Genetic exchange between species within the Neisseria genus is well recognized (Linz et al., 2000) and can have important implications for the management of gonorrhoea, both in terms of detecting gonorrhoea by nucleic acid amplification tests and through the development of gonococcal antimicrobial resistance. For example, cross-reaction of commercial and in-house Neisseria gonorrhoeae nucleic acid amplification tests with commensal Neisseria species has been well documented (Farrell, 1999; Linz et al., 2000; Tabrizi et al., 2011). Furthermore, it is becoming increasingly clear that the acquisition of resistance determinants from commensal Neisseria species is one of the prime pathways leading to N. gonorrhoeae antimicrobial resistance; such determinants include the well-documented N. gonorrhoeae ‘mosaic’ penA sequences, which are thought to have been acquired from commensal Neisseria and are implicated in N. gonorrhoeae resistance to extended-spectrum cephalosporins (Ameyama et al., 2002; Unemo et al., 2012). Here, we report a novel ‘Neisseria meningitidis-like’ mtrR sequence in N. gonorrhoeae isolates with reduced susceptibility to azithromycin.

As part of the ongoing GRAND (Gonorrhoea Resistance Assessment by Nucleic Acid Detection) study of N. gonorrhoeae antimicrobial resistance in Australia (Trembizki et al., 2014a), we are using two assays on the Sequenom MassARRAY iPLEX platform to predict multilocus sequence type (ST) (Trembizki et al., 2014b), and to characterize the genetic resistance determinants of isolates (n=2455) collected throughout Australia in 2012. Among the resistance determinants being investigated are several that affect azithromycin susceptibility: three mutations in the gonococcal mtrR region that affect MtrCDE efflux pump activity [a G45D substitution on the MtrR protein, and two mutations (an adenine deletion and thymine insertion) in the −35 to −10 mtrR promoter region] (Zarantonelli et al., 1999); and two mutations (C2611T and A2059G) in the 23S rRNA genes (Ng et al., 2002; Chisholm et al., 2010).

Upon assaying 397 isolates from Queensland, the majority of isolates provided full single-nucleotide polymorphism profiles (data not shown). However, the Sequenom ‘resistance’ assay failed to characterize mtrR promoter mutations for a cluster of 10 isolates, whereas the MtrR codon 45 was successfully determined for these isolates (wild type). The 23S rRNA gene 2611 and 2059 positions were also characterized and were both wild type for all isolates. Interestingly, this cluster of gonococcal isolates displayed reduced susceptibility/low-level resistance to azithromycin, with MICs of 0.12 mg l\(^{-1}\) for two isolates, 0.5 mg l\(^{-1}\) for three isolates and 1 mg l\(^{-1}\) for five isolates. Based on previous multilocus ST data (Trembizki et al., 2014b), all 10 isolates were found to be ST9363. Application of N. gonorrhoeae multi-antigen sequence typing (NG-MAST) revealed three closely related types: eight of the isolates were of NG-MAST ST6946 and the remaining two were of NG-MAST ST7415 and ST8056. These three NG-MAST types shared the same tlpB allele (29) but different porB alleles (4148, 4446 and 4803); despite being assigned different porB alleles, the types actually shared >99 % nucleotide identity. Thus, it is likely all isolates were of the same strain that had undergone further diversification in the highly variable porB gene. All 10 isolates were from male patients and were derived from swab samples of various sites: urethral (n=5), throat (n=3), rectal (n=1) and penile (n=1).

Suspecting that the failure to characterize the mtrR promoter region was caused by variations in the primer targets of the Sequenom assay, DNA sequencing of the mtrR coding and mtrR–mtrC intergenic regions was performed and identical sequences were obtained for all 10 isolates. Based on the sequencing data, none of the isolates had a G45D substitution in the coding region, or an adenine deletion or thymine insertion in the intergenic region. Sequencing analysis revealed that mismatches were indeed present in the Sequenom assay primer targets, but additionally showed numerous other differences to the wild-type gonococcal mtrR sequence; the mtrR–mtrC intergenic and MtrR protein sequences of all 10 isolates exhibited 76.9 % (Fig. 1a) and 95.8 % (Fig. 1b) nucleotide identity, respectively, with the N. gonorrhoeae FA1090 strain (GenBank accession no. AE004969). Moreover, both sequences demonstrated higher nucleotide identity (94.8 and 99.5 %) with N. meningitidis sequences (GenBank accession nos CP00242 and 002343243, respectively) (Fig. 1), suggesting that these gonococci may have acquired meningococcal mtrR sequences via horizontal genetic exchange and recombination. Based on the lack of recognized 23S rRNA and mtrR azithromycin-resistance determinants, it is also possible that acquisition of these mtrR sequences by these gonococci strains may have influenced susceptibility to azithromycin. This is particularly worrisome, given that dual therapy with azithromycin plus ceftriaxone is now being recommended for the treatment of gonorrhoea, and has only recently been adopted in Queensland.

In summary, the results reveal a novel gonococcal mtrR sequence, presumably obtained from N. meningitidis. While further studies are required to confirm the role of this novel sequence in reduced susceptibility to azithromycin, these isolates nevertheless serve as yet another reminder of the ability of gonococcus to undergo genetic exchange and confound molecular detection methods.
Acknowledgements

The GRAND study is a national study investigating gonorrhoea nucleic acid detection strategies in Australia and is funded by the National Health and Medical Research Council (APP1025517). GRAND study investigators include Rebecca Guy, James Ward, John Kaldor, Basil Donovan, Handan Wand and David Regan from the Kirby Institute, UNSW; Marcus Chen and Christopher Fairley from the University of Melbourne and Melbourne Sexual Health Centre; Nathan Ryder and Jiunn-Yih Su from the Sexual Health and Blood Borne Virus Unit, Northern Territory; and Theo Sloots, Michael Nissen and Stephen Lambert from the Queensland Children’s Medical Research Institute.

Ella Trembizki,1,2 Christine Doyle,3 Amy Jennison,3 Helen Smith,3 John Bates,3 Monica Lahra,4 David Whiley1,2 on behalf of the GRAND study investigators

1Queensland Paediatric Infectious Diseases Laboratory, Queensland Children’s Health Services, Queensland, Australia
2Queensland Children’s Medical Research Institute, The University of Queensland, Queensland, Australia
3Public Health Microbiology, Queensland Health Forensic and Scientific Services, Archerfield, Queensland Australia
4WHO Collaborating Centre for STD, Microbiology Department, South Eastern Area Laboratory Services, Prince of Wales Hospital, Sydney, New South Wales, Australia

Correspondence: David Whiley (d.whiley@uq.edu.au)

Fig. 1. Comparison of the novel mtrR–mtrC intergenic (a) and MtrR amino acid sequences (b) with ‘wild-type’ N. gonorrhoeae and N. meningitidis sequences. The −35 and −10 hexamer mtrR promoter sequences and amino acid position 45 are highlighted. *N. gonorrhoeae FA1090: GenBank accession no. AE004969; †N. meningitidis: GenBank accession no. CP002422; ‡N. meningitidis: GenBank accession no. 002343243.

