Antimicrobial resistance, genetic resistance determinants for ceftriaxone and molecular epidemiology of Neisseria gonorrhoeae isolates in Nanjing, China

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Objectives: Antimicrobial resistance (AMR) in Neisseria gonorrhoeae is a major problem worldwide. This study investigated the AMR, genetic ceftriaxone resistance determinants and molecular epidemiology of N. gonorrhoeae in Nanjing, China.

Methods: N. gonorrhoeae isolates were collected in 2007 (n = 198) and 2012 (n = 80). The susceptibility to ceftriaxone, spectinomycin, ciprofloxacin and tetracycline was determined using an agar-dilution method. The ceftriaxone resistance determinants penA, mtrR and penB were examined using sequencing. N. gonorrhoeae multi-antigen sequence typing (NG-MAST) was performed for molecular epidemiology.

Results: All isolates were resistant to ciprofloxacin, 42.4% produced β-lactamase and 34.9% showed high-level resistance to tetracycline (MIC ≥ 16 mg/L). In total, 5.4% of isolates were resistant to ceftriaxone; however, all of these isolates were obtained in 2007 and the susceptibility to ceftriaxone appeared to have increased. All isolates were susceptible to spectinomycin. No penA mosaic alleles were found. Non-mosaic penA alleles with A501T and G542S alterations, an H105Y alteration in mtrR and an A102D/N alteration in porB1b were statistically associated with decreased susceptibility or resistance to ceftriaxone. The most prevalent NG-MAST sequence types (STs) were ST568 (n = 13), ST270 (n = 9) and ST421 (n = 7). ST270 was the most common ST in isolates with decreased susceptibility or resistance to ceftriaxone.

Conclusions: Ceftriaxone, ideally 500 mg and together with azithromycin (1–2 g), should be recommended for treatment of gonorrhoea in Nanjing, China. However, N. gonorrhoeae strains with resistance to ceftriaxone have been found in Nanjing. NG-MAST and ceftriaxone resistance determinant analysis can be valuable to supplement the antimicrobial resistance surveillance in China, which needs to be further strengthened.

Keywords: gonorrhoea, treatment, penA, mtrR, porB, NG-MAST

Introduction

Gonorrhoea is one of the most prevalent bacterial sexually transmitted infections and a major public health concern globally. In 2008, the WHO estimated the number of new cases of gonorrhoea to be 106 million globally each year.1 In China, in 2012 a total of 95,263 cases of gonorrhoea were reported to the Chinese Center for Disease Control and Prevention (Chinese CDC), and gonorrhoea was ranked as the sixth most common infectious disease.2 Gonorrhoea can result in severe reproductive complications and can also increase the transmission of HIV.3 Gonorrhoea, including its severe complications, causes substantial morbidity and socio-economic consequences.

Neisseria gonorrhoeae has developed antimicrobial resistance (AMR) to most drugs used for treatment. Worryingly, resistance to the extended-spectrum cephalosporins (ESCs), the last remaining options for empirical first-line monotherapy, has now emerged.4–9 Cefixime treatment failures have been verified in Japan,10 Norway,11 the UK,12 Austria,13 France,14 Canada15 and South Africa.16 Accordingly, cefixime has been excluded from the first-line empirical antimicrobial treatment recommendations in Japan,17 the USA18 and Europe.19 The most potent ESC, ceftriaxone, remains effective
for treatment; however, some failures to treat pharyngeal gonorrhea in Japan,20,21 Australia,22–26 Sweden25 and Slovenia26 have been described. Most worryingly, the first extensively drug-resistant \textit{N. gonorrhoeae} strains also with high-level resistance to ceftriaxone, ‘superbugs’, have been verified in Japan,21 France,16 and Spain.27

