Case Report

Two cases of failed ceftriaxone treatment in pharyngeal gonorrhoea verified by molecular microbiological methods

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Diagnostic, genotypic and antibiotic-resistance determinants of *Neisseria gonorrhoeae* were analysed by molecular methods to verify the failure of ceftriaxone treatment in two cases of pharyngeal gonorrhoea. Monoplex assays were needed to define competitive inhibition of a positive *Chlamydia* PCR in a duplex assay. Different *penA* changes were detected in the *N. gonorrhoeae* isolated from the two cases. These were associated with raised ceftriaxone MICs of 0.03 and 0.016 mg l⁻¹, which may have contributed to the treatment failures in these cases.

Introduction

Concerns have recently been expressed regarding public health control of gonorrhoea and the consequent need to eliminate reservoirs of infection, especially pharyngeal infection, through enhanced detection and treatment (Workowski *et al.*, 2008). This disquiet has been heightened by reports of the failure of treatment with oral extended-spectrum cephalosporins in male urethral gonorrhoea caused by multidrug resistant *Neisseria gonorrhoeae* (Muratani *et al.*, 2001; Deguchi *et al.*, 2003; Yokoi *et al.*, 2007; Lo *et al.*, 2008). The gonococci concerned had raised MICs for ceftriaxone, cefixime and cefixime, possessed a mosaic penicillin-binding protein 2 (PBP2) (the principal target site for this group of antibiotics), were of the Ito type X (Ito *et al.*, 2005) and comprised multiple gonococcal subtypes (Ito *et al.*, 2005; Whiley *et al.*, 2007b). Increases in ceftriaxone MICs have also been noted with non-mosaic-gene-based alterations in PBP2 (Ito *et al.*, 2005; Whiley *et al.*, 2007b; Osaka *et al.*, 2008), but the contribution of these lesions to treatment failure is not known. No similar instances of failure to eliminate male urethral *N. gonorrhoeae* have followed therapy with the injectable agent ceftriaxone, and clinical treatment failures from other sites, although reported and traditionally known to be more difficult to treat (Workowski *et al.*, 2008), have not been fully

investigated. A recent study reported organism clearance of 15 pharyngeal *N. gonorrhoeae* with a 1 g intravenous dose of ceftriaxone and where some of the infecting gonococci contained mosaic PBP2 (Muratani *et al.*, 2008).

Detailed investigation of gonococci from patients with treatment failure provides valuable insights into resistance mechanisms, the relevance of existing MIC breakpoints and the spread of resistant gonococci. Treatment failure occurs more frequently with pharyngeal gonorrhoea, even when the infecting organism remains fully sensitive to the antibiotic used (Sathia *et al.*, 2007). Although it is difficult at times to distinguish treatment failure from reinfection, it is important to investigate all such cases for any possibility of emerging resistance that may impinge adversely on treatment outcomes. Two cases are reported here where ceftriaxone failed to eradicate pharyngeal gonococci with altered, but non-mosaic, PBP2, and where diagnostic, genotypic and antimicrobial-resistance determinant testing ultimately relied upon combinations of molecular methodologies.

Case reports

Case 1

A 27-year-old English homosexual man presented to the Sydney Sexual Health Centre in December 2007 with urethral discharge and dysuria 1 week after unprotected fellatio from a casual male partner. There was no history of

Abbreviations: BHQ, black-hole quencher; FAM, 6-carboxyfluorescein; FVU, first void urine; IM, intramuscular; NAAT, nucleic acid amplification test; NG-MAST, *Neisseria gonorrhoeae* multi-antigen sequence typing.

