Identification of *Neisseria gonorrhoeae* isolates with a recombinant *porA* gene in Scotland, United Kingdom, 2010 to 2011

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Three isolates of *Neisseria gonorrhoeae* have been identified in Scotland in 2010 and 2011, which lack sequences in the *porA* pseudogene commonly used as the target for confirmatory gonorrhoea polymerase chain reaction assays. Two isolates were clustered temporally and geographically and have the same sequence type and *porA* sequence. A similar strain was reported in Australia during early 2011. The other Scottish isolate was identified separately and is different in sequence type and *porA* sequence.

Introduction

We report three isolates of two different *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) types in Scotland in 2010–2011 which lack the oligonucleotide binding sites for a *porA* polymerase chain reaction (PCR) in common use as a confirmatory assay for *N. gonorrhoeae* [1].

Nucleic acid amplification tests (NAATs) for *N. gonorrhoeae* are increasingly used in screening and diagnosis of gonorrhoea. They have a number of advantages over culture, particularly increased sensitivity when used on non-invasive and extra-genital specimens and where rapid transport of the specimen to the laboratory is not possible. However, concerns about the specificity of commercially-available NAATs have led to widespread recommendations for the confirmatory testing of reactive specimens [2,3]. This should be performed using a NAAT amplifying a different gene target to the original test.

In Scotland, specimens positive for *N. gonorrhoeae* by NAAT may be referred to the Scottish Bacterial Sexually Transmitted Infections Reference Laboratory (SBSTIRL) for confirmation. In addition, all *N. gonorrhoeae* isolates and those NAAT specimens confirmed locally are referred to SBSTIRL for typing by NG-MAST [4] and antimicrobial susceptibility testing (isolates only). Isolates are stored indefinitely on Microbank beads (Pro-Lab).

Confirmatory *N. gonorrhoeae* NAAT testing at SBSTIRL is performed using a real-time PCR targeting the *porA* pseudogene [1] with an internal inhibition control [5]. Specimens producing indeterminate or negative results are generally tested using Aptima GC (Gen-Probe). However, some referred specimens are insufficient in volume for Aptima GC or are in an incompatible transport medium [6].

In May 2011, two isolates of *N. gonorrhoeae* from the same patient, which harboured a recombinant *porA* gene were reported in Australia [7]. These isolates were NG-MAST type 5377, and were not amplifiable using the PCR primers used also by SBSTIRL.

Patients and isolates

In October 2011, a rectal *N. gonorrhoeae* isolate (GC1) and rectal swab positive by NAAT from the same male patient were referred to SBSTIRL. The NAAT specimen was negative by *porA* PCR, but was insufficient for testing by Aptima GC. A nucleic acid extract of the isolate was tested by the *porA* PCR and was also negative. The identity of the isolate was confirmed as *N. gonorrhoeae* serogroup WII/III by Phadebact Monoclonal GC test (Bactus AB), by carbohydrate utilisation test and by Aptima GC. GC1 was NG-MAST type 5967, and exhibited chromosomal resistance to penicillin, tetracycline and ciprofloxacin, while being sensitive to cefixime, ceftriaxone, azithromycin and spectinomycin. A database search for NG-MAST type 5967, revealed a stored rectal isolate (GC2) from a male patient from the same area of Scotland, diagnosed with gonorrhoea one month previously. The patient reported multiple male partners who remain untraced. There was no NAAT specimen for this patient, and no link was found between him and the previously described patient. GC2 had a similar antimicrobial susceptibility profile to GC1 and also failed to amplify using the *porA* PCR. No further identifications of NG-MAST type 5967 strains have been made in Scotland to date.

A further urethral isolate of *N. gonorrhoeae* (GC3) was identified through a search for *porA*-negative, Aptima GC-positive specimens. The male patient was diagnosed with gonorrhoea in December 2010, by both culture and NAAT, in a different region of Scotland to the previous patients. He reported one male partner who was not traced. GC3 was confirmed to be *N. gonorrhoeae* using the same methods as GC1 and GC2, was serogroup WII/III, NG-MAST type 3149, and exhibited chromosomal resistance to penicillin, tetracycline and ciprofloxacin, while being sensitive to cefixime, ceftriaxone, azithromycin and spectinomycin.

