asymptomatic period, infection status must be confirmed by microbiological examination of faecal specimens and in some cases, if necessary, the determination of an immune response must be included.

Health risk in this context is about the probability of infection following ingestion of *Campylobacter* and the probability of illness. Estimation of health risk is performed after the exposure assessment (Chapter 7), with exposure levels being input to the models used for estimating health risk.

## 8.2 Background

This chapter aims to compare the human health risk resulting from *Campylobacter* following the consumption of the three different types of chicken. It focuses on risk at the consumer level. It links the probability and magnitude of exposure to *Campylobacter* following the consumption of a chicken meal or other related food to possible adverse consequences. The resulting risk is expressed as the risk per serving of meal consumed. Since there are no data available for the consumption of chickens by specific groups (*e.g.* young children), the risk in this study cannot address a specific population. Data on amounts of chicken consumed can be incorporated into the model to arrive at estimates for the general population.

Currently, the models used in other studies are unable to provide a clear estimate of risk due to uncertainties in two key components of the model, namely the impact of undercooking and the impact of cross-contamination (WHO, 2001; Christensen *et al.*, 2001; Bartholomew

*et al.*, 2005 and Nauta *et al.*, 2005b). Since these processes are the ultimate determinant of final exposure of the consumer, the unbounded and irresolvable nature of the uncertainty undermines the establishment of a reliable estimate of consumer risk. In addition, the models related to the issue of antimicrobial resistance in *Campylobacter* have not yet been applied. However, information from those studies still provides useful information for studying potential exposure pathways, and these ways would contribute to the health risk posed by *Campylobacter* associated with the consumption of broiler chickens.

In this chapter estimation of the health risk is based on the dose-response relationship model. The outcomes describe the probability of infection and the probability of illness. In addition, the consequence of antimicrobial resistance is also included in the risk for additional consideration of health risk. The health risk was independently considered based on two criteria:

1) the risk from *Campylobacter* itself
2) the risk from *Campylobacter* with additional antimicrobial resistance.

Hence, the methods for estimating the risk were calculated by two separate consecutive models. Firstly, the risk from *Campylobacter* itself (including all *Campylobacter* isolates with and without antimicrobial resistance) was determined using the dose-response relationship model based on the studies of the joint FAO/WHO working group (WHO, 2001); Christensen *et al.*, (2001) and Nauta *et al.*, (2005b). Secondly, the additional risk from antimicrobial resistant *Campylobacter* was estimated by two separate models, which were a CVM model and a modified model adapted from the current quantitative microbial risk assessment (QMRA) (section 7.2.5). The framework of this chapter is illustrated in Figure 8.2.
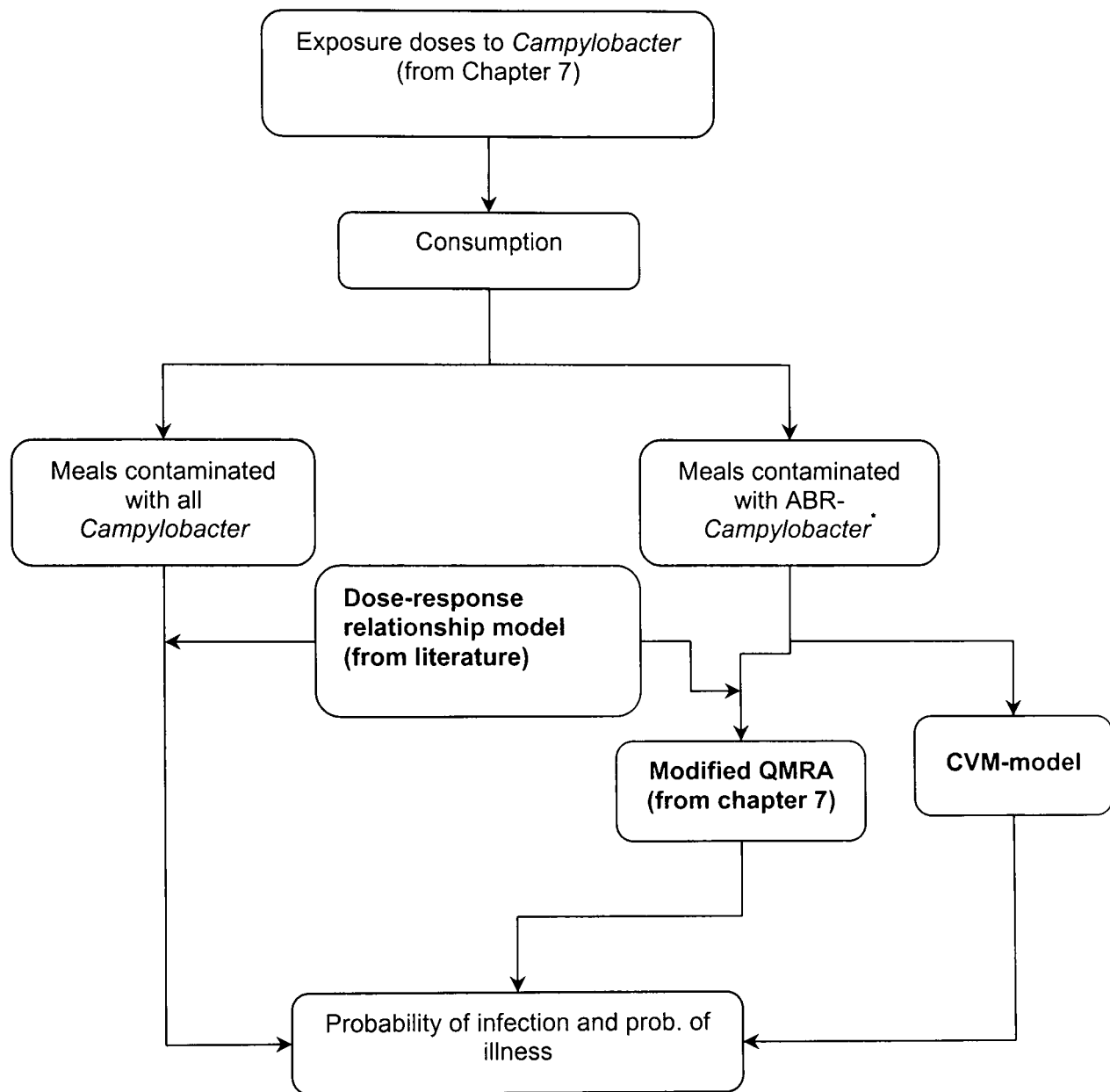
**Figure 8.2** A conceptual framework for health risk characterisation performed in this chapter

## 8.2.1 The Principle of a dose response-relationship model

The consumption of foods contaminated with pathogenic organisms may lead to the ingestion of a number of these pathogens. Any organisms entering the host have a certain probability of survival against the host-defence system and can colonise the digestive system and viable organisms may multiply, thereby achieving an infective dose (concentration). The infection may be asymptomatic if the host is not ill. However, some hosts may become ill and symptoms may vary greatly in severity (Teunis *et al.*, 1996).

The relationship between the ingestion of a certain number of pathogens, *Campylobacter* in this context, and the possible resulting consequences may be described in a number of different ways. The dose is a quantitative measure of the intensity of exposure of a host to the pathogen (Teunis, *et al.*, 1996 and Haas *et al.*, 1999). This number of micro-organisms

entering the digestive system of a host per exposure event (*i.e.* consuming a contaminated chicken meal or salad) may be expressed in different units of functional particles of pathogens (*e.g.* colony-forming unit).

In principle, a dose response relationship may be determined experimentally, with healthy volunteers using various doses of the pathogen of interest. The results are scored and integrated with a mathematical function to predict the probability of infection or disease at the doses occurring in real life situations.

However, these doses are often so low that the probability of infection is then quite small. Studies on large numbers of volunteers would be needed in order to fill this knowledge gap. This would be impossible to do in the real situation. Therefore, most studies estimate the dose using experimental data at high doses for the calibration of the model, resulting in extrapolation to low doses under the circumstance similar to that of a real life situation (Black *et al.*, 1988 and Teunis *et al.*, 1996).

A human volunteer trial for *Campylobacter jejuni* was performed by Black *et al.* (1988). It was performed on 89 adult volunteers who were fed doses of *C.jejuni* ranging from $8 \times 10^2$ to $2 \times 10^9$ organisms. Latter on, Medema *et al.*(1996) used these outcomes from this trial to develop a dose-response relationship for *C.jejuni* infection using a Beta-Poisson model to fit the data. It establishes a relationship between the level of microbial exposure and the likelihood of the probability of illness from ingesting a certain amount of pathogenic organisms which is bound by zero (no effect) and one (complete conversion to adverse state) (Teunis *et al.*, 1996 and Haas *et al.*, 1999). This model assumes that all strains have the same potential to cause human illness (Mead, 2004). The model is based on the chain of transitional probability of the occurring events displayed in Figure 8.3, which distributes as a stochastic process.[12] The susceptibility parameters $\alpha$ and $\beta$ were used to characterise susceptibility and variability of response to the pathogen within a population. As a result, the maximum likelihood estimates for the model were: probability of getting ill ($\alpha$) = 0.145 and probability of not getting ill ($\beta$) = 7.59 (Medema *et al.*,1996).

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[12] A stochastic process is a random function, which a random variable X is defined on a probability space ($\Omega$, Pr) with values in a space of functions F.
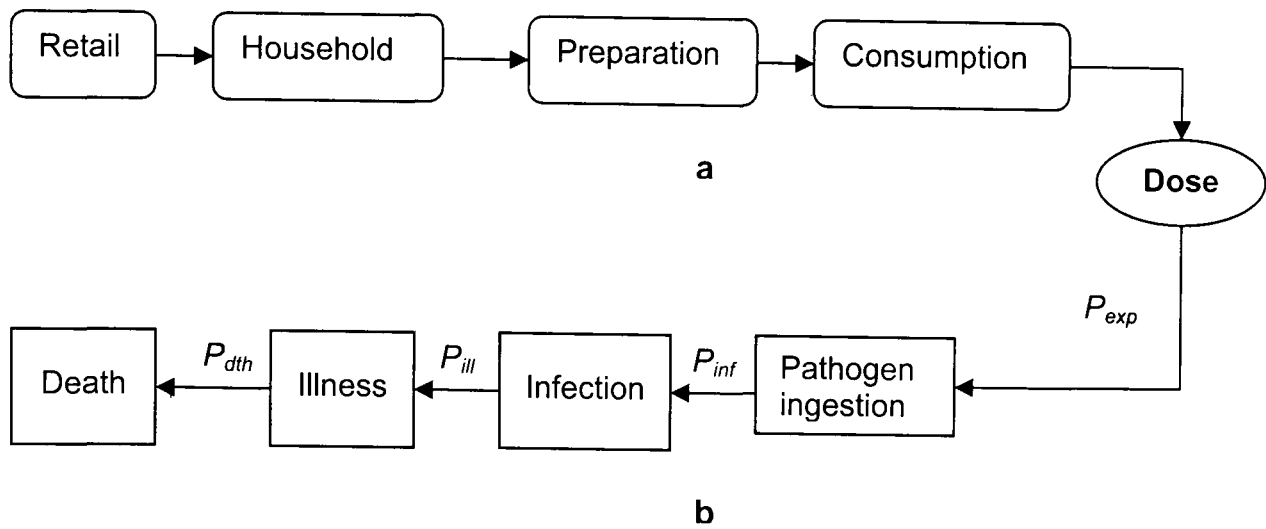
**Figure 8.3** Subsequently events occurring after the exposure of host to pathogen (modified from Teunis *et al*, 1996): a) exposure assessment pathway (Chapter 7) and b) estimation of health risk (Chapter 8)

Although these model parameters ($\alpha$ and $\beta$) have been thought to be overestimated, there are currently no other studies that can provide a better solution. These parameters have been therefore adopted to characterise health risk from *Campylobacter* in a number of reports (WHO, 2001; Christensen *et al.*, 2001 and Nauta *et al*, 2005b).

It was indicated that the infective dose of *C. jejuni* which may cause illness in some individuals is as low as 500 cells (Black *et al.*, 1988). However, a number of studies suggest that host susceptibility is also a key factor influencing infective dose to some degree (Kothary and Babu, 2001). The pathogenic mechanisms of *Campylobacter* are currently still not completely understood. Thereby, when an individual is infected with *Campylobacter*, the precise infective dose of *Campylobacter* is uncertain. It is unknown whether there is a required minimum dose or if a single organism is sufficient.

The basic assumption therefore is that the probability of ingesting precise *j* organisms from an exposure in which the mean dose is *d* organisms is $P_1(j/d)$. The probability of *k* organisms ($\leq j$) surviving the immune system and being able to initiate the infection is $P_2$ *(k/j)*. If the distribution of organisms between doses is random (*i.e.* Poisson distributed) and each organism has an independent and identical probability of surviving the immune defences and initiating infection, the overall probability of *k* organisms surviving to initiate the infection is given by

$$P(k) = \sum_{j=1}^{\infty} P_1(j/d)P_2(k/j) \qquad [8\text{-}1]$$

If the minimum number of the organisms (denoted as $K_{min}$) survives and then initiates infection, the probability of infection can be determined by

$$P_{\text{inf}} = \sum_{k=k_{\min}}^{\infty} \sum_{j=k}^{\infty} P_1(j/d)P_2(k/j) \qquad [8\text{-}2]$$

Based on these assumptions (equations 8-1 and 8-2) and dose-response model parameters ($\alpha$ and $\beta$), the models for estimating health risk related to *Campylobacter* were developed by a number of studies (Medema *et al*, 1996; Teunis *et al*., 1996; Haas *et al*., 1999; Havelaar *et al*., 2000 and Nauta *et al*., 2005b). The model equations are:

1. Probability of infection $\qquad P_{\text{inf}}(d;\alpha,\beta) = 1-[1-p]^d \qquad [8\text{-}3]$

**Where:**

$p$ is the probability of infection from ingestion of one organism

$d$ is the dose ingested

$\alpha$ and $\beta$ are dose-response model parameters (Medema *et al*., 1996)

2. Probability of illness given infection

$$P_{ill/\text{inf}}(d;\alpha,\beta) = 1-(1-Beta(\alpha,\beta)^d) \qquad [8\text{-}4]$$

Nauta *et al*. (2005b) quantified the value of probability of illness given infection ($P_{ill/inf}$) shown in equation 8-4 using the trial data from Black *et al*.(1988). In the trial, there were 29 of 89 volunteers that showed symptom of illness. If a person becomes infected, there is a certain probability that the person will become ill. Thus, the uncertainty about the true value of this probability is described by a beta distribution. The probability of getting ill given infection is Beta ($\alpha,\beta$), when $\alpha$ = 29+1 and $\beta$ = (89-29)+1 (Nauta *et al*., 2005b), therefore;

$$P_{ill/\text{inf}} = 0.33 \text{ [calculated with ~RiskBeta* (29+1, (89-29)+1))} \quad [8\text{-}5]$$

* using @ Risk software release 4.5

Including the studies of Black *et al*. (1988); Havelaar *et al*. (2000) and Nauta *et al*. (2005b), the dose-response model for estimating probability of illness from ingestion of *d* organisms used in this chapter is:

$$P_{ill}(d) = P_{\text{inf}}(d) \times P_{ill/\text{inf}}(d) \qquad [8\text{-}6]$$

**Note:** Details of the development of a dose-response relationship is shown in Appendix 3-1.

# 8.3 Methodology

## 8.3.1 The estimate of the health risk related to *Campylobacter*

From this point "health risk" includes probability of infection and probability of illness and "*Campylobacter* "refers to all *Campylobacter* (with and without antimicrobial resistance).

The health risks following the consumption of a *Campylobacter*-positive chicken was estimated following the exposure assessment (Chapter 7). The exposure doses were input to the model parameters described by Christensen *et al.*, (2001) and Nauta *et al.*, (2005b). Health risks are considered following the consumption of chicken meals and salads. Calculations of these are:

1. Probability of infection from a dose of *Campylobacter* in a chicken meal given gender was calculated by:

$$P_{\inf C/G,E} = 1 - (1 - P_{1C})^{N_{C/G}}, N_{C/G} > 0 \qquad [8\text{-}7]$$

**Where:**

$P_{1C}$     is probability of infection from exposure to one *Campylobacter*.

$N_{C/G}$     is numbers of *Campylobacter* in chicken meal ingested per serving given gender group.

2. Probability of illness from a dose of *Campylobacter* in a chicken meal given gender was calculated by:

$$P_{illC/G,E} = P_{\inf C/G.E} \times P_{ill/\inf C} \qquad [8\text{-}8]$$

**Where:**

$P_{ill/infC}$     is probability of getting ill given probability of infection which can cause adverse consequences. From literature, $P_{ill/infc}$ = Beta (29+1, 89-29+1)= 0.33, adopted from Nauta *et al.*, 2005b.

3. Probability of infection from a dose of *Campylobacter* in salad given gender was calculated by:

$$P_{\inf S/G,E} = 1 - (1 - P_{1C})^{N_{S/G}}, N_{S/G} > 0 \qquad [8\text{-}9]$$

4. Probability of illness from a dose of *Campylobacter* in salad given gender was calculated by:

$$P_{illS/G,E} = P_{\inf S/G.E} \times P_{ill/\inf S} \qquad [8\text{-}10]$$

Model parameters used for above calculations are presented in Table 8.1

**Table 8.1** The parameters for the estimation of health risks of host to *Campylobacter* following the consumption of chicken meals and salads (adopted from Christensen *et al.*, 2001 and Nauta *et al*, 2005b)

| Parameter | Description | Distribution/Expression |
|---|---|---|
| $S_{C/G}$[1] | Average size of chicken serving given gender | Table 8.2 |
| $S_{S/G}$ | Average size of salad serving given gender | Table 8.2 |
| $N_{C/G}$ | Numbers of *Campylobacter* in chicken meal serving given gender | Poisson $(S_{C/G}$ x $^2N_{ins})$ for immediate preparation <br> or Poisson $(S_{C/G}$ x $^3N_{ins/S}$ ) for delayed preparation |
| $N_{S/G}$ | Numbers of *Campylobacter* in salad serving given gender | Poisson $(S_{C/G}$ x $^4N_{salad})$ |
| $P_{1C}$ | Probability of infection from exposure to one *Campylobacter* | 0.018748 [RiskBeta $(0.145,7.59)^5$] |
| $P_{infC/G,E}$ | Probability of infection from a dose in chicken serving given gender, given exposure(E) | $1-(1-P_{1C})^{N_{C/G}}, N_{C/G}>0$ |
| $P_{infS/G,E}$ | Probability of infection from a dose in chicken serving given gender, given exposure(E) | $1-(1-P_{1C})^{N_{S/G}}, N_{S/G}>0$ |
| $P_{illC/G,E}$ | Probability of illness from a dose in chicken serving given gender,E | $P_{infC/G,E}$ x $^6P_{ill/inf}C$ |
| $P_{illS/gender,E}$ | Probability of illness from a dose in salad serving given gender,E | $P_{infS/gender,E}$ x $P_{ill/inf}C$ |

[1] gender of person ingesting meal

[2,3,4] numbers of *Campylobacter* in chicken meals (immediate and delayed preparation) and salad

[5] RiskBeta $(a,\beta)$, the value of $a$ and $\beta$ were obtained from Medema *et al*, 1996

[6] $P_{ill/inf}$ is Beta (29+1, 89-29+1) (from Nauta *et al.*,2005)

**Table 8.2** The average serving size of a chicken meal and salad (based on data from Christensen *et al.*, 2001)

| Consumer group | Serving size (gram) | |
|---|---|---|
| | Chicken meal ($S_{C/G}$) | Salad ($S_{S/G}$) |
| Female | 148.30 | 63.95 |
| Male | 188.15 | 85.78 |

## Calculation procedures

Calculations of the probability of infection and probability of illness associated with the consumption of a chicken meal and salad can be carried out using equations from 8-7 to 8-10. The parameters fed to the models are shown in Tables 8.1 and 8.2. The examples of calculations are demonstrated below:

1. Probability of infection from a dose ($P_{infC/G,E}$) can be calculated following equation [8-7].

$$P_{\inf C/G,E} = 1 - (1 - P_{1C})^{N_{C/G}}, N_{C/G} > 0$$

**Where:**

1.1 $P_{1C}$ = probability of infection from exposure to one *Campylobacter*

→ RiskBeta (α,β), using @Risk release 4.5*, α = 0.145, β = 7.59

→ RiskBeta (0.145, 7.59)

**→ 0.0187**

1.2 $N_{C/G}$ = Numbers of *Campylobacter* in chicken meal serving given gender

→ RiskPoisson (serving size given gender x conc. of *Campylobacter* $g^{-1}$

→ RiskPoisson (148.30 x 0.08 MPM/g) [example for person (female) consumes chicken meal associated with the PICs given T<$T_C$)

**→ 12**

1.3 $P_{infC/G,E}$ = 1-(1-0.0187)$^{12}$

**→ 0.78**

Therefore the probability of infection is 78% when the female group consumes the PIC-chicken meal contaminated with *Campylobacter* with a dose 0.08 MPN/g which was estimated using the model when T <$T_C$.