China has a high burden of gonorrhoea and is also located in the WHO Western Pacific Region, where most gonococcal AMR first emerged. These AMR gonococcal strains have subsequently been transmitted globally.4,7 In China, since 2007 the recommended first-line empirical antimicrobial treatment for uncomplicated gonorrhoea is ceftriaxone (250 g, intramuscularly), cefotaxime (1 g, intramuscularly) or spectinomycin (2 g, intramuscularly). Cefixime (400 mg, orally) or other third-generation cephalosporins are recommended as alternative regimens if clinical experience has proven the given antimicrobial effective.28 Furthermore, despite not being recommended, many other antimicrobials, such as penicillins, macrolides, tetracyclines and fluoroquinolones, are also used for treatment. However, the information regarding AMR (especially ceftriaxone resistance) and molecular characteristics of \textit{N. gonorrhoeae} strains in China remains highly limited. No study regarding genetic resistance determinants for ceftriaxone and the molecular epidemiology of \textit{N. gonorrhoeae} has been performed in our study region in China.

In this study, the susceptibility to ceftriaxone, spectinomycin, ciprofloxacin and tetracycline of \textit{N. gonorrhoeae} isolates collected in 2007 and 2012 in Nanjing was investigated. Furthermore, genetic ceftriaxone resistance determinants, \textit{penA}, \textit{mtrR} and \textit{penB}, were examined and mutation patterns associated with the susceptibility to ceftriaxone were determined using statistical methods. Finally, \textit{N. gonorrhoeae} multi-antigen sequence typing (NG-MAST)76,78 was performed to identify clusters of gonococcal strains and correlations between NG-MAST sequence types (STs) and AMR.

\textbf{Materials and methods}

The work was performed at the National Center for STD Control, Nanjing, China.

\textbf{N. gonorrhoeae isolation and species verification}

A total of 278 \textit{N. gonorrhoeae} isolates were consecutively collected from patients (one isolate per patient) with urogenital gonorrhoea attending the clinic at the National Center for STD Control, Nanjing, China in 2007 (198 isolates) and 2012 (80 isolates). This clinic is one of the 12 sentinel clinics participating in the National Gonococcal Resistance Program in China and captures most of the sexually transmitted disease (STD) cases in the study area. Male urethral swabs and female endocervical swabs were used for sample collection. Selective gonococcal culture media were used to isolate \textit{N. gonorrhoeae}, and identification of Gram-negative diplococci by microscopy, a rapid oxidase reaction and a carbohydrate utilization test were used for species verification of \textit{N. gonorrhoeae}.31 All isolates were stored in skimmed milk at −80°C.

\textbf{Antimicrobial susceptibility testing}

The antimicrobial susceptibility to ceftriaxone, spectinomycin, ciprofloxacin and tetracycline was determined using the agar dilution method, according to recommendations from the WHO.31 Susceptibility and resistance breakpoints from EUCAST (http://www.eucast.org/clinical_breakpoints/) were used. Tetracycline MIC ≥16.0 mg/L was considered as high-level resistance to tetracycline. β-Lactamase (penicillinase)-producing \textit{N. gonorrhoeae} isolates were detected using a nitrocefin solution filter paper test. The 2008 WHO \textit{N. gonorrhoeae} reference strain panel was included in every batch of testing.32

\textbf{DNA extraction}

DNA was extracted from bacterial suspensions using QIAxtractor DX Kits (Qiagen, Hilden, Germany) on a QIAxtractor automated genomic DNA extraction instrument, according to the manufacturer’s instructions (Qiagen, Hilden, Germany).

\textbf{Sequencing of genetic ceftriaxone resistance determinants}

PCRs using previously described primers33 were performed to amplify the ceftriaxone resistance determinants, that is, \textit{penA}, \textit{mtrR} (including the promoter region) and \textit{porB1b} (including the \textit{penB} resistance determinant). Briefly, all PCR amplifications were performed on a GeneAmp PCR 9700 Thermocycler (Applied Biosystems, Singapore). The PCR programme parameters included an initial denaturation step at 94°C for 4 min and 30 subsequent cycles as follows: 2 min at 94°C, 1 min at 55°C and 1 min at 72°C, with finally an extension step at 72°C for 10 min. Genomic DNA from the \textit{N. gonorrhoeae} reference strain FA1090 was used as positive control and distilled water as negative control. PCR amplicons were sequenced at Beijing Genomics Institution (Shenzhen, China) using the same primers as in the PCR amplifications. DNA sequences and deduced amino acid sequences were analysed using BioEdit version 7.1.9. All sequences were compared with the corresponding sequences in the genome sequenced \textit{N. gonorrhoeae} reference strain FA1090.

