Characteristics and dissemination of mosaic penicillin-binding protein 2 harboring multidrug-resistant Neisseria gonorrhoeae with reduced cephalosporin susceptibility in Northern Taiwan

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ABSTRACT

Among 254 Neisseria gonorrhoeae isolates from a STI clinics in Northern Taiwan, 69 isolates were found to contain mosaic penA (MA) gene and associated with elevated cefixime and ceftriaxone MICs. Most of these MA gene-harboring isolates were also resistant to penicillin (71.4%) and ciprofloxacin (100%) and from men who have sex with men (MSM)/bisexual (81.2%).

Three major sequence types (ST835, ST2180 and ST2253) constituted 55.7% of these isolates. The major sequence types harboring mosaic penA gene may represent major sexual networks responsible for the emergence/introduction and the spread of the multiple drug resistant clones in Taiwan.
Neisseria gonorrhoeae infection continues to be one of the most globally prevalent sexually transmitted infections (STIs) (2). The incidence of gonorrhea has increased in Taiwan in the last decade as it has in many other developed countries (4-5). Recently however, the number of N. gonorrhoeae isolates with reduced susceptibility to cephalosporin antibiotics has gradually increased (12, 15-16). Cases of treatment failures have been reported with cefitibuten and cefixime but not with ceftriaxone (8, 14). The major mechanisms of N. gonorrhoeae resistance to oral cephalosporins have been determined to be associated with the presence of a mosaic penicillin-binding protein 2 (PBP2) encoded by the penA gene which was generated by homologous recombination with other related commensal Neisseria species (1, 7), and combined with other determinants (17). We explored the antibiotic susceptibilities, risk characteristics and clonal relatedness of mosaic penA (MA) gene containing gonococcal isolates in Taiwan.

From June 2006 to July 2009, a total of 273 patients, including 254 males and 19 females, were diagnosed with gonorrhea at the Taipei City Hospital, KunMing Branch, in Taiwan. A total of 92.7% (254/274) N. gonorrhoeae isolates were collected from male patients (median age 31, range 17–79); the remaining 20 isolates were from female patients (median age 24, range 14–34). Due to the low percentage of female patients in this population, this study mainly analyzes the male subset of the population with gonorrhea.

The sequencing of the PBP2 gene was conducted to learn the distribution of mosaic penA (MA) genotypes among N. gonorrhoeae clinical isolates in Taiwan. Two distinct MA types were
identified and categorized into MA1 (68 isolates) and MA2 (one isolate) type. The translated amino acid sequence of MA1 gene is identical to the MA allele (GenBank accession no. AB071984) previously identified as mosaic pattern X and associated with reduced susceptibility to cephalosporins (7). The amino acid sequence of MA2 gene is identical to the penA gene uploaded into the NCBI database by Corkill et al. (GenBank accession no. DQ335216), and N. gonorrhoeae isolates with MA2 gene were recently discovered in San Francisco, California (10).

The susceptibility of the 254 isolates to cefixime and ceftriaxone were evaluated by E-test with breakpoints defined as recommended by the Clinical and Laboratory Standards Institute (CLSI) (11). The MICs for cefixime ranged from 0.016 to 0.5 mg/liter, with 5, 26, 21, 15 and 4 isolates having MICs of 0.125, 0.19, 0.25, 0.38 and 0.5 mg/liter, respectively. The distribution of cefixime MICs for the 185 isolates with non-mosaic penA (NM) gene ranged from 0.016 to 0.094 mg/liter except for 2 isolates with MICs of 0.125 mg/liter, respectively. In contrast, the cefixime MICs for the 69 isolates with MA gene ranged from 0.125 to 0.5 mg/liter and constituted 97.2% (69/71) of all isolates having MICs of 0.125 to 0.5 mg/liter. The detection of MA alleles in N. gonorrhoeae clinical isolates could be an effective alternative method for the surveillance of isolates with reduced cefixime susceptibility.

The MICs for ceftriaxone ranged from 0.002 to 0.19 mg/liter, with 14 isolates having MICs of 0.125 mg/liter and one isolate having an MIC of 0.19 mg/liter. The distribution of ceftriaxone MICs for the isolates with NM penA gene ranged from 0.002 to 0.094 mg/liter except for 4 isolates...
with MICs of 0.125 mg/liter. In contrast, the ceftriaxone MICs for the isolates with MA gene ranged from 0.023 to 0.19 mg/liter, with 10 isolates and one isolate having MICs of 0.125 and 0.19 mg/liter, respectively. The presence of mosaic PBP2 seemed to have only partial effect on the reduced ceftriaxone susceptibility. The report of Zhao et al also showed that the mosaic penA gene was one of several resistance determinants that resulted in the reduced susceptibility to ceftriaxone in *N. gonorrhoeae* (17). Ceftriaxone is still highly used for the treatment of gonorrhea in Taiwan. According to the medical records, clinical treatments in this study were successful. However, continuous monitoring of the MIC for ceftriaxone is still critical to detect the emergence of ceftriaxone-resistant gonococcal isolates, which may erode the last resort of gonococcal therapy.

