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LETTER TO THE EDITOR

Identification of an Unusual 16S rRNA Mutation in *Neisseria gonorrhoeae*

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Australian guidelines recommend screening and supplemental assays directed at different targets for molecular detection of *Neisseria gonorrhoeae*, and culture as the preferred test for nongenital sites despite its low sensitivity (1). QML Pathology uses the Aptima Combo 2 assay for screening, with Aptima GC for supplemental testing (Hologic, Marlborough, MA), both of which target the same 16S rRNA gene; the combined use of which the manufacturer considers appropriate because the assays target different regions (2). Cross-reactivity with other *Neisseria* spp. and false-negative reactions have been reported for non-16S rRNA gene-based *N. gonorrhoeae* gene targets (1). In October 2018, a 31-year-old male patient had a routine sexually transmitted infection (STI) screen performed on a throat swab, rectal swab, and urine for *Chlamydia trachomatis* and *N. gonorrhoeae*. The Aptima Combo 2 assay was positive for *N. gonorrhoeae* (RLU 1244), but the Aptima *N. gonorrhoeae* (GC) assay was equivocal (RLU 74) on the throat swab. Throat swabs were collected one week later and submitted for both nucleic acid amplification testing (NAAT) and culture. NAAT by the Aptima Combo 2 assay on the second throat swab was positive (RLU 1331); however, the Aptima *N. gonorrhoeae* (GC) assay was negative (RLU 26). Culture of the second throat swab grew *N. gonorrhoeae* (strain 9987), which was confirmed by phenotypic tests and Vitek MS (bioMérieux, France). The culture isolate was positive for *N. gonorrhoeae* with the Aptima Combo 2 assay (RLU 1290) and by Aptima GC assay (RLU 469). Both the urine and the rectal swab were negative for *N. gonorrhoeae* in the Aptima Combo 2 assay. The strain was whole-genome sequenced on the Illumina NextSeq 500 platform (San Diego, CA), and reads were assembled with Spades (3) (pubMLST identifier [ID] 84143). The strain was confirmed as *N. gonorrhoeae* by Kraken (4) and *rplF* sequence analysis (5) and typed as multilocus sequence type 1893 (MLST-1893), NgSTAR 142, NgMAST 8517; all preexisting profiles in their respective databases. The 16S rRNA sequence showed a single nucleotide polymorphism (G478T) to known sequences for this organism, which was not present in any sequences in the NCBI nucleotide database. This mutation lies within a sequence region listed in the US7172863B1 patent (6), causing an oligonucleotide binding mismatch likely responsible for the negative and equivocal reactions in the supplemental Aptima GC assay. The prevalence of this mutation in the local population is unknown, where 28% of gonococcal notifications have an isolate grown in culture (7), and sequencing of isolates is not routinely performed. Infections may potentially be underreported in areas reliant on this molecular assay for testing. There are three other strains currently in pubMLST with this same 16S sequence (56770, 56772, and 56774), all MLST-1893 and isolated in Spain in 2016, suggesting international dissemination of this strain. Other 16S mutations are associated with spectinomycin resistance (8); however, this strain did not demonstrate spectinomycin resistance (MIC < 64 μg/ml). This report highlights some of the ongoing challenges around the

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selection of molecular diagnostic targets and multitest algorithms for gonorrhea and an advantage of using culture for nongenital site diagnosis.

Accession number(s). The whole-genome sequence has been deposited under SRA accession PRJNA507689. The single nucleotide polymorphism has been deposited in GenBank under reference number MK226480.

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