Determining the in vitro susceptibility of *Neisseria gonorrhoeae* isolates from 8 cities in Guangdong Province through an improved microdilution method

Xingzhong Wu a,b,c,1, Xiaolin Qin a,b,c,1, Jinmei Huang a,b,c, Feng Wang d, Ming Li e, Zhizhou Wu f, Xiaofeng Liu g, Junming Pei h, Shanghua Wu i, Heyong Chen j, Chixing Guo k, Yaohua Xue a,b,c, Sanmei Tang a,b,c, Mingheng Fang a,b,c, Yinyuan Lan a,b,c, Jiangli Ou a,b,c, Zhenmou Xie a,b,c, Yuqi Yu a,b,c, Jieyi Yang a,b,c, Wentao Chen a,b,c, Yunhu Zhao a,b,c, Heping Zheng a

a Dermatology Hospital, Southern Medical University, Guangzhou, Guangdong 510091, China
b Guangdong Provincial Dermatology Hospital, Guangzhou, Guangdong 510091, China
c Guangdong Provincial Center for Skin Diseases and STD Control, Guangzhou, Guangdong 510091, China
d Shenzhen Center for Chronic Diseases Control, Shenzhen, Guangdong 518020, China
e The fifth People’s Hospital of Dongguan, Dongguan, Guangdong 523903, China
f Guangdong Provincial People’s Hospital, Guangzhou, Guangdong 510091, China
g Zhuhai Center for Chronic Diseases Control, Zhuhai, Guangdong 519099, China
h Shantou Dermatology Hospital, Shantou, Guangdong 515041, China
i Shazhong Dermatology Hospital, Jiangmen, Guangdong 512026, China
j Shaoguan Center for Chronic Diseases Control, Shaoguan, Guangdong 512026, China
k Maoming Center for Chronic Diseases Control, Maoming, Guangdong 525099, China
l Panyu Center for Chronic Diseases Control, Guangzhou, Guangdong 511400, China

**ABSTRACT**

A microdilution method for the antibiotic susceptibility testing of *Neisseria gonorrhoeae* was established and improved, and the antibiotic resistance of *N. gonorrhoeae* samples isolated from 8 cities of Guangdong in 2016 was determined. The improved microdilution method was compared with the agar dilution method recommended by the World Health Organization (WHO) Western Pacific Region by testing the susceptibility of 100 clinical *N. gonorrhoeae* isolates. The essential agreement (EA), categorical agreement (CA), very major error (VME), major error (ME), and minor error (MIE) levels of the two methods were analyzed; the acceptable performance rates were measured as follows: ≥90% for EA or CA, ≤3% for VME or ME, and ≤7% for MIE. The EA, CA, VME, ME, and MIE of each method for 7 antibiotics, penicillin, tetracycline, ciprofloxacin, spectinomycin, ceftriaxone, cefixime, and azithromycin, were 96%–100%, 95%–100%, 0–3%, 0–2%, and 0–6%, respectively. The Wilcoxon signed-rank test results indicated 94%–100% agreement between the 2 methods after excluding off-scale values (P > 0.05). The susceptibility of 634 *N. gonorrhoeae* strains to the 7 antibiotics above were tested through the microdilution method. The resistant rates of the isolates against ciprofloxacin, tetracycline, ceftriaxone, spectinomycin, and azithromycin were 99.8%, 88.3%, 53.8%, and 11%, and the percentages of the isolates with decreased susceptibility to ceftriaxone (minimum inhibitory concentration [MIC] ≥0.125 μg/mL) and cefixime (MIC ≥0.25 μg/mL) were 8,8.3%, 53.8%, and 11%, respectively. The findings from this study indicated that the improved microdilution method is an alternative for testing the antimicrobial susceptibility of *N. gonorrhoeae*. The resistance rates of *N. gonorrhoeae* against penicillin, tetracycline, and ciprofloxacin were high. While ceftriaxone, cefixime, and spectinomycin remained effective against *N. gonorrhoeae*, their effectiveness seemed to be decreasing over time. Azithromycin therapy requires timely susceptibility test results.

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1. Introduction

Gonorrhea caused by *Neisseria gonorrhoeae* is one of the most common sexually transmitted diseases (STDs) worldwide. Improper treatment can...
lead to infertility, ectopic pregnancy, and other adverse consequences. Global STD epidemiological studies showed that the incidence of gonorrhea declined from the 1980s to early 1990s but resurged with an upward trend since the late 1990s. In 2012, approximately 78 million gonorrhea cases were estimated worldwide (Newman et al., 2015). In China, 11,470 cases of gonorrhea were reported in 2016, exceeding the number of cases reported in 2015 by 20.8% (NCAIDS et al., 2017). In 2014, 157,410,000 of the population were reported to have gonorrhea in Guangdong, China (Chen et al., 2016).