The overall resistance rate to penicillin and ciprofloxacin was found to be 62.2% (158/254) and 88.6% (225/254) by disk susceptibility tests. The rate of reduced susceptibility of clinical isolates with MA gene to penicillin and ciprofloxacin was higher than that of isolates with NM gene (71.0% vs. 58.9% and 100% vs. 84.3%, respectively) (Table 1). Therefore, the isolates with MA gene not only exhibited reduced susceptibilities to cephalosporins but are also more likely to have multiple drug resistance.

Analysis of demographic features of each patient showed that, among the 69 isolates with MA gene, 56 (81.2%, *P*<0.001) were from MSM/bisexual and 32 (46.4%, *P*<0.001) and 15 (21.7%) were coinfectected with HIV and syphilis, respectively (Table 1). By contrast, the isolates with NM gene were mostly from heterosexual men (67.0%, 124/185) and the proportion of concurrent HIV
(20/185, 10.8%) and syphilis (18/185, 9.7%) infections was lower than isolates with MA gene.

Similar findings involving clinical isolates with reduced cefotaxime susceptibility mainly obtained from MSM patients was also reported in the Netherlands (3). The detection of mosaic penA gene in N. gonorrhoeae may be helpful to identify core groups for transmission of MDR gonococcus and at high risk of concomitantly contracting HIV and syphilis.

The 69 isolates with MA gene were divided by NG-MAST into 29 sequence types (STs). The three major STs were ST2180 (22.9%, 16/70), ST835 (17.1%, 12/70) and ST2253 (15.7%, 11/70), which constituted 55.7% of these isolates. It was discovered that the penA allele of 28 STs was MA1 type, and only one isolate belonging to ST1407 was of MA2 type. The major ST clusters may represent large sexual networks with clonal transmission of specific genotypes. The clonal relationship of isolates of ST835, ST2253 and ST2180 constructed by PFGE correlated well with NG-MAST and supported the high similarity of strains within each major clonal cluster from a common ancestor (data not shown). ST835 with reduced susceptibility to oral cephalosporin and resistance to azithromycin, has been discovered in Australia and Hong Kong, and in Italy, respectively (8, 11, 13) and ST2180 is closely related to ST835. ST1407 harboring the mosaic penA allele MA2 was one of the major STs in Australia and was first discovered in Taiwan (13).

N. gonorrhoeae clinical isolates harboring mosaic penA gene were first identified in 2001 by Ito et al (7). We deduce from a previous report on resistant N. gonorrhoeae strains in Taiwan that this kind of clinical isolate might have emerged in or been introduced to Taiwan from 1999-2003.
Temporal analysis of the strain replacement dynamic of *N. gonorrhoeae* with mosaic *penA* from April 2006 to July 2009 showed that strains of ST835 and ST2180 were predominant in 2006 and were gradually replaced by other STs (ST2253 and ST3082) during 2007-2009 (data not shown). Selection pressure exerted by antibiotic usage or host immunity might have contributed to such a transition. One recent report indicates that the horizontal transfer of the mosaic *penA* from a cefixime reduced susceptibility strain to a cefixime-susceptible strain was proved by an *in vitro* test (9). It might explain why the major *penA* allele, MA1 type, was widely discovered in different STs in this study besides those closely related major clones. Continuous molecular epidemiology may shed more light on the emergence and dissemination of clinical isolates with higher MICs for cephalosporin antibiotics in Taiwan.

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Table 1. Characteristics of male patients infected with isolates that categorized to two PBP2 genotypes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MA type</th>
<th>NM type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>69</td>
<td>185</td>
</tr>
<tr>
<td>Sexual orientation of male patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>13 (18.8)</td>
<td>124 (67.0)</td>
</tr>
<tr>
<td>MSM/bisexual(^b)</td>
<td>56 (81.2)</td>
<td>57 (30.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>4 (2.2)</td>
</tr>
<tr>
<td>Concurrent STI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV(^c)</td>
<td>32 (46.4)</td>
<td>20 (10.8)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>15 (21.7)</td>
<td>18 (9.7)</td>
</tr>
<tr>
<td>Syphilis + HIV</td>
<td>12 (17.4)</td>
<td>10 (5.4)</td>
</tr>
<tr>
<td>Resistance to antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>49 (71.0)</td>
<td>109 (58.9)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>69 (100)</td>
<td>156 (84.3)</td>
</tr>
</tbody>
</table>

\(^a\) MA and NM represent the mosaic *penA* and non-mosaic *penA*, respectively.

\(^b\) There were significant differences among PBP2 genotypes in terms of sexual orientation \((P<0.001)\).

\(^c\) There were significant differences among PBP2 genotypes in patients coinfected with HIV \((P<0.001)\).


