

Title: Development of an impedance-based fast antimicrobial susceptibility test for *Neisseria gonorrhoeae*

Running title: iFAST for Neisseria

Authors: Caitlin N DANIELS¹, Bethany K MARTIN¹, Kazi S NAHAR², Khondaker Miraz RAHMAN³, J Mark SUTTON^{1,3*}, Lucy J BOCK^{1†*}

Addresses: ¹ Antimicrobial Discovery, Development and Diagnostics; Countermeasures Development, Evaluation and Preparedness Division, UK Health Security Agency, Porton Down, Salisbury, Wiltshire, UK, SP4 0JG; ² Department of Natural Sciences, Faculty of Science & Technology, Middlesex University, The Burroughs, Hendon, London NW4 4BT, UK; ³ Institute of Pharmaceutical Science, King's College London, 150 Stamford Street, London SE1 9NH, UK

*To whom correspondence should be addressed: Antimicrobial Discovery, Development and Diagnostics, Vaccine Development and Evaluation Centre, UK Health Security Agency, Porton Down, Salisbury, Wiltshire, UK, SP4 0JG Tel.: +44 (0) 1980 612649; e-mail: mark.sutton@ukhsa.gov.uk, Lucy.Bock@fh-muenster.de

†Current address: FH Münster - University of Applied Sciences, Corrensstraße 25, 48149 Münster, Germany.

21 Abstract

22 Objectives

23 Antimicrobial resistant *Neisseria gonorrhoeae* is a serious threat to global health. Current *N.*
24 *gonorrhoeae* antimicrobial susceptibility testing (AST) is laborious and time consuming (≥ 2
25 days) leading to overprescription of last-resort, broad spectrum antibiotics.

26 In this proof-of-principle study, we developed a fast (< 8 h) phenotypic, impedance-based AST
27 for *N. gonorrhoeae* against frontline and experimental antibiotics.

28 Methods

29 WHO *N. gonorrhoeae* reference strains F, K, O, V, X and Y were exposed to EUCAST
30 susceptibility breakpoint concentrations of ceftriaxone, ciprofloxacin and azithromycin for up
31 to 5 hours and to doubling dilutions of antibiotics for 4 hours. The % cell count of antibiotic
32 exposed compared to unexposed control samples, as measured by impedance-based Fast
33 Antimicrobial Susceptibility Test (iFAST[®]), was used to differentiate between susceptible and
34 non-susceptible strains. Responses to novel antimicrobial compounds, including zoliflodacin
35 and gepotidacin, were also measured by iFAST[®] and compared to published MICs.

36 Results

37 The protocol reported susceptibility profiles concordant with published data for all strain-drug
38 combinations after 4 hours of exposure. Dose-response curves generated using iFAST[®] for
39 most strain-drug combinations were in essential agreement with published Etest MIC values.
40 Novel compounds assessed using iFAST[®] showed higher antimicrobial activity than
41 ciprofloxacin in high level resistant strain WHO X.

42 Conclusions

43 iFAST[®] AST results were concordant with the current clinical standards and measured in
44 under 8 h. This method is therefore promising for the development of a rapid and accurate
45 phenotypic *N. gonorrhoeae* AST.

46 Introduction

47 The bacterium *Neisseria gonorrhoeae* was responsible for an estimated 82.4 million new
48 gonorrhoea infections in 2020.¹ Most infections can be treated with a course of antibiotics
49 when administered promptly and if the infecting strain is susceptible to the antibiotic chosen
50 for treatment. Due to the rise in resistance to previously used antibiotics such as ciprofloxacin
51 and azithromycin, ceftriaxone is the current first-line treatment for the WHO priority pathogen
52 *N. gonorrhoeae*.²⁻⁴ Resistance to this frontline treatment remains low in Europe (0.03%),
53 though extensively drug resistant infections have been reported.^{3, 5} In the UK, to allow for the
54 correct treatment and for surveillance of resistance in the *N. gonorrhoeae* population,
55 antimicrobial susceptibility tests (ASTs) are recommended to be conducted on all treated *N.*
56 *gonorrhoeae*.⁶ Detection of many resistance markers by genomic means remains difficult, as
57 resistance mechanisms are complex and previously undescribed resistance mechanisms
58 would go undetected. Phenotypic ASTs remain the gold standard, though generation of results
59 can take up to seven days for some slow growing isolates of *N. gonorrhoeae*. For this reason,
60 empirical treatment with ceftriaxone is recommended, meaning that there is no last resort
61 antibiotic for *N. gonorrhoeae* treatment.⁴ An AST that can give results within a day would allow
62 identification and use of antibiotics that have high resistance within the population, if the isolate
63 is susceptible, protecting ceftriaxone for use of isolates that are resistant to other antibiotics.

64 In a previous study, we developed an impedance-based fast antimicrobial susceptibility test
65 (iFAST[®]) [7](#). The technology measures the difference in electrical impedance of individual cells
66 that have been treated with antibiotics and compares this with untreated bacteria, incubated
67 for the same time. Bacteria will change the properties of their cell wall or membrane, volume
68 and number of surviving cells in response to exposure to varying concentrations of antibiotic.
69 These responses can be seen in a matter of hours and the measurement of each sample only
70 takes around 1-2 minutes. Susceptibility results can be seen within a working day.