2. Probability of illness from a dose in chicken serving given gender ($P_{illC/gender, E}$) can be consecutively calculated using above result. This is:

$P_{illC/G,E}$ = $P_{infC/G,E}$ x $P_{ill/inf}$ ; where $P_{ill/inf}$ is 0.33

= (0.78 x 0.33)100 = 26.31%

Therefore the probability of illness given probability of infection (78%) is 26.31%

## 8.3.2 Risk model for antimicrobial resistant *Campylobacter*

In this chapter the additional risk from antimicrobial-resistance associated with the consumption of a chicken meal or salad was characterised by two different models: i) the CVM-risk model and ii) a modified dose-response relationship model based on the QMRA model (mQMRA).

## The CVM-risk model

The principle of the CVM-risk model (developed by the Food and Drug Administration-Center of Veterinary Medicine) is explained in section 7.2.5. As described in the CVM-risk model, a relative human risk of antimicrobial resistant *Campylobacter* (ABR-*Campylobacter*) associated with the consumption of chicken is given by;

$$\lambda_i = K_{res} V_i \qquad \text{[8-11]}$$

As the aim of this section is to compare a relative health risk of ABR-*Campylobacter* following the consumption of the PICs, the POCs and the BICs, the values of $\lambda_{PIC}$, $\lambda_{POC}$ and $\lambda_{BIC}$ following the CVM-risk model are as follows;

$$\lambda_{PIC} = K_{res}( A_{PIC} \times S \times F_{pos/PIC} \times P^*_{ABR\,(i)}) \qquad \text{[8-12]}$$

$$\lambda_{POC} = K_{res}( A_{POC} \times S \times F_{pos/POC} \times P_{ABR\,(i)}) \qquad \text{[8-13]}$$

$$\lambda_{BIC} = K_{res}( A_{BIC} \times S \times F_{pos/BIC} \times P_{ABR\,(i)}) \qquad \text{[8-14]}$$

**Where:**

*i* denotes three antimicrobial agents (*i.e.* ciprofloxacin or erythromycin or nalidixic acid).

The meaning of the parameters in equations 8-12, 8-13 and 8-14 are described in Table 8.3

**Table 8.3** The description of model parameters modified from CVM-risk model

| Parameter | Description | Unit | Remark |
|---|---|---|---|
| $A_{PIC}$ | amount of PIC harbouring ABR-*Campylobacter* consumed | pounds | |
| $A_{POC}$ | amount of POC harbouring ABR-*Campylobacter* consumed | pounds | |
| $A_{BIC}$ | amount of BIC harbouring ABR-*Campylobacter* consumed | pounds | |
| S | population size | people | |
| $F_{pos/PIC}$ | fraction of positive-*Campylobacter* chicken (PIC) | | 0.83 (chapter 5) |
| $F_{pos/POC}$ | fraction of positive-*Campylobacter* chicken (POC) | | 0.80 (chapter 5) |
| $F_{pos/BIC}$ | fraction of positive-*Campylobacter* chicken (BIC) | | 1.00 (chapter 5) |
| $P_{ABR\,(i)}$ | proportion of ABR-*Campylobacter* where three antimicrobials taken into account are ciprofloxacin, erythromycin and nalidixic acid) | | chapter 6 |
| $K_{res}$ | an antimicrobial resistance transfer coefficient | | |

However, there are currently no specific data for: i) the association between health risk from *Campylobacter* infection and the consumption of chicken harbouring antimicrobial resistant *Campylobacter* and ii) the consumption of chickens reared from different systems. The amount of chicken consumed was also taken to be the amount of chicken consumed in general. Therefore, the calculations using equations 8-12, 8-13 and 8-14 are carried out by assuming that the value of $A_{PIC}$, $A_{POC}$ and $A_{BIC}$ are the same. As $K_{res}$ is assumed as a

constant for either type of chicken, the relative health risk of ABR-*Campylobacter* is therefore determined by;

$$\lambda_{PIC} \sim F_{pos/PIC} \times P_{ABR\,(i)}$$ [8-15]

$$\lambda_{POC} \sim F_{pos/POC} \times P_{ABR\,(i)}$$ [8-16]

$$\lambda_{BIC} \sim F_{pos/BIC} \times P_{ABR\,(i)}$$ [8-17]

Therefore, health risk (denoted as "$\lambda$") involves the fraction of positive-*Campylobacter* chicken and proportion of ABR- *Campylobacter*. These values (Chapters 7 and 8) were inputted to the equations 8-15, 8-16 and 8-17.

## The development of the modified QMRA model (mQMRA)

As shown in equations 8-15 to 8-17, the parameters input to the CVM model do not include the numbers of *Campylobacter* in chickens. The CVM model is probably less sensitive for comparing the relative risk from the consumption of chicken meals or salad assocated with three different groups of chickens. It is evident that there is a significant difference of the numbers of *Campylobacter* (MPN/g) between these three groups, with the highest level found in the BIC group. Although the resistance rate to erythromycin and nalidixic acid of *Campylobacter* in the PIC, POC and BIC groups were found to be similar (100%), the resistance rate to ciprofloxacin is slightly different. In particular, the POC group was found to be free from ciprofloxacin-resistant *Campylobacter*. Therefore, in order to quantify and compare the health risk from ABR- *Campylobacter* with a specific antimicrobial which is related to the types of chicken consumed, the mQMRA used for antimicrobial resistant *Campylobacter* was customised from the current QMRA model used in Chapter 7 (for details see section 7.2.5, Chapter 7).

Instead of using the initial numbers of *Campylobacter*, the mQMRA considers the number of ABR- *Campylobacter* as a product of the initial number multiplied by the rate of resistance to specific antimicrobial, namely, ciprofloxacin, erythromycin and nalidixic acid, that is;

$$N_{0,ABR} = N_0 \times P_{ABR(i)}$$ [8-18]

Next, the number of ABR- *Campylobacter* ($N_{0,\,ABR}$) was further modelled using equations from 8-7 to 8-10. The calculations were performed similarly to those demonstrated in section 8.3.1.

## 8.3.3 Sensitivity analysis

The models and model parameters used were adopted from the joint FAO/WHO working group and international literature. In the model of heat treatment, internal temperature and exposure time were based on the data from the literature. Internal temperature varied from

60 to 65$^0$C and the exposure time was from 0.5 to 1.5 minutes. Given these data, the heat treatment models used may not fit to the BIC group. As a result, the health risk following the consumption of cooked chicken meal remains high. Currently, there is no further information expressing higher internal temperature or exposure time that those stated above. However, to identify the greatest impact from the changes of variable input, a sensitivity analysis was performed. Rather than using only values from the literature, a new set of values of internal temperature and exposure time were assumed and defined for use, assuming, during heat treatment, amount of time and internal temperature may increase to higher levels than those previously used in the models. Of these values, the mean values were estimated using the probabilistic distribution (using @Risk release 4.5 software). These mean values, presented in Table 8. 4, were fed in to the heat treatment model at T>T$_C$ (In $N_{ins}$= In $N_0$-$(t/D)$. Results of this were presented in section 8.4.3 and discussed in section 8.5.3.

**Table 8.4 Mean values of parameter time ($t_i$) and internal temperature (T)**

| Exposure time (min) | | Internal temperature ($^0$C) | |
|---|---|---|---|
| RiskPERT(min,most likely,max) | Mean value of time (min) | RiskPERT(min,most likely,max) | Mean value of temperature ($^0$C) |
| 0.5,1,1.5* | 1 | 60,64,65 | 63 |
| 1.5,2,2.5 | 2 | 60,64,68 | 64 |
| 2,3,4 | 3 | 62,65,68 | 65 |
| 3,4,5 | 4 | 63,65.5,69.5 | 66 |
| 4,5,6 | 5 | 64.5,66.5,70 | 67 |
| | | 65.5,67.5,71 | 68 |
| | | 66,69,72 | 69 |
| | | 67,70,73 | 70 |
| | | 68,71,74 | 71 |
| | | 70,72,74 | 72 |

# 8.4 Results

It is important to note that this finding was carried out following the assumption and data available. However, the sensitivity analysis was performed to test the reliability of the heat treatment model given $T > T_C$.

## 8.4.1 Health risk of *Campylobacter*

**Determination of the health risk of person consuming a chicken meal prepared immediately**

1) Probability of infection

According to the model and data set, the consumption of chickens purchased from butchers' shops (the BIC group) can cause infection with *Campylobacter* (100%), either cooking with internal temperature[13] lower than $T_C$ ($T_C = 46^0C$) or higher than $T_C$. In the POC group, the probabilities of infection when $T < T_C$ range from 64-72.4% and they decline gradually to 3.7% when $T > T_C$ (Table 8.5). The probability of infection from ingesting these micro-organism attributable for the PIC group shows the lowest value and significantly different from the POCs and the BICs ($p < 0.001$), in particular, if $T > T_C$, the risk was reduced to 0-1.9%. There is no difference of the probability of infection occurring between female and male consumers for all groups of chicken.

**Table 8.5** The probability of infection (%) caused by the consumption of a chicken meal contaminated with *Campylobacter* transferred from three types of chicken given gender and internal temperature at the protected areas

| Type of chicken[1] | Probability of infection (%) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Chicken meal: $T < T_C$[2] | | | Chicken meal: $T > T_C$[3] | | |
| | Female | Male | *p-value* | Female | Male | *p-value* |
| PIC | 20.32 | 26.12 | NS | 0 | 1.88 | NS |
| POC | 64.01 | 72.39 | NS | 3.71 | 3.71 | NS |
| BIC | 100 | 100 | NS | 100 | 100 | NS |
| *p-value** | <0.001 | <0.001 | | <0.001 | <0.001 | |

[1] Pre-packed intensively-reared (PIC), pre-packed organically-reared (POC) and butcher intensively-reared chicken (BIC) group

[2, 3] Internal temperature at the protected area lower or higher than $46^0C$ ($T_C$)

* Using chi-square to analyse the significant difference of probability of infection between three groups

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[13] Internal temperature refers to the temperature at the inside of chicken meat, which is defined as the protected area. *Campylobacter* in this area is thought to be viable.

## 2) Probability of illness

The probability of illness is about 7-9 % following the consumption of the PICs, 21-23 % for the POCs and 33% for the BICs. However, when T>T$_C$, it is less than 1% following the consumption of the PICs, 1-2% for the POCs and 33% for the BICs. Given T>T$_C$, a gradual decrease is found in the probability of illness associated with the consumption of the PICs and the POCs (Table 8.6). The decrease is about 6-8 % in the PICs and 20 % in the POCs. However, the rates found to be associated with the consumption of the BICs were consistent at 33 % whether either model (given T<T$_C$ or given T>T$_C$) was used. So far, the consumption of a chicken meal associated with the BIC group was found to have a significantly higher potential to cause illness than those from the PICs and the POCs ($p$ <0.001).

**Table 8.6** The probability of illness (%) caused by the consumption of a chicken meal contaminated with *Campylobacter* transferred from three types of chicken given gender and internal temperature at the protected areas

| Type of chicken | Probability of illness (%) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Chicken meal: T<T$_C$ | | | Chicken meal: T>T$_C$ | | |
| | Female | Male | *p-value* | Female | Male | *p-value* |
| PIC | 6.70 | 8.62 | NS | 0 | 0.62 | NS |
| POC | 21.12 | 23.88 | NS | 1.22 | 1.22 | NS |
| BIC | 33 | 33 | NS | 33 | 33 | NS |
| *p-value* | <0.001 | <0.001 | | <0.001 | <0.001 | |

## Determination of the health risk for a person consuming salad

### 1. Probability of infection

The results from the consumption of salad related to the POC and BIC groups demonstrate a high probability of infection in both female and male groups (ranged from 95-98% for the POCs and 100 % for the BICs). The values found in the PIC group are significantly lower than the other two groups ($p<0.001$). These values are about 61% for a male group and 51% for a female group. There is no evidence to show a significant difference in probability of infection between female and male groups.

### 2. Probability of illness

The probability of illness related to the consumption of salad contaminated with *Campylobacter* from the uncooked PICs is about 18-26% for men and 18-20% for women; from the POCs is 31-33% for both sexes; and from the BICs is 33% for all age and sex groups. It is evident that in the female group, the difference of the probability of illness following the consumption of salad related to three groups of chicken is significant ($p=0.02$),

whereas there is no significant difference found in the male group. However, in the same group of chicken, there is no significant difference found in both genders. These results are shown in Table 8.7.

**Table 8.7** The health risk caused by the consumption of salad contaminated with *Campylobacter* transferred from three types of chickens

| Type of chicken | Probability of infection (%) | | | Probability of illness (%) | | |
|---|---|---|---|---|---|---|
| | Female | Male | *p-value** | Female | Male | *p-value* |
| PIC | 51.28 | 61.91 | NS | 16.92 | 20.43 | NS |
| POC | 94.88 | 98.16 | NS | 31.31 | 32.39 | NS |
| BIC | 100 | 100 | NS | 33 | 33 | NS |
| *p-value*** | <0.001 | <0.001 | | 0.02 | NS | |

\* Using Fischer's exact test to analyse the significant difference between two groups
\*\*Using Chi-square test to analyse the significant difference between three groups

## Determination of the health risk for a person consuming meals prepared from pre-refrigerated chicken

### 1. A chicken meal

Table 8.8 presents the health risk related to the consumption of chicken meals cooked after the chickens have been stored in a refrigerator. These were found to be highest in the BICs. The results are statistically significant at $p < 0.001$. The health risk related to the PIC and POC groups when immediately prepared and given $T<T_C$ were higher than those found in the delayed preparation. If, however, using the model given $T>T_C$, the outcomes from immediate and delayed preparation were very similar. This is in contrast to the results found in the BIC group, which the phase of preparation (immediate or delayed) and temperature of heat treatment did not change the health risk from *Campylobacter*.

**Table 8.8** The health risk (%) associated with the consumption of a chicken meal prepared from three types of chickens after being stored in a refrigerator

| Type of chicken | Probability of infection (%) | | | | Probability of illness (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | $T<T_C$ | | $T>T_C$ | | $T<T_C$ | | $T>T_C$ | |
| | Female | Male | Female | Male | Female | Male | Female | Male |
| PIC | 1.88 | 3.71 | 0 | 0 | 0.62 | 1.23 | 0 | 0 |
| POC | 12.41 | 15.66 | 0 | 0 | 4.09 | 5.17 | 0 | 0 |
| BIC | 100 | 100 | 100 | 100 | 33 | 33 | 33 | 33 |
| *p-value* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

\* No significant differences of heath risk found between female and male for all groups of chicken

## 2. Salad

Salads contaminated with the uncooked *Campylobacter*-positive BICs stored in a refrigerator have the highest values of health risk to consumer, followed by the POC and the PIC groups, respectively (Table 8.9). In the PICs and POCs, the values from the immediate post preparation consumption group were found to be significantly higher than those from the delayed post preparation consumption group ($p<0.001$). However, in the BIC group, all values were found to be the same whether salad is contaminated with the positive carcasses before or after the chickens were stored in a refrigerator.

**Table 8.9** The health risk caused by the consumption of salad contaminated with *Campylobacter* transferred from three types of chickens after being stored in a refrigerator

| Type of chicken | Probability of infection (%) | | Probability of illness (%) | |
|---|---|---|---|---|
| | Female | Male | Female | Male |
| PIC | 7.29 | 9.03 | 2.41 | 2.98 |
| POC | 32.79 | 41.13 | 10.82 | 13.57 |
| BIC | 100 | 100 | 33 | 33 |
| *p-value* | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

* No significant differences of health risk found between female and male for all groups of chicken

## 8.4.2 Comparison of health risk due to antimicrobial resistant *Campylobacter* between the three groups of chicken

### The health risk determined by the CVM model

The health risk from ABR-*Campylobacter* following the consumption of three groups of chicken can be determined with two different assumptions. These assumptions are based on: i) the rate of resistance to three antimicrobials as a proportion per carcass and ii) the rate of resistance to three antimicrobials as a proportion per isolate (section 6.4.2, Chapter 6).

**Note**: rate /carcass means percentage of chicken carcases harbouring one/more resistant isolates
        rate /isolate means percentage of isolates found to be resistant to a specific antimicrobial

As shown in Table 8.10, given a ciprofloxacin resistance rate per isolate, a health risk ($\lambda_{BIC}$) from the consumption of the BICs is approximately 4.8 times over that related to the PICs ($\lambda_{PIC}$), whereas there is no additional health risk from *Campylobacter* associated with the consumption of the organic chickens. For erythromycin and nalidixic acid, the risks are

slightly different between these three groups of chicken. The value of $\lambda_{BIC}$-erythromycin resistance found in the BICs is higher than those associated with the PIC and POC groups.

When using the resistance rate per carcass (Table 8.10), the risk of ciprofloxacin-resistant *Campylobacter* from the BICs is still higher than those from the PICs. There is no additional health risk from ciprofloxacin resistance related to the consumption of organic chickens. Although the additional risks related to erythromycin and nalidixic acid resistance in these three groups are only slightly different, the highest rate is still found in the BIC group (1.00 for both $\lambda_{BIC}$-erythromycin and $\lambda_{BIC}$-nalidixic acid).

**Table 8.10** The health risk of antimicrobial-resistant *Campylobacter* following the consumption of chicken given three types of chickens and antimicrobials using a CVM model

| Type of chicken | Health risk ($\lambda$): isolate[1] | | | Health risk ($\lambda$): carcass[2] | | |
|---|---|---|---|---|---|---|
| | Ciprofloxacin | Erythromycin | Nalidixic acid | Ciprofloxacin | Erythromycin | Nalidixic acid |
| PIC | 0.02 | 0.83 | 0.83 | 0.07 | 0.83 | 0.83 |
| POC | 0 | 0.80 | 0.76 | 0 | 0.80 | 0.80 |
| BIC | 0.12 | 1.00 | 0.81 | 0.27 | 1.00 | 1.00 |

[1] Health risk determined from the proportion of isolates found to be resistant to tested antimicrobials.

[2] Health risk determined from the proportion of carcasses harbouring one/more resistant isolates

## The health risk determined by the modified QMRA

From this approach the additional health risk refers to the probability of infection and probability of illness associated with ABR-*Campylobacter* following the consumption of a chicken meal or salad.

## 1. A chicken meal

Tables 8.11 and 8.12 show that according to the model the highest additional risks related to *Campylobacter* resistant to three antimicrobials were found in the BIC group (*i.e.* 100% probability of infection and 33% probability of illness) whether the model used is given T<$T_C$ or given T>$T_C$.

The results from the PIC group show very low additional risks related to ciprofloxacin-resistant *Campylobacter* (CR-*Campylobacter*) (< 2% probability of infection). For other two antimicrobials (erythromycin and nalidixic acid), the additional risks were found to be higher than that related to CR-*Campylobacter*. These risks related to ER- and NAR-*Campylobacter* were found to be less than those from the POCs and the BICs, respectively. This becomes clear when using the model given T>$T_C$.

There is no additional risk related to CR- *Campylobacter* from POC-chicken meals. The risks related to other antimicrobial resistances were found to be fairly high (64-72 % for ER-*Campylobacter* and 62-71% for NAR-*Campylobacter*). However, when the internal temperature used is higher than $T_C$, the risks were reduced to very low levels. Levels of additional health risks related to POC-chicken meals were found to be between the levels from the PICs and the BICs (PICs<POCs<BICs), except for that related to ciprofloxacin resistance.

Levels of the additional risks related to ABR- *Campylobacter* show the same whether the levels were taken into account as a proportion of a carcass or of an isolate.

