Clinical *Neisseria gonorrhoeae* isolate with a *N. meningitidis porA* gene and no prolyliminopeptidase activity, Sweden, 2011 – danger of false-negative genetic and culture diagnostic results

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We describe a *Neisseria gonorrhoeae* strain, found in Sweden in 2011, that harbours a *N. meningitidis porA* gene causing false-negative results in PCRs targeting the gonococcal *porA* pseudogene. Furthermore, the strain had no prolyliminopeptidase (PIP) activity that many commercial biochemical kits for species verification in culture rely on. Enhanced awareness of the spread of such strains and screening for them can be crucial.

Gonorrhoea remains a global public health threat and the World Health Organization (WHO) estimated that 88 million new gonorrhoea cases occurred in 2005 [1]. In many laboratories worldwide, commercial or in-house nucleic acid amplification tests (NAATs) have rapidly replaced culture of the aetiological agent *Neisseria gonorrhoeae* for the diagnosis of gonorrhoea. The gonococcal *porA* pseudogene is possibly the most common target in in-house PCRs currently used for primary detection and/or verifying detection of *N. gonorrhoeae* globally. This is because the pseudogene is highly conserved and has so far been considered to be present in all gonococcal strains. It is also sufficiently diverse from the meningococcal *porA* gene, and commensal *Neisseria* species are lacking the *porA* gene/pseudogene [2-5]. However, recently the first case of a clinical *N. gonorrhoeae* isolate was found in Australia, in which the gonococcal *porA* pseudogene was replaced with a *N. meningitidis porA* gene sequence, which caused a false-negative result in a gonococcal *porA* pseudogene PCR [6].

This report describes the identification and detailed characterisation of the second case of a *N. gonorrhoeae* isolate harbouring a *N. meningitidis porA* gene that causes false-negative results in PCRs targeting the *N. gonorrhoeae porA* pseudogene.

Case report

In May 2011, a pharyngeal specimen from a woman in her 30s presenting to a dermatovenerological clinic in Sweden was culture-positive for *N. gonorrhoeae*. The patient had recently had oral sex with a man in Sweden who could not be traced. She had no recent trips abroad. She was given therapy with cefixime (400 mg oral dose) and seven days later a test-of-cure using culture was negative, which indicated a successful treatment. However, it is known that culture, especially of pharyngeal specimens, has a suboptimal sensitivity compared to NAATs [7,8].

Characterisation of the *N. gonorrhoeae* strain with a meningococcal *porA* gene

The *N. gonorrhoeae* isolate was initially identified by typical colonies on selective culture medium, rapid oxidase production, presence of Gram-negative diplococci in microscopy, and two phenotypic species-verifying assays, i.e. an in-house sugar utilisation test and Phadebact GC Monoclonal Test (Bactus AB, Sweden).

When screening 200 clinical gonococcal isolates from 2011 with a PCR targeting the gonococcal *porA* pseudogene [2], the isolate obtained from the case above was repeatedly negative. Nevertheless, the phenotypic methods remained positive for *N. gonorrhoeae*, and additional phenotypic methods such as matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS; bioMérieux, France) and API NH (bioMérieux, France) confirmed this species. However, the isolate did not show any prolyliminopeptidase (PIP) activity in the API NH. According to Etest (bioMérieux, Sweden), the isolate was susceptible to cefixime, ceftriaxone, ampicillin, ciprofloxacin and spectinomycin, but resistant to azithromycin (Table). The isolate was also identified as *N. gonorrhoeae* in

APTIMA Combo 2 and APTIMA GC NAATs (Gen-Probe, United States).

For genetic characterisation, DNA was isolated in the robotised NorDiag Bullet (NorDiag ASA Company, Norway) using BUGS n'BEADS STI-*fast* kit (NorDiag ASA Company). The 16S rRNA gene in the isolate showed 100% sequence identity with other *N. gonorrhoeae* strains in a GenBank BLAST search. The strain was assigned to *N. gonorrhoeae* multi-antigen sequencing typing (NG-MAST) ST2382 (*porB* allele 1480 and *tbpB* allele 4) and multilocus sequence typing (MLST) ST7367 (*abcZ* allele 109, *adk* 39, *aroE* 67, *fumC* 111, *gdh* 148, *pdhC* 153, *pgm* 133), performed as previously described [9,10]. However, two gonococcal *porA* pseudogene PCRs [2,4] gave negative results. Sequencing of the full-length gonococcal *porA* pseudogene, performed as previously described [3], identified instead a meningococcal *porA* gene sequence (94% sequence identity with the genome-sequenced meningococcal reference strain MC58 [11]), which was assigned to meningococcal genosubtype P1.21-6,2-48,35-1 (Table). This meningococcal *porA* sequence had multiple mismatches in the target sequences for both the primers and probe used in the two gonococcal *porA* pseudogene PCRs [2,4]. The monoclonal antibody 4BG4-E7 multivalent PorA (which is described and can be obtained at www.nibsc.ac.uk) verified that the meningococcal PorA protein was also expressed.

