Emergence of a *Neisseria gonorrhoeae* clone showing decreased susceptibility to cefixime in England and Wales

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**Objectives:** The third-generation cephalosporins recommended in national guidelines are amongst the last remaining effective agents for treatment of gonorrhoea. This study characterizes gonococcal isolates with decreased cefixime susceptibility from England and Wales.

**Methods:** A total of 96 isolates of *Neisseria gonorrhoeae* exhibiting cefixime MICs of ≥0.125 mg/L, either collected as part of the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) between 2005 and 2008 (54 from a total of 4649 isolates) or referred to the national reference laboratory in 2008 and 2009 (42 isolates), were tested for susceptibility to a range of antimicrobial agents and were typed using *N. gonorrhoeae* multiantigen sequence typing (NG-MAST).

**Results:** All 96 isolates were also resistant to tetracycline (MIC ≥2 mg/L) and ciprofloxacin (MIC ≥16 mg/L) and 56% showed low-level chromosomal resistance to penicillin. Where data were available, the mean patient age was 31 years, and 88% (83/94) of patients were men. Isolates referred through GRASP were predominantly from men who have sex with men (MSM; 29/44, 66%) and from patients of white British ethnicity (25/43, 58%). The majority of isolates belonged either to sequence type (ST) 1407 (71/96, 74%) or to a highly related ST that shares the *tpbB* allele (allele 110), but with a different *por* allele (20/96, 21%). ST1407 was found in both MSM (22/29, 76%) and heterosexual patients (12/15, 80%) and among all eight isolates from patients reporting sex abroad.

**Conclusions:** The emergence of a clonal group of gonococci showing decreased susceptibility to cefixime in England and Wales highlights the need for continued surveillance.

**Keywords:** surveillance, treatment, molecular typing

**Introduction**

Antimicrobial treatment is the mainstay of public health control of gonorrhoea. In England and Wales treatment guidelines¹ are informed by data collected through the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP).² The use of third-generation cephalosporins, 400 mg of cefixime orally or 250 mg of ceftriaxone intramuscularly, to treat patients with gonorrhoea in clinics that are part of GRASP has increased steadily since 2002, such that 94% of patients with gonorrhoea had been treated with one of these agents in 2009.² The true relationship between treatment failure to either cefixime or ceftriaxone, the susceptibility of the infecting organism and the dosage given is not well understood and consequently arbitrary breakpoints are used, including ≥0.25 mg/L³ and ≥0.125 mg/L.² In 2009, GRASP reported that 1.2% of gonococcal isolates demonstrated decreased susceptibility to cefixime (MIC ≥0.25 mg/L) and 10.6% of isolates exhibited the lower cut-off of MIC ≥0.125 mg/L.² This study aimed to explore the characteristics and molecular epidemiology of gonococcal isolates with decreased susceptibility to cefixime, as defined by the putative breakpoint of ≥0.125 mg/L, and provides the first evidence in England and Wales of the emergence of a multiresistant, predominantly clonal organism that is widely disseminated.
Materials and methods

**Bacterial isolates**

A total of 97 gonococcal isolates were selected as exhibiting decreased susceptibility to cefixime (MICs of \( \geq 0.125 \text{ mg/L} \)) from two archived collections within the Sexually Transmitted Bacterial Reference Laboratory (STBRL), HPA, London: isolates submitted as part of GRASP during 2005 (\( n = 1 \) of 1010), 2006 (\( n = 2 \) of 1250), 2007 (\( n = 17 \) of 1113) and 2008 (\( n = 35 \) of 1276), and 42 isolates that had been referred to STBRL during 2008 and 2009 from any primary diagnostic laboratory in England and Wales as part of their reference service. One isolate from GRASP in 2006 was unavailable for testing (total tested = 96). Gender, age, sexual orientation, ethnicity and sexual contact abroad were collected for patients whose isolates were referred through GRASP, whereas only limited patient information (gender and age) was available for isolates referred to the reference service. This collection included all isolates with decreased susceptibility to cefixime referred during these time periods.