The antibiotic resistance of N. gonorrhoeae is a serious public health issue. Thus, the WHO established the Gonorrhoea Antimicrobial Surveillance Programme (GASP) in 1990. The United States and the European CDC established the Gonococcal Isolate Surveillance Project (GISP) in 1986 and the Euro-GASP in 2000. The WHO presented the Global Action Plan for the Control of Antibiotic Resistance in N. gonorrhoeae in 2012 and the Global Action Plan 2015 to call for global antimicrobial resistance monitoring action. The WHO N. gonorrhoeae antibiotic resistance monitoring program involves the use of agar dilution method for the determination of minimum inhibitory concentration (MIC). However, compared with other methods, the agar dilution method is more cumbersome, time consuming, and laborious. It also requires a heavy workload, is incapable of detecting resistant isolates in real time, and requires storing the isolates in —70 °C temperature or liquid nitrogen for batch tests. The Etest method was developed in 1988 by AB Biodisk (Sweden) to directly determine the MICs of clinical isolates (Sanchez et al., 1992) and showed as an alternative to agar dilution testing to determine the susceptibility of N. gonorrhoeae to ceftriaxone, cefixime, and cefpodoxime (Enriquez et al., 2016; Liu et al., 2014; Shende et al., 2016). However, other studies had found that the consistency rates among the results of Kirby–Bauer (KB), Etest, and agar dilution methods for N. gonorrhoeae susceptibility testing were only 72.2%–73.3% for penicillin and 37.5% for ceftriaxone, ciprofloxacin, and tetracycline (Dai and Yin, 2010; Peng Bihua et al., 2011; Pan et al., 1998). Moreover, the consistency rate between the results of the Etest and the agar method for spectinomycin was 65%, indicating a significant difference between the 2 methods (Cao Wenling et al., 2000). The microbroth dilution method is a convenient and rapid MIC test routinely used for bacteria except for fastidious N. gonorrhoeae. In 1984, Shapiro et al. compared a microdilution method by using IsoCite X as an enrichment material for testing the antibiotic susceptibility of N. gonorrhoeae against the agar dilution method. They found no significant difference between the 2 methods, but most of the strains tested through the microdilution method had lower MICs for ampicillin and penicillin than those tested through agar dilution (Shapiro et al., 1984). Geers et al. modified their approach by using supplement C as enrichment, resulting in 94.3% MIC agreement for penicillin, 93.5% for spectinomycin, and 98.4% for ceftriaxone (Geers and Donabedian, 1989). However, the resistance compared above lacked consistency. The Guangdong Provincial Centre for Skin Diseases and STIs Control had been participating N. gonorrhoeae resistance surveillance as one of the laboratories of the WHO Western Pacific Regional (WPR) Resistance Surveillance Program since 1996. To strengthen the surveillance in Guangdong Province, we have established a network including 8 cities’ sentinels in 2008. Our early surveillance results by agar dilution method indicated high resistant rates of penicillin, tetracycline, and ciprofloxacin among different regions. Moreover, new features of penicillinase-producing N. gonorrhoeae African-type plasmid epidemic strains were detected (Zheng et al., 2003; Zheng et al., 2014; Zheng et al., 2015). To facilitate N. gonorrhoeae resistance surveillance in the network, the microdilution method was improved and used for the detection of the antibiotic susceptibility of 634 N. gonorrhoeae strains isolated from the 8 cities of Guangdong Province in 2016.

2. Materials and methods

2.1. N. gonorrhoeae strains

N. gonorrhoeae isolates were collected from the monitoring laboratories located in the Pearl River Delta region (Guangzhou, Shenzhen, Dongguan, Zhuhai, and Jiangmen) and the eastern (Shantou), northern (Shaoquan), and western (Maoming) regions of Guangdong. A total of 634 N. gonorrhoeae strains were isolated from the STD clinic patients of the 8 cities from January 1 to December 31, 2016. The specimens were collected by inserting a 2–3-cm swab into the male urethra or a 2–cm swab into female cervix and gently scraping the mucosa by rotating the swab for 5–10 s. Conococci were isolated in selective Thayer–Martin agar medium and confirmed by Gram staining, oxidase and catalase assays, and sugar fermentation test. All stains were preserved in skimmed milk and stored at —70 °C before they were sent to the Guangdong Provincial Centre for Skin Diseases and STIs Control for susceptibility testing.

Six different reference strains were used as quality controls. The WHO N. gonorrhoeae reference strains, namely, D. G. J. L. K. P. and A, were provided by Dr. Yueping Yin from the National Center for STD Control, Nanjing, China.