In this proof-of-principle study, we applied this technology to AST of *N. gonorrhoeae*. Antibiotics that are currently used to treat *N. gonorrhoeae* infections, ceftriaxone, ciprofloxacin and azithromycin, as well as new antimicrobials zoliflodacin and gepotidacin and two novel antimicrobials developed in our laboratory were used to test this methodology on six WHO reference strains.

Methods

Bacterial strains and reagents

WHO *N. gonorrhoeae* reference strains F, K, O, V, X and Y⁸ were stored at -80°C in glycerol prior to culture. Ceftriaxone (CRO), ciprofloxacin (CIP) and azithromycin (AZM) were purchased from Sigma-Aldrich. Antibiotic solutions were prepared according to supplier guidelines each week and stored at 4°C. Novel compounds were dissolved in DMSO, then diluted in ddH₂O.

Media and culture conditions

N. gonorrhoeae strains were plated on Chocolate PolyVitex™ agar (bioMérieux, France) and incubated at 3-5% CO₂ at 37°C for 15 h, before culturing in liquid supplemented Brain-Heart Infusion (sBHI) medium: BHI (EO Labs, UK) supplemented with 1% v/v BBL™ IsovitaleX™ Enrichment (Fisher Scientific, BD 211876) and 4.2% sodium bicarbonate (sBHI) on the day of use.⁹

A loopful of bacterial culture from the confluent part of the overnight agar plate was suspended in 3mL prewarmed (37°C) sBHI media, vortexed and incubated for 30 - 60 minutes at 37°C, 250rpm. The liquid culture was diluted in saline and cell/mL were counted using iFAST®- (iFAST Diagnostics Ltd, Southampton), cells were diluted to 5x10⁵ cfu/mL in 1mL sBHI and grown statically at 37°C or exposed to breakpoint or doubling dilutions of ceftriaxone, ciprofloxacin and azithromycin or novel compounds in sBHI. After every hour for 5 hours (time course assay) or only after 4 hours of exposure (all other assays), samples were taken and

measured on iFAST. Susceptible breakpoint concentrations were defined by the European Committee on Antimicrobial Susceptibility Testing,¹⁰ and minimum inhibitory concentrations (MIC) were defined by the WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections.⁸

Impedance Cytometry Workflow

Samples were analysed as previously described.¹¹ Briefly, bacterial suspensions were diluted 1:10 in sBHI containing 2µm carboxylated polystyrene beads (Sigma L3030) and measured on the iFAST for 2 minutes at a flow rate of 30µL/min. For the novel compound analysis an automated system from iFAST® Diagnostics was used, which measures for only 30 seconds. Impedance data was analysed using MATLAB (2023a) and FlowJo (v10.10.0). The 2µm calibration beads were used to normalise the impedance data (diameter 2, opacity 1, phase 0). Cells were gated to exclude beads and electrical noise. Sample exclusion criteria were quality checked for the following features: a) control 1 (C1) <1900 cells, b) bead count >2-fold difference to C1, c) technical anomalies of read-out. Total event count was determined and the % cell count compared to control calculated. The data was exported to Microsoft 365 Excel (v2208), where it was linked to published MICs⁸ and sorted into susceptible and resistant per antibiotic. Using the Real Statistics Resource Pack software (Release 8.9.1 www.real-statistics.com), t-tests and ANOVA were calculated on % cell count compared to control.

Results

Exposure Time Determination

To establish the minimum antibiotic exposure time to generate reliable results on iFAST®, logarithmically growing cultures of susceptible WHO F and resistant WHO X were exposed to EUCAST breakpoint or ECOFF concentrations of ceftriaxone, ciprofloxacin and azithromycin (Table 1) in liquid culture for five hours with samples measured every hour (Figure 1).

The unexposed controls were viable at every timepoint, whereas exposed populations became non-viable over time. Published E-test susceptibility profiles were used to define susceptible and resistant strains (Table 1).⁸ At 1 hour, there was no significant reduction in cell count compared to the control for any of the susceptible bacterial cultures (F-CRO, F-CIP, F-AZM, X-AZM, Figure 1). After 2-, 3-, 4- and 5-hours exposure, all susceptible WHO F samples (F-CRO, F-CIP, F-AZM) showed significant reduction in cell count compared to control. WHO X exposed to azithromycin (X-AZM) also showed a reduction in cell count compared to control at every time point after 1 h, which became significant at 5 hours of exposure. None of the resistant WHO X samples showed a significant decrease in % cell count compared to control at any timepoint as measured on iFAST[®] (Figure 1, Table S1). Based on these results and to allow measurement of antimicrobial susceptibility within a working day (8 hours), a 4 h exposure timepoint was selected for further development of the method.

Breakpoint AST

Proceeding with a 4 h exposure protocol, the susceptibility profiles of an increased number of *N. gonorrhoeae* strains, WHO F, K, O, V, X, and Y, were assessed against the same antibiotics using iFAST[®] (Figure 2, Table S2). These strains have a variety of susceptibilities and resistance mechanisms (Table 1, Figure S1 and ⁸).