Sequencing of *porA*

The *porA* gene was sequenced bidirectionally using the primers described by Whiley et al. [7] (Figure). Basic Local Alignment Search Tool (BLAST) searches were performed via National Center for Biotechnology Information (NCBI), GenBank. Sequences were aligned using Seqscape software (Applied Biosystems).

Sequences from GC1 and GC2 were identical, and very similar to the sequence previously reported [7]. The sequence from GC3 was quite different from these, but the primer sites for the *porA* PCR were again missing and the sequence aligns most closely with a *porA* sequence from *N. meningitidis*.

Discussion

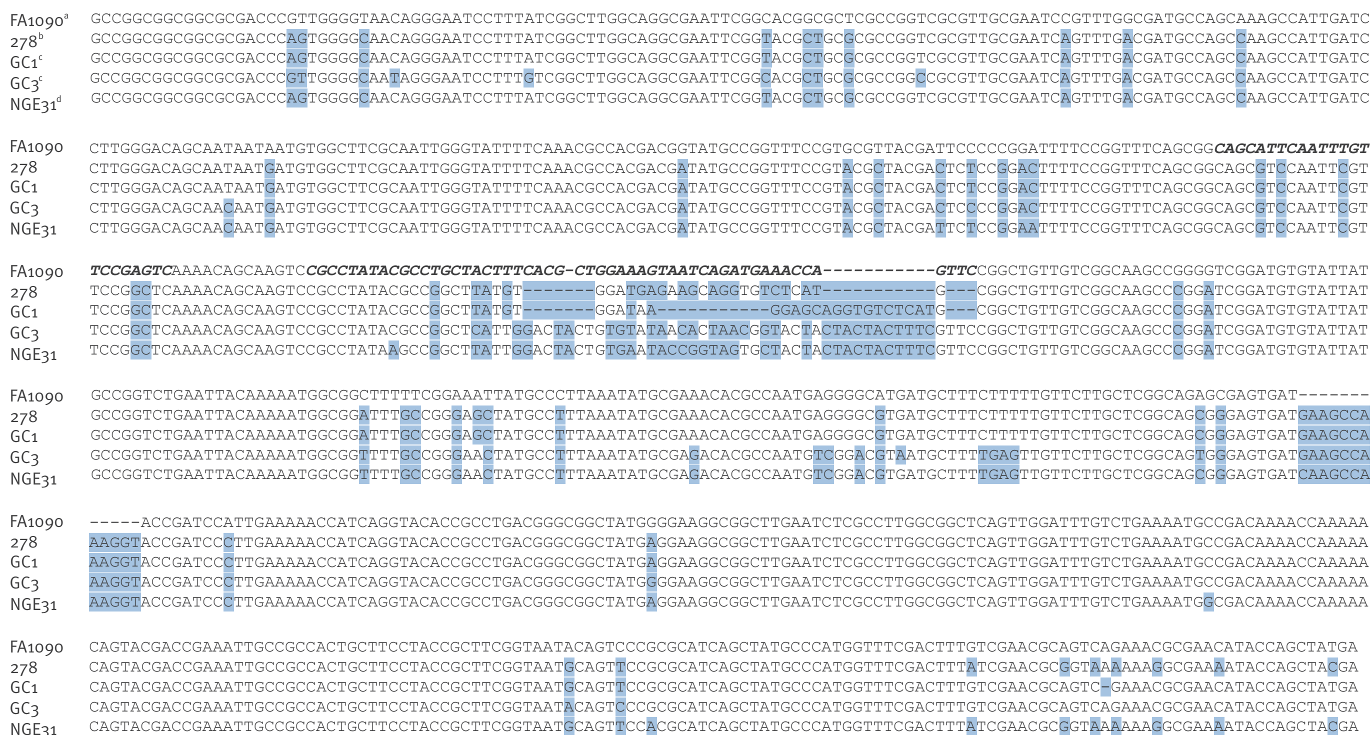
Similarly to the strain reported in Australia, the *N. gonorrhoeae* strains that we identified in this study have undergone an apparent recombination event with *N. meningitidis* in the *porA* region and therefore lack the sequences targeted by a published PCR assay [1] which may be commonly used in reference laboratories.

In contrast to the *porA* of *N. meningitidis*, the related sequence in *N. gonorrhoeae* is an unexpressed pseudogene. Whilst the consequently low selection pressure appears to have produced a rather conserved sequence, the apparent lack of function may make it vulnerable to mutation, including recombination with *porA* genes of other *Neisseria* species that may coexist with *N. gonorrhoeae*.

