Surveillance of gonococcal antimicrobial susceptibility resulting in early detection of emerging resistance

Janice Yee Chi Lo1*, King Man Ho2 and Angus Chun Tim Lo1

1Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region, China; 2Social Hygiene Service, Public Health Services Branch, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region, China

*Corresponding author. Tel: +852-2319-8254; Fax: +852-2776-5758; E-mail: janicelo@dh.gov.hk

Received 10 December 2011; returned 12 January 2012; revised 19 January 2012; accepted 23 January 2012

Objectives: To undertake laboratory and clinical surveillance of gonococcal antimicrobial susceptibility to various therapeutic agents in Hong Kong, so as to monitor for emerging resistance and to inform on appropriate choice of empirical therapy.

Methods: Trends in susceptibility of gonococci to ceftriaxone, spectinomycin, ceftibuten and azithromycin were monitored over time. Isolates with reduced susceptibility to oral extended-spectrum cephalosporins were further characterized by detection of the mosaic penA gene and typing by Neisseria gonorrhoeae multi-antigen sequence typing (NG-MAST). Correlation with clinical and epidemiological findings was undertaken on isolates positive for the mosaic penA gene.

Results: Trends in susceptibility of gonococci to ceftriaxone, spectinomycin and ceftibuten remained stable between 2005 and 2010. In 2010, 30.3% of tested strains were not susceptible to azithromycin. The percentage of gonococcal strains harbouring the mosaic penA gene increased from 1.0% during April to December 2010 to 8.2% during January to September 2011 (P<0.0001). Review of available clinical records showed that, out of 35 patients infected by strains positive for the mosaic penA gene, 30 had laboratory-documented treatment failure.

Conclusions: This study showed that ceftriaxone and spectinomycin remained effective against gonorrhoea in Hong Kong. There was an alarming increase in strains with reduced susceptibility to oral extended-spectrum cephalosporins associated with clinical treatment failure. One-third of gonococcal isolates were non-susceptible to azithromycin. The need to switch to agents other than oral extended-spectrum cephalosporins for empirical treatment is imminent. Continued surveillance with strain characterization is essential to monitor the effectiveness of currently recommended therapy.

Keywords: Neisseria gonorrhoeae, cephalosporins, treatment failure, surveillance

Introduction

Neisseria gonorrhoeae remains an important agent of sexually transmitted infections worldwide. Effective empirical treatment is essential to prevent sequelae and further transmission. However, as this organism has demonstrated the propensity to develop antimicrobial resistance under selective pressure, continued surveillance is essential to ensure the sustained effectiveness of empirical antimicrobial agents. Clinical treatment failure and resistance of N. gonorrhoeae to extended-spectrum cephalosporins, the agents most commonly recommended globally for empirical treatment, have already been reported. The WHO has warned that gonorrhoea might eventually become untreatable. The first strain of N. gonorrhoeae with high-level resistance to ceftriaxone has recently been characterized. In order to control this infection, the WHO has developed recommendations for comprehensive surveillance.

The origin of global emergence of gonococcal antimicrobial resistance has mostly been mapped to the Western Pacific Region, where Hong Kong is located. In Hong Kong, since abandoning penicillin and ofloxacin for empirical treatment for gonorrhoea in 1985 and 1997, respectively, due to emergence of resistance, oral cephalosporins have been in use, with a low incidence of treatment failure. The current study describes the surveillance of gonococcal antimicrobial susceptibility in Hong Kong for early detection of emerging resistance, and to inform on the appropriate choice of empirical therapy.
Methods

Clinical isolates of *N. gonorrhoeae*

The public health laboratory in Hong Kong provides microbiological diagnostic services to public and private sectors, and also provides laboratory support to all government sexually transmitted disease (STD) clinics in different geographical regions in Hong Kong. It has also been an active member of the WHO Western Pacific Region Gonococcal Antimicrobial Surveillance Programme (WPR GASP) for many years.15

Culture for *N. gonorrhoeae* in patients attending STD clinics was performed by near-patient inoculation of male urethral or female endocervical specimens onto modified Thayer–Martin medium (MTM) as previously described.5,16 Identification of *N. gonorrhoeae* isolates was undertaken by conventional methods.17 Isolates of *N. gonorrhoeae* were preserved in a commercial bead system at −70°C. Use of the isolates for the current study formed an integral part of the surveillance of antimicrobial resistance of *N. gonorrhoeae* in Hong Kong.

