Cell envelope damage of *Neisseria gonorrhoeae* after 15-min beta-lactam exposure enables rapid antimicrobial susceptibility testing

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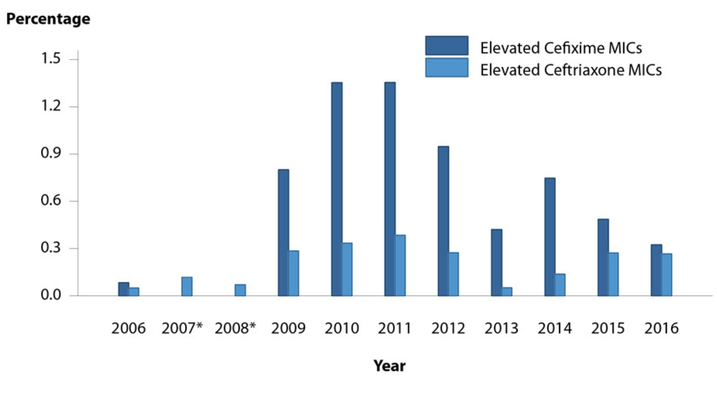
Abstract

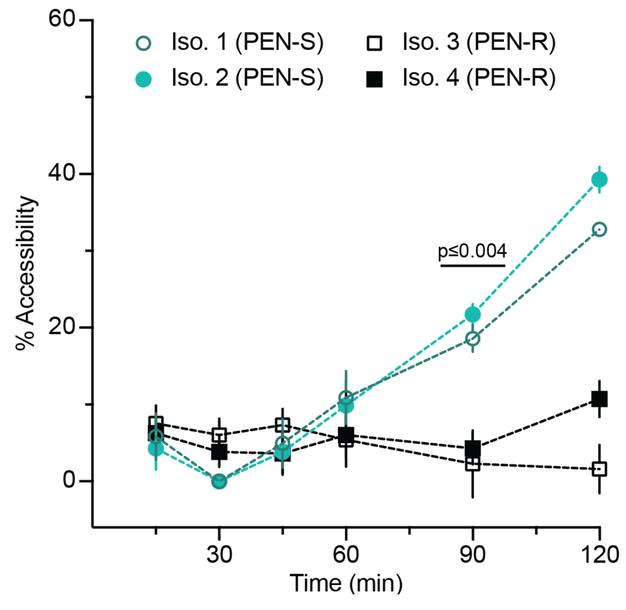
Designing diagnostic tools to perform phenotypic antimicrobial susceptibility testing (AST) at the point-of-care (POC) is a vital step in tackling the global threat of antimicrobial resistance. Gonorrhea infections with resistance to the first-line dual therapy have already emerged, highlighting the impending threat of untreatable gonorrhea. A rapid, phenotypic AST could enable evidence-based (instead of empirical) therapy and improve surveillance. The focus of this work is to develop innovative strategies to measure the phenotypic antimicrobial susceptibility of *Neisseria gonorrhoeae (Ng)* clinical isolates after just 15-30 min of exposure with an antibiotic. We focused on the duration of the exposure step because it remains the bottleneck for phenotypic AST with fastidious and slow-growing microorganisms, such as *Ng*. We use a nucleic acid readout because it provides high sensitivity and specificity. Our long-term goals include building fully integrated POC devices that determine the phenotypic response to antibiotic of a specific pathogen rapidly. By designing techniques that allow us to rapidly determine the antibiotic phenotype, evidence-based prescription of antibiotics will become possible.



Gonococcal Isolate Surveillance Project (GISP), 2006–2016[1]

Accessibility AST (aAST)

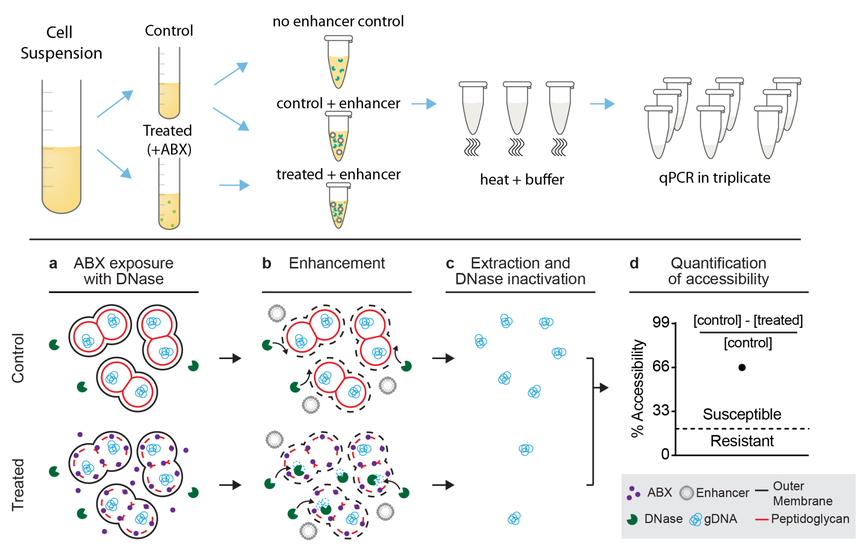
Our lab has been developing rapid, phenotypic ASTs using ultrasensitive measurements of changes in nucleic acids (NA) in response to an antibiotic.[2] However, fastidious and slow-growing organisms (such as *Ng*) remain challenging. Here, we hypothesized that an NA-based approach could work on *Ng* exposed to beta-lactams, such as penicillin (PEN), because these antibiotics degrade the cell walls of susceptible bacteria, leading to cell lysis over long exposures. Degrading DNA from lysed cells enables us to measure the susceptibility to the beta-lactam.