**Table 8.11** Probability of infection of person consuming chicken harbouring ABR-*Campylobacter*

| Type of chicken | Type of antimicrobial | Probability of infection (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T<T_c$ | | | | $T>T_c$ | | | |
| | | /carcass | | /isolate | | /carcass | | /isolate | |
| | | F | M | F | M | F | M | F | M |
| PIC | Ciprofloxacin | 1.9 | 1.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Erythromycin | 21.8 | 26.1 | 21.8 | 26.1 | 0 | 1.9 | 0 | 1.9 |
| | Nalidixic acid | 21.8 | 26.1 | 21.8 | 26.1 | 0 | 0 | 1.9 | 1.9 |
| POC | Ciprofloxacin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Erythromycin | 64.0 | 72.4 | 64.0 | 72.4 | 3.7 | 5.5 | 3.7 | 5.5 |
| | Nalidixic acid | 64.0 | 72.4 | 61.9 | 70.8 | 3.7 | 3.7 | 5.5 | 3.7 |
| BIC | Ciprofloxacin | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Erythromycin | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | Nalidixic acid | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 8.12** Probability of illness of person consuming chicken harbouring ABR-*Campylobacter*

| Type of chicken | Type of antimicrobial | Probability of illness (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T<T_c$ | | | | $T>T_c$ | | | |
| | | /carcass | | /isolate | | /carcass | | /isolate | |
| | | F | M | F | M | F | M | F | M |
| PIC | Ciprofloxacin | 0.6 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Erythromycin | 7.2 | 8.6 | 7.2 | 8.6 | 0 | 0.6 | 0 | 0.6 |
| | Nalidixic acid | 7.2 | 8.6 | 7.2 | 8.6 | 0 | 0.6 | 0 | 0.6 |
| POC | Ciprofloxacin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Erythromycin | 21.1 | 23.9 | 21.1 | 23.9 | 1.2 | 1.8 | 1.2 | 1.8 |
| | Nalidixic acid | 21.1 | 23.9 | 20.4 | 23.4 | 1.2 | 1.8 | 1.2 | 1.2 |
| BIC | Ciprofloxacin | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Erythromycin | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| | Nalidixic acid | 33 | 33 | 33 | 33 | 33 | 33 | 33 | 33 |

## 2. Salad

Table 8.13 shows that salad contaminated with ABR-*Campylobacter* transferred from the uncooked BICs exhibited the highest health risk whether the resistance is to ciprofloxacin or erythromycin or nalidixic acid (100% probability of infection and 33% probability of illness). The results from the PIC groups show lower risks than other two groups, except for the risks related to CR- *Campylobacter*. There is no risk related to CR-*Campylobacter* following the consumption of POC-salad.

**Table 8.13** Health risks of person consuming salad contaminated with ABR-*Campylobacter*

| Type of chicken | Type of antimicrobial | Probability of infection | | | | Probability of illness | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | /carcass | | /isolate | | /carcass | | /isolate | |
| | | F | M | F | M | F | M | F | M |
| PIC | Ciprofloxacin | 5.5 | 7.2 | 1.9 | 1.9 | 1.8 | 2.4 | 0.6 | 0.6 |
| | Erythromycin | 49.4 | 60.4 | 49.4 | 60.4 | 16.3 | 19.9 | 16.3 | 19.9 |
| | Nalidixic acid | 49.4 | 60.4 | 49.4 | 60.4 | 16.3 | 19.9 | 16.3 | 19.9 |
| POC | Ciprofloxacin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Erythromycin | 94.9 | 98.1 | 94.9 | 98.1 | 31.3 | 32.4 | 31.3 | 32.4 |
| | Nalidixic acid | 94.9 | 98.1 | 94.0 | 97.7 | 31.3 | 32.3 | 31.0 | 32.3 |
| BIC | Ciprofloxacin | 100 | 100 | 100 | 100 | 33 | 33 | 33 | 33 |
| | Erythromycin | 100 | 100 | 100 | 100 | 33 | 33 | 33 | 33 |
| | Nalidixic acid | 100 | 100 | 100 | 100 | 33 | 33 | 33 | 33 |

## 8.4.3 Sensitivity of the model

The changes of the variables input, time and internal tempaerature, have the great imact on the outcomes. When the mean value of time increases, the health risk can be reduced to very low. Similarly, increase of the mean value of internal temperature greatly affects the health risk. The results are presented in Tables 8.14 - 8.17 and Figures 8.4 and 8.7.

**Table 8.14** The exposure dose and health risk following a change of time ($t_i$) given internal temperature at the protected area 63$^0$C

| Parameter function of $t_i$ [RiskPERT(min,most likely,max)] | Time ($t_i$) (min) | Dose (MPN/g) | Prob. infection (%) | | Prob. illness (%) | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | Female | Male | Female | Male |
| 0.5,1,1.5* | 1 | 75.16 | 100 | 100 | 33 | 33 |
| 1.5,2,2.5 | 2 | 4.49 | 100 | 100 | 33 | 33 |
| 2,3,4 | 3 | 0.27 | 53.09 | 61.18 | 17.52 | 20.19 |
| 3,4,5 | 4 | 0.02 | 3.71 | 5.5 | 1.2 | 1.8 |
| 4,5,6 | 5 | 0.001 | 0 | 0 | 0 | 0 |

* The amount of time previously used for the risk modelling presented in chapter 7

**Table 8.15** The exposure dose and health risk following a change of internal temperature ($^0$C) given a time of 1 minute that the protected area exposes to heat

| Parameter function of T ($^0$C) [RiskPERT(min,most likely,max)] | Temperature ($^0$C) | Dose (MPN/g) | Prob. infection (%) | | Prob. illness (%) | |
|---|---|---|---|---|---|---|
| | | | Female | Male | Female | Male |
| 60,64,65 | 63 | 75.16 | 100 | 100 | 33 | 33 |
| 60,64,68 | 64 | 44.73 | 100 | 100 | 33 | 33 |
| 62,65,68 | 65 | 24.2 | 100 | 100 | 33 | 33 |
| 63,65.5,69.5 | 66 | 11.69 | 100 | 100 | 33 | 33 |
| 64.5,66.5,70 | 67 | 4.94 | 100 | 100 | 33 | 33 |
| 65.5,67.5,71 | 68 | 1.78 | 99.32 | 100 | 32.78 | 33 |
| 66,69,72 | 69 | 0.53 | 77.57 | 84.92 | 25.6 | 28.02 |
| 67,70,73 | 70 | 0.13 | 30.2 | 36.5 | 9.97 | 12.05 |
| 68,71,74 | 71 | 0.02 | 5.51 | 7.3 | 1.82 | 2.41 |
| 70,72,74 | 72 | 0.003 | 0 | 1.9 | 0 | 0.006 |

**Table 8.16** The exposure dose and health risk following a change of internal temperature ($^0$C) given a time of 2 minutes that the protected area exposes to heat

| Parameter function of T ($^0$C) [RiskPERT(min,most likely,max)] | Temperature ($^0$C) | Dose (MPN/g) | Prob. infection (%) | | Prob. illness (%) | |
|---|---|---|---|---|---|---|
| | | | Female | Male | Female | Male |
| 60,64,65 | 63 | 4.49 | 100 | 100 | 33 | 33 |
| 60,64,68 | 64 | 1.58 | 98.85 | 99.65 | 32.62 | 32.88 |
| 62,65,68 | 65 | 0.47 | 72.9 | 81.09 | 24.06 | 26.76 |
| 63,65.5,69.5 | 66 | 0.11 | 26.12 | 31.51 | 8.62 | 10.39 |
| 64.5,66.5,70 | 67 | 0.02 | 5.52 | 7.3 | 1.82 | 2.4 |
| 65.5,67.5,71 | 68 | 0.003 | 0 | 0 | 0 | 0 |

**Table 8.17** The exposure dose and health risk following a change of internal temperature ($^0$C) given a time of 3 minute that the protected area exposes to heat

| Parameter function of T ($^0$C) [RiskPERT(min,most likely,max)] | Temperature ($^0$C) | Dose (MPN/g) | Prob. infection (%) | | Prob. illness (%) | |
|---|---|---|---|---|---|---|
| | | | Female | Male | Female | Male |
| 60,64,65 | 63 | 0.27 | 53.09 | 61.18 | 17.51 | 20.19 |
| 60,64,68 | 64 | 0.06 | 14.05 | 18.75 | 4.6 | 6.2 |
| 62,65,68 | 65 | 0.009 | 1.87 | 3.7 | 0.006 | 1.2 |
| 63,65.5,69.5 | 66 | 0.001 | 0 | 0 | 0 | 0 |

## A chicken meal: immediate cooking with T>T$_C$

### 1) Increase of amount of time that the protected area is exposed to the heat given an internal temperature of 63$^0$C
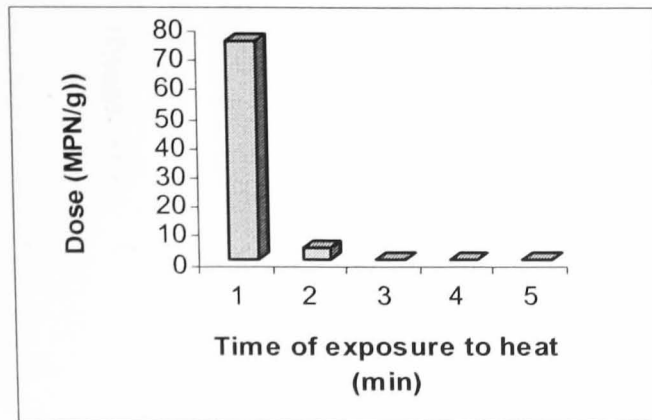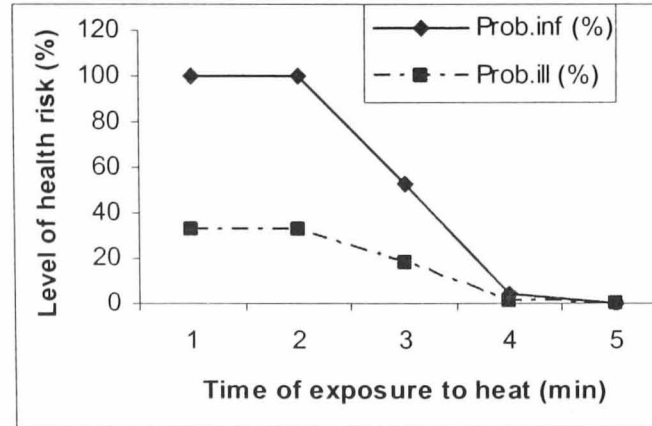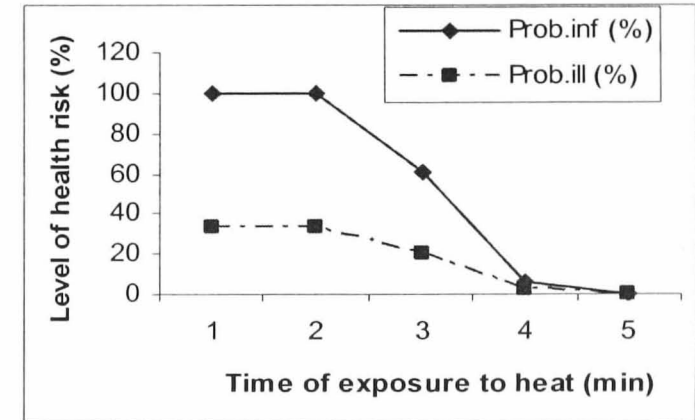


a) Dose of *Campylobacter* (MPN/g)　　　　b) Health risk female group　　　　c) Health risk male group

**Figure 8.4** Level of health risk when increasing time of exposure to heat treatment given internal temperature 63$^0$C

### 2) Increase of internal temperature given 1 minute that the protected area is exposed to the heat
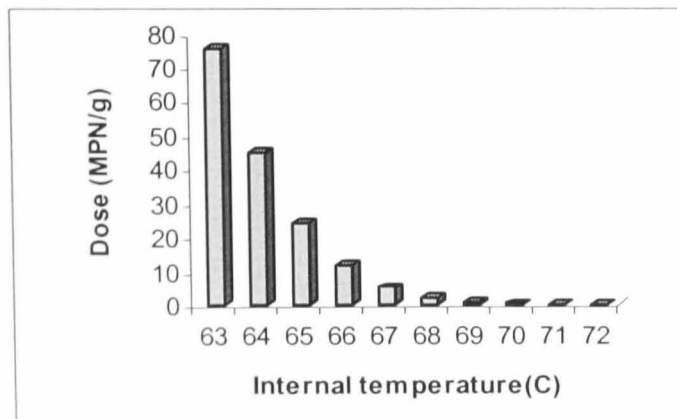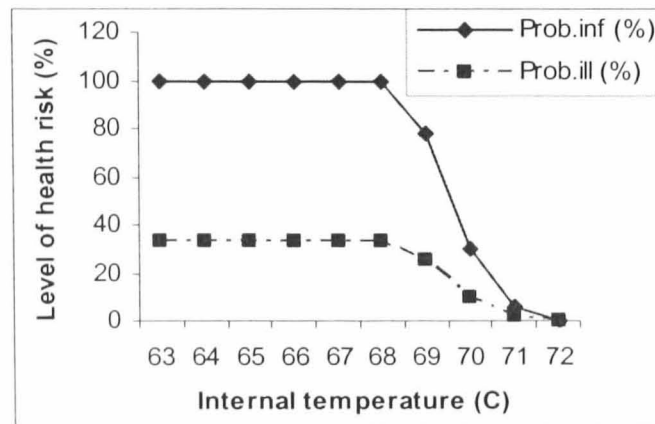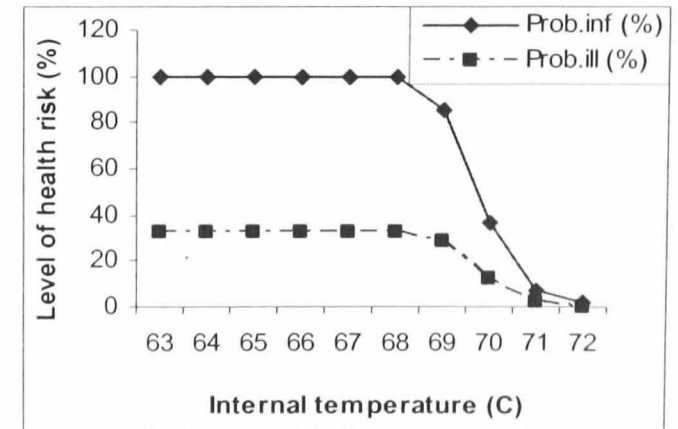


a) Dose of *Campylobacter* (MPN/g)　　　　b) Health risk female group　　　　c) Health risk male group

**Figure 8.5** Level of health risk when increasing internal temperature given 1 minute of exposure time to heat treatment

**3) Increase of internal temperature given 2 minutes that the protected area is exposed to the heat**



a) Dose of *Campylobacter* (MPN/g)

b) Health risk female group

c) Health risk male group
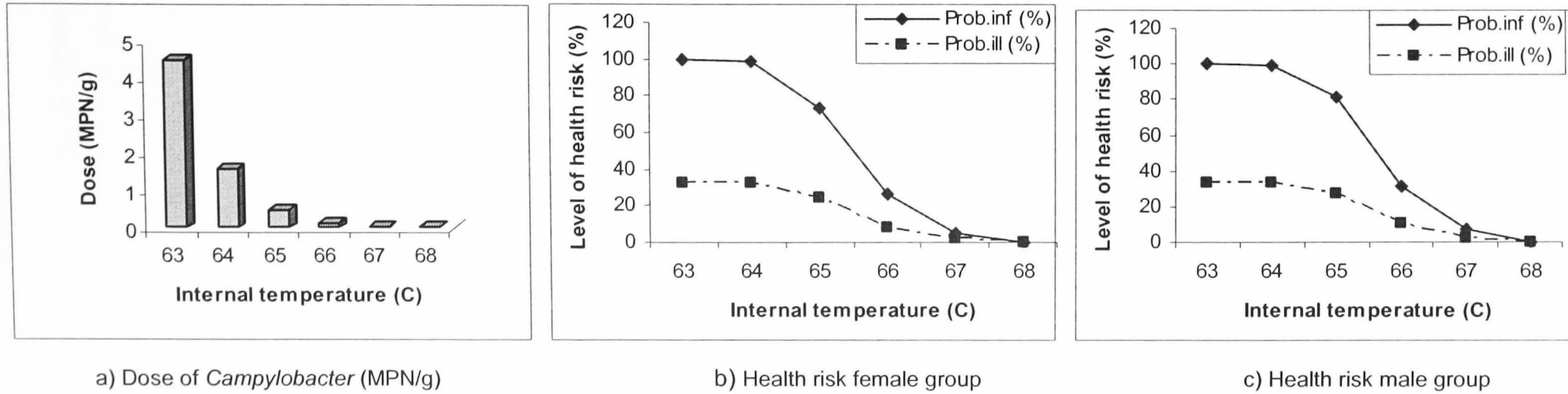
**Figure 8.6** Level of health risk when increasing internal temperature given 2 minutes of exposure time to heat treatment

**4) Increase of internal temperature given 3 minutes that the protected area exposes to the heat**



a) Dose of *Campylobacter* (MPN/g)

b) Health risk female group

c) Health risk male group
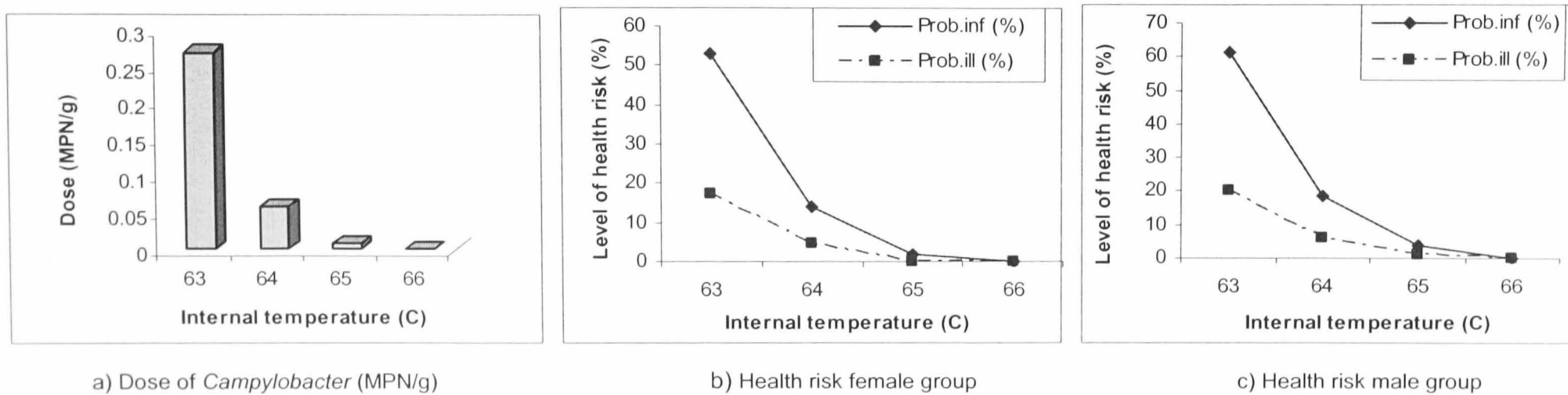
**Figure 8.7** Level of health risk when increasing internal temperature given 3 minutes of exposure time to heat treatment

# 8.5 Discussion

The health risks from *Campylobacter* are considered in relation to probability of infection and illness. These negative consequences can be induced by exposure to potentially hazardous organisms at different levels on the health of consumer. Both the probability of infection and probability of illness are taken into account. The risk is dependent on the circumstances which are influenced to various degrees by the pathogens, host characteristics and food matrix including condition of ingestion. It is important that any consideration of risk to human health from *Campylobacter* (with and without antimicrobial resistance) focuses on the moment occurring at **the time of consumption of the food (chicken or salad)**.

## 8.5.1 The comparison of relative health risk associated with three types of chicken

The results from the BIC group harbouring very high numbers of *Campylobacter* with high antimicrobial resistant isolates show the association between the probability of infection and the exposure dose of potential (viable) *Campylobacter*. The consumption of a BIC-meal therefore may result in high risk of infection.

It appears that the value of probability of illness related to the BIC consumptions based on probability of infection ($P_{ill/inf}$) use in the model is very high (33%). This may be as attributed to a bias due to the insufficiency of the human exposure study (Teunis *et al.*, 1999; Teunis *et al.*, 2005 and Vialette *et al.*, 2005) and of other data (*i.e.* cooking parameters, transfer coefficients related to hygiene practices in a private kitchen).

If elevated internal temperatures ($63^0$C-$72^0$C) are achieved for a sufficient length of time (1-5 minutes), the probability of illness related the BICs can be reduced from 33% to <1%. Clearly, the use of a risk model and data must be carefully considered. Ineffective risk model or insufficient data may lead to an inaccurate outcome. A sensitivity analysis has hightlighted the need for careful consideration of the model parameters and data used (*i.e.* the mean values of internal temperature and time).

As stated before, this study aims to investigate the relative a health risk of *Campylobacter* following the consumption of three groups of chicken, which were supplied from the different rearing systems and distributed from a slaughterhouse with different packaging methods. Thus, the dose-response relation model is still able to reliably demonstrate **the difference of health risk induced between these three types**. As a result, the consumption of chicken meals or salad associated with the BIC group would cause the infection and illness with a higher probability than that related to the PIC and the POC groups. It is also to state that the consumption of the PIC causes lowest consequences, in particular, after being cooked. The

consumption of salad contaminated with the uncooked chicken contributes a higher risk than that from a chicken meal properly cooked.