Antimicrobial susceptibility testing

Penicillin, ciprofloxacin and tetracycline susceptibility testing was performed using the disc diffusion test in accordance with CLSI guidelines.18 The disc diffusion test for ceftibuten was performed according to a previously published protocol.9 Prior to September 2006, MICs of spectinomycin and ceftriaxone were determined using the agar dilution method according to the manufacturer’s instructions for Adatab (Mast Diagnostics, Bootle, UK). From September 2006 onwards, MICs of spectinomycin and ceftriaxone were determined using the Etest (bioMérieux, Durham, USA) according to the manufacturer’s instructions. Cefixime and azithromycin MIC determination using the Etest was initiated in August 2009 and June 2010, respectively. Antimicrobial susceptibility results for spectinomycin, ceftriaxone and cefixime were interpreted according to CLSI guidelines,18 and for azithromycin according to EUCAST guidelines.19 Cefixime and azithromycin susceptibility results were interpreted as previously published.9 Quality control for antimicrobial susceptibility testing was in accordance with CLSI guidelines together with use of WHO control strains.20 The laboratory also participated fully in the WHO WPR External Quality Assessment Programme during the entire study period.

Genetic characterization

Detection of the presence of the mosaic penA gene was by means of PCR and was followed by nucleotide sequencing of the gene.21 Phylogenetic analysis of penA gene sequences was undertaken using the program Lasergene 9.0 (DNASTAR Inc., Madison, USA). Gonococcal strains were defined as being positive for the mosaic penA gene by the presence of the amino acid substitutions I312M, V316T and G545S.21 The amino acid sequence patterns of penicillin-binding protein (PBP) 2 were classified according to published references.22,23 Molecular typing of *N. gonorrhoeae* strains was performed on all isolates harbouring the mosaic penA gene using *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST).24 All sequencing reactions were undertaken with both forward and reverse primers using an automatic sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA).

Clinical management of patients with gonorrhoea

Apart from clinical history, epidemiological information including sexual exposure history was collected from patients attending government STD clinics. The standard empirical treatment for patients with on-site positive microscopy for genital *N. gonorrhoeae* infection, as indicated by the presence of intracellular Gram-negative diplococci, was a single 400 mg oral dose of ceftributen under direct supervision, and a request to attend follow-up after 1 week. On return of the patient, a second specimen was obtained for on-site microscopy and for *N. gonorrhoeae* test of cure (TOC) culture. Returning patients with on-site positive microscopy suggestive of *N. gonorrhoeae* infection and putative ceftributen treatment failure were given a single-dose intramuscular injection of either 250 mg of ceftrixone or 2 g of spectinomycin, while a repeat culture for *N. gonorrhoeae* was undertaken.

Statistical analysis

The χ2 test was used for statistical analysis, and P<0.05 was considered statistically significant.

Results

Surveillance of antimicrobial susceptibility of gonococcal isolates

The susceptibility of gonococci to ceftriaxone, spectinomycin and ceftributen between 2005 and 2010 is shown in Tables 1 and 2. Azithromycin MIC results were available from June to December 2010, and out of 485 strains tested the proportions susceptible

Table 1. Trends in *N. gonorrhoeae* ceftriaxone and spectinomycin MICs from 2005 to 2010