For ceftriaxone and ciprofloxacin, there was a highly significant difference in the % cell counts compared to the control between susceptible and resistant strains (T-test P-value=0.00019 and P=0.000005, respectively), with all susceptible values <40% cell count of control after 4 h exposure (Figure 2). For azithromycin the significance is low (P=0.05) for the difference between those with an MIC on or below the ECOFF and those above, possibly due to a single resistant strain being compared to five susceptible ones. The highest mean % cell count compared to control for a susceptible strain was 36% of control (V-CRO) and the lowest in a resistant strain was 58% (V-CIP). An accurate cut-off is therefore likely to be between these two values and to vary between antibiotics as shown previously.¹¹

148

149 Dose-Response Analysis

150 Having shown concordance between qualitative (susceptible/resistant) and published
151 quantitative (MICs) susceptibility profiles, we wanted to investigate whether the iFAST®-
152 protocol could be used to determine MICs in agreement with published values faster than
153 current gold standard methods. Strains WHO X and WHO O were selected for the
154 development of this iFAST®-based electrical MIC (eMIC)¹² protocol, to cover a range of MICs.
155 The MIC concentration and at least two doubling dilutions above and below were tested for
156 each strain-antibiotic combination.⁸ Cell count decreases plateaued at 20-30% of control,
157 coinciding with essential agreement with their published MIC, for nearly all tested
158 combinations (Figure 3). The exceptions were strain WHO O exposed to ceftriaxone and WHO
159 X exposed to ciprofloxacin, which reproducibly showed a significant decrease in cell counts
160 three doubling dilutions below the estimated MIC of 64mg/L (published MIC is >32mg/L; Figure
161 3, Table S3). The lack of change for O-CRO may be linked to the number of concentrations
162 tested here.

163

164 Dose Response (eMICs) of novel compounds measured on iFAST

165 To establish whether these electronic MICs (eMICs) could also show susceptibility to novel
166 compounds, extended eMICs for zoliflodacin and gepotidacin compared to ciprofloxacin were
167 tested on WHO X, which contains mutations conferring resistance to fluoroquinolones,
168 tetracycline, sulphonamides, extended-spectrum cephalosporin and increased efflux of
169 macrolides and β -lactams.⁸ Both new compounds showed a clear, reproducible dose-
170 response curve (Figure 4, Table S4), which showed WHO X to be much more susceptible to
171 both zoliflodacin (purple) and gepotidacin (orange) than ciprofloxacin (blue). The decrease in
172 cell count for all compounds plateaued at 20-30% of the control. For ciprofloxacin this was at
173 32-64 mg/L, coinciding with the published MIC of >32. Zoliflodacin and gepotidacin showed

very similar dose response curves with predicted eMICs of between 0.25 and 1 mg/L⁸. This matches the published MIC for gepotidacin (0.5mg/L)⁸, but is slightly higher than that of zoliflodacin (0.064mg/L)⁸. These results show promise for the iFAST method in establishment of susceptibility for a variety of compounds.

Despite the recent identification of zoliflodacin, and gepotidacin, new antibiotic leads are still urgently needed for *N. gonorrhoeae*. Screening of experimental compounds is often difficult for *N. gonorrhoeae* as they may be available in small amounts and have limited diffusion through agar, making it difficult to use standard methods. To explore the utility of iFAST[®] for testing novel compounds against *N. gonorrhoeae*, the eMIC of two novel compounds currently under evaluation for a range of Gram-negative species, BL7 and L22, were compared to ciprofloxacin.¹³ As expected, the eMIC trends for BL7 (black) and L22 (brown) were notably different to ciprofloxacin (Figure 4). For BL7 and L22 the decrease in cell count plateaued at 16mg/L and 4mg/L respectively, again at around 20% of the control, indicating that these novel compounds have a lower MIC than ciprofloxacin (>32mg/L) against WHO X. This shows the utility of the methods described here to accelerate discovery and evaluation of new antimicrobial candidates for *N. gonorrhoeae*, as well as support more rapid ASTs.

Discussion

In this proof-of-principle study three current gonococcal antibiotics, with different mechanisms of action, as well as two recently licensed therapeutics, were tested against up to six WHO *N. gonorrhoeae* reference strains, demonstrating the potential of the novel iFAST[®] technology as a platform for both rapid *N. gonorrhoeae* AST and eMIC protocols. The technology, currently in development for the rapid diagnosis of AMR bloodstream and urinary tract infections (iFAST Diagnostics Ltd),¹¹ has here been successfully adapted for use with fastidious, Gram-negative STI etiological agent, *N. gonorrhoeae*.

Currently, not many phenotypic ASTs are being developed for *N. gonorrhoeae*. Most involve expensive consumables, equipment and complex algorithms for interpretation. Molecular analyses include the use of a DNA-crosslinker dye that only enters cells which have been damaged by antibiotics. Quantitative real time PCT (qPCR) is then performed on extracted DNA, as crosslinked DNA will not be amplified.¹⁴ Similarly, Melendez *et al*¹⁵ quantify bacterial growth by the qPCR of the *OPA* gene in the presence and absence of antibiotics and Khazaei *et al*¹⁶ conduct qPCR of a number of genes. Direct phenotypic detection methods of cell survival following antibiotic exposure are described by Kuipers *et al*¹⁷, who developed a higher through-put agar dilution method by adapting the traditional method to a 96-well format, and by Somajo *et al*¹⁸, who developed a flow cytometry AST for *N. gonorrhoeae*. All of these methods are able to perform an AST within less than eight hours, but require expensive consumables and equipment for qPCR or flow cytometry or are cheaper, but no faster than the current gold standard.¹⁷ iFAST® opens the opportunity of a fast, high-throughput, semi-automated method without the requirement for expensive consumables.¹⁹

The *N. gonorrhoeae* strains used in this study were consistently grown in liquid media by adding 4.2% sodium bicarbonate to the media,⁹ negating the need for growth in a CO₂ atmosphere during antibiotic exposure. This allowed much easier, faster and cheaper handling of samples and could positively impact on the ease of use of this method in diagnostic laboratories.

