Phenotypic and genetic characterization of the first two cases of extended-spectrum-cephalosporin-resistant Neisseria gonorrhoeae infection in South Africa and association with cefixime treatment failure

David A. Lewis1–3*, Charlotte Sriruttan4, Etienne E. Müller1, Daniel Golparian5, Lindy Gumede1, Donald Fick6, Johan de Wet7, Venessa Maseko1, Jennifer Coetzee4 and Magnus Unemo5

1Centre for HIV and Sexually Transmitted Infections, National Institute for Communicable Diseases, National Health Laboratory Service, 1 Modderfontein Road, Sandringham 2192, South Africa; 2Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Park Town 2193, South Africa; 3Division of Medical Microbiology, University of Cape Town Medical School, Anzio Road, Observatory 7925, South Africa; 4Department of Clinical Microbiology, Ampath National Laboratory Services, 166 Witch Hazel Street, Highveld Park, Centurion 0157, South Africa; 5WHO Collaborating Centre for Gonorrhoea and Other STIs, Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden; 6Meldene Medicross Clinic, Cnr 3rd Avenue and Main Street, Melville 2109, South Africa; 7Springs Medicross Clinic, 1 Nigel Road, Selection Park, Springs 2213, South Africa

*Corresponding author. Tel: +27-11-555-0468; Fax: +27-11-555-0470; E-mail davidl@nicd.ac.za

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Objectives: To describe the phenotypic and genetic characteristics of the first two cases of extended-spectrum cephalosporin (ESC)-resistant Neisseria gonorrhoeae in South Africa, one of which was associated with verified cefixime treatment failure.

Patients and methods: Two ESC-resistant N. gonorrhoeae isolates were cultured from the urethral discharge of two men who have sex with men (MSM). One man reported a persistent urethral discharge that had failed to respond to previous therapy with oral cefixime. Agar dilution MICs were determined for eight antibiotics. β-Lactam-associated resistance mutations were identified through PCR-based amplification and sequencing for several key genes: penA, mtrR and its promoter, porB1b (penB), ponA and pilQ. For molecular epidemiological characterization, full-length porB gene sequencing, N. gonorrhoeae multiantigen sequence typing (NG-MAST) and multilocus sequence typing (MLST) were performed.

Results: Both isolates were resistant to cefixime, ciprofloxacin, penicillin and tetracycline and intermediate/resistant to azithromycin, but susceptible to ceftriaxone, gentamicin and spectinomycin. Both isolates had the type XXXIV penA mosaic allele in addition to previously described resistance mutations in the mtrR promoter (A deletion), porB1b (penB) (G101K and A102N) and ponA1 (L421P). For molecular epidemiological characterization, full-length porB gene sequencing, N. gonorrhoeae multiantigen sequence typing (NG-MAST) and multilocus sequence typing (MLST) were performed.

Conclusions: Both isolates were resistant to cefixime, ciprofloxacin, penicillin and tetracycline and intermediate/resistant to azithromycin, but susceptible to ceftriaxone, gentamicin and spectinomycin. Both isolates had the type XXXIV penA mosaic allele in addition to previously described resistance mutations in the mtrR promoter (A deletion), porB1b (penB) (G101K and A102N) and ponA1 (L421P). Both isolates had an identical NG-MAST sequence type (ST4822) and MLST sequence type (ST1901).

Keywords: gonorrhoea, mosaic penA genes, cefpodoxime, ceftriaxone

Introduction

Neisseria gonorrhoeae, the Gram-negative bacterium responsible for the sexually transmitted infection (STI) gonorrhoea, has a remarkable propensity to acquire resistance determinants. Historically the gonococcus has developed resistance to several classes of antimicrobial agents including, most recently, third-generation extended-spectrum cephalosporins (ESCs),1–3 The emergence and global spread of gonococcal strains with decreased susceptibility or resistance to ESCs from the western
Pacific region to Europe and beyond is now a major public health concern. The epidemiological application of highly discriminatory molecular typing methods, such as N. gonorrhoeae multi-antigen sequence typing (NG-MAST), as well as those genetic typing methods that are able to best reflect the evolution of gonococcal strains by virtue of lower discriminatory ability, such as multilocus sequence typing (MLST), has identified gonococcal clones that are important for driving transmission of multidrug-resistant gonorrhoea within international networks.