Discussion

There is one previously published report from Australia on a *N. gonorrhoeae* isolate that lacks the highly conserved gonococcal *porA* pseudogene [6]. We describe here the identification and characteristics of a *N. gonorrhoeae* isolate from Europe lacking the gonococcal *porA* pseudogene. The results from the present study together with the data from the Australian report [6] show that gonococcal strains can harbour a *N. meningitidis porA* sequence instead of the gonococcal *porA* pseudogene that causes false-negative results using *N. gonorrhoeae porA* pseudogene PCRs [2,4], which are commonly used in many laboratories globally. The isolate described in the present study also lacked PIP activity, which might challenge the species verification in culture if commercial biochemical kits such as API NH, RapID NH, Gonocheck II, Bacticard Neisseria and Neisseria Preformed Enzyme Test (PET) are used [12]. These kits are used worldwide and rely entirely or in part on the gonococcal PIP activity. This is of major concern, in particular because global transmission of PIP-negative gonococcal strains has previously been described [12]. The isolate described in the present study was assigned to MLST ST7367 (differing in two of the seven alleles from the previously described strain from Australia [6], i.e. which had *aroE* 170 and *pgm* 65) and to NG-MAST ST2382 (differing from the previously described strain from Australia [6] by 65 bp in a sequence alignment of the *porB* alleles and by 1 bp in the *tbpB* allele). Accordingly, this clone was not identical to the gonococcal clone reported from Australia, which was assigned to MLST ST1901 and NG-MAST ST5377 [6]. Thus it is clear that more than one gonococcal clone has acquired a meningococcal *porA* sequence, most likely through horizontal gene transfer and subsequent recombination.

It is worrying that the sexual contact of the present case could not be traced and this gonococcal strain could therefore be circulating in a larger sexual network. The findings of the present study have prompted us to carry out systematic screening of isolates from the past 10 years, which is currently ongoing.

In conclusion, the identification of a *N. gonorrhoeae* isolate harbouring a *N. meningitidis porA* gene as well as lacking PIP activity highlights the limitations and challenges using NAATs for diagnosis of gonorrhoea as well as in species verification in culture diagnostics for gonorrhoea. The presence of these two genetic changes in the same strain, which allow the strain to escape commonly used diagnostic tests, clearly illustrates how versatile the *N. gonorrhoeae* species is. Enhanced awareness of the spread of such strains is needed, and screening for them can be crucial. The opportunities to use combinations of different diagnostic methods (such as NAAT and culture) and multi-target NAATs in a laboratory remain exceedingly valuable.

Table

Characteristics of a *Neisseria gonorrhoeae* strain harbouring a *N. meningitidis porA* gene that causes false-negative results in gonococcal *porA* pseudogene PCRs, Sweden, 2011

NG-MAST	MLST	PIP activity[a]	Ampicillin MIC (mg/L)	Ciprofloxacin MIC (mg/L)	Spectinomycin MIC (mg/L)	Ceftriaxone MIC (mg/L)	Cefixime MIC (mg/L)	Azithromycin MIC (mg/L)	*porA*[b]	*porA* genosubtype
ST2382	ST7367	Negative	0.064	<0.002	8	0.002	<0.016	8	94% MC58	P1.21-6, 2-48,35-1

MIC: minimum inhibitory concentration (Etest was used); MLST: multilocus sequence typing; NG-MAST: *Neisseria gonorrhoeae* multi-antigen sequence typing; PIP: prolyliminopeptidase.

[a] The *N. gonorrhoeae* strain did not show any prolyliminopeptidase (PIP) activity, which might challenge the species-verification in culture if commercial biochemical kits are used that rely entirely or in part on the gonococcal PIP activity, such as API NH, RapID NH, Gonocheck II, Bacticard Neisseria and Neisseria Preformed Enzyme Test (PET). This is of particular concern because global transmission of PIP-negative gonococcal strains has previously been described [12].

[b] The *porA* gene in the *N. gonorrhoeae* strain showed 94% sequence identity with the *porA* gene in the genome-sequenced *N. meningitidis* reference strain MC58 [11].

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