Although we compared various metrics of exposed cells (changes in diameter, phase and opacity), the metric of % cell count compared to unexposed control population was the best predictor of susceptibility. This is consistent with our previous observations for *Enterobacterales* exposed to various UTI-relevant antibiotics¹⁹. What the changes in other metrics indicate, in terms of antibiotic mechanisms of action and bacterial responses to these antibiotics, remains to be elucidated.

EUCAST guidelines state that azithromycin is always to be used in conjunction with another effective agent and therefore does not have a defined breakpoint. The iFAST® azithromycin

breakpoint assay was able to differentiate between susceptible and high level resistant, but with low significance. This finding is consistent with other studies^{18, 20} and may be linked to the low number of samples tested here, with only one azithromycin resistant strain. The azithromycin eMIC did show that MIC determination was consistent with published data, with both tested strains dropping to about 20% cell count compared to untreated control at the published MIC of azithromycin. Measuring eMICs, rather than single breakpoint concentrations, may allow iFAST[®] to give accurate eMICs for azithromycin in future. The eMICs gave results within 2- to 4-fold of the published MICs for all antibiotics and strains. A better definition of the cut-off in reduction of cell count compared to control or, more likely, a metric that describes at which concentration the reduction in cell count plateaus, would improve alignment with published MICs.

Overall, the iFAST[®] assay developed here shows results consistent with current gold-standard *N. gonorrhoeae* agar MIC testing in less than eight hours. It shows further promise for use as a screening tool with novel compounds, which are much needed despite the advances made with zoliflodacin, and gepotidacin and other promising compounds.²¹ When screening novel compounds for anti-gonococcal activity, agar-based MIC assays are time-consuming and expensive to perform. The label-free test used here provides greater speed and higher throughput and, additionally, indications of modes of action and mechanisms of resistance, as can be seen in the distribution of diameter, opacity and phase of the cells (Figure S1, S2), mirroring responses in other Gram-negative organisms.¹¹ This can give further insight for the development and susceptibility testing of anti-gonococcal compounds. iFAST[®] could also be used as a companion diagnostic for susceptibility testing of either existing compounds in high predicted resistance settings or for watch/reserve use of novel compounds. The surveillance programmes across many nations could also benefit from having a more automated system, rather than the laborious agar dilutions currently used. Thus, the iFAST[®] technology could be used for multiple applications.

As with all other methods mentioned, currently, the *N. gonorrhoeae* isolates still need to be grown up for 24 hours before the AST testing, meaning that the complete AST is not carried out in <8 hours. However, as only about 20,000 cells are measured in the iFAST® technology, in future, protocol optimisation may allow for liquid culture using sodium bicarbonate and antibiotic exposure directly from sample swab, further reducing the time to AST results. The limit of detection on iFAST® is still being evaluated, but initial data suggest that 200µL of <5x10⁶/mL is sufficient for reliable susceptibility testing and so, very small amounts of viable bacteria are required for this assay.

In conclusion, the iFAST® protocol produces *N. gonorrhoea* AST results consistent with published values in <8 h from culture. If the data is shown to be equally promising with a range of clinical isolates, in addition to the WHO reference strains used here, it could become a rapid testing method for antibiotic susceptibility testing in gonococcal samples. Future work will focus on expanding strain and antimicrobial compound analysis to optimise metrics for susceptibility definitions for *N. gonorrhoeae* on iFAST®. The technology has advantages in being label free, not requiring expensive consumables such as qPCR reagents and the platform being suitable for a range of AST applications with other bacteria. Depending on the costs of the instrumentation and availability of tests that have been through regulatory approval, this could offer an attractive option to support improved evidence-based antibiotic prescribing in clinical laboratories.

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285 Transparency Declarations
286 None to declare. iFAST Diagnostics is a company established to commercialise the iFAST
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289 References