The main mechanism for decreased susceptibility and resistance to third-generation ESCs is alteration of the penA gene encoding penicillin-binding protein 2 (PBP 2). Acquisition of a penA mosaic allele and non-mosaic penA alleles with mutations at position A501 have been associated with increased ESC MICs. Furthermore, specific mutations in the promoter and/or coding sequence of the mtrR gene increase the expression of the MtrCDE efflux pump and hence the MIC for the strain. The mtrR determinant is most often a single adenine (A) deletion in the 13 bp inverted repeat of the promoter region, while other mutations within the coding sequence of MtrR, such as G45D, are less common. Mutations in the porB1b (penB) gene that alter amino acids G101 and A102 in the PorB1b porin result in further increased ESC MICs. However, some mutations involved in chromosomally mediated high-level penicillin resistance, such as the penA1 allele (encoding a L421P-altered PBP 1) and mutations in pilQ, have yet to be shown to substantially increase ESC MICs. At least one resistance determinant remains unknown. Until 2008, Botswana was the only African country using an ESC, specifically a single 250 mg intramuscular dose of ceftriaxone, for the treatment of presumptive gonorrhoea. With the recent rise in the prevalence of fluoroquinolone resistance in southern Africa, there has been a regional move, initiated in South Africa (2008) and Namibia (2009), to replace fluoroquinolones with single-dose oral cefixime. This article describes the phenotypic and genetic characterization of the first two verified cases of N. gonorrhoeae resistant to oral third-generation ESCs in South Africa. One of these cases represents the first confirmed in-country gonorrhoea treatment failure with internationally recommended first-line ESC treatment, which is the first strictly verified in Africa. Both these gonococcal infections were acquired by individuals belonging to a high-frequency transmitting population, specifically men who have sex with men (MSM).

Patients and methods

Patients

Two MSM, one in his 50s and the other in his mid-20s, presented to Johannesburg-based private general practitioners (GPs) with locally acquired purulent urethral discharges in May (patient A) and July (patient B) 2012. While further details were lacking for patient A, patient B gave a history of persistent urethral gonorrhoea that had not responded to two recent treatments with single-dose oral cefixime. Both GPs sent urethral pus swabs to the Ampath laboratory for microscopy, culture and susceptibility testing. It is not known if either patient had asymptomatic gonorrhoea at other anatomical sites at the time their urethral infections were treated. As both patients were lost to follow-up, it was impossible to obtain further clinical information and, importantly, neither clinical nor microbiological cure could be confirmed at the relevant anatomical sites.

Laboratory identification of N. gonorrhoeae

The Ampath laboratory identified the urethral isolate from each patient as N. gonorrhoeae by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF; Bruker Daltonik GmbH, Bremen, Germany). The isolates were subsequently sent to the Centre for HIV and STIs at the National Institute for Communicable Diseases (NICD), where confirmatory identification was undertaken using the Phadebaclc Monoclonal GC test (Becton AB, Huddinge, Sweden), which also differentiates gonococci into either a W1 or a WII/III serogroup, and the APTIMA GC assay (GenProbe Inc., San Diego, CA, USA).

Antimicrobial susceptibility testing

Initial antimicrobial susceptibility testing was performed by disc diffusion according to CLSI guidelines. A nitrocefin chromogenic test (Oxoid, Basingstoke, UK) was used to detect β-lactamase production. At the NICD, MIC values were determined initially by Etest (bioMérieux, Marcy l’Etoile, France) for cefixime, cefpodoxime and ceftriaxone, and subsequently by agar dilution for a panel of eight antibiotics. Agar dilution MIC assays were performed using medium composed of GC agar (National Health Laboratory Service, Sandringham, South Africa) plus 1% BBL IsoVitalex enrichment (Becton Dickinson, Le Pont de Clai, France). The 2008 WHO N. gonorrhoeae reference strains F, G, K, N, O and P were used as quality controls. EUCAST clinical breakpoints (version 1.3) for N. gonorrhoeae were used to interpret MICs for most antibiotics. As internationally accepted breakpoints have not yet been established for gentamicin, susceptibility definitions were based on recommendations from Daly et al.

DNA isolation

Genomic DNA was extracted from the two clinical N. gonorrhoeae isolates and the WHO N. gonorrhoeae reference strains F and K, using an X-tractor Gene automated DNA extractor (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions.

Mosaic penA gene PCR screening

The presence of a mosaic sequence in the penA gene that encodes for a mosaic PBP 2 was determined by a previously described PCR assay. The WHO reference strains F and K were used in the assay as negative and positive controls, respectively. Successful amplification of a mosaic penA sequence, as well as an internal control amplification product, was demonstrated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Sequencing of the penA gene and other key resistance determinants

The full-length penA gene was PCR amplified and sequenced as previously described. PCR-based amplification and sequencing of additional resistance determinants, i.e. the mtrR (including its promoter), porB1b (penB), penA and pilQ genes, were performed as described previously.
Molecular epidemiological typing

For molecular epidemiological characterization, reflecting both micro- and macro-epidemiology, NG-MAST, full-length porB gene sequencing and MLST were performed, as described previously.8,16,20–22 NG-MAST allele numbers and a sequence type (ST) number for each gonococcal isolate were assigned through the NG-MAST database (http://www.ng-mast.net). For MLST, the seven N. gonorrhoeae housekeeping genes (abcZ, adk, eae, fumC, gdh, pdhC and pgm) were amplified by PCR and sequenced, as described previously.12,22 Allele numbers of the MLST loci and the MLST STs were assigned at the Neisseria MLST database (http://pubmlst.org/neisseria).23

