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Note

Genotyping *Neisseria gonorrhoeae gyrA* and *penA* antimicrobial genes from remnant *Neisseria gonorrhoeae* positive Cepheid Xpert® clinical specimens – A feasibility study

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ABSTRACT

Neisseria gonorrhoeae (NG) has developed resistance to most antibiotics, making it increasingly difficult to treat. Previous studies have predicted antimicrobial NG susceptibility based on the antimicrobial gene target DNA gyrase subunit A (*gyrA)* codon serine 91 and the penicillin-binding protein 2 (*penA*) using Roche Cobas® and Hologic APTIMA™ clinical specimens. We studied whether similar methods could be used on remnant NG-positive Cepheid Xpert® specimens.

Neisseria gonorrhoeae (NG) is an increasingly common sexually transmitted infection and becoming more difficult to treat (Centers for Disease Control and Prevention, 2014). Globally, antimicrobial resistant NG is a major problem. The CDC has designated antimicrobial resistant NG as one of the three most urgent threats to public health (Centers for Disease Control and Centers for Disease Control and Prevention, 2015). In response to that threat, researchers have developed molecular assays to predict antimicrobial resistance (Melendez et al., 2018) based on the presence of mutations in antimicrobial target genes. Those assays may aid in individualizing treatment options for individuals with NG infection. Targeted antimicrobial treatment options may reduce the selection pressure on NG and slow down the continued emergence of resistant NG infections (Buono et al., 2012).

Investigators have genotyped the NG DNA gyrase subunit A (*gyrA)* gene, the target of fluoroquinolones like ciprofloxacin, and the penicillin-binding protein 2 (*penA)* gene, the target of third-generation cephalosporins like cefixime, in NG isolates as well as in clinical specimens from Hologic Aptima™ and Roche Cobas® NG detection assays (Donà et al., 2016; Hemarajata et al., 2016; Whiley et al., 2012; Wong et al., 2017). In order to expand the use of those assays to different testing platforms, we conducted a pilot study to determine if we could genotype the NG antimicrobial target genes *gyrA* and *penA* in remnant Cepheid CT/NG Xpert® clinical specimens (Wong et al., 2017).

We tested 61 frozen (−80 °C), de-identified, remnant pharyngeal, rectal, urine, and vaginal NG-DNA positive specimens previously self-collected between May 2017 and January 2019 as part of the U.S. National Institutes of Health Adolescent Trials Network study (ATN) (Shannon et al., 2019). Specimens were tested with the Cepheid Xpert ® CT/NG Assay (Cepheid, Sunnyvale, CA), a qualitative real-time PCR assay, approved by the Food and Drug Administration for genital, pharyngeal, and rectal specimens.

Specimens were thawed at room temperature and 100 μL of NG DNA was extracted from a total volume of 200 μL from each specimen using the Roche® MagNA Pure System (Roche, Indianapolis, Indiana). We then used the Roche LightCycler 480 (Roche, Indianapolis, Indiana) to produce a high resolution, multiplex melt curve analysis for the detection of the *gyrA* codon serine 91 mutation and the *penA* mosaic XXXIV allele (Wong et al., 2017). The fluorescent labels of the detection probes for the *penA* mosaic XXXIV target (Cyanine-5 dye) differed from that of *gyrA* (LightCycler® 640 probe) so that both genes could be detected simultaneously at various wavelengths. For internal controls we used isolates with previously confirmed presence of the NG mutant *gyrA,* the NG wild type *gyrA,* and the mosaic *penA* XXXIV allele. Lastly, we presented the frequency of the *gyrA* serine 91 mutation and the *penA* XXIV allele by anatomic site: rectum, pharynx, urethra, and vagina.

Among the 61 NG positive clinical specimens, 52% (32/61) were successfully genotyped (Table 1): *gyrA* serine 91 mutant (16/32, 50%), wildtype (16/32, 50%), mosaic *penA* XXXIV (2/32, 6.3%), and non-

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Table 1
Anatomical distribution & genotype of 32 Cepheid Xpert® remnant *Neisseria gonorrhoeae* specimens.