- 290 1. WHO. Gonorrhoea (Neisseria gonorrhoeae infection). Geneva, Switzerland: WHO, 2025.
- 291 2. Fifer H, Ismail MA, Soni S *et al*. British Association of Sexual Health and HIV UK National
292 Guideline for the Management of infection with Neisseria gonorrhoeae , 2025. *International Journal*
293 *of STD & AIDS* 2025.
- 294 3. Jacobsson S, Cole MJ, Schröder D *et al*. Antimicrobial resistance in Neisseria gonorrhoeae and
295 its risk groups in 23 European countries in 2022 within the European Gonococcal Antimicrobial
296 Surveillance Programme (Euro-GASP): a retrospective observational study. *The Lancet Regional Health*
297 *- Europe* 2025; **54**: 101318.
- 298 4. WHO. Updated recommendations for the treatment of Neisseria gonorrhoeae, Chlamydia
299 trachomatis and Treponema pallidum (syphilis), and new recommendations on syphilis testing and
300 partner services. 2024; 108.
- 301 5. Maubaret C, Caméléna F, Mrimèche M *et al*. Two cases of extensively drug-resistant (XDR)
302 Neisseria gonorrhoeae infection combining ceftriaxone-resistance and high-level azithromycin
303 resistance, France, November 2022 and May 2023. *Eurosurveillance* 2023; **28**.
- 304 6. Suzy Sun PN, Anna Vickers, Rachel Pitt, Michel X. Doumith, Sandra David, Sandhya Vivekanand,
305 Zdravko Ivanov, Zeynab Yusuf, Fahima Rashid, James Johnson, Kirsty Bennet, Emma Callan, Michelle
306 Cole, Hamish Mohammed, Katy Sinka, Sarah Alexander, Helen Fifer. Gonorrhoea: guidance, data and
307 analysis - GRASP report: data to August 2024. In: UKHSA, ed: UK Health Security Agency, 2010.
- 308 7. Spencer DC, Paton TF, Mulroney KT *et al*. A fast impedance-based antimicrobial susceptibility
309 test. *Nat Commun* 2020; **11**: 5328.
- 310 8. Unemo M, Sanchez-Buso L, Golparian D *et al*. The novel 2024 WHO Neisseria gonorrhoeae
311 reference strains for global quality assurance of laboratory investigations and superseded WHO N.
312 gonorrhoeae reference strains-phenotypic, genetic and reference genome characterization. *The*
313 *Journal of antimicrobial chemotherapy* 2024; **79**: 1885-99.
- 314 9. Spence JM, Wright L, Clark VL. Laboratory maintenance of Neisseria gonorrhoeae. *Curr Protoc*
315 *Microbiol* 2008; **Chapter 4**: Unit 4A 1.
- 316 10. Testing TecoAS. Breakpoint tables for interpretation of MICs and zone diameters, version
317 12.0. 2022.

11. Lucy J Bock DCS, Bethany K Martin, Caitlin N Daniels, X Dyball, Charlotte K Hind, Matthew E Wand, Collette E Allen, Robert C Read, H Morgan, J Mark Sutton. Rapid impedance-based Antimicrobial Susceptibility Testing (iFAST) of Enterobacterales in urinary tract infections. *Journal of Infection* 2025; **submitted**.

12. Spencer D, Li Y, Zhu Y *et al*. Electrical Broth Micro-Dilution for Rapid Antibiotic Resistance Testing. *ACS Sens* 2023; **8**: 1101-8.

13. Laws M, Hind C, Nahar K *et al*. Efflux Resistance Breakers: A New Technology to Overcome Efflux-Mediated Resistance in Multidrug Resistant Bacteria. *ChemRxiv*, 2025.

14. Tjandra KC, Ram-Mohan N, Abe R *et al*. Rapid molecular phenotypic antimicrobial susceptibility test for *Neisseria gonorrhoeae* based on propidium monoazide viability PCR. Cold Spring Harbor Laboratory, 2023.

15. Melendez JH, Chen F-E, Wang T-H. Antimicrobial susceptibility testing of *Neisseria gonorrhoeae* using a phenotypic-molecular assay and lyophilized antimicrobials. *Diagnostic microbiology and infectious disease* 2022; **102**: 115590.

16. Khazaei T, Barlow JT, Schoepp NG *et al*. RNA markers enable phenotypic test of antibiotic susceptibility in *Neisseria gonorrhoeae* after 10 minutes of ciprofloxacin exposure. *Scientific Reports* 2018; **8**.

17. Kuipers JE, Seidel L, Garnier NE *et al*. *Neisseria gonorrhoeae* antimicrobial susceptibility testing: application of traditional agar dilution to a high-throughput 96-well microtiter assay. *Microbiology Spectrum* 2025; **13**.

18. Somajo S, Nilsson F, Ekelund O *et al*. Detection of antimicrobial resistance in <5 h in *Neisseria gonorrhoeae* isolates using flow cytometry-proof of concept for seven clinically relevant antimicrobials. *The Journal of antimicrobial chemotherapy* 2024; **79**: 815-9.

19. Bock LJ, Spencer DC, Martin BK *et al*. Rapid impedance-based Antimicrobial Susceptibility Testing (iFAST) of Enterobacterales in urinary tract infections. *J Infect* 2025; **91**: 106549.

20. Chen SC, Liu JW, Wu XZ *et al*. Comparison of Microdilution Method with Agar Dilution Method for Antibiotic Susceptibility Test of *Neisseria gonorrhoeae*. *Infect Drug Resist* 2020; **13**: 1775-80.

21. GARDP backs Debiopharm's Swiss-developed compound to treat drug-resistant gonorrhoea infections. Geneva and Lausanne: GARDP and Debiopharm, 2025.

347

348 Tables

349 **Table 1:** Published susceptibility profiles of the strains used in this study ⁸.