Two penicillin-susceptible (PEN-S) and two penicillin-resistant (PEN-R) clinical Ng isolates are exposed to PEN in the presence of exogenous nuclease (DNaseI), which degrades accessible DNA from damaged or lysed cells. Phenotypic differences arise after 90 min of PEN exposure.

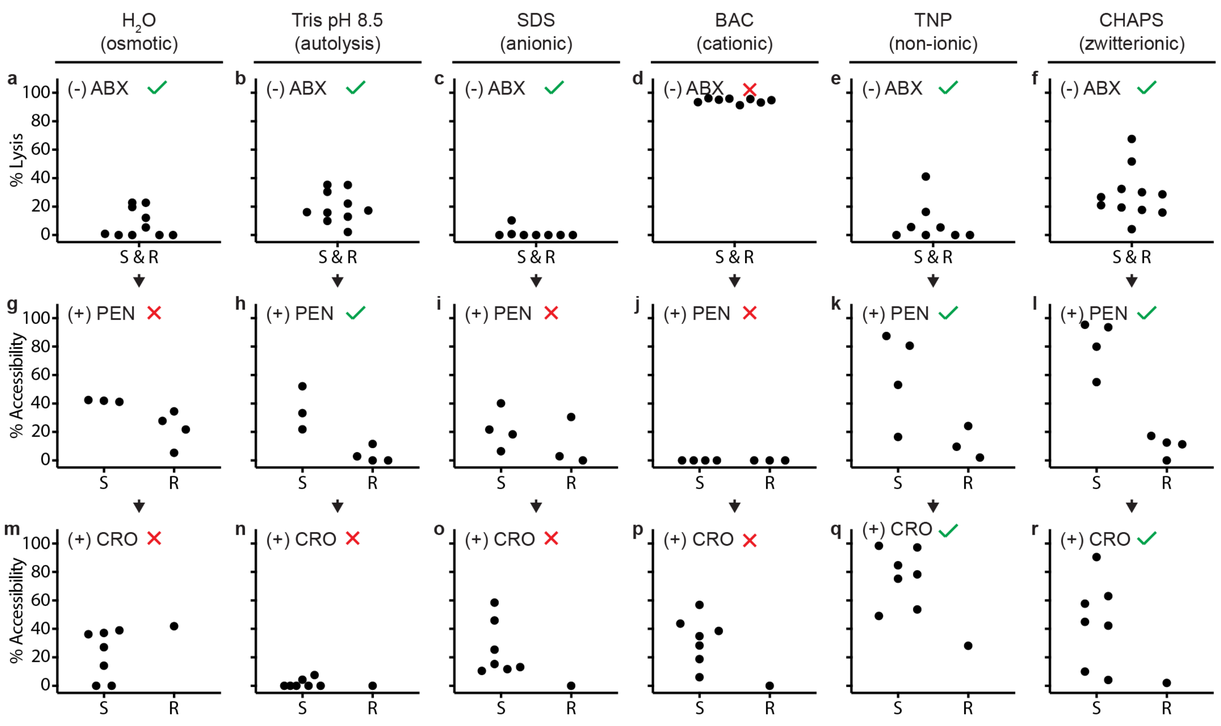
aAST with enhancement step

Building from previous phenotypic AST developments in the lab[2], the workflow for an "accessibility AST" (aAST) is shown. The beta-lactams target the peptidoglycan cell wall and the enhancer targets the outer membrane, allowing antibiotic damage to be measured after short antibiotic exposures. Nucleases digest accessible nucleic acids from cells with cell wall and outer membrane damage. Quantification of nuclease-accessible nucleic acids allows a phenotypic assessment of susceptibility of *N. gonorrhoeae* to the beta-lactam.



$$qPCR\ \%\ Lysis = \left(1 - 2^{(Cq_{DNaseI} - Cq_{enhancer})}\right) * 100$$
$$qPCR\ \%\ Accessibility = \left(1 - 2^{(Cq_{control} - Cq_{treated})}\right) * 100$$

