Can residual urethral swabs be used for detection of *Neisseria gonorrhoeae* and antimicrobial resistance markers?

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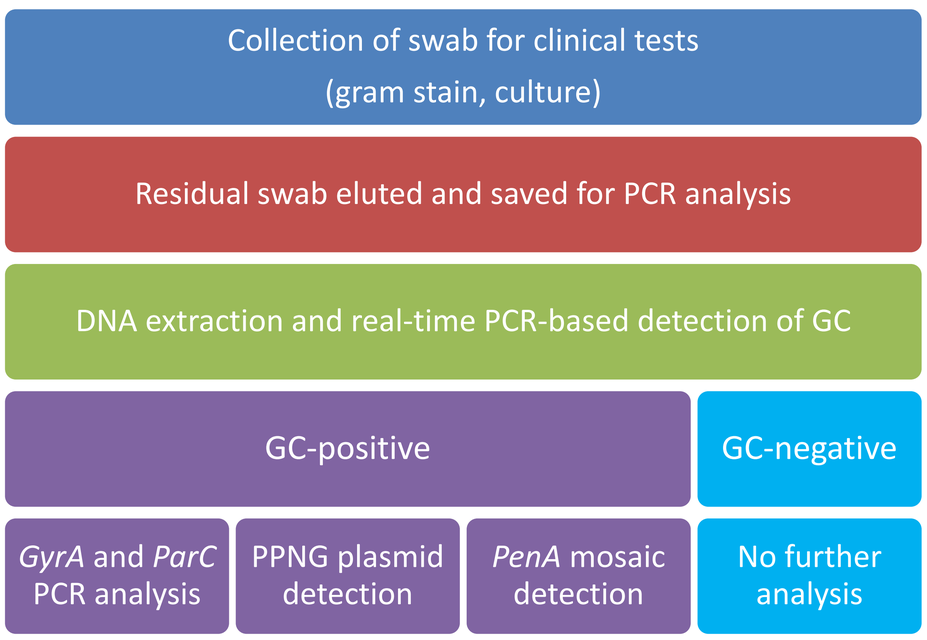
Background

Neisseria gonorrhoeae (GC) is the second most commonly reported bacterial sexually-transmitted infection (STI) worldwide. Rapid diagnosis and treatment with effective antimicrobials are critical for disease management and prevention of outbreaks. However, the use of nucleic acid amplification tests (NAATs) prevents antimicrobial susceptibility testing thus limiting efforts for antimicrobial stewardship and surveillance. In the prevent study, we have investigated whether residual urethral swabs collected at a local STI clinic can be used for the molecular detection of GC and associated antimicrobial resistance markers.

Methods

The de-identified urethral swabs used in this study were collected from patients undergoing gonorrhea testing at the Baltimore City Health Department (BCHD). Briefly, a single urethral swab was collected and used for gram stain analysis and cultures. The residual swab was then given a study number, re-hydrated in 500 µL of autoclaved water, and stored frozen for future PCR analyses. A 200 µL aliquot of each sample was then extracted for DNA on the Magna Pure LC instrument (Roche Diagnostics, Indianapolis, IN), and the resulting DNA sample tested blinded by PCR for the presence of GC DNA. GC-positive samples were further tested for genetic markers associated with decreased susceptibility to three different classes of antimicrobials, using previously described real-time PCR assays. Characterization of decreased susceptibility to quinolones was mediated by detection of mutant sequences in the *GyrA* and *ParC* region; testing for the plasmid in penicillinase-producing *N. gonorrhoeae* (PPNG), which confers resistance to penicillin, and direct detection of the *PenA* sequences associated with reduced susceptibility to extended-spectrum cephalosporins. Following PCR analyses, PCR results were compared to culture results.



Study design and analysis

Results

- 150 urethral swabs were analyzed by culture and PCR
- Sensitivity of the PCR assay for detection of GC using residual swabs was low (62.5%)

	Culture-positive	Culture-negative	Total
PCR-positive	**25**	**5**	**40**
PCR-negative	**15**	**105**	**120**
Total	**40**	**110**	**150**

Comparison of culture to PCR-based detection of GC directly from residual urethral swabs

- Of the 25 GC-positive by PCR, 6 (24%) had mutations in the *GyrA* or *ParC* gene, which are associated with decreased suscpetibility to quinolone.
- One sample (4%) was identified as PPNG.
- No markers associated with decreased susceptibility to extended-spectrum cephalosporins were detected.

Conclusions

- Sensitivity of PCR for detection of GC and resistance markers in residual urethral swabs was low; use of a separate swab for PCR-based analysis may increase sensitivity.
- Prevalence of genetic markers associated with quinolone resistance is high in this cohort of samples.
- Extended-spectrum cephalosporins appears to be an effective treatment option in this population based on the lack of strains with altered mosaic *PenA* sequences.

References and Acknowledgments

Goire N, Nissen MD, LeCornec GM, Sloots TP, Whiley DM. Diagn Microbiol Infect Dis. 2008. 61: 6-12.

Giles J, Hardick J, Yuenger J, Dan M, Reich K, Zenilman J. J Clin Microbiol. 2004. 42: 3281-3.

Goire N, Freeman K, Tapsall JW, Lambert SB, Nissen MD, et al. J Clin Microbiol. 2011. 49: 513-8.

Ochiai S, Ishiko H, Yasuda M, Deguchi T. J Clin Microbiol. 2008. 46: 1804-10.

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