WHO strain name NCTC strain name	bacterial strain												EUCAST breakpoints	
	F		K		O		V		X		Y		mg/L	
	MIC	WHO	MIC	WHO	MIC	WHO	MIC	WHO	MIC	WHO	MIC	WHO	S≤	R>
Ceftriaxone	<0.002	S	0.008	S	0.032	S	0.064	S	2	HLR	1	HLR	0.125	0.125
Ciprofloxacin	0.004	S	>32	HLR	0.008	S	>32	HLR	>32	HLR	>32	HLR	0.03	0.06
Azithromycin (ECOFF)	0.125	S	0.25	S	0.25	S	>256	HLR	0.5	I	1	R	1	

350 MIC as defined by ⁸; WHO – susceptibility as defined by ¹⁰; S – Susceptible; HLR – High level of resistance; I –

351 susceptible, increased exposure; R – resistant; ECOFF – no defined breakpoint for azithromycin, so WT ECOFF

352 used in this study

Figures

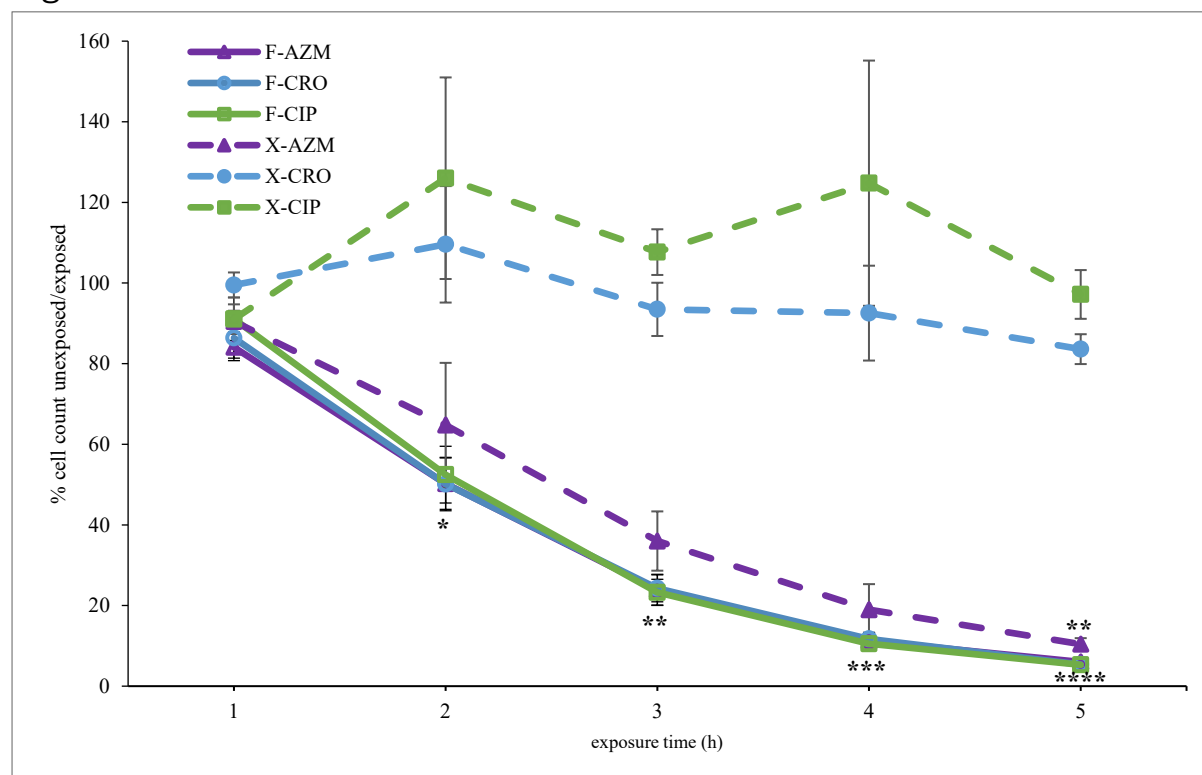
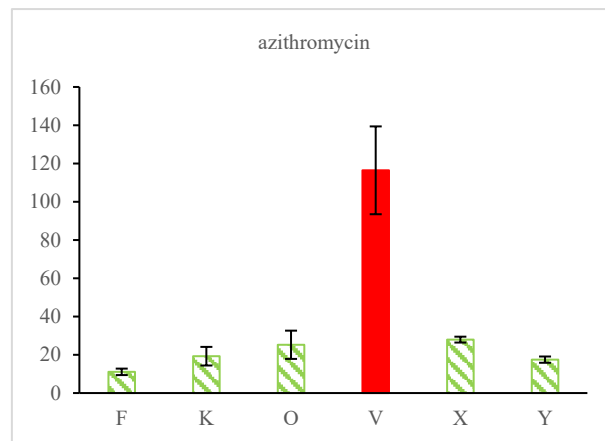
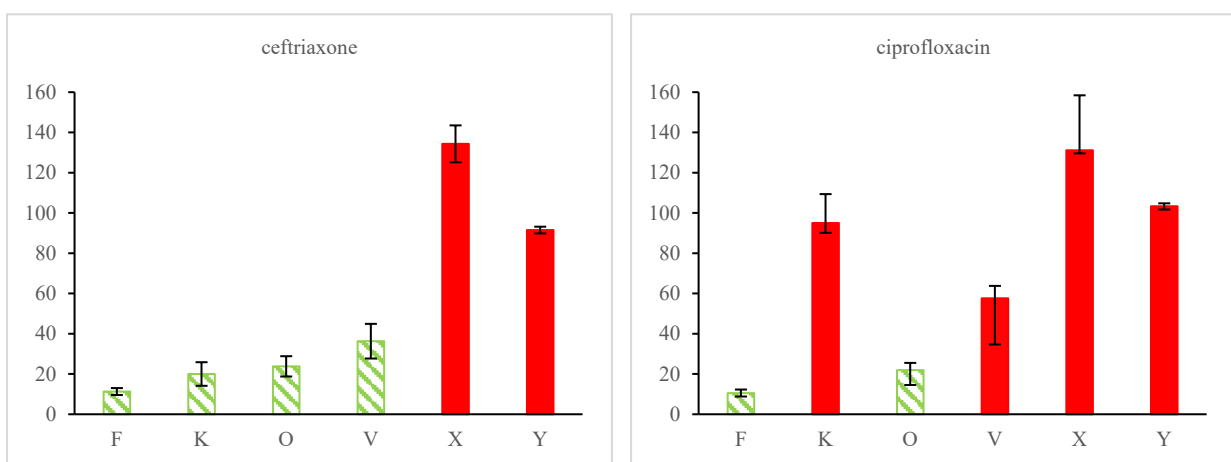