Results and discussion

Both isolates were non-susceptible to cefpodoxime when susceptibility was assayed by either disc diffusion or Etest®-based MIC determination (patient A, MIC 0.75 mg/L; patient B, MIC 1 mg/L). Agar dilution MIC assays determined that both isolates were resistant to cefixime (MIC 0.25 mg/L), ciprofloxacin (MIC 16 mg/L), penicillin (MIC 4 mg/L, β-lactamase negative) and tetracycline (MIC 4 mg/L).17 Patient A’s isolate exhibited intermediate susceptibility to azithromycin (MIC 0.5 mg/L), while patient B’s isolate was resistant (MIC 1 mg/L).17 Both isolates were susceptible to ceftriaxone (patient A, MIC 0.125 mg/L; patient B, MIC 0.064 mg/L). Gentamicin (MIC 0.5 mg/L) and spectinomycin (MIC 32 mg/L)17,18

Both isolates were found to have a mosaic penA gene by PCR screening. Subsequent full-length penA gene sequencing showed that these two isolates contained the identical penA mosaic allele XXXIV, which has been correlated with decreased susceptibility/resistance to third-generation cephalosporins and treatment failure with cefixime in many countries.2,4,24,25 Sequencing of additional gonococcal resistance determinants showed that both isolates contained identical mtrR and porB1b (penB) alterations, namely A deletion in the inverted repeat of the mtrR promoter and two consecutive amino acid alterations in PorB1b (G101K and A102N).2,8,9 The two isolates also possessed the penA1 allele but had wild-type pilQ genes.

Both South African isolates belonged to the W11/III1I sero-group and had identical STs by NG-MAST (ST4822) and MLST (ST1901). The two isolates did, however, differ by a single nucleotide polymorphism in the full-length porB gene: patient A’s isolate had a G and patient B’s isolate had an A in position 86. The penA mosaic allele XXXIV has previously been shown to be associated with MLST ST1901 and NG-MAST ST1407.2 ST4822 differs from ST1407 by only one nucleotide in the porB allele and by 15 nucleotides in the tbpB allele. Accordingly, ST4822 may be an evolved subtype of the gonococcal clone ST1407. Gonococci with MLST ST1901 and NG-MAST ST1407 STs form a multidrug-resistant gonococcal clone, characterized by decreased susceptibility/resistance to cefixime, that is spreading worldwide.2,4,22,26 The previously reported ceftriaxone treatment failure in Slovenia and a number of reports of cefixime treatment failure in several European countries, as well as Canada, were due to this gonococcal clone or its evolving subtype.2,4,22,26–28

The high-risk sexual behaviour of some MSM, particularly those with multiple sexual partners who travel abroad extensively, may significantly impact the global transmission of multidrug-resistant gonococcal populations through their participation in international sexual networks. The observation that both our isolates, acquired by MSM in Johannesburg, are possibly genetically linked to MSM sexual networks in Europe and North America is consistent with the international spread of multidrug-resistant N. gonorrhoeae isolates among MSM.5,6,27,29 Indeed, only a few months after the detection of these two gonococcal isolates, a third N. gonorrhoeae isolate exhibiting similar decreased susceptibility or resistance to oral cephalosporins and also possessing NG-MAST ST4822 was obtained from a third MSM patient attending a clinic in Cape Town (D. A. Lewis, unpublished observation).

The STI syndromic management approach, which has been rolled out across much of Africa, typically ignores the sexual healthcare needs of MSM, in whom pharyngeal and ano-rectal gonococcal infections are typically asymptomatic. Both ‘fellatio’ (oro-penile) and ‘rimming’ (oro-anal) sexual activities may predispose MSM to acquiring pharyngeal gonorrhoea, which has a lower cure rate than ano-genital gonorrhoea.30 Owing to differential antibiotic concentrations at genital and pharyngeal sites, those gonococci residing in the pharynx are potentially at a survival advantage and may persist through periods of antibiotic therapy, which may also select for antibiotic-resistant clones.3,30,31 In addition, occupation of the pharyngeal niche provides an increased opportunity for gonococcal cells to exchange DNA, through transformation, with commensal Neisseria species inhabiting the same mucosal surface. If oral sexual intercourse is indeed facilitating the emergence and transmission of gonococci with decreased susceptibility/resistance to cephalosporins, then more effort is certainly required to diagnose and treat pharyngeal gonorrhoea in key populations, such as MSM.2,3,31

The fact that both our isolates were detected in a private laboratory serves as a reminder of the paucity of STI specimens being sent to South Africa’s public sector laboratories as a consequence of implementing the syndromic management approach. Both cases highlight the extreme vulnerability of South Africa’s public health system in terms of ability to identify and characterize such multidrug-resistant strains, particularly in the context of MSM transmission networks. Vigilance for recurrent infections and treatment failures, with proactive laboratory testing of clinical specimens, is essential for appropriate patient management. Several public health agencies, including WHO, have emphasized the importance of establishing early warning systems within countries or geographical regions to detect and track the spread of ESC-resistant gonorrhoea.32 This will be a significant challenge for most African countries, where there is an urgent need to strengthen laboratory capacity for N. gonorrhoeae diagnosis and improve the quality of gonococcal antimicrobial susceptibility testing.

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