\textbf{Molecular epidemiological typing}

NG-MAST was performed as described previously.29 The NG-MAST STs reported in this paper have been partially presented previously.16

\textbf{Statistical analysis}

An independent sample \(t\)-test was applied to compare mean ceftriaxone MICs of isolates with the presence of different resistance determinants. For \(\chi^2\) test analysis, all isolates were divided into two groups. Accordingly, one group represented isolates with decreased susceptibility or resistance to ceftriaxone [isolates \((n=87)\) with ceftriaxone MICs of 0.125–0.5 mg/L (72 isolates with MICs=0.125 mg/L, 14 isolates with MIC=0.25 mg/L and 1 isolate with MIC=0.5 mg/L)] and one group included isolates with susceptibility to ceftriaxone [isolates \((n=191)\) with ceftriaxone MICs of \(0.004–0.06\) mg/L]. \(\chi^2\) tests were used to compare the presence of different patterns of ceftriaxone resistance determinants between the isolates with decreased susceptibility or resistance to ceftriaxone and the isolates with susceptibility to ceftriaxone. \(P\) values <0.05 were considered as statistically significant. SPSS version 13.0 (SPSS Inc., USA) was used for the statistical analysis.

\textbf{Ethics approval}

The study protocol was reviewed and approved by the Medical Ethics Committee at the Institute of Dermatology, the Chinese Academy of Medical Sciences & Peking Union Medical College and the National Center for Sexually Transmitted Disease Control, Nanjing, China (approval number 2011-KY-003). Written informed consent was obtained from all of the participants or the caretakers of the minors/children involved in our study.
Antimicrobial resistance and resistance determinants in Nanjing

Results

Sociodemographic characteristics and antimicrobial susceptibility

Out of the 278 patients, 232 (83.5%) were males and 46 (16.5%) were females. The age ranges of the males and the females were 15–85 years (mean ± SD, 36.4 ± 11.1 years) and 20–63 years (mean ± SD, 35.2 ± 11.5 years), respectively.

All (100%) N. gonorrhoeae isolates were resistant to ciprofloxacin (MIC >0.064 mg/L), 47.5% (n=94) of isolates in 2007 and 30.0% (n=24) in 2012 produced β-lactamase resulting in high-level resistance to penicillin, 33.3% (n=66) of isolates in 2007 and 38.8% (n=31) in 2012 were highly resistant to tetracyclines (MIC ≥16 mg/L), 15 (5.4%, 95% CI, 3.3%–8.7%) of isolates (all from 2007) were resistant to ceftriaxone (MIC >0.12 mg/L; Table 1), and no isolates were resistant to spectinomycin (MIC >64 mg/L). Furthermore, the proportion of isolates with ceftriaxone MIC=0.125 mg/L (exactly at the resistance breakpoint) was 39.4% (95% CI, 32.3%–46.3%) in 2007 and 11.3% (95% CI, 6.0%–20.0%) in 2012. Gonococcal isolates with this ceftriaxone MIC have previously resulted in failures in treating pharyngeal gonorrhoea,4,23–26 and can be considered as having a decreased susceptibility to ceftriaxone. The proportion of isolates with decreased susceptibility or resistance to ceftriaxone (ceftiraxone MIC ≥0.125 mg/L) was higher among older patients (>36 years of age, χ²=5.428, P=0.02) and among females (χ²=3.805, P=0.051). The mean ceftriaxone MIC of isolates from the older patient group (>36 years of age) was also significantly higher than that of younger patients (P=0.014).