This suggests that the degree of a health risk at the time of consumption is associated with the concentration of *Campylobacter* in the carcass before and after cooking. However, this assumes that the organisms can survive the human immune system and initiate an infection (Mead, 2004). Moreover, this is clearly influenced by pathogens and food matrix, with host characteristics assumed to be constant. It is important to note that these results provide the information for general population (not for a specific at risk groups), suggesting that if it is to be used for a vulnerable group, young children, elderly people, immuno-compromised host, *etc.*, the current dose response model may not be reliable. Since the immune system of these groups may be deficient or lower than that of healthy group, the dose that can initiate an infection is likely to be lower than that for the general population. However, it is unlikely to that a dose-response model for these individual groups could be developed from current epidemiological data because of the sporadic nature of *Campylobacter*. This concern has been recognised in several studies (Anderson *et al.*, 2001; Adak *et al.*, 2002; Adak *et al.*, 2005). The probabilistic approaches have been applied to the QMRA model in attempt to include both uncertainty and variability due to the lack of data or some limitations of the approaches (Teunis *et al.*, 1997 and Kang *et al.*, 2000).

It is interesting to note that the consumption of salad contaminate with uncooked chickens may cause a higher risk to human health than eating a chicken meal. The consequence of cross-contamination may be a key factor in increasing or decreasing health risk. Thereby, the magnitude of the risk is highly dependent on individual food-handling practices (Christensen *et al*, 2001; Rosenquist *et al.*, 2003; Bartholomew *et al.*, 2005 and Nauta *et al.*, 2005b). Individuals vary greatly in the stringency of their kitchen hygiene, so the data on personal hygiene in the kitchen should be taken into account. Since there are currently few studies on food-preparation practices in private kitchens, it is difficult to quantify precisely doses related to cross-contamination, which affect the level of the risk. However, with probabilistic approaches applied to the estimations, these findings can support the notion that a great deal of health risk following the consumption of a contaminated chicken or other food commodity could be eliminated or reduced with good hygiene practice in the private kitchen (Bartholomew *et al.*, 2005 and Nauta *et al.*, 2005b). In addition, a proper cooking of a chicken alters the health risk significantly, in particular, on carcasses harbouring low concentrations of *Campylobacter*. For the carcasses harbouring high concentrations, bearing a high risk, it may be necessary to combine several approaches in order to reduce the risk. Although, a decrease in the numbers of *Campylobacter* occurs after refrigeration, other consequences which have not been investigated and elucidated may come to light following refrigeration. Using the storage under refrigeration to reduce the number of organism should not be recommended to consumers.

## 8.5.2 The comparison of relative health risk related to antimicrobial-resistant *Campylobacter*

Estimation of health risk related to antimicrobial resistant *Campylobacter* involves numerous factors. It requires a large number of data. It is difficult to directly relate contributions from antimicrobial resistance in *Campylobacter* to human health following the consumption of chicken. Until now there is no evidence to demonstrate a direct relationship between antimicrobial resistance in animals and in humans. Several studies showed that antimicrobial resistance in humans comes from a number of sources (*i.e.* overuse of microbials in humans, environment). Currently, there has been one study that performed a probabilistic risk assessment for fluoroquinolones resistant *Campylobacter* (FQ-*Campylobacter*) (Bartholomew *et al.*, 2005). However, this recent work, using the CVM-risk model quantified the relationship between amount of chicken harbouring ABR-*Campylobacter* consumed and a number of cases of human campylobacteriosis with fluoroquinolones resistance phenomena. It appears that both prevalence of a positive-*Campylobacter* chicken and a proportion of FQ- *Campylobacter* in the chickens were considered at the retail point (at which the carcass is a raw product), even before cooking. Herein, the CVM model does not focus on the risk at the time of consumption and thus does not reflect to the real situation. Nonetheless, at present, no adequate and appropriate model is available for predicting the health risk of antimicrobial-resistance *Campylobacter* at the time of consumption.

Health risk related to antimicrobial resistant *Campylobacter* in this context was considered as not only the additional risk to human campylobacteriosis, but also the comparative risk following the consumption of three different types of chicken. Therefore, two models were used in parallel for assessing the health risk. Whilst, the CVM model was selected because it is only a quantitative risk model currently used for FQ- *Campylobacter*, the new modified model was developed for quantifying health risk related to the exposure dose at the time of consumption.

Using the CVM model, the health risk from the resistant isolates (to either erythromycin or nalidixic acid) of *Campylobacter* isolated from the BICs is high and is different from the other two groups (PICs and POCs) around 20%. There is no difference found between the PICs and POCs for these antimicrobials, except for ciprofloxacin resistance. It is become clear that as the organic chickens are free from ciprofloxacin-resistant *Campylobacter*, there is no additional health risk related to ciprofloxacin resistance to be concerned about.

The modified model (mQMRA) considers the health risk at the time of consumption of the chicken. The results found are very interesting. The health risks of CR- or ER- and NAR-*Campylobacter* following the consumption of the PICs and POCs diminish rapidly with heat treatment, in particular, when using the model given $T > T_C$. Notably, after cooking the additional health risk of CR- *Campylobacter* following the consumption of the PICs is zero,

being equal to that found to be associated with the organic chicken (completely free from CR- *Campylobacter*). In addition, the health risks related to ER- and NAR- *Campylobacter* following the consumption of salad contaminated with *Campylobacter* from the PICs are lower than those found contaminated with *Campylobacter* from the POCs and BICs. This can be explained by the initial lowest number of *Campylobacter* isolated from the PICs compared with other two groups.

The additional health risk of these three antimicrobial resistant *Campylobacter* associated with the consumption of a chicken meal or salad associated with the BICs remains high. This suggests that the numbers of *Campylobacter* in the raw carcasses play a significant factor for the estimate of a health risk to consumers. If the carcass harbours low concentrations of the organism and it is treated with the proper cooking or food-handling, the health risk of the antimicrobial resistant *Campylobacter* can be diminished to as low a level as that found in the carcass free from the antimicrobial resistant isolates. In contrast, a carcass harbouring high level of this organism remains a high risk associated with antimicrobial resistance to be concerned about. However, the dose-response model used for estimating health risk was based on one human exposure study. It is possible that the model and availabilities of data are thus leading to an inaccurate estimate. The results from a sensitivity analysis support this notion.

Compared with the CVM model, the mQMRA model provides a sounder basis for the estimation of the risk at the time of consumption. Interestingly, both models indicated that the consumption of the BICs can cause high risk to human health in terms of illness and failure of antimicrobial treatment. Whilst the result from the CVM model suggests that the consumption of both the PICs and BICs (intensively reared chicken) can cause an infection with *Campylobacter* resistant to ciprofloxacin, the mQMRA shows that after heat treatment the risk of CR- *Campylobacter* related to the PIC group is completely eliminated. Furthermore, the CVM model showed that the consumption of the PICs can cause higher additional risk related to CR-, ER- and NAR- *Campylobacter* than those by the POCs. In contrast, the mQMRA showed that the consumption of the POCs may lead to more health risk regarding ER- and NAR- *Campylobacter* than that by the PICs.

Whilst the CVM model seems to estimate the health risk at the upper bound (a conservative approach for the worst case) (Anderson *et al.*, 2003 and Cox and Popken, 2004), the mQMRA using the probabilistic approach includes both the best case and the worst case. Different findings due to use of different models can introduce a conflict for consumers. If this is distributed to the public without clearer explanation, consumers would be confused with this information. The results from the CVM model could additionally lead to public concern on the effectiveness of antimicrobial therapy for campylobacteriosis because ciprofloxacin is the drug of choice in treatment of campylobacteriosis and broad-spectrum

antimicrobials used for the infection with an additional complication, in particular, in the vulnerable group (Helms *et al.*, 2005).

It is difficult to compare findings here to other studies because most studies have not carried out the probabilistic health risk assessment for ABR- *Campylobacter* following the consumption of chickens. In addition, none of those attempted to determine the comparative risk between intensively and organically reared chickens. Moreover, whether the carcass is sold as a pre-packaged or unwrapped product has not been taken into account. In other words, there is no evidence of comparison of cross-contamination occurring at the retail point. Thereby, the cross-contamination after the processing at slaughterhouse has not been considered clearly.

Each model has limitations as it is constrained by unresolved uncertainties. It appears that no robust evidence has verified the direct transfer of antimicrobial resistant *Campylobacter* from animals to humans via the consumption of foods (Bartholomew *et al.*, 2005). Ideally, in doing so, it is necessary to use an effective surveillance system to track both resistant organisms and antimicrobial use, providing the information on antimicrobial use which should be able to relate back to the information on resistance. In addition, there is a need to collect definite consumption data and information of what product is thought to be the major sources of *Campylobacter* and antimicrobial resistance. It is useful to consider the health risk from *Campylobacter* with and without antimicrobial resistance using different models and assumptions. One model or assumption can provide supportive data or information for the other. For example, Hurd *et al.*(2004) and Cox (2005) applied the event hazard or fault scenario to determine a public health consequences of antimicrobial use in animals. This method is a deterministic risk assessment which requires a great number of extensive scientific and numeric data. Bartholomew *et al.*, (2005) considered the event obtained from reported campylobacteriosis related to the consumption of chickens at consumer level. The former provides the overview of the problem. The latter focuses on a specific group.

## 8.5.3 The sensitivity of the model parameters

In spite of scarcity of data, the risk can be further predicted using the modelling approaches. However, the result must be interpreted following the assumptions and conitions fed to the model. In addition, the sensitivity of a change in parameter to the outcome must be considered. This notation can be demonstrated so far with the previous results of health risk related to the consumption of the BIC-meals. The very high health risk was found to be related to the BIC group harbouring high numbers of *Campylobacter*. However, the risks were characterised using the assumptions and data from the literature. There are two parameters involving the heat treatment model: i) time ($t_i$) that the protected area exposes to the internal heat and ii) internal temperature (T) reaching the protected area. The ranges of time of exposure to internal heat fed into the model are 0.5-1.5 minutes [Risk Pert (0.5,1,1.5),

mean value ~ 1 minute] The ranges of internal temperature fed into the model are 60 – 65°C [ RiskPert (60,64,65), mean value~ 63°C]. There are currently no other studies investigating the effect of cooking with longer time and higher internal temperature on *Campylobacter* carried out in chickens.

The health risks related to the BIC group can be reduced to as low a level as those found in the PICs and the POCs when the time or internal temperature was slightly increased compared to the values previously used. This suggests that the health risk is sensitive to a change of the variable time and temperature. Therefore, the models selected for use are sensitive to variation in the inputs. However, this demonstration generated using mathematical equations and is not a real situation. More studies related to the association of time, internal temperature and *Campylobacter* level in the protected area are required. It is questionable whether it is possible to increase the heat treatment or time of cooking sufficiently high as to be able to reach the point as demonstrated above without deterioration of the meals. It is noted that, changing of time and temperature does not affect the health risk related to a chicken meal given $T<T_C$ and salad.

## 8.6 Summary

The health risks (probability of infection and probability of illness) were calculated using exposure doses to *Campylobacter* (with and without antimicrobial resistance) calculated in Chapter 7. The calculations were performed based on the assumptions from the literature related to establishment of probability of infection and probability of illness. Additional data (*e.g.* seving size of a chicken meal) were obtained from other studies. The results in this chapter are as follows:

1. When comparing the three types of chicken using currently available data, the risk of *Campylobacter* associated illness related to the consumption of chicken meals from the BICs is higher (33% probability) than that related to the POCs (1-24% probability) and the PICs (0-7% probability). However, this is the worst case scenario. The best case scenario is that the elevated internal temperatures (63°C-72°C) can be achieved for a sufficient length of time (1-5 minutes). It results in the reduction of the health risk associated with the BICs from 33% to <1% (section 8.4.3 and Figures 8.4 to 8.7). It becomes clear that the risk model and data fed to the calculation must be carefully selected as they may result in an inaccurate outcome.

2. The consumption of salad contaminated with *Campylobacter* transferred from a *Campylobacter*-positive PIC and POC is more likely to cause illness than that related to an adequately cooked chicken meal (16-20% for the POCs, 31-32% for the POCs and 33% for the BICs. However, this contamination is related to the uncooked chicken, suggesting that the reduction of health risk can be achieved by good hygiene practices in the kitchen.

3. Using the CVM-model, the additional health risk associated with ABR-*Campylobacter* is found to be highest following the consumption of meals prepared from the BICs (Table 8.10). However, the CVM model considers the association between the prevalence of *Campylobacter*–positive chicken at retail outlet and the numbers of cases of campylobacteriosis with antimicrobial resistance. This does not represent real health risk occurring at the time of consumption. It could be therefore the worst case scenario of health risk associated with ABR-*Campylobacter*.

4. Using the mQMRA, the risk of ABR-*Campylobacter* associated illness is found very high following the consumption of meals related to the BICs (33% probability of illness). However, if internal temperature and time of thermal at the protected area are increased to higher levels than those stated in the assumptions, the health risk related to the BIC can also be reduced.

5. There is no apparent additional health risk related to ciprofloxacin resistance following the consumption of chicken meals prepared from organic chickens. The consumption of the PICs- and the BICs-chicken meals may lead to *Campylobacter* infection with antimicrobial resistance. The risk related to ciprofloxacin resistance related to the PIC was lower than that related to the BICs when using current data (<1% for the PIC and 33% for the BICs). The health risk related to antimicrobial resistance following the consumption of chicken meals can be reduced to a very low probability if elevated internal temperatures ($63^0$C-$72^0$C) are achieved for a sufficient length of time (1-5 minutes).

The results suggest that the risk model and data used must be carefully selected as they may result in an inaccurate outcome. It may be misleading and lead to an inappropriate interventions for the mitigation of the health risk. The banning animal antimicrobials to reduce selection pressure for resistance in bacteria may cause an increase numbers and consequently in the incidence of illness. However, if antimicrobials were allowed to be freely used on the farm, over time *Campylobacter* may develop high resistance genes and resulting in *Campylobacter* load in chicken can referring to high numbers. This could potentially lead to a considerably higher risk to human health. The effectiveness of an intervention for the management of risk related to *Campylobacter* with and without antimicrobial resistance may involve several factors. It cannot be resolved by a single intervention, for example banning antimicrobial use in animal or controlling farm practices, without also regulating at the point of sale. Finally, it is important to understand the effect of any intervention on other foodborne infections such as *Salmonella* and *E. coli* in order to achieve an intregrated approach.

# CHAPTER 9

# Potential Interventions for the Management of Health Risk related to *Campylobacter* and Antimicrobial Resistance in Poultry Production

## 9.1 Background

### 9.1.1 General

Although *Campylobacter* infection does not cause severe illnesses (except for the vulnerable groups) unlike those caused by *E.coli O157:H7* or *Salmonella*, it has been recognised as the most common pathogen amongst these foodborne pathogens found to be associated with enteritis. This is also found similar in the UK, where *Campylobacter* is currently the highest identified causes of bacterial infectious intestinal disease (IID)(*Campylobacter jejuni* 77.3%, *Salmonella* 20.9%, *Escherichia coli O157:H7* 1.4%). A high prevalence of positive *Campylobacter* has been found in poultry. The economic burden (*i.e.* medical costs and productivity losses) due to *Campylobacter* infection is large (Bean and Griffin, 1990; Bryan and Doyle, 1995; Buzby and Roberts, 1997; Adak *et al.*, 2002; Bull *et al.*, 2003; ACMSF, 2004 and Burgess, *et al.*, 2005). This confluence of human health and *Campylobacter* (zoonotic bacteria) associated with the consumption of poultry has been a growing public health issue. In addition, the emergence of antimicrobial resistance resulting in failure of antimicrobial therapy for this organism has introduced an additional potential risk to human health (Engberg *et al.*, 2004). It has been proposed that antimicrobial use in poultry husbandry may induce the development of resistance to antimicrobials in *Campylobacter* species with the risk that this resistance may pass to humans via the food chain (Endtz *et al.*, 1991; Loovern *et al.*, 2001; Sarah, 2002; Luber *et al.*, 2003; Randall *et al.*, 2003 and Wagner *et al.*, 2003). However, contribution of *Campylobacter* with antimicrobial resistance to human health from the consumption of poultry, mainly chicken, is not yet elucidated.

Several sources of *Campylobacter* infection and antimicrobial resistance in humans have been considered following the evidence identified by epidemiological data and case control studies (Endtz *et al.*, 1991; Evans *et al.*, 1998 and Helms *et al.*, 2005). Evidently, chickens may constitute one of the major sources of human campylobacteriosis. In addition, the association between the consumption of *Campylobacter*-positive chicken and the causation of illness in humans has been indicated by several studies (Bryan and Doyle, 1995; Corry and Atabay, 2001 and Coker *et al.*, 2002). Other studies have demonstrated an association between the strains of *Campylobacter* in human infection and those found in retail chickens (Smith *et al.*, 1999; Kramer *et al.*, 2000 and FSA, 2003b). Chicken meals contaminated with *Campylobacter* usually look, smell and taste normal due to the odourless characteristic of this organism. Thus, the *Campylobacter*-positive chicken cannot be detected by visual

inspection (Koidis and Doyle, 1983). At the consumer level, the ingestion of undercooked chicken and improper handling of raw chicken have been described as important factors in *Campylobacter* infection (Worsefold and Griffith, 1997 and Christensen *et al.*, 2005).

Heat, particularly through cooking, has long been the principal method of eliminating pathogens in food (James and Corry 2000; and Solow *et al.*, 2003). Various heat treatment procedures have been suggested for decontamination of raw meat, *e.g.* hot water treatment (dipping or spraying), steam at atmospheric, high or reduced pressure, high intensity dry heat or microwave heating. Clearly, hot water treatment can significantly reduce the numbers of bacteria only after relatively long contact times and high temperatures (Corry and Atabay, 2001). However, some of these thermal approaches may have residual effects on appearance and taste. Both steam and microwave heating cannot treat the surface of poultry meat without causing some surface deterioration (Göksoy *et al.*, 2000 and Corry and Atabay, 2001).

The application of irradiation, *i.e.* gamma rays, electron beams, is an effective method for eliminating *Campylobacter* in poultry products after processing. It can also be applied to warm, chilled or frozen carcasses. However, this method is relatively expensive and is unattractive to consumers (Corry and Atabay, 2001). Although post processing, storage under refrigeration or freezing conditions will reduce the numbers of micro-organism in chicken products (Anderson *et al.*, 2003), this method is not recommended due to other concerns, *e.g.* quality, taste, nutrient losses.

During meal preparation at home, individuals can be exposed to *Campylobacter* from a fresh chicken through a number of pathways. Different studies have demonstrated that during meal preparation, cross-contamination through hands and work surfaces (*e.g.* cutting boards, utensils, *etc.*) is the key to widespread dissemination of *Campylobacter* (Christensen *et al.*, 2005).

Currently, a numbers of countries, including, Denmark, Canada, the Netherlands, New Zealand, Norway, the U.K. and the USA, have implemented control measures to reduce the prevalence of *Campylobacter* in poultry throughout the food chain from farm to consumer and subsequently the incidence and burden of illness in humans (Anderson *et al*, 2001; Anderson *et al*, 2003; ACMSF, 2004; Batz *et al*, 2004; Christensen *et al.*, 2005 and Nauta *et al*, 2005b). In some countries, risk management interventions were undertaken following formal risk assessment, whilst in others risk management has been performed without assessing the risk. However, in other countries (*e.g.* New Zealand) the risk profile of *Campylobacter* led to no further intervention (Lake *et al*, 2003). Most studies focused mainly on health risk from *Campylobacter* infection related to the consumption of chicken. There were only few studies that considered the risk of *Campylobacter* infection in conjunction with the antimicrobial resistance phenomenon. Furthermore, almost all management options were developed for intensively produced poultry rather than for extensive poultry (free-range

or organic flocks). This may have limited the choice of interventions to be implemented according to the criteria of rearing procedures (UK-DEFRA, 2005).

Some intervention options currently proposed for the control of foodborne pathogens in general were considered in this chapter. It is important to note that this study does not intend to provide a risk management strategy.

## 9.1.2 Current risk intervention options for the control of *Campylobacter* and other enteric pathogens in poultry production

Proposed risk intervention options for the control of foodborne pathogens including *Campylobacter* in poultry production include standards (*e.g.* good agricultural practices), guidelines and other control measures aimed at reduction of human illness resulting from infection with foodborne pathogens, *e.g.Campylobacter, Salmonella*, through the food chain A range of interventions for the control of *Campylobacter* in poultry have been developed and recommended by a number of organisations (FSAI, 2001; CAC, 2002a and FSA, 2003a). Preventative intervention can take place at any stage from farm to slaughterhouse, including transportation. The various options for preventative intervention ideally require using the best available data on the probability and distribution of risk and how risk can be reduced most effectively and efficiently.

Ideally, there should also be specific options for the control of *Campylobacter* that are appropriate to a particular rearing method, *i.e.* intensive or extensive (free range/ organic) rearing (WHO, 2004). These control measures would be more practical if designed for particular rearing practices. With the current state of poultry rearing practices, it is not possible to establish a precise option for intensive or extensive systems. Most interventions are formed as general guidelines for poultry production and thus can be applied to any foodborne pathogens at any stage of production. For example, hygiene measures can be generally applied to control *Campylobacter* or *Salmonella* or *E.coli* colonisation in birds on the farm or to control cross-contamination at the slaughterhouse (ACMSF, 2004). Bio-security[14] practices have been introduced to both intensive and extensive farm in order to reduce the flock prevalence of foodborne pathogens. However, for extensive farms, allowing birds to be outside during daytime, some practices, such as human traffic control[15] and access restriction, need to be adjusted before implementation. In addition, to date, there is no specific control measure or intervention available at the consumer level.