Anatomical site	*penA* XXXIV absent (*N* = 30)		*penA* XXXIV present (*N* = 2)	
	gyrA MT[a] (*N* = 15)	*gyrA* WT[b] (N = 15)	*gyrA* MT[a] (N = 1)	*gyrA* WT[b] (N = 1)
	n (%)	n (%)	n (%)	n (%)
Rectal	9 (28.1)	8 (25.0)	1 (3.1)	0
Pharyngeal	3 (9.4)	3 (9.4)	0	1 (3.1)
Urethral	2 (6.3)	3 (9.4)	0	0
Vaginal	1 (3.1)	1 (3.1)	0	0

[a] MT = Mutant.
[b] WT = Wildtype.

mosaic *penA* (30/32, 94.7%). Overall, five out of six urine specimens (83%), 18/26 rectal samples (69%), 2/5 vaginal (40%) and 7/24 pharyngeal (30%) were genotyped. The table summarizes the frequency of *penA* and *gyrA* genotypes by anatomic site (rectal, pharyngeal, urethral, and vaginal).

We tested remnant Cepheid Xpert® urogenital and extragenital specimens for the NG *gyrA* serine 91 mutation and mosaic *penA* XXXIV allele. In this pilot study, we showed that it is feasible to detect antimicrobial resistance genes in a small batch of remnant samples. We successfully genotyped about half of the previously collected and stored specimens that included both genital and extragenital samples. We show that genotyping of antimicrobial targets using remnant Cepheid Xpert ® clinical specimens is feasible, but additional improvements, particularly in pharyngeal specimens, are needed.

Of the successfully genotyped samples, half had a mutated *gyrA* gene predicting decreased susceptibility or resistance to ciprofloxacin. Another study found a similar prevalence of the *gyrA* resistance mutation among patients infected with NG (Bhatti et al., 2017). Genotyping the *gyrA* gene can help predict susceptibility to ciprofloxacin, which may allow for the use of ciprofloxacin for treatment and avoidance of last line antibiotics like ceftriaxone (Allan-Blitz et al., 2017).

Previous studies have shown that isolating NG genetic material from genital samples is more efficient than extragenital samples and there are differences in *gyrA* assay performance in different anatomical sites. In a recent publication from our team, *gyrA* was successfully genotyped in a higher proportion of urine/genital samples (76.4%), followed by rectal (67.2%) and pharyngeal samples (36.1%) (Ellis et al., 2019). In addition, another study tested 73 NG genital and extragenital samples and conducted sequencing of different gene targets (*por*B, *tbp*B) (Whiley et al., 2010). That study managed to successfully genotype all urogenital samples, 85% (11 of 13) of rectal samples and 57% (4 of 7) pharyngeal samples. Even though the latter group studied different genes, both studies showed high success in genotyping urogenital samples whereas extragenital specimens were more difficult to genotype.

The bacterial load and presence of other *Neisseria* species might affect the performance of NG genotyping in clinical specimens. Whiley et al. (2010) observed cross-reaction between commensal, non-gonococcal *Neisseria* species in pharyngeal samples, which reduced the specificity of their assay. It is possible that saprophytic *Neisseria* species of the throat are cross-reacting and inhibiting the PCR reaction. An additional reason for a lower success rate of genotyping could be the differences in bacterial load in the different anatomical sites. Bacterial load may be lower in extragenital sites compared to genital, and even lower in pharyngeal compared to rectal (Bissessor et al., 2011).

Our study was limited by its modest sample size, which limits the generalizability of our findings and does not allow us to conduct comparisons on the assay performance between the different anatomical sites. In addition, NG isolates were not concurrently tested for phenotypic susceptibility, the gold standard for measuring antimicrobial susceptibility.

It is possible to use targeted genotyping of NG specimens as a novel means to strengthen antimicrobial stewardship in the rising threat of multi-drug resistant *Neisseria gonorrhoeae*. Our findings suggest that a variety of remnant specimen types may be used for successful genotyping of the antimicrobial target genes, however additional research is needed to improve detection performance. Large commercial CT/NG assay manufacturers should invest in the development of assays that predict antimicrobial susceptibility in NG infections in both genital and extragenital samples.

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Declaration of Competing Interest

None.

References

Allan-Blitz, L.T., Wang, X., Klausner, J.D., 2017. Wild-type gyrase a genotype of neisseria gonorrhoeae predicts in vitro susceptibility to ciprofloxacin: a systematic review of the literature and meta-analysis. Sex. Transm. Dis. https://doi.org/10.1097/OLQ.0000000000000591.

Bhatti, A.A., Allan-Blitz, L.T., Castrejon, M., Humphries, R.M., Hemarajata, P., Klausner, J.D., 2017. Epidemiology of Neisseria gonorrhoeae gyrase a genotype, Los Angeles, California, USA. Emerg. Infect. Dis. https://doi.org/10.3201/eid2309.170215.