Presence of ceftriaxone genetic resistance determinants and associations with decreased susceptibility and resistance to ceftriaxone

The NG-MAST STs, the MIC of ceftriaxone and the presence of ceftriaxone genetic resistance determinants (penA, mtrR and penB (specific mutations in porB1b)) among the ceftriaxone-resistant isolates (n=15) are shown in Table 1. Six penA alleles were found among these 15 isolates, i.e. penA alleles V (n=2), XVII (n=2) and XIII (n=2), new allele 1 (n=1), new allele 6 (n=7) and new allele 8 (n=1). None of these penA alleles was a mosaic allele. However, substitutions of A501 in the penicillin-binding protein 2 (PB2), which have also been associated with decreased susceptibility or resistance to ceftriaxone,6,14,27,35–38 were common among these isolates (13/15, 86.7%), of which 7 (46.7%) isolates contained an A501T alteration and 6 (40.0%) isolates contained an A501S alteration. Furthermore, PB2 G542S alteration was also prevalent (13/15, 86.7%), while PB2 P551S was rare (2/15, 13.3%) (Table 1). Both of these PB2 alterations have been previously suggested to be associated with decreased susceptibility to ceftriaxone.39 Also, when examining all isolates (n=278), no penA mosaic alleles were found. Nevertheless, 68 (24.5%) isolates contained a PB2 P551T alteration and 101 (36.3%) isolates had a PB2 A501V alteration (Table S1, available as Supplementary data at JAC Online). The MICs of ceftriaxone for the isolates with PB2 A501T and A501S alterations ranged from <0.016 to 0.25 mg/L (mean MIC, 0.103 ± 0.064 mg/L) and from 0.016 to 0.5 mg/L (mean MIC, 0.075 ± 0.062 mg/L), respectively. The mean ceftriaxone MIC of the A501T group was significantly higher than that of the A501 wild-type (WT) group (P<0.001) (Table S1). The prevalence of the PB2 G542S alteration was 38.1% (106/278) among all isolates. The mean ceftriaxone MIC of the PB2 G542S group was significantly higher than that of the G542 WT group (P<0.001; Table S1). Overall, the PB2 A501T and G542S alterations were significantly more common among the isolates with decreased susceptibility or resistance to ceftriaxone (42.5% and 58.6%, respectively) as compared with the ceftriaxone-susceptible isolates (16.2% and 28.8% respectively; P<0.001). The PB2 P551S/L alteration was only present in the G542 WT group. No significant difference in the mean ceftriaxone MIC or proportion in isolates with decreased susceptibility or resistance

Table 1. N. gonorrhoeae isolates with resistance to ceftriaxone (>0.125 mg/L) isolated in Nanjing, China (n=15)