other sexual contact in the prior 3 months. A Gram stain of a urethral discharge showed numerous polymorphonuclear leukocytes and Gram-negative intracellular diplococci, and the patient was treated with a 250 mg intramuscular (IM) dose of ceftriaxone (Table 1). Urethral and throat samples collected from the patient on this presentation subsequently yielded *N. gonorrhoeae* from culture on modified New York City medium, but a rectal swab, taken at the same time, did not. Rectal swabs and a first void urine (FVU) were also collected and sent for *Chlamydia trachomatis* PCR testing using Roche Amplicor, a duplex nucleic acid amplification test (NAAT) for both *C. trachomatis* and *N. gonorrhoeae*. Both these initial rectal and urine samples were reported as negative for *C. trachomatis*. At follow-up, 1 week later, the symptoms had almost entirely resolved and the patient had abstained from sexual contact. No investigations were undertaken on this presentation. He next represented 17 days after his initial treatment with increasing urethral tingling, and again denied any further sexual contact. Gram staining of a mucoid urethral discharge showed more than 10 polymorphonuclear leukocytes per high-powered microscope field ($\times 400$), but no Gram-negative diplococci. He was given 1 g oral azithromycin, and repeat samples were taken from the urethra and throat for the culture of *N. gonorrhoeae*. A second FVU sample for repeat *C. tracho-*

omatis NAAT was now positive, and his throat culture again grew *N. gonorrhoeae*. However, both his urethral culture and his urine, for the NAAT for *N. gonorrhoeae* using the Amplicor assay, were negative. One week later he received 500 mg IM ceftriaxone prior to returning to England. All three gonococcal isolates (one each from the urethra and pharynx on his first visit, and one pharyngeal isolate 17 days post treatment) were indistinguishable when genotyped by *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) (Martin *et al.*, 2004). They were of NG-MAST sequence type 5, were penicillin and quinolone resistant (MICs 1 and 16 mg l⁻¹, respectively, as determined by the methods of the Australian Gonococcal Surveillance Programme), but ceftriaxone susceptible according to currently accepted parameters (MIC 0.03 mg l⁻¹), and of Ito type VII (Ito *et al.*, 2005) based on *penA* sequencing (Whiley *et al.*, 2007b). An assay for mosaic *penA* genes (Whiley *et al.*, 2007a) was negative. The original and the second FVU samples were then retested in parallel with both the original Amplicor *C. trachomatis* NAAT and with two additional monoplex assays. One monoplex real-time *C. trachomatis* NAAT targeted the *C. trachomatis* cryptic plasmid and was kindly provided by Dr Gerry Harnett, PathCentre, Nedlands, Perth, Western Australia. Briefly, the reaction mix (total volume 25 µl) consisted of 12.5 µl QuantiTect probe PCR master mix

Table 1. Pattern of diagnostic test positivity by pathogen type, test sample and test method, and over time for case 1, case 2 and the single contact of case 2

The tests were as follows: NAAT duplex, Roche Amplicor duplex assay for *C. trachomatis* and *N. gonorrhoeae*; NAAT monoplex, assays for each agent – for *C. trachomatis* a real-time assay targeting the *C. trachomatis* cryptic plasmid and a conventional PCR analysed by gel targeting a separate site, and for *N. gonorrhoeae* a real-time NAAT targeting the *porA* pseudogene.

Patient	Day	Test	Sample type/pathogen				
			CT		NG		
			FVU	FVU	Ts	Us	HVS
Case 1	1	NAAT duplex	–	+			
		NAAT monoplex	+	+			
		Culture			+	+	
	17	NAAT duplex	+	–			
		NAAT monoplex	+	–			
Case 2†		Culture			+	–	
	Index case	Culture			+		+
		Spouse			+	+	
	Index case	Culture			+		–
		Spouse			–	–	
	Index case	Culture			–		–

CT, *C. trachomatis*; HVS, high vaginal swab; NG, *N. gonorrhoeae*; Ts, throat swab; Us, urethral swab.

*Positive isolates indistinguishable by genotyping.

†Index case, female index case; spouse, husband of index case.

‡Positive isolates indistinguishable by genotyping.