Figure 1. 4hr exposure to antibiotic breakpoint concentration is sufficient to measure phenotypic resistance profiles in *N. gonorrhoeae* WHO reference strains F and X. 5×10^5 cells/mL of strains F (through lines) and X (dashed lines) were exposed to EUCAST susceptibility breakpoint concentrations of ceftriaxone (CRO, 0.125mg/L, blue circles), ciprofloxacin (CIP, 0.03mg/L, green squares), and azithromycin (AZM, 1mg/L, purple triangles) at 37°C. Cell counts were measured on the iFAST every hour for 5 h and compared to an unexposed control (n=3). Error bars are $\pm$ SEM. Tukey-HSD significance denoted by * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), **** ($P \leq 0.0001$). See Table S1 for data.



365



366 **Figure 2. Susceptibility profiles of *N. gonorrhoeae* can be accurately identified after 4 h**
 367 **breakpoint concentration exposure to three frontline antibiotics using iFAST®.** 5×10^6 cells/mL of
 368 reference strains F, K, O, V, X, and Y were exposed to breakpoint concentrations of ceftriaxone
 369 (0.125mg/L), ciprofloxacin (0.03mg/L), and azithromycin (1mg/L) for 4 h at 37°C, measured on iFAST®
 370 and compared to unexposed controls (n=3). Published susceptibility classifications⁸ are denoted by bar
 371 design: susceptible = green diagonal stripes, high level resistant = red. Error bars are ±SEM. See Table
 372 S2 for data, figure S1 for scatter plots.