**Susceptibility testing**

Antimicrobial susceptibility was determined by the agar dilution technique using Diagnostic Sensitivity Test (DST) agar (Oxoid, Basingstoke, UK), supplemented with 5% lysed horse blood (TCS Biosciences, Buckingham, UK) and 1% Vitox (Oxoid). The antimicrobial agents tested were: ciprofloxacin, 0.002–32 mg/l; penicillin, 0.03–4 mg/l; spectinomycin, 2–64 mg/l; tetracycline, 1–32 mg/l; ceftriaxone, 0.002–0.125 mg/l; and cefixime 0.002–0.25 mg/l. An inoculum of 10^5 cfu/ml in saline was applied with a multipoint inoculator. Plates were incubated for 48 h in 5% CO_2 at 36°C and the MIC was determined as the lowest concentration that inhibited growth of Neisseria gonorrhoeae. Quality control was performed using WHO gonococcal control strains (WHO A–E), TR01 (resistant to tetracycline) and 81-10 (reduced susceptibility to ciprofloxacin). A control strain exhibiting decreased susceptibility to cefixime was not initially available and failure to include such a strain is a limitation of the study. However, an extended WHO panel which has since been published and WHO strains K and L,\(^7\) which have a penA mosaic and an AS01 mutation, will be included in future studies. Penicillinase production was determined using nitrocefin, a chromogenic cephalosporin (Oxoid).

Etest (Bio-Stat Ltd, Stockport, UK) were also used to determine antimicrobial susceptibility according to the manufacturer’s instructions. Briefly, a gonococcal suspension of approximately 10^6 cfu/ml (turbidity equivalent to that of a 0.5 McFarland standard) was inoculated on GC agar (Becton Dickinson, Oxford, UK) supplemented with 1% IsoVitalex. Etest strips were applied and MICs were recorded following incubation at 36°C in 5% CO_2 for 18–20 h.

Isolates referred to the reference service were initially tested for susceptibility to cefixime, ceftriaxone and azithromycin using Etest and then subsequently tested additionally to penicillin, tetracycline, ciprofloxacin and spectinomycin using agar dilution as above. Isolates from the GRASP survey were initially tested for susceptibility to all antibiotics using agar dilution. Those showing decreased susceptibility to azithromycin, cefixime and ceftriaxone were subsequently confirmed by Etest and the Etest results were included in this study.

**Sequence-based typing**

All isolates were typed using N. gonorrhoeae multiantigen sequence typing (NG-MAST), which differentiates strains on the basis of sequence variation in two hypervariable genes, por and tbpB, following a previously described method.\(^5\) All sequence analyses were performed in BioNumerics version v5.1 (Applied Maths, Kortrijk, Belgium), including a comparison of por sequence similarity using neighbour-joining clustering.

**Results**

Susceptibility testing of the 96 isolates exhibiting decreased susceptibility to cefixime to other antimicrobials is shown in Table 1. Among the 96 patients, gender was known for 94, of whom 83 (88%) were men. The overall mean patient age was 31.3 years (range 17–67 years). Of the 54 GRASP patients, sexual orientation was known for 44, of whom 29 (66%) were men who have sex with men (MSM), and ethnicity was known for 43, of whom 25 (58%) were of white British ethnicity. Information was available for 43 patients on whether or not they had had sex abroad in the past 3 months, and 8 patients (19%) reported sex abroad: 4 in Western Europe, 1 additionally in South America, 2 in Spain, 1 in Portugal and 1 in an unknown country.

NG-MAST of the 96 isolates showed that 71 (74%) were indistinguishable (ST1407, where ST stands for sequence type) and a further 20 isolates (21%) shared the same tbpB allele (allele 110) with ST1407, but differed at the por locus (Table 2). Of these 91 isolates, with the exception of the por allele 2604 (ST4238) observed in two isolates (Table 2), all other por alleles were \( >99\% \) similar (Figure 1), indicating that 93% (89/96) of isolates with raised cefixime MICs were ST1407 or a closely related type. Of the isolates for whom sexual orientation was known, the 29 from MSM all shared the tbpB allele (allele 110), 22 (76%) belonging to ST1407 and the remainder differing in por alleles only. The 15 isolates known to be from heterosexual patients all shared the tbpB allele (allele 110) and 12 (80%) belonged to ST1407. All eight individuals reporting sex abroad were infected with isolates belonging to ST1407.

**Discussion**

The current study presents the first evidence that decreased susceptibility to cefixime has arisen in England and Wales from the dissemination of a relatively clonal group of organisms. These organisms are circulating among MSM and heterosexual patients, some of whom reported sex abroad.

