Rapid AMR diagnostics via recombinase polymerase amplification – our experiences to date

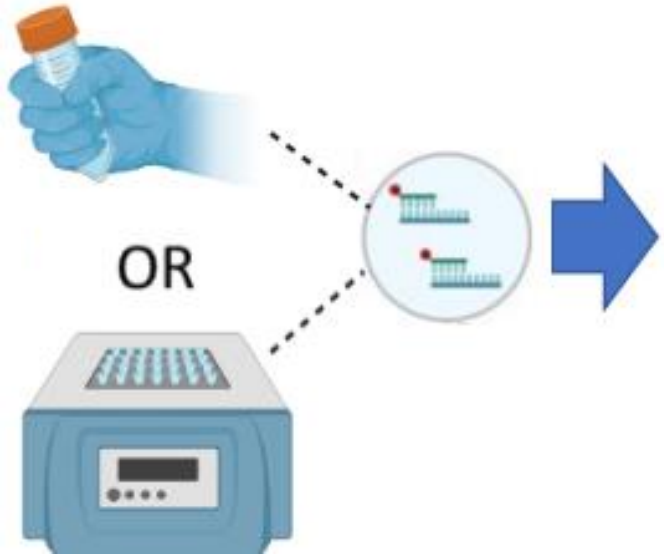
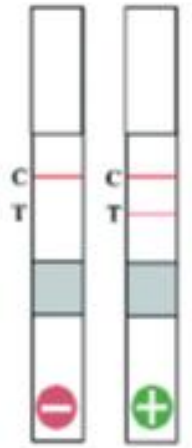
Dr Nicole Ertl

The University of Queensland – Centre for Clinical Research

Background

- Early detection of antimicrobial resistance (AMR):
  - Targeted treatment
- Potential of isothermal amplification tests closer to/at point-of-care
  - Fast turn-around time, sensitive and specific, versatile, easy to carry out and read, no specialised equipment
- Our experiences developing AMR tests using recombinase polymerase amplification (RPA)

Methods

Targets	Extract DNA	Amplify	Detect
• *Neisseria gonorrhoeae*[1] • Ciprofloxacin susceptibility[1] • ESBLs[2] • AmpC[2] • Carbapenemases	E.g. • Commercial kit • Boiled preparation • Rapid extraction reagent		
Time:	**10-30 min**	**10-15 min**	**5 min**

1. Ayfan *et al.* 2022, *J Antimicrob Chemother*, 77(11):2933-2936; 2. Ertl *et al.* 2023, *JAC Antimicrob Resist*, accepted

Results

Test parameters	*N. gonorrhoeae*[1]	Ciprofloxacin[1]	ESBLs[2]	AmpC[2]	Carbapenemases
Diagnostic target(s)	*porA*	*gyrA* wildtype	*bla*$_{\text{CTX-M group 1}}$ *bla*$_{\text{CTX-M group 9}}$	*bla*$_{\text{CMY-2}}$	*bla*$_{\text{KPC}}$ *bla*$_{\text{IMP}}$ *bla*$_{\text{OXA-48}}$
Amplification temp (°C)	39	39	39	39	39
Detection	lateral flow	lateral flow	lateral flow	lateral flow	lateral flow
Analytical sensitivity (copies/reaction)	11.4	247.4	≤ 27.3	3.4	≤ 17.7
Clinical sensitivity (%)[a]	87.5	83.3	100*	100	-
Clinical specificity (%)[a]	-	100	≥ 94.1	100	≥ 97.9
Time to result (min)	15	15	15	15	15-20

[a]compared to PCR and/or Sanger sequencing
*only *bla*$_{\text{CTX-M group 1}}$ clinical sensitivity could be determined

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- Similar sensitivity and specificity as PCR
- Rapid (sample to result: ≤ 30 min)
- Easy to adapt
- Easy readout
- No specialised equipment / hand incubation[1]

- SNP detection difficult (tolerant to mismatches)
- Increase user friendliness by decreasing number of steps
- Manufacturing issues

1. Ayfan *et al.* 2022, *J Antimicrob Chemother*, 77(11):2933-2936

Conclusion

- Potential as closer to/at point of care test:
  - Rapid sample to result when coupled with rapid extraction method
  - No specialised equipment or extensive training needed
- Potential to increase testing accessibility in low resource environments and low income countries
- Continue collaborating with industry with aim to take our research into practice

Disclosure of interest

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Contact

Dr Nicole Ertl
nicole.ertl@uq.edu.au

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