The Codex Committee on Food Hygiene (CAC, 2002a) recommended control measures for the control of *Campylobacter* in poultry and management options for implementation in the

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[14] Bio-security means keeping farm units secure from the introduction of new diseases, and minimising the spread of disease within herd.
[15] Human traffic control is to restrict visitors entering the range or building.

various stages of production from farm to table. Table 9.1 summaries assumptions and statements of interventions based on the current information and knowledge (CAC, 2002a and CAC, 2002b). It may be noted that: i) there are the differences and limitations in these approaches and ii) these interventions are general practices that can be applied for any foodborne pathogens.

**Table 9.1** The control measures for foodborne pathogens as applied for the control of *Campylobacter* in poultry (adapted from CAC , 2002a)

| Staging of process | Intervention |
|---|---|
| Farm | **Extensive (free range/organic)**<br>♦Promotion and application of good agricultural practice[*] (GAP) for structural and general measures[16], operational measures[17]<br>♦Control of antimicrobial and chemical usage<br>♦Vaccination<br>♦ Phage treatment (experimentation)<br>**Intensive rearing**<br>♦Biosecurity and hygiene measures<br>   - house construction and ventilation<br>   - environmental hygiene surrounding poultry houses<br>   - cleaning and disinfection<br>   - water<br>   - visitors/vehicles<br>   - physical barriers<br>   - rodent and pet control, wild birds<br>   - thinning/crate hygiene<br>   - number flock per farm<br>   - single species farms<br>   - pre-slaughter testing |
| Transport | ♦Control of the breach of biosecurity and hygiene addressing<br>   - catching hygiene and crates<br>   - trucks/drivers<br>   - stress-time/distance<br>   - segregation of flock |
| Slaughter | ♦ Testing on entry<br>♦ Reduction of cross-contamination<br>   - scheduling (end of day)/ channelling; in combination with decontamination<br>   - Good manufacturer practice (GMP)/Good hygiene practice (GHP)[*]<br>   - carcass treatment (decontamination)<br>   - Packaging<br>♦ End product testing<br>♦ Application of HACCP (Hazard Analysis Critical Control Point) |
| Retail | ♦ Hygiene measures for processing at retail<br>♦ Packaging control (packaged/unpackaged) |
| Domestic kitchen and catering | ♦ Promotion of GHP particularly focusing on<br>   - cross-contamination between food commodity<br>   - personal hygiene during food preparation and cooking. |

**\*Note:**

**Good agricultural practice (GAP)** is the methods of land use which can best achieve the objectives of agronomic and environmental sustainability. GAPs are described in several different Codes of Practice designed by producers organizations (*e. g* COLEACP), importers and retailers consortia (*e.g.* BRC, FPC, CIMO, EUREP) and Government bodies

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[16] Structural and general measures, *e.g.* poultry house, visitors
[17] Operational measures, *e.g.* cleaning, disinfection, insecticide control

representing consumers (*e.g.* UK Food Standards Agency). Many UK supermarkets have in addition their own codes of practice which their suppliers must satisfy. American retailers use a different standard called SQF 2000, which is based on HACCP (NRI, 2003).

**Good Manufacturing Practice (GMP)** is the minimum sanitary and processing requirements for food companies. GMPs are the part of quality assurance that ensures products are consistently produced and controlled to the quality standard appropriate to their intended use and as required by the marketing authorisation (WHO, 1997).GMPs are not designed to control specific hazard, but are intended to provide guidelines to help processors' produce safe and wholesome product (FDA, 1999)

**Good hygiene practice (GHP)** is a set of practices, essential to support HACCP programme. It includes all control measures of food safety during the manufacture, storage and distribution that ensure the minimisation or prevention of contamination of the food from excessive micro-organisms, physical and chemical contamination (CIEH, 2004).

## 9.2 Current risk intervention practices and questions

Potential interventions as applied for the control of *Campylobacter* from production to consumption have been proposed by several organisations. The main objectives of these options are the reduction of *Campylobacter* prevalence and cross-contamination in poultry. However, most interventions have not considered other additional consequences, for example, antimicrobial resistance (CAC, 2002a; WHO, 2002c; ACMSF, 2004; WHO, 2004 and Batz *et al*, 2004).

The mitigation for antimicrobial- resistant *Campylobacter* is however approached separately by different organisations. Since most studies suggested that the rates of resistance to certain antimicrobials have been increasing following the introduction of antimicrobials to animal husbandry, the precautionary principle has been introduced to mitigate health risk related to antimicrobial resistant *Campylobacter*. The principle is that banning or prudent antimicrobial use may achieve a reduction of human risk from antimicrobial resistant *Campylobacter* (Endtz *et al.*, 1991; Evans *et al.*, 1998; Teuber, 2001; Bartholomew *et al.*, 2005 and Helms *et al.*, 2005).

Contrary to this assumption, Phillips *et al.* (2004) and Cox (2005) demonstrated potential human health benefits of antimicrobial use in food-producing animals as well as postulating that withdrawing antimicrobials may increase the incidence of campylobacteriosis. It is important to note that this controversy may result from the different predictive models used, assumptions or data and information available, but it could also result from a failure to consider the risk tradeoffs that occurs in this circumstance (Graham and Wiener, 1995).

The risk model for fluoroquinollone-resistant *Campylobacter* (FQ-*Campylobacter*) proposed by the center of veterinary medicine (Bartholomew *et al.*, 2005) considered the health risk related to FQ-*Campylobacter* following the consumption of chicken harbouring resistant isolates. The impact was calculated by using the numbers of cases of human campylobacteriosis per year and the rate of FQ-resistance in raw chicken carcasses. Actually, the chicken is consumed as a cooked meal. Thus, it is possible that the outcome is overestimated because cooking (heat treatment) is able to reduce the numbers of *Campylobacter* whether it is susceptible or resistant to antimicrobials, resulting in reduction of health risk.

Cox (2005) estimated health risk associated with antimicrobial resistance by using the basic logic of multiplicative approach. The approach was based on the association between the expected incremental numbers of adverse human health consequences per year caused by an antimicrobial resistance and those prevented by an antimicrobial use in chicken. The result demonstrated that continued use of virginiamycin in chickens would prevent at least thousands of times more illness days. The application of the precautionary principle (withdrawing antimicrobial use) is thought to be a non-specific approach and would rather contribute other unexpected consequences, for example, increase of the incidence of illness in consumers.

Risk intervention options for the control of *Campylobacter*, whether it is susceptible or resistant to antimicrobials should be based on knowledge of relevant factors, *i.e.* principles, assumptions, data available, socio-economic impact, cost-benefit consideration, acceptability, culture, attitude, environment impact and risk transfer phenomena. A number of questions arise from current risk management options (Table 9.1) and a number of recent reports of how the risk mitigation options were implemented in some countries (Denmark, Netherlands, and the United Kingdom) (Christensen *et al.*, 2001; ACMSF, 2004; Batz *et al.*, 2004 and Nauta *et al.*, 2005b). From these the following issues emerge:

### 1) Maintenance and consistency

The recommendations or suggestions or regulations proposed require high responsibility and incorporation among regulators, stakeholders and workers to carry on the practices following the guidelines. It is difficult to maintain the persistence of workers in continuing the good practice. Therefore, long term maintenance would be difficult. For example, following on-farm biosecurity practice, changing footwear believed to be important for preventing the entry of *Campylobacter* into broiler flocks is required for every person who accesses to the farm. This recommendation would be strictly carried out at the begining; however, it would probably be relaxed as time goes by.

## 2) Difference in practice between countries

The systems of production in one country are usually different to some degree from those in others, *e.g.* size of industry, climate. Changing footwear, for instance, may not be effective on an extensive farm since birds in this farm are allowed to be outside in daytime (UK-DEFRA, 2005). Again, single species farms may be possible for an extensive farm. However, this would not be able to protect the extensive birds from other animals from outside, in particular, wild animals.

## 3) Inter-relationship between the control measures

The options have been set up for an individual part of the pathway of poultry production from farm to consumption (*e.g.* on farm, slaughterhouse, transport). However, the control of pathogenic contamination in poultry (chicken) meat leading to infection in humans involves many factors. Some factors are interconnected and may be either synergistic or antagonistic. If each risk management option is constructed without reference to the previous or following step, some evidence may be missing and this might impair effectiveness. For example, during the transport of birds from farm to slaughterhouse, the control measures are not clearly documented. It is possible that a flock negative at the exit of a farm becomes a positive flock at the entrance of a slaughterhouse from cross-contamination (Slader *et al.*, 2002).

Correctly followed biosecurity practice specifies that transport crates must be washed in a soak tank water (ACMSF, 2004). However, there is no guidance on how to discard the wash water after use. It can be therefore assumed that if the water is discarded into the environment without decontamination, cross-contamination could occur. Figure 9.1 shows the cycle of *Campylobacter* in the human-chicken-environment pathway.
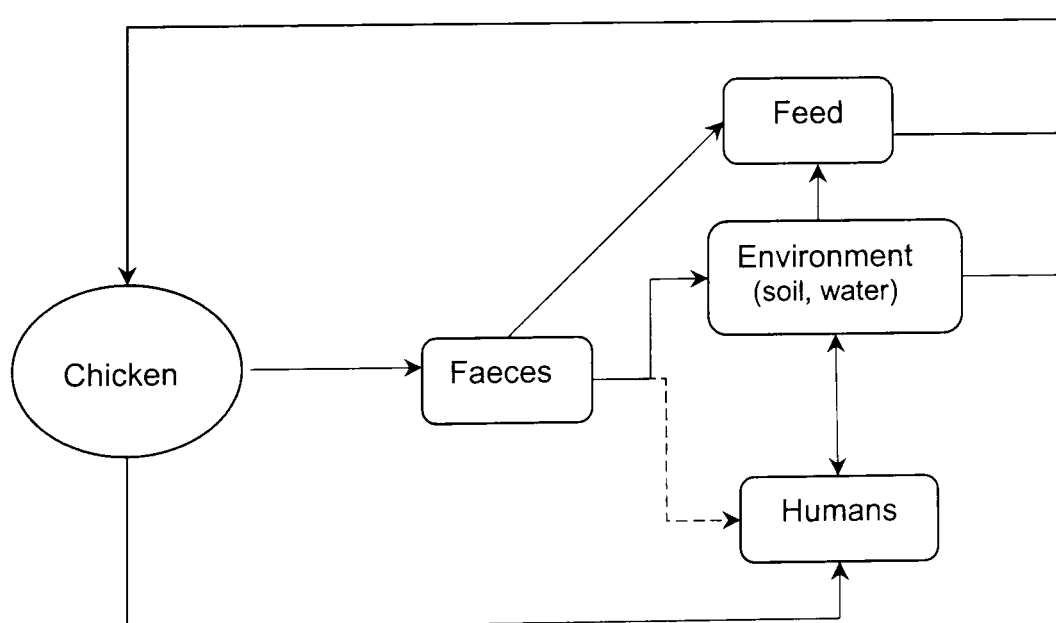


**Figure 9. 1** Human-chicken-environment pathway of *Campylobacter*

## 4) Availability of up to date current scientific data

Recommendations or regulations may not rely on current scientific data. In general, testing and channelling [18] of positive birds is recommended for slaughter and processing in slaughterhouses. Usually, extensive (organic and free range) birds are processed before intensive birds on the same day. El-Shibiny *et al.*, (2005) as well as other studies (including this study) showed that the organic chicken harbours higher numbers of *Campylobacter* than that found in intensively reared chicken. Channelling by processing the extensively reared bird before the intensive may increase cross-contamination of *Campylobacter* rather than reducing it. It is therefore necessary to review and update data consistently.

Additionally, at present there are no practical and specific control measures for antimicrobial resistant *Campylobacter* in poultry. Most recommendations only involve the development and establishment of a surveillance programme on general non-human usage of antimicrobial agents and a surveillance programme on antimicrobial resistance in bacteria from animals to humans (EMEA, 1999; FAO, 1997; FSAI, 2001 and WHO, 2004).

The outcome of this study (probability of infection and probability of illness from ABR-*Campylobacter* presented in Chapter 8) indicate that the most important factor causing the health risk to humans is the number of organisms found in chicken carcasses. A chicken carcass harbouring ABR-*Campylobacter*, but with low numbers, would be less of health risk than a carcass harbouring high numbers of *Campylobacter* susceptible to antimicrobial agents. The withdrawal of antimicrobial use in food-producing animal would not therefore be an appropriate solution. In the light of this, it is necessary to re-consider mitigation options for antimicrobial resistant *Campylobacter*, in particular, for organically reared chickens which tend to be exposed to higher environmental levels of *Campylobacter* (El-Shibiny *et al.*, 2005 and Chapters 7 and 8).

## 5) Enforcement law and practice

Currently, most countries in the European Economic Area and North America issue guidance or Regulations or Acts for food hygiene and safety at the retail point or other equivalent premises, to enforce and ensure that food products are safe for consumers (Montforts *et al.*, 2004). However, in reality, effective enforced control relies on authorised officers in terms of inspection, enforcement and managerial responsibility. The findings from the BIC group (unwrapped intensively reared chickens purchased from butcher's shops) (Chapters 5 to 8) suggest a need to ensure that control measures are undertaken competently. The qualifications of food inspection officers are important. The food inspection officers should comprehend their task. For example, sampling in accordance with

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[18] Channelling use for holding and uploading food animal during slaughtering process in order to prevent cross-contamination.

microbiological examination must be undertaken by the officer who is properly trained in the appropriate techniques.

## 6) Consumer guidance

A number of studies from several countries have reported programmes for mitigation of poultry consumption related to health risk from *Campylobacter* and other foodborne pathogens (Christensen *et al.*, 2001; ACMSF, 2004; Batze *et al.*, 2004 and Nauta *et al.*, 2005b). Few interventions have been developed and implemented on the farms, at slaughterhouses or, even, at retail premises. None of these have addressed consumer perception. The subject of health risk is yet to be directly and meaningfully communicated to consumers. For example, some countries campaign on personal hygiene of food handling in the household. However, there are no monitoring measures in place to ensure that the risk is perceived transparently by public (Frewer, 2004 and Renn, 2004).

As such, it would not improve personal hygiene practices during food preparation, becuase most suggestions or recommendations are too abstract and unclear for consumers. For example, it has been stated that *Campylobacter* infection is related to the consumption of undercooked chicken (Adak, *et al.*, 2005). It is then suggested that chicken must be cooked properly. This suggestion is ambiguous and leaves the public without clearer explanation of which is the correct level of cooking for chickens.

In addition, the mitigations do not focus on a specific group, *i.e.* young children or immunocompromised host. It is well recognised that members of these groups are vulnerable, and tend to develop complications after infection. In particular, the current risk assessment models are developed from feeding trials on healthy volunteers, who may have better immune function. It is therefore necessary to consider special risk management options for these particular groups.

## 7) Cost benefit analysis

Cost benefit analysis has not been taken into account meaningfully. The implementation of potential control systems always implies financed investment. Any additional costs resulting from interventions must be put onto the cost of final products and consumers eventually. In addition, since broiler chicken production is extremely price competitive, this would inevitably encourage illegal products (from illegal slaughterhouse or unknown sources of chicken), which may be cheaper in price, to be introduced to the markets. It is evident that the consumption of chickens from unknown sources may carry an increased risk to humans compared with pre-packaged properly labelled and identified chickens. This consideration is supported by the results of the BIC group expressed in Chapters 5-8.

# 9.3 Factors which should influence interventions

Intervention options in this study are considered in parallel with the current options presented in Table 9.1 and the rationales generated from the findings of this study. It is important to note that the risk assessment in this study is calculated for the general population. Special needs groups such as young children, elderly people, pregnant women or immuno-compromised hosts require better stringent regimes. However, more information is required in order to generate risk models for these specific groups.

This study focuses on the comparison of the relative risks from *Campylobacter* (with and without antimicrobial resistance) to human health from the consumption of different groups of chicken (PIC, POC or BIC). The implications for risk management are considered based on: i) the results from Chapters 7 and 8 related to *Campylobacter* with and without antimicrobial resistance at a consumer phase and ii) literature

On the basis of the findings of this study (Chapters 7 and 8), the relevant results include the following:

1. The most significant factors influencing health risk associated with *Campylobacter* are the number of organisms, personal hygiene practices and cooking procedures. Cooking procedures and personal hygiene in food preparation in a private kitchen can significantly alter the health risk from the consumption of *Campylobacter*-positive chicken.

2. The consumption of salad contaminated with *Campylobacter* transferred from a positive uncooked carcass can cause higher risk than that related to a chicken meal properly cooked.

3. The health risk associated with antimicrobial-resistant *Campylobacter* (ciprofloxacin, erythromycin and nalidixic acid) is found to be highest following the consumption of meals prepared from the BIC group harbouring the high numbers of *Campylobacter* with high resistance rate to antimicrobials.

4. The consumption of meals prepared from organic chickens is free from health risk related to ciprofloxacin resistance. However, the risks related to the other two antimicrobials (erythromycin and nalidixic acid) are still high, being equal to that following the consumption of intensively reared chicken whether the carcass is wrapped or not.

5. The health risk related to antimicrobial resistance following the consumption of meals prepared from PIC can be reduced to very low probability if the meal is cooked with the sufficient heat, which must be reach the area protected from heat of the carcass, such as, drumsticks.

6. The health risk in the general population associated with the consumption of positive- *Campylobacter* chickens is not influenced by the age and sex of the consumer.

As a result of above assumptions and findings from previous chapters, the primary factors influencing risk intervention options for *Campylobacter* at the consumer level can be summarised as follows:

1. The number of *Campylobacter* in the carcass is the most important factor causing the risk to human health. Therefore, the reduction of the number of *Campylobacter* in chicken is the critical control point of the health risk associated with the consumption of chicken in this context.

2. Antimicrobial resistance would cause additional risk to human health if the chicken carcass harbours *Campylobacter* with a high resistance rate as well as a high number of organisms.

3. Cross-contamination rather than undercooking is the main source of *Campylobacter* for a person consuming a meal prepared from chicken. Proper meal preparation can effectively reduce the risk. In particular, if the carcass harbours low numbers of organisms, the numbers can be reduced to level below the infective dose.

The evidence suggests that the health risk caused by *Campylobacter* is related to not only the prevalence of positive chickens but also the number of bacteria in each carcass and ultimately the dose ingested. Reduction of prevalence of *Campylobacter* contamination of carcasses and of the concentration of contamination has broadly equal importance in terms of risk reduction. However, the objective of achieving *Campylobacter*-free chickens is improbable without very vigorous and expensive control measures.

The intervention component in this study is concerned with reducing health risk caused by *Campylobacter* to as low as reasonably achievable (ALARA) taking into account economic considerations. Using the rationales stated above, mitigation options can be constructed following two scenarios: i) Reduction of the prevalence and number of *Campylobacter*, and ii) Reduction of the rate of resistance to antimicrobial agents. The first scenario can be carried out throughout the whole pathway from farm to consumption. As antimicrobial use on farms is recognised as the predominant factor for antimicrobial resistance in *Campylobacter*, the potential intervention for reducing the rate of resistance to antimicrobials must mainly focus on implementation on the farm.

