Molecular Determination of Antimicrobial Resistance in *Neisseria gonorrhoeae* Positive Remnant Aptima Urine Specimens Indicates Emerging Resistance to Cephalosporins and Azithromycin in Saskatchewan, Canada

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Background

N. gonorrhoeae (NG) is classified as a superbug because it has developed resistance to all classes of antibiotics used for treatment, including extended spectrum cephalosporins (ESCs) and macrolides such as azithromycin. The later two antibiotics are used in combination therapy to combat antimicrobial resistance (AMR) development. Thus the surveillance of AMR in NG is critical to ensure that appropriate therapies are recommended. The shift from culture-based methods to nucleic acid amplification tests (NAAT) to detect NG infections does not permit AMR detection. Whole genome sequencing methods have been utilized to predict AMR from *N. gonorrhoeae* cultures and not from NAAT specimens. We evaluated a PCR approach to establish the AMR profiles to 6 antibiotics of 50 Ng positive Aptima remnant NAAT specimens. The AMR strain types were also evaluated.

Methods

Results

1). Viral RNA mini kit showed improved DNA concentration and 260/230 ratio.

Figure 1A: Comparison of DNA concentrations extracted using approach 1 and approach 2

Figure 1B: Comparison of 260/230 ratio for the DNA extracted with two different protocols. Black dotted line indicating recommended 260/230 ratio.

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2). The utilization of approach 2 was more efficient for amplification of non-amplifiable Aptima urine specimens:

	% of amplicons obtained	% of Sequences aligned with NGSTAR database
Approach 1	41.7% (146/350)	97% (142/146)
Approach 2	100% (204/204)	93% (189/204)

3). Emergence of reduced susceptibility to ESCs and Azithromycin resistance in *N. gonorrhoeae*:

Figure 2: A phylogenetic tree representing grouping of Aptima urine specimens based on respective *penA* type.

The two main clusters are marked as A & B. Columns indicate the mutation patterns for each molecular determinants. Purple represents presence of the determinant and orange its absence. Gray indicates specimens possessing *porB1a* rather than *porB1b*. [†] NAC= New Allelic Combination, NGSTAR. The complete profile of 14/50 samples could not be ascertained.

Conclusions

- QIAGEN Viral RNA mini kit improved DNA quality and quantity from NAAT specimens.
- In contrast to the AMR determination from bacterial cultures, this study predicted emerging resistance to cephalosporins and azithromycin in Saskatchewan, with high levels of ciprofloxacin resistance.
- AMR surveillance can be increased by predicting AMR in NAAT specimens.