<table>
<thead>
<tr>
<th>Antimicrobial susceptibility result, MIC (category according to CLSI)</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ceftriaxone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.03 mg/L (susceptible)</td>
<td>1317 (77.7)</td>
<td>1142 (78.3)</td>
<td>1009 (72.9)</td>
<td>1100 (82.0)</td>
<td>903 (69.6)</td>
<td>726 (76.7)</td>
</tr>
<tr>
<td>0.06–0.125 mg/L (susceptible)</td>
<td>377 (22.2)</td>
<td>315 (21.6)</td>
<td>375 (27.1)</td>
<td>242 (18.0)</td>
<td>392 (30.2)</td>
<td>221 (23.3)</td>
</tr>
<tr>
<td>0.25 mg/L (susceptible)</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (0.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Spectinomycin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤16 mg/L (susceptible)</td>
<td>1670 (98.5)</td>
<td>1434 (98.4)</td>
<td>1383 (99.9)</td>
<td>1342 (100)</td>
<td>1296 (99.9)</td>
<td>944 (99.7)</td>
</tr>
<tr>
<td>32 mg/L (susceptible)</td>
<td>22 (1.3)</td>
<td>24 (1.6)</td>
<td>1 (0.1)</td>
<td>0 (0)</td>
<td>1 (0.1)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>64 mg/L (intermediate)</td>
<td>3 (0.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Number of strains tested</strong></td>
<td>1695</td>
<td>1458</td>
<td>1384</td>
<td>1342</td>
<td>1297</td>
<td>947</td>
</tr>
</tbody>
</table>

Downloaded from https://academic.oup.com/jac/article-abstract/67/6/1422/687841 by guest on 06 Apr 2019
Surveillance of gonococcal isolates with reduced susceptibility to oral extended-spectrum cephalosporins

To enhance detection of gonococcal isolates with reduced susceptibility to oral extended-spectrum cephalosporins, laboratory criteria were adopted as follows: non-susceptibility to ceftibuten using the 30 μg disc diffusion test (zone diameter <30 mm) or exhibition of cefixime MIC >0.064 mg/L. All such isolates were analysed for the presence of the mosaic penA gene and the number of positive isolates between April 2010 and September 2011 is shown in Table 3. Among a total of 78 strains harbouring the mosaic penA gene, 29 pairs were pre- and post-ceftibuten treatment isolates from the same patients. For the remaining 20 isolates, one pre-treatment isolate was recovered from a patient whose post-ceftibuten TOC specimen was positive for Gram-negative diplococci during on-site microscopy, but was culture negative. Five patients given standard empirical oral ceftibuten had TOC specimens after 1 week yielding negative culture results for N. gonorrhoeae. Nine patients did not return for follow-up. Four patients received 250 mg of intramuscular ceftriaxone during the first clinic visit due to severe symptoms on presentation. One patient received a 1 week course of oral doxycycline as empirical treatment for chlamydia based on on-site positive microscopy for pus cells without Gram-negative diplococci, and was clinically asymptomatic during follow-up, with the TOC specimen negative for N. gonorrhoeae by culture.

Characteristics of gonococcal isolates harbouring the mosaic penA gene

The PBP 2 amino acid sequence patterns and NG-MAST types of gonococcal isolates harbouring the mosaic penA gene are shown in Table 3 by month of specimen reception by the laboratory. Out of the 78 isolates, for the 29 patients with pre- and post-ceftibuten treatment isolates, as the pairs of isolates were identical, only pre-treatment isolates were included in Table 3. Information on a total of 49 isolates is thus presented. Regarding antimicrobial susceptibility, penicillin-intermediate and -resistant results were detected in 14 and 35 strains, respectively, with one strain among the latter demonstrating β-lactamase activity. All strains were resistant to ciprofloxacin, while 39 were intermediate and 10 resistant to tetracycline. All strains were susceptible to spectinomycin, while 41 were susceptible and 8 intermediate to azithromycin. MIC values of ceftriaxone were 0.016 mg/L (2 strains), 0.032 mg/L (12 strains), 0.064 mg/L (26 strains) and 0.125 mg/L (9 strains). The disc diffusion test for ceftibuten yielded zone diameters of 20–31 mm, while cefixime MIC values ranged from 0.125 to 0.5 mg/L.
Emerging gonococcal antimicrobial resistance

Discussion

*N. gonorrhoeae* has repeatedly demonstrated propensity for developing resistance to recommended empirical antimicrobial agents, such that surveillance of its antimicrobial susceptibility pattern and continued vigilance for new potential therapeutic options are essential. The WHO has accorded a high priority to addressing this problem.10,25 Findings from the current study showed that the MIC levels of gonococci in Hong Kong for ceftriaxone have been relatively stable from 2005 to 2010. For azithromycin, which has been considered as a potential alternative agent for treatment of gonorrhoea, the non-susceptibility rate was 30% in 2010, making this agent unsuitable for consideration as empirical therapy in our setting.