<table>
<thead>
<tr>
<th>Isolation year</th>
<th>NG-MAST ST</th>
<th>MIC of ceftriaxone (mg/L)</th>
<th>penA alteration</th>
<th>A501 alteration</th>
<th>G542 alteration</th>
<th>P551 alteration</th>
<th>mtrR alteration</th>
<th>adenine (A) deletion in promoter region</th>
<th>porB1b alteration</th>
<th>G101 alteration</th>
<th>A102 alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>1612</td>
<td>0.25</td>
<td>new 6</td>
<td>T</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
<td>yes</td>
<td>yes</td>
<td>K</td>
<td>G</td>
</tr>
<tr>
<td>2007</td>
<td>1691</td>
<td>0.25</td>
<td>V</td>
<td>WT</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
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<td>yes</td>
<td>D</td>
<td>G</td>
</tr>
<tr>
<td>2007</td>
<td>1790</td>
<td>0.25</td>
<td>new 1</td>
<td>V</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
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<td>yes</td>
<td>K</td>
<td>G</td>
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<tr>
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<td>1790</td>
<td>0.25</td>
<td>new 6</td>
<td>T</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
<td>no</td>
<td>yes</td>
<td>K</td>
<td>D</td>
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<tr>
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<td>S</td>
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<td>H105Y</td>
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<td>yes</td>
<td>D</td>
<td>G</td>
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<td>1943</td>
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<td>WT</td>
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<td>H105Y</td>
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<td>K</td>
<td>D</td>
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<td>2279</td>
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<td>T</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
<td>yes</td>
<td>yes</td>
<td>K</td>
<td>D</td>
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<td>2288</td>
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<td>A39T</td>
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<td>yes</td>
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<td>D</td>
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<td>2007</td>
<td>2416</td>
<td>0.25</td>
<td>XVII</td>
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<td>yes</td>
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<td>new 6</td>
<td>T</td>
<td>S</td>
<td>WT</td>
<td>H105Y</td>
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<td>yes</td>
<td>K</td>
<td>D</td>
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<tr>
<td>2007</td>
<td>8785</td>
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<td>XIII</td>
<td>V</td>
<td>WT</td>
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<td>G45D</td>
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<td>yes</td>
<td>K</td>
<td>D</td>
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<tr>
<td>2007</td>
<td>8795</td>
<td>0.25</td>
<td>XIII</td>
<td>V</td>
<td>WT</td>
<td>S</td>
<td>H105Y</td>
<td>yes</td>
<td>yes</td>
<td>K</td>
<td>D</td>
</tr>
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</table>
to ceftriaxone and the isolates with susceptibility to ceftriaxone was found between the P551S/L group and P551 WT group \((P=0.901\) and \(P=0.562\), respectively; Table S1).

A single nucleotide (A) deletion in the inverted repeat of the promoter region of the \(mtrR\) gene was observed in 14 (93.3\%) of the isolates with resistance to ceftriaxone \((n=15)\) and in 272 (97.8\%) of all the isolates \((n=278)\); Table 1 and Table S1). Amino acid alterations in MtrR were found in 261 (93.9\%) isolates. Accordingly, 11 (4.0\%) isolates contained an A39T alteration, 64 (23.0\%) isolates a G45D alteration, 24 (8.6\%) isolates a D79I alteration and 186 (66.9\%) isolates an H105Y alteration \[notably, in 11 (73.3\%) of the 15 isolates with resistance to ceftriaxone\].

In 15 (7.6\%) of the 195 ceftriaxone-resistant isolates, amino acid alterations at G101 or A102 were detected in 274 (98.6\%) isolates and G101/A102 double mutation was observed in 252 (90.6\%) isolates. The mean MIC of ceftriaxone was significantly higher in the A102D alteration group than in the A102 WT group \((P=0.001)\) and A102D alteration was also significantly associated with isolates with decreased susceptibility or resistance to ceftriaxone \((P=0.003)\). No significant difference in mean ceftriaxone MIC or proportion was observed for any other \(porB1b\) mutations (Table S1).

### Molecular epidemiological characterization

The 278 \(N.\) gonorrhoeae isolates were assigned to 163 different NG-MAST STs. The most prevalent ST was ST568 \((n=16)\), followed by ST270 \((n=10)\), ST421 \((n=7)\), ST1866 \((n=7)\), ST1766 \((n=6)\), ST2288 \((n=5)\) and ST2318 \((n=5)\). Furthermore, 6 STs were represented by four isolates, 9 STs by three isolates, 30 STs by two isolates and 111 STs by single isolates (Table 2).34 The ceftriaxone-resistant isolates (MIC >0.125 mg/L) were assigned to ST2288 \((n=1)\), ST1612 \((n=1)\), ST1691 \((n=1)\), ST1790 \((n=2)\), ST1868 \((n=1)\), ST1943 \((n=1)\), ST2279 \((n=1)\), ST2416 \((n=1)\), ST7085 \((n=1)\), ST8757 \((n=1)\), ST8772 \((n=1)\), ST8780 \((n=1)\), ST8785 \((n=1)\) and ST8795 \((n=1)\). Of the most prevalent STs, seven (87.5\%) of the ST270 isolates shared an identical A501T and G542S altered PBP2 allele \(\text{new 6}\), a single nucleotide \(A\) deletion in the \(mtrR\) promoter, H105Y alteration in MtrR and G101K/A102D alterations in \(porB1b\).