The sequences obtained from GC1 and GC2 are identical, and circumstantial evidence suggests that they may have been acquired as part of the same chain of transmission. No further epidemiologically connected cases have been identified and there is no known

Figure

Alignment of *porA* nucleotide sequences derived from Scottish isolates of *Neisseria gonorrhoeae* with the *porA* sequence of *Neisseria gonorrhoeae* FA1090 strain and with *porA* sequences of *Neisseria meningitidis* strains, United Kingdom, 2010–2011



Shaded characters indicate differences to the *Neisseria gonorrhoeae* FA1090 strain *porA* pseudogene sequence.

[a] *Neisseria gonorrhoeae* strain FA1090, *porA* pseudogene; GenBank accession AJ223447. Italicised regions indicate polymerase chain reaction primer and probe binding sites.

[b] *Neisseria meningitidis* strain 278, *porA* gene; GenBank accession GQ173789.

[c] *porA* sequence derived from an isolate *of Neisseria gonorrhoeae* in this study.

[d] *Neisseria meningitidis* strain NGE31, *porA* gene; GenBank accession AF226348.

history of sex abroad or with a person from outside Scotland from either patient. However, the histories supplied by the patients are incomplete.

Isolate GC3 was NG-MAST type 3149, which is not uncommon in Scotland, with sixteen isolates identified by SBSTIRL to date since July 2010, of which GC3 was the fourth to be found. All fifteen other NG-MAST type 3149 isolates are either *porA* PCR-positive or are from patients episodes where there was also a NAAT specimen which was *porA* PCR-positive. It is therefore possible that the *porA* recombination event occurred either in the patient from whom the isolation of GC3 was made, or within a very short chain of transmission. It is very likely, from the history reported by the patient, that this infection was acquired in Scotland from someone resident in Scotland, who has unfortunately not been identified.

NG-MAST type 5967, as represented by isolates GC1 and GC2, comprises alleles por 3558 and transferrin binding protein B (tbp) 4. These alleles are 99.8% and 99.7% similar to alleles por 1297 and tbp 983, respectively (representing in each case one nucleotide difference), which make up NG-MAST type 5377, the sequence type of the *porA*-recombinant strain reported in Australia [7]. In contrast, alleles por 1903 and tbp 110, which make up NG-MAST type 3149 are 92.5% and 79.8% similar to por 3558 and tbp 4, respectively. This represents significant sequence divergence and provides additional evidence that strain GC3 is unrelated to GC1, GC2 and the previously-reported strain.

All patients reported in Scotland and Australia were either men who have sex with men (MSM), or were infected rectally. The most likely site of co-colonisation with *N. gonorrhoeae* and *N. meningitidis*, and therefore of genetic exchange, is the pharynx, which is also the least amenable site to successful eradication of *N. gonorrhoeae* and is a frequent site of infection in MSM. It is notable that we have not so far identified pharyngeal infections with these unusual strains, but important that they are recognised if and when they occur in future.

No partners of any of the patients identified in Scotland are known to have been traced and tested or treated. While important for the interruption of gonorrhoea transmission and a mainstay of the public health response to sexually transmitted infections, partner notification remains a challenge in settings where contacts are frequently anonymous or semi-anonymous.

Due to the isolation of *N. gonorrhoeae*, all three patients were correctly diagnosed and adequately treated despite any difficulty with NAAT confirmation. The antimicrobial susceptibility pattern of all three isolates is typical of gonococci seen regularly in Scottish patients. None of the *N. gonorrhoeae* NAAT tests in use in Scotland for primary diagnosis target the *porA* gene [8] and therefore it appears that false-negative results are unlikely with these strains.

There is a small likelihood that patients exist who have been infected with *N. gonorrhoeae* strains similar to those described, in whom culture was unsuccessful and the original NAAT result was unconfirmed. The SBSTIRL records are currently being reviewed with the help of referring laboratories to attempt to identify such patients, and this work to date suggests that they are very few, if any.

We recommend that laboratories performing *porA*-based PCR to confirm positive *N. gonorrhoeae* NAAT results consider the use of a third NAAT, with an alternative target gene where the confirmatory assay is negative. This third target could alternatively be included as a duplex with the *porA* assay.

Laboratories and clinicians alike should be alert to the propensity of *N. gonorrhoeae* to develop unusual variations in genotype, as well as the well-established phenotypic variations..

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