Bissessor, M., Tabrizi, S.N., Fairley, C.K., Danielewski, J., Whitton, B., Bird, S., ... Chen, M.Y., 2011. Differing Neisseria gonorrhoeae Bacterial Loads in the Pharynx and Rectum in Men Who Have Sex with Men: Implications for Gonococcal Detection, Transmission, and Control. Journal of Clinical Microbiology 49 (12), 4304–4306. https://doi.org/10.1128/JCM.05341-11.

Buono, S., Wu, A., Hess, D.C., Carlson, J.S., Rauch, L., Philip, S.S., Barry, P., Bernstein, K., Klausner, J.D., Pandori, M.W., Oct 2012. Microbial Drug Resistance. https://doi.org/10.1089/mdr.2011.0229. ahead of print.

Centers for Disease Control and Prevention, 2014. Reported STDs in the United States: 2013 National data for chlamydia, gonorrhea, and syphilis. In: CDC Fact Sheet, https://doi.org/10.1037/a0029416.Socioeconomic.

Centers for Disease Control, Centers for Disease Control and Prevention, 2015. Sexually Transmitted Disease Surveillance. 2011. Centre of Disease Control and Prevention. https://www.cdc.gov/std/stats14/default.htm.

Donà, V., Kasraian, S., Lupo, A., Guilarte, Y.N., Hauser, C., Furrer, H., ... Endimiani, A., 2016. Multiplex Real-Time PCR Assay with High-Resolution Melting Analysis for Characterization of Antimicrobial Resistance in Neisseria gonorrhoeae. Journal of Clinical Microbiology. https://doi.org/10.1128/jcm.03354-15.

Ellis, O., Hemarajata, P., Shahkolahi, A., Masinde, G., Buchs, K., Humphries, R.M., Klausner, J.D., 2019. A multisite implementation of a real-time polymerase chain reaction assay to predict ciprofloxacin susceptibility in Neisseria gonorrhoeae. Diagn. Microbiol. Infect. Dis. 94 (3), 213–217. https://doi.org/10.1016/j.diagmicrobio.2018.12.018.

Hemarajata, P., Yang, S., Soge, O.O., Humphries, R.M., Klausner, J.D., 2016. Performance and verification of a real-time PCR assay targeting the gyrA gene for prediction of ciprofloxacin resistance in Neisseria gonorrhoeae. J. Clin. Microbiol. https://doi.org/10.1128/JCM.03032-15.

Melendez, J.H., Hardick, J., Barnes, M., Barnes, P., Geddes, C.D., Gaydos, C.A., 2018. Molecular characterization of markers associated with antimicrobial resistance in Neisseria gonorrhoeae identified from residual clinical samples. Sex. Transm. Dis. https://doi.org/10.1097/OLQ.0000000000000755.

Shannon, C.L., Keizur, E.M., Fehrenbacher, A., Wood-Palmer, D., Ramos, W., Koussa, M., ... Klausner, J.D., 2019. Sexually Transmitted Infection Positivity Among Adolescents With or at High-Risk for Human Immunodeficiency Virus Infection in Los Angeles and New Orleans. Sexually Transmitted Diseases 46 (11), 737–742. https://doi.org/10.1097/OLQ.0000000000001056.

Whiley, D.M., Goire, N., Ray, E.S., Limnios, A., Lambert, S.B., Nissen, M.D., Sloots, T.P., Tapsall, J.W., 2010. Neisseria gonorrhoeae multi-antigen sequence typing using non-cultured clinical specimens. Sex. Transm. Infect. 86 (1), 51–55. https://doi.org/10.1136/sti.2009.037689.

Whiley, D.M., Goire, N., Lahra, M.M., Donovan, B., Limnios, A.E., Nissen, M.D., Sloots, T.P., 2012. The ticking time bomb: escalating antibiotic resistance in Neisseria gonorrhoeae is a public health disaster in waiting. J. Antimicrob. Chemother. https://doi.org/10.1093/jac/dks188.

Wong, L.K., Hemarajata, P., Soge, O.O., Humphries, R.M., Klausner, J.D., 2017. Real-time PCR targeting the penA mosaic XXXIV type for prediction of extended-spectrum-cephalosporin susceptibility in clinical Neisseria gonorrhoeae isolates. Antimicrob. Agents Chemother. https://doi.org/10.1128/aac.01339-17.