### Discussion

Gonorrhoea and its control remain major public health concerns globally and clearly also in Nanjing, China. In the present study, an exceedingly high prevalence of resistance to the previously recommended first-line antimicrobials ciprofloxacin, penicillin and tetracycline was observed. This is in accordance with previous

### Table 2. NG-MAST STs and MICs (mg/L) of ceftriaxone for \(N.\) gonorrhoeae \((n=278)\) isolated in Nanjing, China in 2007 and 2012

<table>
<thead>
<tr>
<th>NG-MAST ST</th>
<th>(\leq0.008)</th>
<th>0.015</th>
<th>0.030</th>
<th>0.060</th>
<th>0.125</th>
<th>0.250</th>
<th>0.500</th>
<th>Total no. of isolates</th>
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<td>7</td>
<td>6</td>
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<td>4</td>
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<td>STs ((n=9)) represented by three isolates</td>
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<td>10</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>STs ((n=30)) represented by two isolates</td>
<td>5</td>
<td>9</td>
<td>20</td>
<td>19</td>
<td>7</td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Unique ST ((n=111))</td>
<td>2</td>
<td>6</td>
<td>33</td>
<td>37</td>
<td>28</td>
<td>5</td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>Total no. of isolates</td>
<td>4</td>
<td>21</td>
<td>79</td>
<td>87</td>
<td>72</td>
<td>14</td>
<td>1</td>
<td>278</td>
</tr>
</tbody>
</table>

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Antimicrobial resistance and resistance determinants in Nanjing

studies from other countries in the WHO Western Pacific Region.41–45 None of these antimicrobials is now recommended for first-line empirical therapy of gonorrhoea in China; however, the use of these antimicrobials in the treatment of gonorrhoea due to, e.g. over-the-counter use and self-treatment, is highly probable. The results of the present study support the current first-line treatment in China. Ceftriaxone and spectinomycin (if no pharyngeal infection is suspected) can continue to be recommended for the treatment of gonorrhoea in Nanjing, which is also in concordance with data from the National Gonococcal Resistance Program in China.15 Fifteen isolates with ceftriaxone MICs of 0.25–0.5 mg/L were identified in the present study, but no treatment failures for urogenital gonorrhoea have been verified at those MICs internationally. Nevertheless, based on the described failures to treat pharyngeal gonorrhoea,20–26 the in vitro ceftriaxone resistance identified in the present study (5.4%) and the overall cephalosporin resistance situation in the WHO Western Pacific Region41–45 and basically worldwide4–9,15,16,21–27,35–38,43,45,47,48 increasing the ceftriaxone dose to 500 mg and/or adding azithromycin (1–2 g) to the recommended first-line treatment should be considered, which is in line with the dual antimicrobial treatment regimens recommended in the USA18 and Europe.19 These dual antimicrobial treatment regimens also effectively eradicate any concomitant Chlamydia trachomatis infection. Furthermore, exclusion of other third-generation cephalosporins such as cefotaxime and cefixime from the recommended treatment should also be considered. There is an urgent need to maintain and further strengthen the N. gonorrhoeae AMR surveillance in Nanjing as well as in other regions of China, to monitor the AMR trends in a timely manner (with focus on the third-generation cephalosporins), to identify emergence of new resistance, to develop evidence-based treatment guidelines and develop strategies to prevent and mitigate the further spread of AMR N. gonorrhoeae strains locally and nationally.

Notably, older age of the gonorrhoea patients was associated with higher ceftriaxone MIC mean and higher proportion of decreased susceptibility or resistance to ceftriaxone. This may be due to increased amount of travel and, accordingly, exposure to AMR N. gonorrhoeae strains, but also to increased exposure to antimicrobials (self-treatment with antimicrobials is exceedingly common in China), which have been previously reported as risk factors for fluoroquinolone-resistant N. gonorrhoeae.46 This is a major concern that not only compromises the surveillance programmes but also adds a high selective pressure on N. gonorrhoeae to further develop AMR as well as on other bacterial species, which can share resistance genes with N. gonorrhoeae. Consequently, the antimicrobial stewardship in China needs to be strengthened and antimicrobials should only be accessible through prescriptions from medical doctors.