## 9.4 Intervention scenario

Following the results of the risk assessment (Chapters 7 and 8), this study further explored the mitigation options recommended for foodborne pathogen. These options are selected and therefore applied for the control of poultry production in relation to reduction of health risk from *Campylobacter*. However, the limitations in data available prevent the application of

a full process of risk management including economics and public perception. Following the risk decision-making process presented in Figure 9.2, the potential intervention options (Block "B") were considered against two scenarios, which are: i) scenario 1: reduction of health risk related to all *Campylobacter* isolates and ii) scenario 2: reduction of health risk related to antimicrobial resistance in *Campylobacter*.
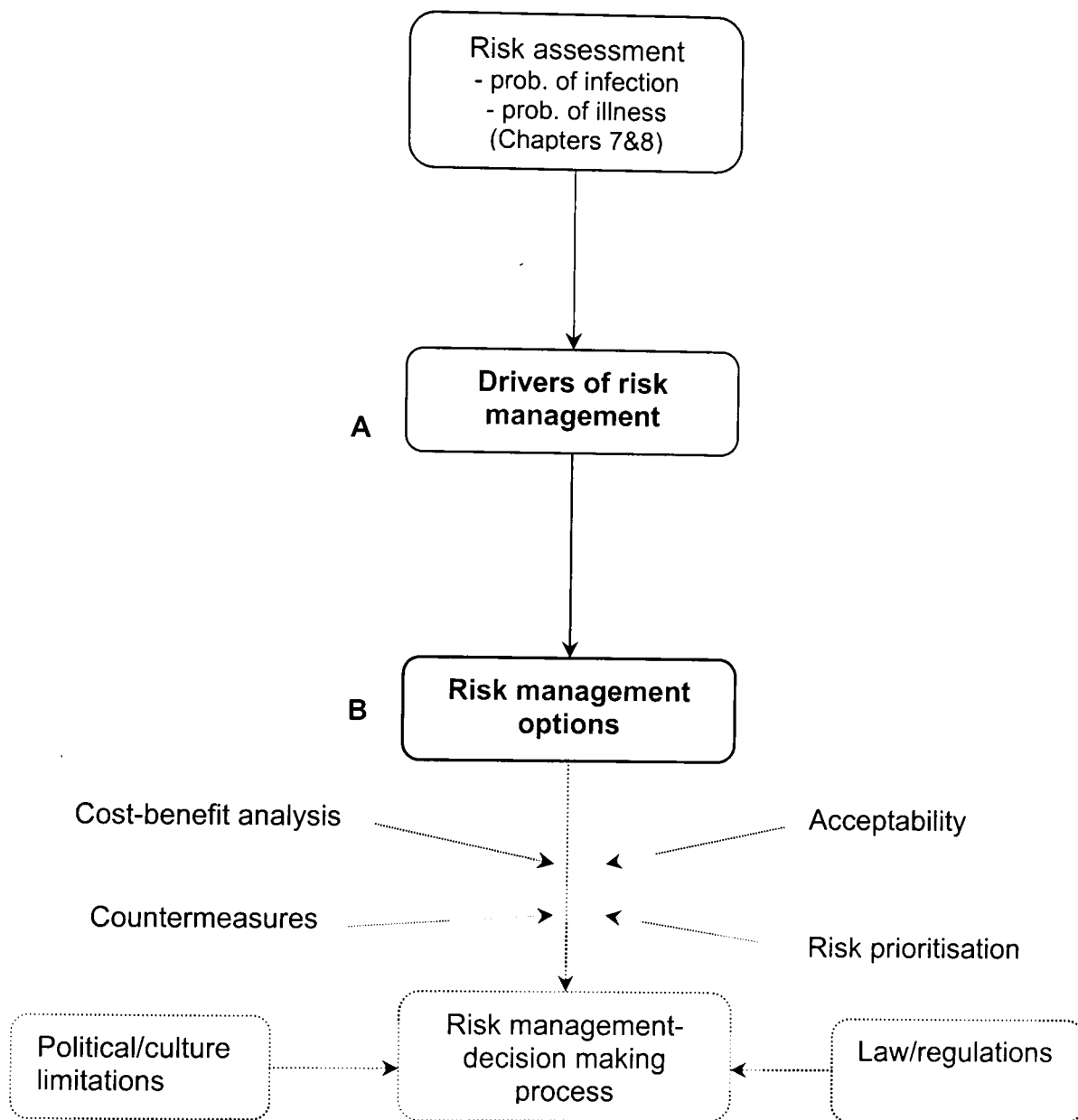


**Figure 9.2** A framework of the risk decision-making process

## 9.4.1 Scenario 1: Reduction of health risk related to all *Campylobacter* isolates

As explained in section 9.3, the mitigation for health risk of *Campylobacter* associated with the consumption of chicken can be performed by reducing either the prevalence or the number of organisms, or both. Shanker *et al.* (1990) found that after the first bird was infected with *Campylobacter*, widespread dissemination of this organism can take place

throughout the whole flock within 4-7 days. Thus, the reduction in numbers is easier to be achieved than the reduction in prevalence (in particular, the achievement of free-*Campylobacter* chicken). Under this scenario, the risk management options focus on the reduction of **Campylobacter load** on the carcass. The reduction of *Campylobacter* load can be considered from farm to consumption as shown in Figure 9.3.
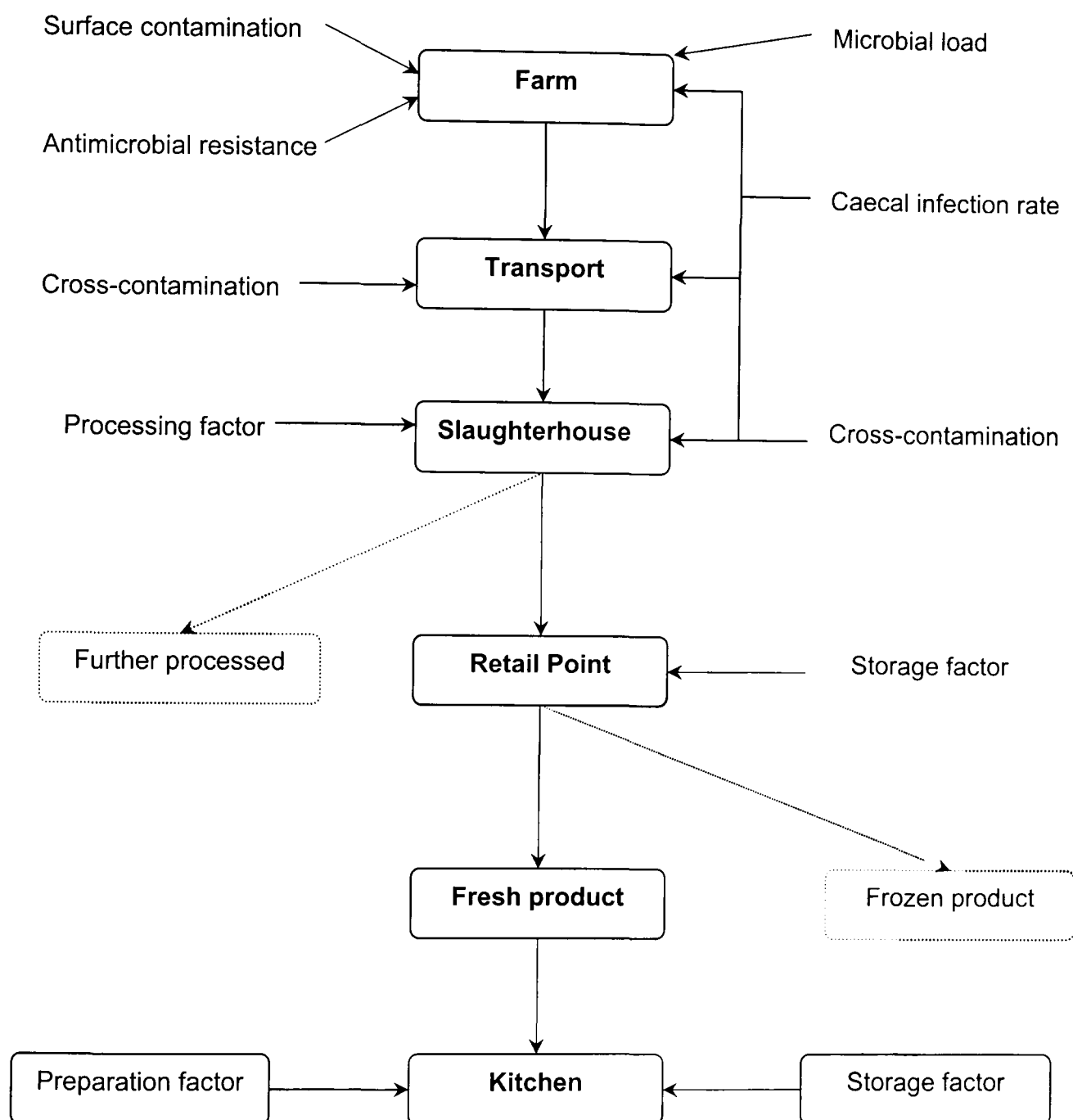


**Figure 9.3** A scheme of scenarios in reducing the *Campylobacter* load and antimicrobial resistance in the chicken.

The recommendations presented in the following issues were constructed based on the interventions generally recommended for the control of foodborne pathogens (e.g. *Salmonella*) in poultry production. The aim of reduction the microbial load, *Campylobacter* in this context, in chicken from production to consumption is now considered at each production stage.

# Rearing practices on farm

## a) On farm- Extensive rearing

**Recommendations are:**

1. Under the condition regarding the regulations for broiler chickens designated as organic products, biosecurity and GAP are essential measure for the control of *Campylobacter* on farm.

2. Vaccination is not practical and would add more economic burdens for producers. More studies are required to ascertain whether: i) vaccination is able to positively prevent the colonisation of *Campylobacter* in broiler chickens; ii) vaccination can have negative effect(s); and iii) if the benefit of vaccination can surpass the additional cost.

3. If possible, the environment of the farm (water, soil, feed and litter) must be inspected and monitored periodically. However, it is necessary to re-evaluate the need for monitoring. If the results are somewhat constant, inspection can become occasional.

4. Low-cost husbandry and veterinary support is a necessity for poultry production, in particular, small scale farms. Farmers must be instructed regarding proper husbandry procedures in order to improve skills and understanding of the current situation.

5. The study of the potential for transmission of infection between animal husbandry and wildlife, thought to be one of significant sources of *Campylobacter*, is urgently needed. This is particularly pertinent for extensive rearing systems in order to clarify how such relationships influence the microbial load. This would help in the development of practical risk management for an extensive farm.

6. The use of dietary modification has been proposed for reducing *E.coli O157:H7* population in cattle (Russell and Rychlik, 2001). Probiotics, *e.g.*fructo-oligosaccharides, can promote growth of the native microflora which can eliminate pathogenic bacteria from the intestine (Zopf and Roth, 1996). This may be an alternative procedure to promote intestinal health of birds (Callaway *et al.*, 2003). However, it needs more studies to identify the mechanism which affects the organisms in the gut.

## b) On farm- Intensive rearing

**Recommendations:**

1. The biosecurity or GAP or GHP is very effective for the control of cross-contamination (Mead, 2004). The control must extent to what will occur following

these practices, in particular, residual waste discarded into environment. Decontamination strategies must be included. However, further studies are required to understand the need and establishment of the treatment system for the environmental pollution on the farm. It is clear that it will cost more money to implement this treatment system. This also requires cooperation from several sectors, *e.g.* veterinary, scientist, ecologist, stakeholder, *etc*, which may not be easy to achieve. The attitude of people who have to follow the instruction may determine the degree of success of the intervention.

2. Although, intensively reared broilers are reared for commercial purposes, they are living and sensitive. To safeguard welfare[20] and avoiding suffering, a wide range of needs must be taken into account. Maintaining a good indoor environment in the building is essential for broiler welfare. Examples of important factors in the building are stocking density, live-weight of birds, ventilation rate, indoor temperature and type and management of drinkers. Fresh air and sunlight are beneficial for the health of birds. It necessary to apply stringent control to rearing conditions, for example, low stocking density of birds in a house in order to prevent overcrowded causing breast blisters and leg problems and eventually infection.

3. Dietary modification (similar to recommendation 6 of on-farm extensive rearing)

## Slaughtering and processing

The cross-contamination by *Campylobacter* from skin, hides or the alimentary tract cannot be completely eliminated during slaughtering procedures as currently practiced. However, implementation of the appropriate measures would help to minimise such contamination. In the United Kingdom, either domestic[21] or export-approved slaughterhouses[22] are governed by the Meat Hygiene and Inspection Regulations. The regulations provide guidelines for the processes of slaughter and cutting of carcasses. These are supervised by authorised officers of the local authority, who are trained for the purpose. The legislation controlling the production of meat is largely directed towards the reduction of the risk to humans from pathogenic organisms carried by animals. They tend to concentrate on the reduction of pathogenic organisms in meat and meat products, and therefore attempt to anticipate the routes by which meat could potentially be contaminated by this specific group of organisms.

Although, in practice, many slaughterhouses find it difficult to comply with the requirements, in order to gain legal recognition they have to adopt the recommended interventions. These are described in Table 9.1. As these interventions are essential measures for micro-organisms, in such a way the control of *Campylobacter* must follow the standards of the

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[20] The welfare of animal is defined as regards its attempts to cope with its environment, which may lead to disease and injury.
[21] Domestic slaughterhouse producing meat is not for export
[22] Export-approved slaughterhouse producing meat is for export

regulations. The efficiency of implementation of the approved control measures as standard operating procedures must be consistently high at all stages and all times. This needs to be governed by legislation via food authorised officers as well as the control measures being communicated to all personnel throughout the entire production.

It is more likely that the regulations will be stringently followed by medium or large premises, which are usually licensed. For small scale premises, it is difficult to complete the standard operating procedures without involving monetary investment as well as understanding the control measures. Most operate with old machines or old systems. **It is vital to reinforce** small scale premises to strictly adopt at least a basic quality assurance to the process.

Although the current control measures for *Campylobacter* have been recommended by a number of organisations, this study reinforces the need for recommendations by focusing on key factors arising from the findings in Chapters 7 and 8. These factors are:

### *1) Channelling procedure*

Usually, most slaughterhouses operate the processing of both intensively and extensively reared chicken by applying channelling procedures, in which the extensive birds will be processed before the intensive ones. It appears that the extensively reared chickens highly expose to micro-organism, resulting in harbouring higher numbers of micro-organism. Therefore, if the objective of the channelling is for the control of cross-contamination of micro-organism, the intensively reared birds should be processed before the extensive ones. For example, as the extensively reared chickens harbour higher numbers of *Campylobacter*, this type of channelling and its objective must be carefully re-considered.

### *2) Hygiene measures*

For premises that cannot afford to change equipment, proper cleaning and sanitary treatment of equipment can decrease cross-contamination. Basic hygiene practices can be applied to achieve this goal with less cost. The intervention measure for hygiene practices requires that some stages of the entire process are identified as critical point for transmission of micro-organisms. From the literature reviews shown in Chapter 7, the stages of slaughter and processing considered as critical points of cross-contamination include incoming birds at the entrance of slaughter facility, scalding, de-feathering, evisceration and packaging and chill. The suggestions are as follows:

### Incoming birds

Usually birds entering plant are coated with excreta. It is therefore necessary to remove all foreign materials, including faeces. The contamination at this early stage of the process can block the passage of the equipment or contaminate the equipment at the following stages, e.g. scalding. Therefore, incoming birds must be cleaned by chlorinated rinse.

## Scald- tank

The scalder[23] is the most important area of processing in which cross-contamination with micro-organism sheds from the gut of birds (Humphrey and Lanning, 1987) frequently occurs. A scald tank (Figure 9.4) always accumulates excreta. If the water does not flow properly (Figure 9.5), this can become a source of micro-organism including *Campylobacter* transferred to successive birds (Cason and Shackelford, 1999; and Russell, 2002).

## Recommendations:

1. It is essential to control the flow of scald water and ensure a rapidly moving flow as counter-current direction (Figure 9.6).
2. The temperature of the scald water must be as high as possible (should be over $60^0C$) without causing breast striping.
3. Fresh water must be re-added into the scald-tank frequently.
4. A Multi-tank scalder is more effective in reducing microbial concentration remaining on the carcasses.
5. Cleaning and sanitation of the scald tank must be carried out regularly and frequently to eliminate the organic material in the scalder.
6. Further research is necessary into the development of the system of scald-tank, which can mix the scald water properly, reducing bacteria suspended in the scalder.

**Figure 9.4** Scald water containing a high concentration of excreta.

(taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

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[23] Scalder is a tank containing hot water in which the birds are submerged to soften feather follicles for feather removal

**Figure 9.5** Scald water that is not flowing and acts as a bath.

(taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

**Figure 9.6** A counter-current water flow

## De-feathering

The level of cross-contamination in the carcass entering the defeathering stage is associated with the previous stage of the process, scalding. If the water in a scald tank is dirty from organic materials, accumulating high numbers of micro-organisms, it may increase cross-contamination to the carcasses. If high levels of contaminants are presented on the carcasses, these contaminants can be driven into the feather follicle during picking.

## Recommendations:

1. Pre-de-feathering processes (*e.g.* at the entrance of incoming bird, scalding) must be strictly controlled.
2. Regular cleaning of working areas or equipment is very important. Feathers removed from the carcasses must be discarded properly and the surrounding must be cleaned up regularly. Although the presence of feathers and feather follicles does not make a significant difference in carcass bacterial contamination immediately after defeathering or in spoilage bacteria after 1 week of refrigeration (Cason *et al.*,2004), poor cleaning in the de-feathering stage (Figure 9.7) could be a source of micro-organisms over time and it may increase the cross-contamination.

**Figure 9.7** De-feathering system after scalding

(taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

## Evisceration

After being killed, scalded and defeathered, the carcass head, shanks and viscera are removed. Inedible viscera or guts[24] are removed by loosening the crop[25]. In small farms these viscera are manually eviscerated (Figure 9.8). If the gut is torn, the exterior of the carcass can be contaminated with faeces (Figure 9.9). A torn crop or a crop removal machine can be a source of contamination (Figures 9.10 and 9.11). One gram of gut content can carry $10^9$ bacteria (Mead, 2004). In some countries, at this stage washing of the carcass is permitted in order to clean off faecal content.

## Recommendations:

1.  Proper feed withdrawal[26] before processing will help reduce tears and faeces in the gut and a crop.
2.  Washing carcasses with chlorinated water after gut removal may be necessary.
3.  Unwanted viscera must be removed from the working area using a hygienic process, for instance, using water to continually wash the viscera away.
4.  Proper training for individuals who works at this point in order to ensure correct evisceration, avoiding gut tears.
5.  This stage must be inspected carefully and frequently. Carcasses found to be contaminated with gut content must be condemned immediately.

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[24] Viscera include intestines, oesophagus, spleen, reproductive organs and lungs.
[25] A crop (ingluvies) is an enlargement or out pouching of the oesophagus proximal to the proventriculus, or glandular stomach and functions primarily in a food storage role for avian species.
[26] Feed withdrawal refers to the total length of time the bird is without feed before processing. This includes the time the birds are in the house without feed, as well as the time the birds are in transit and in the live hold area at the plant.

**Figure 9.8** Evisceration in a small scale plant

(taken from http://attra.ncat.org/attra-pub/poultryprocess.html#Feather: 22 December 2005)

**Figure 9.9** Intestinal cuts and torn intestines and nicked vent due to improperly functioning venting machine (taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

**Figure 9.10** Faecal material in crop of chicken

(taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

**Figure 9.11** Crop removal machine

(taken from http://pubs.caes.uga.edu/caespubs/pubcd/B1222.htm. 19 December 2005)

## Packaging

The findings from this study have identified differences between the pre-packaged and unwrapped carcasses indicating that packaging may influence the process of cross-contamination.

## Recommendations:

1. Carcasses should be packed as soon as they are washed and then properly chilled.
2. The pre-packaged product must be stored at a strictly controlled temperature ($4^0$C or below).
3. The pre-packaged product must be labelled in a manner that accurately describes the product and does not mislead the consumer. Labelling should be regulated and the unwrapped unlabelled carcasses must not be distributed to the market.

## Retail outlets[27]

Poultry products at a retail point are displayed in several different ways. Whole carcasses are often displayed without refrigeration at open market stalls, particularly, in the developing world. Refrigerated products are displayed in shops and other retail units unpackaged or over-wrapped on plastic trays. Meat and poultry are sometimes sold from mobile refrigerated shops. In developing countries small scale producers may sell by bartering for other consumable items (Silverside and Jones, 1992).

The BIC group were found to harbour not only the highest numbers of *Campylobacter* but also highest rate of antimicrobial resistance. This is consistent with the open presentation of

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[27] Retail outlet is where a consumer directly purchases a dressed or eviscerated chicken carcass.

the unwrapped carcass causing high cross-contamination. If no labelling is provided, it should be assumed that the carcass is supplied by unlicensed or illegal premises.

**Recommendations:**

1. Premises which sell or offer raw meat or poultry must register with the local food authority as licensed premises.
2. At least one person working in the shop or outlet must be trained in food hygiene.
3. Poultry product must be pre-packed with labelled before being distributed from the slaughterhouse. Unwrapped carcasses should not be displayed or packaged at a retail point.
4. In mix businesses, raw meat products must be handled or displayed in accordance with hygienic requirements. Individual meat products must be separately put on the certain area of the shelf. This is for prevention of cross-contamination among these products.
5. Individual meat products, if possible, must be situated in an individual tray to prevent the leakage of liquid from the carcasses or other meat. This is in particular necessary for the unwrapped poultry products.
6. Jeremiah and Gibson (1997) found that the light used for displaying meat products can raise the surface temperature of exposed products, resulting in increasing growth of pathogen. It is therefore Intensity and duration of display illumination must be monitored and control.
7. Authorised food officers must regularly inspect and, if possible, check the premises that do not comply with the regulations.

## Consumers

Medeiros *et al.* (2001) described that far essential food handling behaviours consumers must be educated, including personal hygiene, proper cooking and prevention of cross-contamination. The evidence indicates that food handling and cooking in a kitchen at home can dramatically reduce the probability of infection and probability of illness related to foodborne pathogen including *Campylobacter* in carcasses. As presented in Chapters 7 and 8. After cooking, the health risk from *Campylobacter* can be reduced to be very low levels. Currently, these issues have been introduced to the public by focusing on good hygiene practices (FSA, 2005). For example:

1. Personal hygiene guidelines (washing hands, covering hairs during food preparation, *ect.*)
2. Cleaning work surfaces and equipmen, for example:
• Cutting boards should be used separately among raw meat, fish poultry and vegetables. It must be cleaned and dried up immediately after each use. A wooden cutting board should be visually checked to assure that food is not getting imbedded in cracks or crevices.