Selection of enhancers

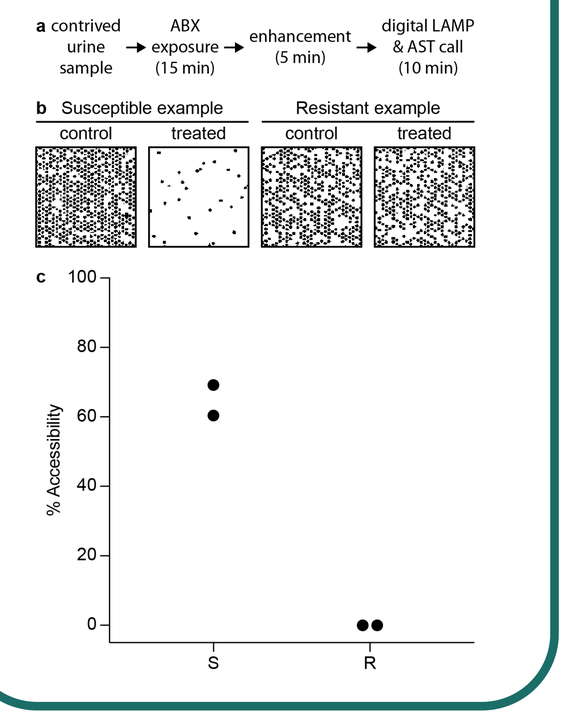


Six enhancers were tested for the potential to enhance the readout of the aAST. Experiments are shown (left) to compare enhancers given to the cells after a 15 min exposure to two different beta-lactam antibiotics, penicillin (PEN) or ceftriaxone (CRO). Exposure to each enhancer totaled 5-10 min. Enhancers were evaluated for minimization of background lysis and correct categorical separation of antibiotic susceptibility. Osmotic stress, TRIS buffer pH 8.5[3] to induce autolysis, and several surfactants were compared. DNA was measured with real time qPCR in technical triplicates.

The amount of DNA digested, represented on these plots as a percentage, is representative of the damage done to the cell wall and outer membrane. Checkmarks indicate enhancers that met our criteria and X's indicate enhancers that do not meet our criteria.

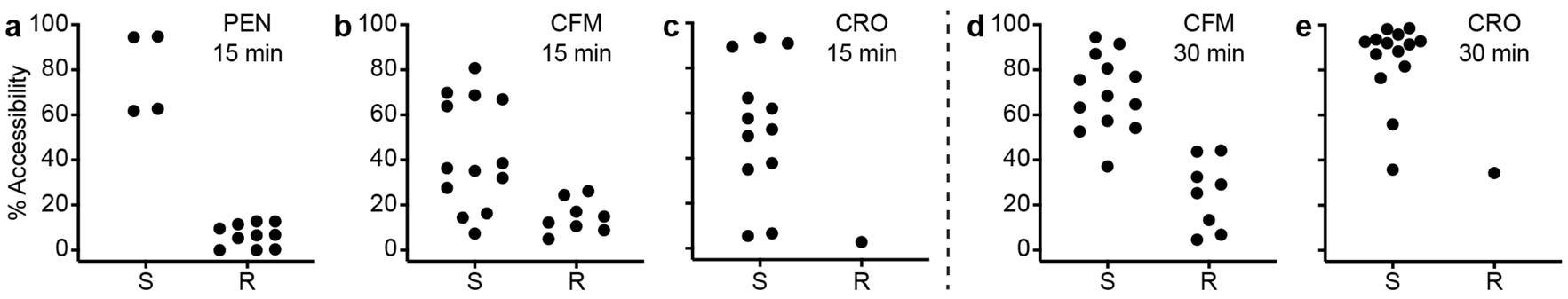
Contrived urine samples aAST in 30 minutes

Using contrived samples (clinical isolates of Ng spiked into healthy human urine), we performed an experiment as a proof-of-concept of the speed of the steps of the aAST method. The aAST was run with a 15-min penicillin exposure and a 5-min enhancement step using CHAPS. Rapid DNA amplification was achieved with isothermal digital LAMP. All four clinical isolates were correctly categorized (S=susceptible; R=resistant by EUCAST breakpoints). The sum of all steps was <30 min, illustrating the potential of aAST for use in a POC diagnostic.



Enhanced aAST

Categorical agreement for clinical isolates exposed to penicillin (PEN) ceftriaxone (CRO) and cefixime (CFM) is achieved after extending the antibiotic incubation time from 15 min to 30 min of exposure, with a 5 min secondary incubation with CHAPS to enhance accessibility.



(a-c) PEN, CFM, and CRO data represent 3-10 biological replicates of 15-minute antibiotic exposure to a clinical isolate. 100% categorical agreement is achieved for PEN.
(d-e) Exposures of isolates to CFM and CRO with 30-minutes of antibiotic exposure show improvements of categorical agreement, with one minor error each.
S = susceptible; R = Resistant by EUCAST breakpoints

CARB-X

References

(1) CDC, 2018 [https://www.cdc.gov/std/stats16/gonorrhea.htm]
(2) Nathan G. Schoepp, Travis S. Schlappi, Matthew S. Curtis, Slava S. Butkovich, Shelley Miller, Romney M. Humphries, Rustem F. Ismagilov. 2017. "Rapid pathogen-specific phenotypic antibiotic susceptibility testing using digital LAMP quantification in clinical samples."
(3) Hebeler, B. H., & Young, F. E. 1975. "Autolysis of Neisseria gonorrhoeae."

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