CRISPR-based diagnostics for resistance guided therapy of gonorrhea at the point-of-care

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Background: The extensive use of antibiotics in the field of sexually transmitted infections (STIs) has contributed to the development of antimicrobial resistance in *Neisseria gonorrhoeae*. Currently, only intramuscular ceftriaxone is recommended for empirical treatment of gonorrhea and extensively drug-resistant strains have already emerged. Ideally, diagnostic tests should facilitate stewardship of both older and newer antimicrobials to preserve effective treatments. As no such test currently exists, our goal is to develop an affordable and rapid test for resistance-guided therapy of gonorrhea.

Methods: We used our novel CRISPR-based diagnostic assay, which combines nucleic acid amplification techniques with CRISPR detection for optimal sensitivity and specificity of single nucleotide polymorphisms encoding antibiotic resistance. We identified and optimized gRNAs and primers to detect multiple STI and resistance targets.

Results: We demonstrated that our assay has PCR-like sensitivity, and detects samples with cycles to threshold (Ct) of 37 in under 10 minutes. Our assay detected targets for *Chlamydia trachomatis, Trichomonas vaginalis* and *Neisseria gonorrhoeae*. High specificity of CRISPR for detection of single nucleotide polymorphisms with ciprofloxacin-susceptible *Neisseria gonorrhoeae*, shows the suitability of CRISPR-Cas12 assays for resistance guided therapy. Finally, we demonstrated that our assay can be easily integrated into an affordable battery-operated device for decentralized use.

Conclusion: Our assay enables resistance-guided therapy at the point-of-care, providing oral treatment alternatives to ceftriaxone. Its compatibility with existing molecular point-of-care instruments could enhance current testing platforms, while its versatility and specificity ensure fast adaptation to evolving targets and resistance markers.