Rapid AMR diagnostics via recombinase polymerase amplification - our experiences to date.

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Background: Early detection of antimicrobial resistance (AMR) is pivotal to enhancing management, including targeting treatment, pinpointing patients requiring test-of-cure and/or isolating infected individuals. Isothermal amplification tests are well recognized for potential use at point-of-care settings due to their ease of use and limited technical requirements. Our laboratory has recently developed a range of rapid AMR tests utilizing isothermal recombinase polymerase amplification (RPA), and here we provide an overview of experiences to date.

Methods: RPA based assays coupled with lateral flow detection (RPA-LFD) have so far been developed for detection of *Neisseria gonorrhoeae* and associated ciprofloxacin resistance, as well as various other clinically important genes harbored by other Gram-negative bacteria; CTX-M, CMY-2, KPC, IMP and OXA-48.

Results: The RPA-LFD assays offer high analytical sensitivity, typically less than 100 copies per reaction, and can (depending on the DNA extraction step) be performed in less than 30 minutes. Our *N. gonorrhoeae* RPA assay, for example, provided a positive percentage agreement (PPA) of 87.5% compared with a commercial *N. gonorrhoeae* nucleic acid amplification test for detection of *N. gonorrhoeae*, and correctly identified ciprofloxacin-susceptible infections albeit at lower sensitivity (83%). The latter was achieved without any equipment, and incubating samples in the palm of one's hand. Hurdles include RPA's tolerance for sequence mismatches, and also identifying a suitably rapid DNA extraction step.

Conclusion: While some issues still need to be overcome, RPA-LFD in combination with rapid sample preparation methods have considerable potential for point-of-care application, particularly in low resource environments due to their fast sample to result turnaround time and independence from specialized equipment and personnel. Development and optimizations are ongoing.

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