• Kitchen dishcloths, sponges and aprons must be washed daily. Hand-washed dishes should be air-dried as repeated using towels to dry them will spread germs.

• Cutlery and utensils previously used for raw meat should be washed before the next use.

• If meat, poultry or seafood was in a marinade, bring marinade to a boil for at least 3 minutes before using as a sauce.

3. Special care should be taken when cooking for a special groups (children, elderly people or immuno-compromised person), ensuring a proper temperature and time for cooking.

4. Refrigerator rules should be implemented. This should include: i) checking temperature since correct temperature can preserve food commodity and reduce the risk of food poisoning and ii) putting poultry on the bottom shelf of the refrigerator and separate from cooked food.

Although a number of recommendations have been made available to public, it is evident that the public does not comprehend the messages thoroughly. This may be because of: i) lack of clear evidence to illustrate the consequences resulting from improper food handling or preparation, ii) non-specific instruction (e.g. proper temperature, washing cutting board regularly), iii) demanding requirements (e.g. keeping raw meat separately from cooked food and vegetables is merely impossible for some families), iv) insufficient information related to the real situation in a private kitchen regarding the food handler or preparer and persons consuming meal and v) public perception on health risk from Campylobacter with and without antimicrobial resistance following the consumption of chicken.

## Recommendations:

1. Since public perception, which may depend greatly on the way in which information is presented, influences food selection, the information from risk assessment must be transparently conveyed to the public. Information from credible sources is more likely to influence public perception of a risk than is information from sources that lack this attribute. Based on what consumers perceive, they would be able to decide what their preferences are.

2. The recommendations advised to public must provide meaningful, relevant and accurate information. Their relevance to kitchen hygiene must be clear and practical so that consumers will want to apply them in kitchens, for example, what would be the sufficient temperature for a proper cooking, how often the cutting board needs to be cleaned.

3. More research is needed on personal behaviours during food preparer in a kitchen at home in order to construct the meaningful and precise recommendations which consumers can be clearly understood. This includes experiments which reflect the real situation during cooking, e.g. temperature, time, etc.

4. Consumers' participation may be necessary for the development of risk communication messenges, strengthening the link and comprehension improving the perception.

## 9.4.2 Scenario 2: Reduction of health risk related to antimicrobial resistance in *Campylobacter*

Since the emergence of antimicrobial resistance in foodborne pathogens, the use of antimicrobial agents in food-producing animal husbandry has been strictly controlled. Some countries adopted the precautionary principle, banning antimicrobial use, to mitigate the risk. To date there have not been any interventions that can effectively mitigate the risk related to antimicrobial resistance in foodborne pathogens. More importantly, both studies related to antimicrobial resistance in foodborne pathogens and mitigation for *Campylobacter* are not harmonised and continuous, varying from working group to working group or countries to countries.

The findings from Chapters 7 and 8 indicate that the health risk from antimicrobial resistant *Campylobacter* depends on the numbers of organisms rather than the rate of resistance. Banning antimicrobial use in food animal husbandry may not be effective for risk management without causing other risks. However, it remains necessary to control the use of antimicrobial agents in animal husbandry. This is because increasing resistance to antimicrobial of pathogens threatens the potential action of antimicrobial agents and may cause the failure of treatment for infection in patients, especially vulnerable groups.

Risk management options for scenario 2 considered alongside the findings in Chapters 7 and 8 can be constructed with reference to the numbers of organism and the rate of resistance to antimicrobials. Firstly, reduction in the number of *Campylobacter* can be achieved by following the steps in scenario 1. Secondly, the mitigation for the reduction of the rate of antimicrobial resistance in *Campylobacter* can be achieved by concentrating on the use of antimicrobial agents on the farm. This is because the use of antimicrobials on the farm is thought to be a major contributing factor in antimicrobial resistant-*Campylobacter* (Ge *et al.*, 2003 and Hurd *et al.*, 2004).

The precautionary principle may not be appropriate for the health risk related to antimicrobial resistant *Campylobacter* following the consumption of chicken. Cox (2002) compared the economic costs of the different risk management interventions for *Campylobacter* by using the four scenarios of management options (Table 9.2) in order to calculate the costs for the implementation and illness reduction. It is evident that banning antimicrobial agent (enrofloxacin) is not an economic option, whereas adding chlorinated water or using antimicrobial spray on the farm (reducing the numbers of organisms) can significantly

decrease the cost. Ball and Goats (1996) suggested that it is necessary to consider where the overall risk benefit is and what is achievable.

**Table 9.2** Comparison of cost and benefit of the intervention cost for reducing microbial health risk (adopted from Cox, 2005)

| Scenario | Implementation cost (m) | Illness cost (m) | Net cost (m) |
|---|---|---|---|
| Ban enrofloxacin | 11.7 | 9.8 | 21.5 |
| additional chlorinated water | 120.1 | -253.7 | -133.6 |
| antimicrobial spray | 105.3 | -296.8 | -191.5 |
| Irradiation | 810.9 | -305 | 505.9 |

## Recommendations:

1. If banning antimicrobial use in animal husbandry is to be enforced through legislation, further studies are required in order to identify whether it can significantly reduce the risk from antimicrobial resistant bacteria to humans. This would provide a relatively simple framework for consideration of the tradeoffs between the reduction of campylobacteriosis (with and without antimicrobial resistance) and the reduction of antimicrobial resistant campylobacteriosis.

2. It has been recognised that sources of antimicrobial resistant pathogens poses risks to human health via a number of routes including overuse or misuse in humans. The control measure of prudent antimicrobial use should include the use in humans in the community, hospitals and the use in animals.

3. The control measure for antimicrobial use presents a dilemma. On the one hand, due to an impact of antimicrobial resistance associated with antimicrobial use in veterinary practice on human health, withdrawal of antimicrobial use is required. On the other hand, withdrawal of antimicrobial use on the farm may increase infection with pathogens in poultry and subsequent health risk to humans following the consumption of poultry.

In this context, the control measure of antimicrobial use in animal on the farm should be considered in two separate ways, which are:

1. A short-term mitigation in relation to the reduction of the numbers of foodborne pathogens is that certain antimicrobial agents may be permitted in use on the farm in order to reduce the infection with pathogens in broilers. This requires: i) clarification of classes and types of antimicrobial agents permitted, ii) record of the use, *i.e.* names of agent and of flock administrated with the certain antimicrobial and duration of the use and iii) review and monitor at least annually regarding the appropriate use of

antimicrobial agents and the prevalence of a positive flock with and without antimicrobial resistance.

2. A long-term mitigation aims at prevention of the increase of antimicrobial resistance rate in foodborne pathogens. Although antimicrobial agents used in poultry may reduce the numbers of pathogens, over time these organisms may develop the resistance to antimicrobials frequently used. This could result in diminishing the potential action of the antimicrobial and then it may not be able to reduce the numbers of micro-organisms. Thus, the health risk from pathogens including *Campylobacter* may increase, whereas, the efficiency of antimicrobials for the treatment of infections may decrease. Therefore long-term mitigation must consider: i) antimicrobial agents permitted to be used in animals must not be the same or equivalent type as those used in humans, ii) further researches on alternative agents able to reduce the numbers of micro-organisms without inducing the development of the resistance in micro-organisms are required in order to minimise antimicrobial use in animal husbandry, iii) other control measures to reduce the numbers of micro-organisms must be enforced and be applied from production to consumption and iv) an effective surveillance system is necessary.

3. The information on antimicrobial use and its impact must be efficiently disseminated to all interested parties. It is important that farmers must be included as members of the working groups. In this context, farmers should be informed and educated about antimicrobial use and relative risk to humans and the environment. An effective exchange of information would enhance the understanding of this situation between scientists, veterinary, regulators and farmers. Clear understanding will lead the parties to the right issue of concern, for example:

♦ Scientist or veterinary presents the association between antimicrobial use on the farm and the development of antimicrobial resistance.

♦ Regulator explains the strategies of the control measures.

♦ Farmer demonstrates real situation of what occurs on the farm.

♦ Economist compares cost and benefit of the implementations.


More importantly, cooperative relationships provide a larger number of data and relevant information which are essential to develop robust interventions. Thus, an efficient and effective risk management option would have likely achieved.

4.   More studies are needed in order to;

♦ identify the sources of antimicrobial-resistant *Campylobacter* in humans.

♦ elucidate the association between antimicrobial-resistant *Campylobacter* in humans and antimicrobial-resistant *Campylobacter* in animals.

♦ develop a robust and responsive infrastructure for the surveillance of animal and human health associated with *Campylobacter* with and without antimicrobial resistance.

♦ estimate the costs and benefits of interventions.

## 9.5 Conclusion

The effectiveness of the various interventions may be measured in terms of the achievement of targets/goals and the benefits. Whilst the achievement can be analysed using cost-effectiveness technique, the estimation of the benefits involves monetary values which could then be incorporated into cost-benefit analysis (Bennett, *et al.*, 2003).

Since multiple entry points exist for foodborne pathogens from production to consumption, multifaceted intervention approaches are required to successfully control contamination of poultry during the various phases in the food chain. The control measures used for preventing colonisation on farms include hygiene practices, biosecurity, immunisation, dietary management and antimicrobial use, and biological control procedures. Cross-contamination in the processing plant can be reduced through good manufactural practices, temperature controls, chemical interventions, water replacements and counter-flow technology in the scalder and chiller, and equipment maintenance. Food handling at retail outlets and by consumers (*i.e.*, storage at the proper temperature and adequate cooking) are the final critical control points from farm to consumer (Callaway, *et al.*, 2003).

Although these measures can be generally applied for the control of all foodborne pathogens, the characteristics of each pathogen must be taken into consideration and an understanding that contamination can be introduced into foods at numerous points along the food chain is requires (Callaway, *et al.*, 2003). For example, i) cross-contamination with *Salmonella* does not affected by chilling in the same manner as *Campylobacter*, chilling practices cannot effectively reduced cross-contamination of *Salmonella*; but can reduce the numbers of *Campylobacter*, ii) *Campylobacter* cannot mutiply during processing unlike *E. coli* and *Salmonella* and therefore preventing faecal contamination at the beginning of processing may be the most effective practices in controlling cross-contamination of *Campylobacter*. Good agricultural practices (GAPs), good manufactural practices (GMPs) and good hygiene practices (GHPs) (Note of Table 9.1) are the most successful intervention in controlling cross-contamination and can be implemented for the control of all foodborne pathogens at any stage of the food chain with less monetary involvement (Bennett *et al.*, 2003). Other interventions such as immunisation, dietary management, competitive exclusion, use of bacteriophage have been contemplated and are currently under investigation (Hovde *et al.*, 1999; Huff *et al.*, 2001 and Callaway *et al.*, 2003).

In conclusion, potential intervention options in this study were constructed based on the results of the risk assessment (Chapters 7 and 8) and the control measures for the control of foodborne pathogens from production to consumption recommended by other studies. These relate to the reduction in health risk associated with *Campylobacter* by reducing the

numbers of *Campylobacter* in the chickens. The interventions in scenario 1 (section 9.4.1) aim at the reduction of the *Campylobacter* load in chickens. The mitigations can efficiently decrease the numbers of organisms through the full process from farm to consumption. Options are therefore discussed at each stage. The interventions of scenario 2 (section 9.4.2) focus on the practices on the farm. However, the interventions of the scenario 1 are still useful for the reduction of the health risk of antimicrobial resistant *Campylobacter*. The options were recommended as short term and long term interventions.

# CHAPTER 10
## Conclusion

Insufficient information related to *Campylobacter* infection has generated the uncertainties in the development of the control measures. Risk tradeoffs stimulate fluctuations between permission and prohibition of antimicrobial use in animal husbandry. This induces ambiguity in the shared understanding amongst scientists, regulators, producers and consumers.

This study evaluates the relative risk of *Campylobacter* (with and without antimicrobial resistance) associated with the consumption of intensively and organically reared chickens using quantitative microbial risk assessment (QMRA). It attempts to identify and demonstrate the key factors in the consideration of the potential interventions for the management of health risk. These are discussed below.

## 10.1 Isolation and enumeration methods

Consideration of risk related to *Campylobacter* focuses on health impact on humans at the time of consumption. As only **viable** *Campylobacter* can cause infection, conventional culture methods for isolation and enumeration used in this study are therefore appropriate for determining prevalence of *Campylobacter*-positive chicken. Although molecular methods thought to have reliability, they are sensitive to not only viable but also the dead cells. Therefore, they are likely to overestimate for the prevalence of infection.

Clearly, conventional culture methods are imperfect measurements, generating the errors. However, these errors can be minimised with the appropriate application of mathematical and statistical methods as demonstrated in Chapter 5. This will be useful for a large scale works or routine work where molecular methods are not available or are too expensive to perform. The application of mathematics and statistics can also be used for the adjustment, without extra cost, of isolation rates thought to be underestimated. Nonetheless, sufficient primary data is still required. This highlights the need for the establishment of internationally accepted standard methods and data bank for *Campylobacter*. The MPN method has been found to be the most sensitive culture method but at the same time it needs to be revised as it is time and material consuming. The large volume of samples containing micro-organisms used in the MPN method needs to be treated before disposal. This is costly and will be long term problem. The modified MPN method developed in this study overcomes some of the undesirable problems above and addresses the principle of waste minimisation. However, it needs to be further evaluated for the reliability and an accurate approach for its interpretation should be established. Estimation of the accurate values of MPN can be resolved using the maximum likelihood estimation.

In addition, the different results of isolation rates for *Campylobacter* found in the different parts of the chicken (meat & skin, cavity and tail) indicate that sampling procedures may greatly influence the error of measurement.

## 10.2 Perspective on antimicrobial resistance in *Campylobacter*

Two patterns of the minimum inhibitory concentration (MIC) distribution were found between three antimicrobials. Theses were: i) very low MIC and ii) very high MIC. The very low MICs were found to be associated with ciprofloxacin, whose equivalent antimicrobials have been prohibited to be used in animal husbandry. The high MICs were associated with the other two antimicrobials (erythromycin and nalidixic acid), whose equivalent forms have been used for treatment of sick birds and as growth promoters. This evidence may indicate that if no effective control measures in antimicrobial use on the farms, *Campylobacter* may develop resistance to ciprofloxacin in the future similar to what is found for erythromycin and nalidixic acid. The evidence found in the organic group which is free from ciprofloxacin resistance highlights this notion. In other word, the current low-MIC found against ciprofloxacin may represent a point in time similar to that found in the past for erythromycin and nalidixic acid.

However, consideration for the control measures must take into account evidence obtained from different sources and should address complex issues. Conflicts arise from implementation of the interventions currently used for the reduction of *Campylobacter* level on one hand and for the reduction of antimicrobial resistance in *Campylobacter* on the other. These originated from: i) lack of co-operation amongst working groups, ii) lack of availability of data due to partial information and iii) lack of internationally accepted standard methods and surveillance programmes. Although conflicts resulting from these tradeoffs are inevitable, they can be minimised by reducing gaps in the availability of data and information in terms of scientific findings, socio-economic issues, policy demography and politic. Health risk associated with this pathogen cannot be completely eliminated without causing other consequences. Hence, if necessary, the mitigation approaches must be flexible to some degree and address both short term and long-term planning.

## 10.3 The usefulness of risk analysis on food safety management for *Campylobacter*

This study considers the relative health risk associated with the consumption of three types of chicken which are raised in two different rearing systems. It also considers whether the stringent controls are able to reduce exposure and therefore health risk. In this context, the estimate of the exposure dose to *Campylobacter* (with and without antimicrobial resistance was considered in relation to the effect of the heat treatment (cooking) and the *Campylobacter* number transfer coefficients for the number of *Campylobacter* in chicken

meals and salads. The exposure dose establishes the likelihood of a population being harmed by *Campylobacter*. Reduction in the numbers of *Campylobacter* in chicken using simple and low cost interventions is likely to greatly mitigate that risk

In this context, quantitative microbial risk assessment is very useful for the evaluation of the risk from foodborne pathogens (including *Campylobacter*) and the additional consequences related to antimicrobial resistance. It can identify main factors and where the interventions can be most effective. It has the potential to provide evidence for risk managers to set priorities for risk identification and risk management options. However, the scarcity of current data generates significant uncertainty and often results in controversy amongst stakeholders. The main differences originate from the different predictive models used and assumptions or data and information available. They could also result from a failure to consider risk tradeoffs. Thus, the risk models adopted for use need to be clearly described and limitations and gaps in the data taken into account.

Some considerations arising from this study suggest unexpected conclusions related to antimicrobial resistant *Campylobacter*. The results were interpreted and discussed based on the assumptions applied to the each of the models used. It is clear that some factors are not included due to lack of data. Despite the lack of data and the uncertainties, this study can demonstrate how risk assessment can be applied to microbial food safety management. It can then be incorporated into risk management and the decision making process for the selection of the best course of action and the implementation of appropriate control measures.

Food risk management is a plausible strategy to be implemented for the control of *Campylobacter* (with and without antimicrobial resistance). It is a useful process that balances conflicts arising between interests groups. The outcome of food risk assessment and food risk management can lead to the development of standards, guidelines and other recommendations for food safety. This may change the way nations approach food safety and public health decisions.

Risk decision making is a conditional process which requires the dissemination of information. The interaction between relevant factors must be transparent. This process would benefit from the sharing of local knowledge and expertise. Cooperation amongst organisations is extremely important.

In theory, the information gathered in recent studies is sufficient to initiate the process of construct international strategies which can be implemented in different types of husbandry (*i.e.* small-scale or large scale farming). However, in practice, it is difficult to implement these strategies due to conflicts of interests amongst scientists, regulators and stakeholders as

well as members of the public. The different perceptions of risk do not facilitate understanding amongst these groups. Nevertheless, the risk management options currently used can be the core of further development of specific mitigation options and these may be revised in future.

## 10.4 Conclusion and recommendations

1.  Current methods for Isolation, enumeration and antimicrobial susceptibility testing used worldwide should be harmonised and it is necessary to establish internationally accepted standard methods. These standard methods should be implemented appropriately depending on the type and study objective.
2.  Appropriate mathematical and statistical methods should be included in all studies and as some limitations can be addressed using the appropriate approaches and complex, expensive lab methods may not be necessary.
3.  QMRA for estimating heath risk related to antimicrobial resistant *Campylobacter* should be re-considered. A revised model is necessary for the assessment and it will contribute to an effective risk management.
4.  Further studies on several aspects as addressed in previous chapters are required to reduce the gaps in the availability of data.

## 10.5 Further prospects

I would consider this work as the preliminary for my future work in Thailand. A number of issues arising from this study ignited my interest and enthusiasm for the application of risk analysis and related subjects (*e.g.* Bayesian statistics). In this light I would keen to:

1.  apply risk assessment to other aspects of food safety management in Thailand where control systems may be different from the system in the UK. These differences involving demography, tradition, culture, climate and other related issues greatly influence risk assessment and risk management;
2.  explore the usefulness of the potential of mathematical and statistical methods for the application of microbial risk assessment where, due to the characteristics of micro-organisms, uncertainties play a bigger role than in chemical risk assessment;
3.  introduce risk communication to in future evaluation of food safety management.