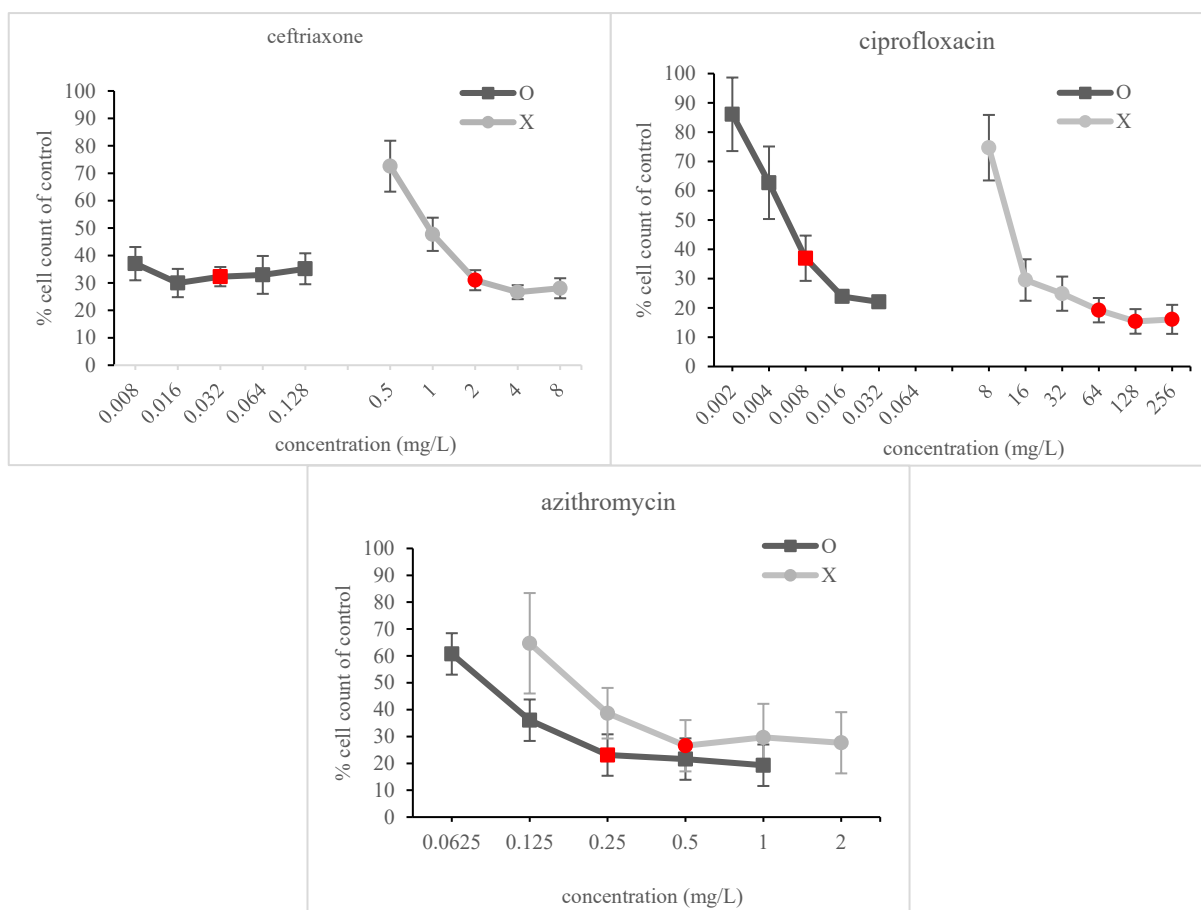


Figure 3. iFAST® electrical minimum inhibitory concentration (eMIC) assay results for *N. gonorrhoeae*. 5×10^6 cells/mL of strains O (dark grey, filled squares) and X (light grey, filled circles) were exposed to doubling dilutions of ceftriaxone, ciprofloxacin and azithromycin for 4 h at 37°C. Cell counts were measured on iFAST® and compared to unexposed control ($n=3$). Published agar dilution-derived MIC values denoted by red marker.⁸ Error bars are $\pm$ SEM. See Table S3 for data, Figure S2 for scatter plots.

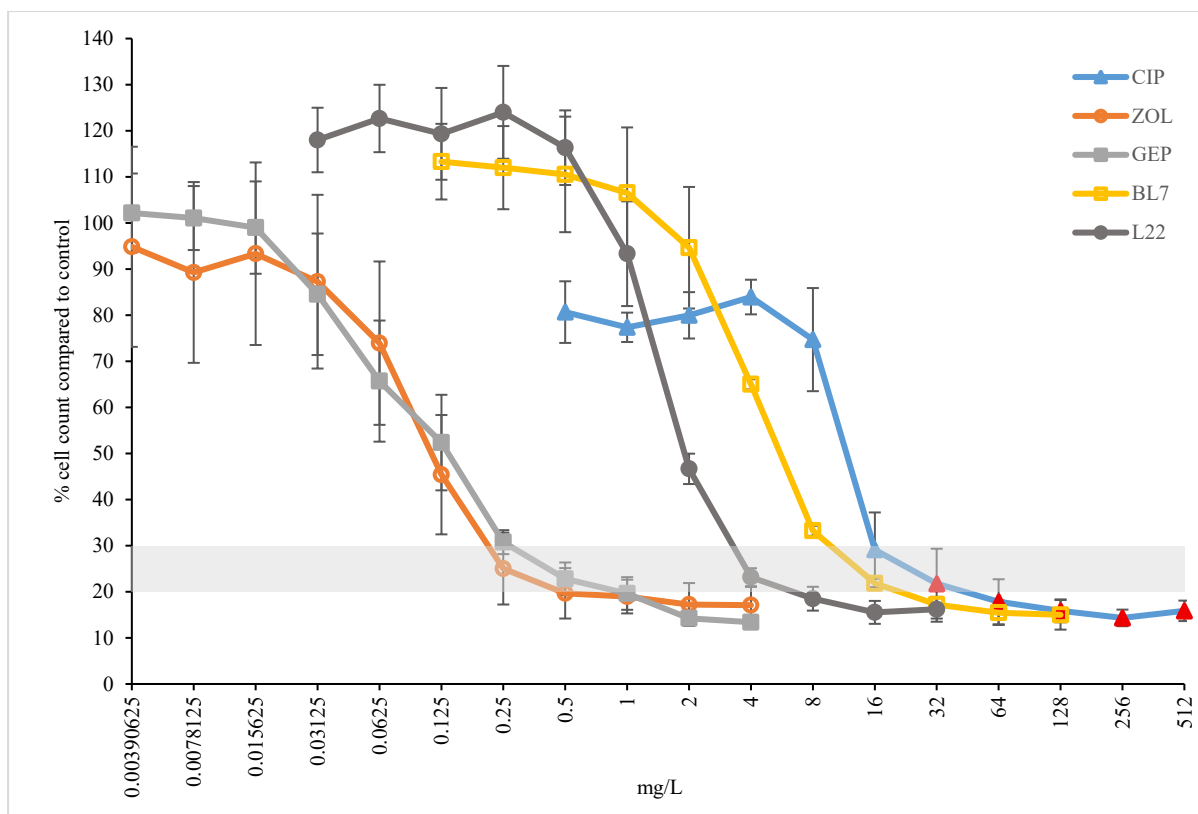


Figure 4. iFAST[®] technology can be used to investigate novel compound activity against *N. gonorrhoeae*. 5×10^6 cells/mL of strain WHO X were exposed to doubling dilutions of ciprofloxacin (blue, closed triangles), zoliflodacin (ZOL, orange, open circles), gepotidacin (GEP, light grey, closed squares) and novel compounds BL7 (yellow, open squares) and L22 (dark grey, closed circles) for 4 h at 37°C. Cell counts were measured on iFAST[®] and compared to unexposed control (n=3). Error bars are $\pm$ SEM. Grey bar indicates zone where dose response curves plateau at 20-30% cell count of control, likely indicating eMICs. See Table S4 for data.