Treatment failure has recently been reported in England and Wales,\(^1\) with isolates with MICs of 0.19–0.25 mg/L. Failure in this MIC range is consistent with Monte Carlo simulation modelling.
Gonococcal decreased susceptibility to cefixime

In this study, only a small number of patients reported sexual contact outside England and Wales, but all were infected with ST1407, suggesting possible importation. However, the frequency of ST1407 among gonococcal isolates that are susceptible to cefixime in England and Wales is unknown and the lack of NG-MAST data globally makes it impossible to establish the route of emergence of this clone. Nevertheless, the current study showed that the isolates identified in GRASP in 2005 and 2006 were distinct NG-MAST types (STs 437 and 835) from the clonal group that emerged from 2007 onwards. The later-emerging ST1407 and related STs may have been imported from elsewhere and disseminated widely, or may have emerged de novo as a result of selection pressure created by increased use of third-generation cephalosporins, most notably cefixime, to treat gonorrhoea in the UK.2

The potential for treatment failure would not appear to be confined to any one region of England and Wales, given the widespread geographical distribution of gonococci with decreased susceptibility to cefixime observed to date, and is found in both heterosexuals and MSM. Furthermore, these infections may be difficult to treat with alternative therapies as all isolates encountered to date exhibited resistance to previously used therapeutic agents. Currently these isolates appear susceptible to the alternative agent, ceftriaxone, using the European Committee on Antimicrobial Susceptibility Testing (EUCAST)3 internationally accepted breakpoint of 0.25 mg/L, although GRASP reported 0.3% of isolates exhibiting MICs of ≥0.125 mg/L in 2009,5 and there has been a recent report of a strain exhibiting high-level resistance.10

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Table 2. NG-MAST types of 96 gonococcal isolates with cefixime MICs ≥0.125 mg/L in relation to year of isolation and geographical distribution

<table>
<thead>
<tr>
<th>ST</th>
<th>por</th>
<th>tbpB</th>
<th>No. of isolates (%)</th>
<th>Year</th>
<th>Distribution (regions of Englanda and Walesb)</th>
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<tr>
<td>1407</td>
<td>908</td>
<td>110</td>
<td>71 (74)</td>
<td>2007–09</td>
<td>all regionsa</td>
</tr>
<tr>
<td>3149</td>
<td>1903</td>
<td>110</td>
<td>5 (5)</td>
<td>2007–09</td>
<td>south-east and north-westa</td>
</tr>
<tr>
<td>3422</td>
<td>2073</td>
<td>110</td>
<td>4 (4)</td>
<td>2008</td>
<td>London and north-easta</td>
</tr>
<tr>
<td>3127</td>
<td>1901</td>
<td>110</td>
<td>2 (2)</td>
<td>2008</td>
<td>north-westa</td>
</tr>
<tr>
<td>3708</td>
<td>2236</td>
<td>110</td>
<td>2 (2)</td>
<td>2008</td>
<td>north-east and southa</td>
</tr>
<tr>
<td>4238</td>
<td>2604</td>
<td>110</td>
<td>2 (2)</td>
<td>2008</td>
<td>Londona</td>
</tr>
<tr>
<td>3499</td>
<td>2115</td>
<td>110</td>
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<td>2008–09</td>
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</tr>
<tr>
<td>3709</td>
<td>2237</td>
<td>110</td>
<td>1 (1)</td>
<td>2008</td>
<td>Londona</td>
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<tr>
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<td>2043</td>
<td>110</td>
<td>1 (1)</td>
<td>2009</td>
<td>north-westa</td>
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<tr>
<td>4237</td>
<td>2603</td>
<td>110</td>
<td>1 (1)</td>
<td>2009</td>
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<td>90</td>
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<td>Londona</td>
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<tr>
<td>2322</td>
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</tr>
<tr>
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<td>2180</td>
<td>10</td>
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<td>2009</td>
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<tr>
<td>835</td>
<td>566</td>
<td>10</td>
<td>1 (1)</td>
<td>2006</td>
<td>southb</td>
</tr>
<tr>
<td>437</td>
<td>14</td>
<td>4</td>
<td>4 (1)</td>
<td>2005</td>
<td>Londona</td>
</tr>
</tbody>
</table>

aRegions of England; bregions of Wales.

Figure 1. Neighbour-joining clustering showing similarity of por alleles in gonococci with reduced susceptibility to cefixime (MICs ≥0.125 mg/L). por alleles in bold font represent STs with tbpB allele 110.

which has indicated that, with current regimens, peak serum cefixime concentrations are not maintained at a sufficient level for long enough to ensure successful eradication of gonococcal infections exhibiting MICs of 0.125 mg/L or greater.2 The rate of decreased susceptibility to oral cephalosporins and associated treatment failure is particularly high in Asia, most notably in Japan.7 Decreased susceptibility to cefixime and ceftriaxone has been linked with ST1407 and the presence of the penA mosaic in Sweden,9 suggesting wider circulation of this clone.
Transparency declarations
None to declare.

Disclaimer
The views expressed in this publication are those of the authors and are not necessarily those of the UK Department of Health.

References