(Qiagen), 0.4 μM each forward (5'-CGGTTTTCCTC-GATGATTGTA-3') and reverse (5'-CGAGCGGAAAAT-GAAATTACTCA-3') primers, 0.16 μM minor groove binder (MGB) TaqMan probe (5'-FAM-TTCAGCGCTAC-ACACG-MGB-BHQ-3'; Applied Biosystems) and 5.0 μl specimen extract. The second additional monoplex *C. trachomatis* assay was a conventional PCR (where the PCR products are analysed by gel-based methods) used to identify variant *C. trachomatis* strains possessing a 377 bp deletion of the Roche assay target site (Ripa & Nilsson, 2006; Lynagh *et al.*, 2007). The original urine sample remained negative in the AMPLICOR *C. trachomatis* NAAT and was positive in the second sample, whereas both first and subsequent urine samples were positive with the two other assays. The initial, but not the subsequent, urine sample was positive for *N. gonorrhoeae* in both the AMPLICOR and a monoplex in-house real-time NAAT (Whiley *et al.*, 2004; Goire *et al.*, 2008). A high *N. gonorrhoeae* infectious load was inferred in the original sample by both the low cycle number (26) for test positivity in the monoplex NAAT and the heavy growth of *N. gonorrhoeae* in culture. In contrast, the high *C. trachomatis* cycle number (38) in the in-house real-time monoplex assay on the first urine sample indicated a low infective inoculum and the probability of competitive inhibition of amplification of the *C. trachomatis* DNA in the presence of disproportionate gonococcal nucleic acid content (Whiley *et al.*, 2008).

Case 2

A 34-year-old Australian married woman presented to her general practitioner at 9 weeks post-vaginal delivery with a vaginal discharge present for 4 days after her last sexual contact with her husband (Table 1). When *N. gonorrhoeae* was cultured from a vaginal swab by a private practitioner, both she and her 36 year-old husband were referred to the Short Street Sexual Health Clinic for confirmation of the diagnosis and for treatment. Gram staining of the copious vaginal discharge showed abundant polymorphonuclear leukocytes and Gram-negative intracellular diplococci. Her high vaginal swab and throat swab collected at this presentation both subsequently yielded *N. gonorrhoeae* in culture. Her husband reported inflammation of the glans penis but denied urethral symptoms. On examination he had meatitis and a scanty muco-purulent discharge, which showed abundant polymorphonuclear leukocytes and Gram-negative intracellular diplococci when Gram stained. His urethral swab and throat swab cultures both yielded *N. gonorrhoeae*. Both husband and wife were treated with 250 mg IM ceftriaxone and were advised to abstain from sexual contact until a negative test-of-cure culture was obtained. After 2 weeks they reported no further sexual contact, and the husband's urethral and throat cultures were negative for *N. gonorrhoeae*. However, he had also been prescribed unspecified antibiotics elsewhere for a middle-ear infection. The woman's endocervical culture was negative, but her throat culture again yielded *N.*

gonorrhoeae. A further dose of 1 g IM ceftriaxone cleared this persisting infection on subsequent culture. All the gonococcal isolates from the wife and husband were indistinguishable, were of NG-MAST sequence type 2740, and were penicillin and quinolone resistant (MICs 1 and 16 mg l^{-1} , respectively) but susceptible to ceftriaxone (MIC 0.016 mg l^{-1}). All isolates were Ito type V based on *penA* sequencing and negative in the screening assay for mosaic *penA* genes.

Discussion

These cases have some instructive features from both microbiological and clinical perspectives. Treatment failure occurred with the pharyngeal infections, whereas intercurrent *N. gonorrhoeae* infections were successfully cleared from genital sites in two patients. In each instance, reinfection of the oropharynx was clinically unlikely, and the original and repeat pharyngeal isolates were indistinguishable by NG-MAST genotyping. Additional molecular testing also distinguished treatment failure from possible reinfection in case 1. An initially negative *C. trachomatis* NAAT that became positive upon retesting 2 weeks later, a result that was confirmed using two additional monoplex *C. trachomatis* assays, suggested that, despite repeated denials of re-exposure, reinfection may have been the cause of recurrent symptoms. However, the use of the additional monoplex NAATs for *C. trachomatis* indicated that this was probably due to competitive inhibition of the original *C. trachomatis* PCR by the much higher load of *N. gonorrhoeae* in a duplex NAAT. The positive result obtained with the initial urine sample using monoplex *C. trachomatis* NAATs was not affected by this phenomenon.