Previous studies investigating the ESC resistance determinants in N. gonorrhoeae have focused on three genes: penA, encoding the lethal target for β-lactam antimicrobials PBP2; mtrR, regulator of the MtrC–MtrD–MtrE efflux pump; and porB, encoding the major outer membrane porin.4,11,13–16,21,25–27,33,35–39,47,48 In the present study, all these genes were investigated and decreased susceptibility or resistance to ceftriaxone was significantly associated with A501T and G542S alterations in PBP2, H105Y alteration in MtrR and A102D alteration in PorB1b. Even stronger associations were found between combinations of these three alterations (double alteration or triple alteration) and decreased susceptibility or resistance to ceftriaxone (data not shown). However, the ceftriaxone MICs of the isolates with double or triple alteration varied substantially, i.e. from <0.016 to 0.5 mg/L, which illustrates the difficulty in predicting decreased susceptibility or resistance to ceftriaxone using solely genetic methods. As an example, a multiplex PCR targeting all the four mutations mentioned above for prediction of decreased susceptibility or resistance to ceftriaxone in the current isolates would only detect 15 out of the 87 isolates with decreased susceptibility or resistance to ceftriaxone (sensitivity, 17.2%; specificity, 95.8%). This might reflect the different presence or absence of epistatic mutations4,38,48 and/or the unknown non-transformable ‘factor X’4,14,21,38,48 in those isolates. Importantly, the type of statistical identification of AMR determinants used in the present study can be biased by, for example, clonal spread of specific AMR strains and epistatic or unknown resistance determinants, and the importance of any new genetic AMR determinant needs to be verified in appropriate subsequent laboratory experiments. In many previous studies, mosaic PBP2 alleles have been strongly associated with decreased susceptibility or resistance to ESCs4,10,11,13–16,20,21,23–27,35–38,43,45,47,48. However, in Nanjing no isolate with a mosaic PBP2 allele was found. Instead, several new non-mosaic PBP2 alleles were found and the ‘new allele 6’ was very common in isolates with decreased susceptibility or resistance to ceftriaxone. This new allele 6 contained PBP2 A501T and G542S alterations, which have been previously reported to be associated with decreased susceptibility to ceftriaxone.19 The present study showed a high prevalence of gonococcal isolates with decreased susceptibility or resistance to ceftriaxone in Nanjing, which is only one of the 12 sentinel sites of the Chinese national gonococcal antimicrobial susceptibility programme. It could be valuable to strengthen the gonococcal AMR surveillance in China by also including determination of genetic AMR determinants and molecular epidemiological characteristics of at least a subset of the N. gonorrhoeae isolates.

NG-MAST analysis showed a diversified population of N. gonorrhoeae in Nanjing, China, with 163 different NG-MAST STs among the 278 isolates. The high number of unique STs (n = 111) and STs that were only described in our previous study (n = 88) may be associated with the highly limited number of NG-MAST studies in China.46 ST1407, a successfully spread clone that has caused many cefixime and ceftriaxone treatment failures internationally,4,11,13–16,26 was not identified in the present study.

In conclusion, ceftriaxone should be recommended for the treatment of gonorrhoea in Nanjing, China. However, N. gonorrhoeae strains with resistance to ceftriaxone have been found in Nanjing. Accordingly, increasing the ceftriaxone dose to 500 mg and/or adding azithromycin (1–2 g) to the recommended first-line treatment should be considered, which is in line with the dual antimicrobial treatment regimens recommended in the USA18 and Europe.19 These dual antimicrobial treatment regimens also effectively eradicate any concomitant Chlamydia trachomatis infection. NG-MAST and ceftriaxone resistance determinants analysis can be valuable to supplement AMR surveillance in China.

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Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).