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# Appendix 1

## Media composition

**Appendix 1-1:** Formular composition of Bolton broth

| Formula | gm/litre |
|---|---|
| Meat peptone | 10 |
| Lactalbumin hydrolysate | 5.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Alpha-ketoglutaric acid | 1.0 |
| Sodium pyruvate | 0.5 |
| Sodium metabisulphite | 0.5 |
| Sodium carbonate | 0.6 |
| Haemin | 0.01 |
| pH 7.4 ± 0.2 | |

**Appendix 1-2:** Composition of the selective supplement

| Type of antibiotic | mg/vial | Action |
|---|---|---|
| Cephalosporin (cefoperazone) | 10 | inhibits *Enterobacter* spp., *Serratia* spp., *Pseudomonas aeruginosa* , some *Proteus* spp., *Yersinia enterocolitica* |
| Trimethoprim | 10 | inhibits *Proteus* spp. and gram positive cocci |
| Vancomycin | 10 | inhibits gram positive cocci |
| Cycloheximide | 25 | inhibits yeasts and moulds. |

**Appendix 1-3:** A composition of mCCAD and the supplement

| Formula | gm/litre | Type of antibiotic | mg/vial |
|---|---|---|---|
| Nutrient Broth No.2 | 25.0 | Cefoperazone | 16 |
| Bacteriological charcoal | 4.0 | Amphotericin B | 5 |
| Casein hydrolysate | 3.0 | | |
| Sodium desoxycholate | 1.0 | | |
| Ferrous sulphate | 0.25 | | |
| Sodium pyruvate | 0.25 | | |
| Agar | 12.0 | | |
| pH 7.4 ± 0.2 | | | |

**Appendix 1-4:** Preparation of two-fold of concentration of antimicrobial

| Final plate conc.(mg/L) | Stock used(mg/L) | Vol. per 100ml | Vol. per 20ml(µl) |
|---|---|---|---|
| 1024 | 10000 | 10.24 ml in 90 ml | 2048 µl in 18 ml* |
| 512 | | 5.12 ml in 95 ml | 1024 µl in 19 ml* |
| 256 | | 2.56 ml in 97.5 ml | 512 µl |
| 128 | | 1280 µl in 99 ml | 256 µl |
| 64 | | 640 µl | 128 µl |
| 32 | | 320 µl | 64 µl |
| 16 | | 160 µl | 32 µl |
| | | | |
| 8 | 1000 | 800 µl | 160 µl |
| 4 | | 400 µl | 80 µl |
| 2 | | 200 µl | 40 µl |
| Final plate conc.(mg/L) | Stock used(mg/L) | Vol. per 100ml | Vol. per 20ml |
| 1 | 100 | 1 ml | 200 µl |
| 0.5 | | 500 µl | 100 µl |
| 0.25 | | 250 µl | 50 µl |
| 0.125 | 10 | 1.25 ml | 250 µl |

**Appendix 1-5: The 0.5 McFarland standard** is prepared by added 0.5ml of 0.048M $BaCl_2$ (1.17%w/v $BaCl_2$. $2H_2O$) to 99.5ml of 0.18M $H_2SO_4$(1%w/v) with constant stirring. It was distributed into a screw cap bottle of the same size and with the same volume as those used in growing the broth cultures.

## Appendix 2
## Number of purchasing days

| Type of chicken | Shop's Name | Branch | No. of purchasing day |
|---|---|---|---|
| PIC | M&S<br>Morrison<br>Sainsbury<br>Tesco<br>Waitrose | Bounds Green<br>Camden Town<br>East Barnett<br>Endfield Town<br>Finchley<br>Holoway<br>Ponders End<br>Wood Green | 25 |
| POC | M&S<br>Morrison<br>Sainsbury<br>Tesco<br>Waitrose | Bounds Green<br>Camden Town<br>East Barnett<br>Endfield Town<br>Finchley<br>Holoway<br>Ponders End<br>Wood Green | |
| BIC | Butchers'shop | Finchley<br>Hackney<br>Holoway<br>Palmmers Green<br>Ponders End<br>Seven Sisters<br>Wood green | 15 |

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# Appendix 3

# Model Equations

**Appendix3- 1:** A dose –response- relationship model

A dose response model estimates a relationship between the level of microbial exposure and the likelihood of occurrence of adverse consequence. The basic assumption is that the probability of ingesting precisely $j$ organisms from an exposure in which the mean dose is $d$ organisms is $P_1(j/d)$. The probability of $k$ organisms ($\leq j$) surviving the immune system and able to initiate the infection is $P_2(k/j)$. If the distribution of organisms between doses is random and hence it is Poisson distributed and that each organism has an independent and identical probability of surviving the immune defences and initiating infection. The overall probability of $k$ organisms surviving to initiate the infection is given by

$$P(k) = \sum_{j=1}^{\infty} P_1(j/d)P_2(k/j)$$

If infection occurs when the minimum number of the organisms (denoted as $K_{min}$) survives and then initiating infection, the probability of infection may be given by

$$P_{inf} = \sum_{k=k_{min}}^{\infty} \sum_{j=k}^{\infty} P_1(j/d)P_2(k/j)$$

It is noted that $K_{min}$ may not be a single number but may in fact be a probability distribution (Haas *et al.*, 1999). By using the exponential dose-response model, the model for estimating the dose can be simulated in which assumes that the distribution of organisms between doses is random (Poisson distribution). It is as well the organism has the independent and identical probability of survival, defined as $r$, and the single hit theory is applied, $K_{min}$ equals 1. In addition, the survival is the binomial distribution. Therefore, the probability of infection can be re-written as

$$P_{inf} = \sum_{k=k_{min}}^{\infty} \sum_{j=k}^{\infty} [\frac{d^j}{j!}e^{-d}][\frac{j!}{k!(j!-k)}(1-r)^{j-k}r^k$$

This can be written in terms of $d$ and $r$;

$$P_{inf} = \sum_{k=k_{min}}^{\infty} \frac{(dr)^k e^{-dr}}{k!} \sum_{j=k}^{\infty} \frac{[d(1-r)]^{j-k}}{j-k!} e^{-d(1-r)}$$

As considering the second summation of equation, which is $\sum_{j=k}^{\infty} \frac{[d(1-r)]^{j-k}}{(j-k)!}e^{-d(1-r)}$ , this has

the form of a Poisson distribution, where

$$P(n) = \frac{e^{-\mu}\mu^n}{n!}$$

It is therefore the summation of a Poisson series, that is $\sum_{n=0}^{\infty} \frac{e^{-\mu}\mu^n}{n!}$ , As such, the summation

is equal to unity. Hence, as a function of the dose, $d$, is given by

$$P_{inf}(d;r) = \sum_{k=k_{min}}^{\infty} \frac{(dr)^k e^{-dr}}{k!}$$

Given that the summation of a Poisson series is equal to unity. This can be re-written as

$$P_{\text{inf}}(d;r) = 1 - \sum_{k=0}^{k=k_{\min}-1} \frac{(dr)^k e^{-dr}}{k!}$$

As the single-hit theory applied, $k_{\min} = 1$, this simplifies to give the equaton above. This is referred to the exponential dose response relationship.

$$P_{\text{inf}}(d;r) = 1 - e^{-rd}$$

The model assumes a constant survival probability; however, there may be variation in the probability of infection given host responses and pathogenicity of organisms. Therefore, the probability of the organisms surviving immune defences and initiating infection, that is r, is assumed to follow a beta-Poisson, then;

$$P_{\text{inf}}(d;\alpha,\beta) = 1 - [1 + \frac{d}{\beta}]^{-\alpha}$$

$$P_{\text{inf}}(d;\alpha,\beta) = 1 - [1 - p]^d$$

Here $p$ is the probability of infection from ingestion of one organism, d is the dose ingested. Given $p \sim$ beta $(\alpha,\beta)$ where the parameter $\alpha,\beta$ are the maximum likelihood estimates obtained from Medema et al. ($\alpha = 0.145, \beta = 7.589$) the probability of illness given infection is obtained as;

$$P_{\text{ill}/\text{inf}}(d;\alpha,\beta) = 1 - (1 - Beta(\alpha,\beta)^d)$$

**Appendix3- 2:** The Gibbs sampler (taken from Joseph et al, 1995)

For two diagnostic tests, the full conditional distribution are as follows:

$$Y_1 / u,\pi,S_1,C_2,S_2,C_2 \sim Binomial(u, \frac{\pi S_1 S_2}{\pi S_1 S_2 + (1-\pi)(1-C_1)(1-C_2)})$$

$$Y_2 / v,\pi,S_1,C_1,S_2,C_2 \sim Binomial(v, \frac{\pi S_1(1-S_2)}{\pi S_1(1-S_2) + (1-\pi)(1-C_1)C_2})$$

$$Y_3 / w,\pi,S_1,C_1,S_2,C_2 \sim Binomial(w, \frac{\pi(1-S_1)S_2}{\pi(1-S_1)S_2 + (1-\pi)C_1(1-C_2)})$$

$$Y_4 / x,\pi,S_1,C_1,S_2,C_2 \sim Binomial(x, \frac{\pi(1-S_1)(1-S_2)}{\pi(1-S_1)(1-S_2) + (1-\pi)C_1C_2})$$

$\pi / u,v,w,x,Y_1,Y_2,Y_3,Y_4 \alpha_\pi,\beta_\pi \sim Beta(Y_1 + Y_2 + Y_3 + Y_4 + \alpha_\pi N - (Y_1 + Y_2 + Y_3 + Y_4 + \beta_\pi)$

$S_1 / Y_1,Y_2,Y_3,Y_4 \alpha_{S_1},\beta_{S_1} \sim Beta(Y_1 + Y_2 + \alpha_{S_1}, Y_3 + Y_4\beta_{\pi 1})$

$C_1 / u,v,w,x,Y_1,Y_2,Y_3,Y_4 \alpha_{C_1},\beta_{C_1} \sim Beta(w + x - (Y_3 + Y_4) + \alpha_{C_1} u + v - (Y_1 + Y_2) + \beta_{C_1})$

$S_2 / Y_1,Y_2,Y_3,Y_4 \alpha_{S_2},\beta_{S_2} \sim Beta(Y_1 + Y_3 + \alpha_{S_2}, Y_2 + Y_4\beta_{S_2})$

$C_2 / u,v,w,x,Y_1,Y_2,Y_3,Y_4 \alpha_{C_2},\beta_{C_2} \sim Beta(v + x - (Y_2 + Y_4) + \alpha_{C_2} u + w - (Y_1 + Y_3) + \beta_{C_2})$

# Appendix 4

## Exposure assessment

**Appendix 4-1** The probability of infection (%) following the consumption of a chicken meal prepared from three types of chicken given age, gender and internal temperatures at the protected areas.

| Age | PIC T<Tc M | F | PIC T>Tc M | F | POC T<Tc M | F | POC T>Tc M | F | BIC T<Tc M | F | BIC T>Tc M | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <18 | 18.8 | 18.8 | 0 | 0 | 58.9 | 58.9 | 3.7 | 3.7 | 100 | 100 | 100 | 100 |
| 18-29 | 34.1 | 21.8 | 1.9 | 0 | 82.8 | 64.7 | 5.5 | 3.7 | 100 | 100 | 100 | 100 |
| 30-65 | 26.1 | 21.8 | 1.9 | 0 | 72.9 | 65.4 | 5.5 | 3.7 | 100 | 100 | 100 | 100 |
| >65 | 24.7 | 23.3 | 1.9 | 1.9 | 70.8 | 66.6 | 3.7 | 3.7 | 100 | 100 | 100 | 100 |

**Appendix 4-2** The probability of illness (%) following the consumption of a chicken meal prepared from three groups of chicken given age, gender and internal temperatures at the protected areas.

| Age | PIC T<Tc M | F | PIC T>Tc M | F | POC T<Tc M | F | POC T>Tc M | F | BIC T<Tc M | F | BIC T>Tc M | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <18 | 6.2 | 6.2 | 0 | 0 | 19.4 | 19.4 | 1.2 | 1.2 | 33.0 | 33.0 | 33.0 | 33.0 |
| 18-29 | 11.2 | 7.2 | 0.6 | 0 | 27.3 | 21.4 | 1.8 | 1.2 | 33.0 | 33.0 | 33.0 | 33.0 |
| 30-65 | 8.6 | 7.2 | 0.6 | 0 | 24.1 | 21.6 | 1.8 | 1.2 | 33.0 | 33.0 | 33.0 | 33.0 |
| >65 | 8.2 | 7.7 | 0.6 | 0.6 | 23.4 | 22.0 | 1.2 | 1.2 | 33.0 | 33.0 | 33.0 | 33.0 |

**Appendix 4-3** The probability of infection and illness following the consumption of salad contaminated *Campylobacter* transferred from Three types chicken.

| Source Age | Probability of infection (%) PIC M | F | POC M | F | BIC M | F | Probability of infection (%) PIC M | F | POC M | F | BIC M | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <18 | 45.4 | 45.4 | 92.1 | 92.1 | 100 | 100 | 18.4 | 18.4 | 30.4 | 30.4 | 33.0 | 33.0 |
| 18-29 | 69.6 | 52.2 | 99.3 | 95.4 | 100 | 100 | 26.4 | 20.7 | 32.8 | 31.5 | 33.0 | 33.0 |
| 30-65 | 64.0 | 53.1 | 98.6 | 96.7 | 100 | 100 | 24.2 | 20.9 | 32.5 | 31.6 | 33.0 | 33.0 |
| >65 | 62.6 | 53.1 | 98.3 | 95.5 | 100 | 100 | 23.7 | 21.1 | 32.4 | 31.6 | 33.0 | 33.0 |

**Appendix 4-4** The probability of infection (%) following the consumption of a chicken meal and salad contaminated with *Campylobacter* given group of chicken, age, gender and temperature

| Age | PIC T<Tc M | F | PIC T>Tc M | F | POC T<Tc M | F | POC T>Tc M | F | BIC T<Tc M | F | BIC T>Tc M | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <18 | 55.7 | 55.7 | 45.4 | 45.4 | 96.8 | 96.8 | 92.4 | 92.4 | 100 | 100 | 100 | 100 |
| 18-29 | 80.0 | 62.6 | 70.2 | 52.2 | 99.9 | 98.4 | 99.3 | 95.6 | 100 | 100 | 100 | 100 |
| 30-65 | 73.4 | 63.3 | 64.6 | 53.1 | 99.6 | 98.5 | 98.6 | 95.8 | 100 | 100 | 100 | 100 |
| >65 | 71.9 | 64.0 | 63.3 | 54.0 | 99.5 | 98.5 | 98.4 | 95.7 | 100 | 100 | 100 | 100 |

**Appendix 4- 5**The probability of illness (%) following the consumption of a chicken meal and salad contaminated with *Campylobacter* given group of chicken, age, gender and temperature

| | Source of contamination | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PIC | | | | POC | | | | BIC | | | |
| | T<T$_c$ | | T>T$_c$ | | T<T$_c$ | | T>T$_c$ | | T<T$_c$ | | T>T$_c$ | |
| Age | M | F | M | F | M | F | M | F | M | F | M | F |
| <18 | 18.4 | 18.4 | 15.0 | 15.0 | 31.9 | 31.9 | 30.1 | 30.5 | 33.0 | 33.0 | 33.0 | 33.0 |
| 18-29 | 26.4 | 20.7 | 23.2 | 17.2 | 33.0 | 33.0 | 32.8 | 31.6 | 33.0 | 33.0 | 33.0 | 33.0 |
| 30-65 | 24.2 | 21.0 | 21.4 | 17.5 | 32.9 | 32.5 | 32.5 | 31.6 | 33.0 | 33.0 | 33.0 | 33.0 |
| >65 | 23.7 | 21.1 | 21.0 | 18.0 | 32.9 | 32.5 | 32.5 | 31.6 | 33.0 | 33.0 | 33.0 | 33.0 |

**Appendix 4- 6** The probability of illness (%) following the consumption of an immediately cooked and delayed cooked chicken (PIC) meal given age, gender and internal temperature

| | Immediate preparation | | | | Delayed preparation | | | |
|---|---|---|---|---|---|---|---|---|
| Age | T<Tc | | T>Tc | | T<Tc | | T>Tc | |
| | M | F | M | F | M | F | M | F |
| <18 | 6.2 | 6.2 | 0 | 0 | 0.06 | 0.06 | 0 | 0 |
| 18-29 | 11.2 | 7.2 | 0.06 | 0 | 1.2 | 0.06 | 0 | 0 |
| 30-65 | 8.6 | 7.2 | 0.06 | 0 | 1.2 | 0.06 | 0 | 0 |
| >65 | 8.2 | 7.7 | 0.06 | 0.06 | 1.2 | 1.2 | 0 | 0 |

**Appendix 4-7** The probability of illness (%) following the consumption of salad contaminated with *Campylobacter* isolated from PIC given age and gender.

| | Immediate preparation | | Delayed preparation | |
|---|---|---|---|---|
| Age | M | F | M | F |
| <18 | 15.0 | 15.0 | 1.8 | 1.8 |
| 18-29 | 17.2 | 23.0 | 2.4 | 4.1 |
| 30-65 | 17.5 | 21.1 | 2.4 | 3.5 |
| >65 | 17.5 | 20.7 | 2.4 | 3.5 |

**Appendix 4-8** The probability of illness (%) following the consumption of an immediately cooked and delayed cooked chicken (POC) meal given age, gender and internal temperature

| | Immediate preparation | | | | Delayed preparation | | | |
|---|---|---|---|---|---|---|---|---|
| Age | T<Tc | | T>Tc | | T<Tc | | T>Tc | |
| | M | F | M | F | M | F | M | F |
| <18 | 19.4 | 19.4 | 1.2 | 1.2 | 3.5 | 3.5 | 0 | 0 |
| 18-29 | 27.3 | 21.4 | 1.8 | 1.2 | 6.7 | 4.1 | 0 | 0 |
| 30-65 | 24.1 | 21.2 | 1.8 | 1.2 | 5.2 | 4.6 | 0 | 0 |
| >65 | 23.4 | 22.0 | 1.2 | 1.2 | 5.2 | 4.6 | 0 | 0 |

**Appendix 4-9** The probability of illness (%) associated with the consumption of salad contaminated with *Campylobacter* contributable for POC given age and gender.

| | Immediate preparation | | Delayed preparation | |
|---|---|---|---|---|
| Age | Male | Female | Male | Female |
| <18 | 30.4 | 30.4 | 9.5 | 9.5 |
| 18-29 | 32.8 | 31.5 | 16.0 | 11.2 |
| 30-65 | 32.5 | 31.6 | 14.3 | 11.2 |
| >65 | 32.4 | 31.5 | 14.0 | 11.2 |

**Appendix 4-10** The probability of illness (%) following the consumption of an immediately cooked and delayed cooked chicken (BIC) meal given age, gender and internal temperature

| Age | Immediate preparation | | | | Delayed preparation | | | |
|---|---|---|---|---|---|---|---|---|
| | T<Tc | | T>Tc | | T<Tc | | T>Tc | |
| | M | F | M | F | M | F | M | F |
| <18 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 |
| 18-29 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 |
| 30-65 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 |
| >65 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 | 33.0 |

**Appendix 4-11** Probability of illness of person consumed chicken harbouring ciprofloxacin-resistant *Campylobacter* after heat treatment given age, gender and group of chicken.

| Age (year) | Internal temperature of cooking (T): T<$T_c$ | | | | | | Internal temperature of cooking (T): T>$T_c$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Probability (%): M | | | Probability (%): F | | | Probability (%): M | | | Probability (%): F | | |
| | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC |
| <18 | 6.2 | 0 | 33 | 6.2 | 0 | 33 | 0 | 0 | 33 | 0 | 0 | 33 |
| 18-29 | 11.2 | 0 | 33 | 7.2 | 0 | 33 | 0 | 0 | 33 | 0 | 0 | 33 |
| 30-65 | 8.6 | 0 | 33 | 7.2 | 0 | 33 | 0 | 0 | 33 | 0 | 0 | 33 |
| >65 | 8.2 | 0 | 33 | 7.7 | 0 | 33 | 0 | 0 | 33 | 0 | 0 | 33 |
| Average | 8.55 | 0 | 33 | 7.08 | 0 | 33 | 0 | 0 | 33 | 0 | 0 | 33 |

**Appendix 4-12** Probability of illness of person consumed chicken harbouring erythromycin-resistant *Campylobacter* after heat treatment given age and gender.

| Age | Internal temperature of cooking (T1): T<$T_c$ | | | | | | Internal temperature of cooking (T): T>$T_c$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Probability (%): M | | | Probability (%): F | | | Probability (%): M | | | Probability (%): F | | |
| | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC |
| <18 | 6.2 | 19.4 | 33 | 6.2 | 19.4 | 33 | 0 | 1.2 | 33 | 0 | 1.2 | 33 |
| 18-29 | 11.2 | 27.3 | 33 | 7.2 | 21.4 | 33 | 0.6 | 1.8 | 33 | 0 | 1.2 | 33 |
| 30-65 | 8.6 | 24.1 | 33 | 7.2 | 21.6 | 33 | 0.6 | 1.8 | 33 | 0 | 1.2 | 33 |
| >65 | 8.2 | 23.4 | 33 | 7.7 | 22.0 | 33 | 0.6 | 1.2 | 33 | 0.6 | 1.2 | 33 |

**Appendix 4-13** Probability of illness of person consumed chicken harbouring nalidixic acid-resistant *Campylobacter* after heat treatment given age and gender.

| Age | Internal temperature of cooking (T1): T<$T_c$ | | | | | | Internal temperature of cooking (T): T>$T_c$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Probability (%): M | | | Probability (%): F | | | Probability (%): M | | | Probability (%): F | | |
| | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC | PIC | POC | BIC |
| <18 | 6.2 | 18.7 | 33 | 6.2 | 18.7 | 33 | 0 | 1.2 | 33 | 0 | 1.2 | 33 |
| 18-29 | 11.2 | 26.8 | 33 | 7.2 | 20.7 | 33 | 0.06 | 1.8 | 33 | 0 | 1.2 | 33 |
| 30-65 | 8.6 | 23.4 | 33 | 7.2 | 20.9 | 33 | 0.06 | 1.2 | 33 | 0 | 1.2 | 33 |
| >65 | 8.2 | 22.8 | 33 | 7.7 | 21.4 | 33 | 0.06 | 1.2 | 33 | 0.06 | 1.2 | 33 |