It has been established that antibiotic clearance of *N. gonorrhoeae* from the pharynx is less readily achieved than from other infected sites (Workowski *et al.*, 2008). Improved eradication of pharyngeal gonorrhoea was reported when cephalosporin therapy was combined with oral azithromycin, e.g. for concurrent infection with *C. trachomatis* (Sathia *et al.*, 2007). Azithromycin was used sequentially in case 1, but not at all in case 2. However, although these isolates were 'susceptible' to ceftriaxone *in vitro* by current criteria, their ceftriaxone MICs were substantially higher (0.03 and 0.016 mg l^{-1}) than those originally reported in 'wild-type' gonococci (MICs ≤ 0.0005 mg l^{-1}) (Tapsall & Phillips, 1995). Clearance of *N. gonorrhoeae* from the pharynx of case 2 was confirmed following a second and higher (1 g) dose of ceftriaxone. Additionally, the increases in MICs noted were associated with altered PBP2, the main target site for ceftriaxone. Although a mosaic PBP2, which has been associated with failed treatment in male urethral gonorrhoea, was not present, this raises the possibility that other, non-mosaic, PBP2 lesions, known to also increase ceftriaxone MICs (Ito *et al.*, 2005; Whiley *et al.*, 2007b; Osaka *et al.*, 2008), may contribute to treatment failures at this site known to pose treatment difficulties.

Although only two instances of treatment failure were described here, they suggest the need for systematic, prospective studies on the possible effects of raised ceftriaxone MICs on treatment failure in pharyngeal gonorrhoea. A recent report from Japan (Muratani *et al.*, 2008) of the eradication of *N. gonorrhoeae* in 15 cases of pharyngeal gonorrhoea was achieved by means of a 1 g dose of ceftriaxone given intravenously, one considerably in excess of doses recommended elsewhere, but similar to the 1 g dose ultimately required for organism clearance in case 2. It would be most inconvenient to administer intravenous therapy for this purpose in most Australian clinics.

It is acknowledged that there is an intrinsic difficulty in achieving universal elimination of not only *N. gonorrhoeae*, but also other pathogenic *Neisseria* such as *Neisseria meningitidis* (Simmons *et al.*, 2000), from the oropharynx, not just with ceftriaxone but with any antibiotic. Thus, this report may simply represent that small proportion of cases where treatment failure is observed in pharyngeal gonorrhoea even where the infecting organism is fully sensitive to ceftriaxone. Equally, these cases may indicate that elevated ceftriaxone MICs may contribute to increased rates of treatment failure in pharyngeal gonorrhoea more often than is realized. Test-of-cure cultures are not now universally performed, even in pharyngeal gonorrhoea, so that other cases of failed eradication of pharyngeal gonorrhoea may exist but remain undetected.

Recent emphasis on improved gonococcal disease control and the need to eliminate reservoirs of pharyngeal gonorrhoea suggest that studies on these phenomena, including resistance data and the effects of the use of combined antibiotic treatment (Workowski *et al.*, 2008) or increased doses of existing treatment regimens (Muratani *et al.*, 2008), would be helpful to further clarify treatment options and the reasons for treatment failure. However, this report also indicates some of the difficulties associated with the investigation of possible treatment failure, and the need to distinguish treatment failure from reinfection through close clinical and laboratory collaboration. While we propose to examine prospectively cases of pharyngeal gonorrhoea, and correlate clinical and laboratory data in any situation of possible treatment failure, only 66 cases of culture-confirmed pharyngeal gonorrhoea, 60 in men and 6 in women, were diagnosed in the laboratory from samples referred our two clinics (Short Street Sexual Health Clinic and Sydney Sexual Health Centre) in 2007. We also note that the Japanese study was only able to investigate properly 15 cases of pharyngeal gonorrhoea over 2 years (Muratani *et al.*, 2008). This suggests that the combined contributions of other centres may be needed to clarify this issue.

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