In Hong Kong, the treatment failure rate of a single 400 mg dose of oral ceftibuten has remained consistently low since its use as the first-line treatment for gonorrhoea from 1997,14 with a figure of 3.4% (42/1228) from October 2006 to August 2007.9 In the current study, there was a drastic increase in the rate of gonococcal strains positive for the mosaic *penA* gene, from 1.0% (7/677) during April to December 2010 to 8.2% (71/861) in the first 9 months of 2011 (*P*<0.0001). There was no apparent geographical clustering or epidemiological linkage among the patients harbouring such isolates. A number of different NG-MAST types were represented, as shown in Table 3. Among 35 patients who received oral ceftibuten treatment and returned for follow-up with TOC specimens, only five were documented to have cleared the infection. The alarming increase in the rate of isolates harbouring the mosaic *penA* gene and the strong association of such strains with ceftibuten treatment failure has prompted our STD clinics to review the empirical treatment strategy, with consideration of using intramuscular ceftriaxone as the first-line empirical treatment.

This study demonstrated the practical utility of the ceftibuten disc diffusion test in combination with ceftriaxone MIC testing for the detection of isolates harbouring the mosaic *penA* gene. This strategy proved to be more clinically relevant than using ceftriaxone mapping data. In 2010, out of a total of 947 gonococcal isolates in Hong Kong, 429 (45.3%) had ceftriaxone MIC values of ≤0.03 mg/L, 297 (31.4%) of 0.03 mg/L, 201 (21.2%) of 0.06 mg/L and 20 (2.1%) of 0.125 mg/L. Among the 221 strains (23.3%) with ceftriaxone MIC values >0.06 mg/L, considered as a threshold for ‘decreased susceptibility’ to extended-spectrum cephalosporins for surveillance purpose,20 only 10 were shown to harbour the mosaic *penA* gene, which has been shown to be the major genetic determinant conferring reduced susceptibility to oral extended-spectrum cephalosporins.26 Nevertheless, ceftriaxone MIC data would continue to be valuable to detect strains with reduced susceptibility to injectable extended-spectrum cephalosporins, both for patient management and for public health surveillance.11

The utility of oral extended-spectrum cephalosporins as empirical treatment for *N. gonorrhoeae* in Hong Kong has apparently come to a close. Although spectinomycin is demonstrating effectiveness according to in vitro testing, its widespread use has been associated with rapid development of clinical resistance.55 Azithromycin, with a non-susceptibility rate of 30%, would not be an appropriate option. Ceftriaxone is apparently the most appropriate agent according to findings in the current study, despite the disadvantages of injection compared with oral therapy. Nevertheless, documentation of the first resistant strain underlined the necessity for continued surveillance.11 Other alternative agents also had to be considered, such as carbapenems and aminoglycosides, for which antimicrobial susceptibility data should be collected for their evaluation as potential alternative treatment options under the looming threat of untreatable gonococcal infections.25

Acknowledgements

We would like to acknowledge the technical staff of the Microbiology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region, for their expert technical support. We also wish to dedicate this work to the late Dr John Tapsall, for many years a driving force behind global gonococcal surveillance efforts, and Director of the WHO Collaborating Centre for STD, Microbiology Department, South Eastern Area Laboratory Services, Prince of Wales Hospital, Sydney, New South Wales, Australia.

Funding

The work was supported by the Department of Health, Hong Kong Special Administrative Region, China.

Transparency declarations

None to declare.

References


17 WHO. Laboratory Diagnosis of Gonorrhoea. WHO Regional Publications, South-East Asia Series, No. 33. Regional Office for South-East Asia, 1999.