2.2. Preparation of liquid medium for N. gonorrhoeae

Beef extract (10%), fetal bovine serum (5%), peptone (1.5%), and agar (0.1%) were added to a basal broth (35 g/L). The mixture was autoclaved at 121 °C for 15 min. After cooling, 1% gonococcal growth additive (British, Thermo Fisher Scientific, lot number 1865022) was added, and 10 mL of the medium was added to each bottle.

2.3. Preparation of antibiotic microplate

Penicillin, tetracycline, ciprofloxacin, azithromycin, and ceftriaxone were provided by the National Institutes for Food and Drug Control, while spectinomycin and cefixime were supplied by the Dalian Meilun Biotechnology Co., Ltd.

The working solutions of the seven antibiotics were added to the wells in column 1 of each row, and an identical 2-fold serial dilution was made from column 2 to column 12 of the sterile 96-well ELISA microplate. The final concentration range was 0.015–32 μg/mL for penicillin and ciprofloxacin, 0.002–1 μg/mL for ceftriaxone, 0.008–1 μg/mL for cefixime, 4–128 μg/mL for spectinomycin, 0.25–32 μg/mL for tetracycline, and 0.03–8 μg/mL for azithromycin. H1 and H2 were used as growth controls; H3 and H4 were used as reagent blank controls. The antibiotic microplates were freeze-dried and vacuum packaged, then stored at —20 °C for 6 months.

2.4. Preparation of antibiotic agar plate

The antibiotic agar plate was prepared as recommended by the WHO Western Pacific N. gonorrhoeae Resistance Surveillance Program Collaborative Group (WHO, 1992). The medium used was GC agar (British, Thermo Fisher Scientific) supplemented with 10% defibrinated sheep blood. The final concentrations of the seven antibiotics were the same as those used in the microdilution method described above.
2.5. Antibiotic susceptibility testing

_N. gonorrhoeae_ clinical and reference strain suspensions were adjusted to 0.5 McFarland. Turbidity was measured with a turbidimeter. For the microdilution method, bacterial suspension (150 μL) was added to _N. gonorrhoeae_ liquid medium. Exactly 100 μL of the mixture was distributed into each well of the 96-well microplate containing antibiological agents. The H1 and H2 wells without antibiotics served as growth controls, whereas H3 and H4, each containing 100 μL of nutrient broth, served as reagent blank controls. The plate was incubated at 35 °C at 5% CO2 for 24 h. Absorbance (i.e., optical density [OD]) was read with an ELISA reader (Thermo-Scientific) at the wavelength of 525 nm. According to the principle of EP12-A2 “Qualitative Test Evaluation Method User Agreement: Proposal Guide” (CLSI/NCCLS, 2010), the OD average value in H3 and H4 was recorded as the reagent blank controls. The cutoff value was determined by using the formula H3OD + H4OD. AS/CO value of <1 was set as no growth but confirmed by eye reading. The MIC of each antibiotic was recorded. Quality control with the reference strains was included in each run.

Using the agar dilution method as a reference was recommended by the WHO-WPR Resistance Surveillance Programme (WHO, 1992). The bacterial suspension at 0.5 McFarland was inoculated through multipoint inoculator (UK) in each antibiotic agar plate and incubated at 35 °C at 5% CO2 for 24 h. The MIC is the smallest amount of each antibiotic that limits visible microbial growth on the plate read by eye according to the criteria of WHO. Quality control with the reference strains was included in each run. Before reading the MIC, we need to confirm that the MIC of the positive quality control is within the quality control range. There is no visible microbial growth on the negative plate.

Antimicrobial susceptibilities were interpreted according to the criteria provided by the WHO WPR Resistance Surveillance Programme guidelines (WHO, 1992). For penicillin and ciprofloxacin, isolates with MICs of ≤0.10 mg/L were classified as resistant, those with MICs of 0.06–0.50 mg/L as intermediate susceptibility, and those with MICs of 0.03 mg/L as susceptible. Isolates with MICs of ≥0.5 mg/L to tetracycline were classified as susceptible and those with MICs of ≥1.0 mg/L as resistant. Isolates with MICs of 0.06 mg/L to spectinomycin were classified as susceptible and isolates with MICs of ≥0.128 mg/L as resistant. Isolates with MICs of ≥0.125 mg/L to ceftriaxone were classified as decreased susceptibility and isolates with MICs of ≤0.06 mg/L as susceptible. Isolates with MICs of ≥0.25 mg/L to cefixime were classified as decreased susceptibility and isolates with MICs of ≥0.125 mg/L as susceptible (WHO, 2012). Isolates with MICs of ≥1.0 mg/L to azithromycin were classified as resistant and isolates with MICs of ≤0.5 mg/L as susceptible (Lahty and Enriquez, 2015).

2.6. Analysis of consistency between 2 methods

The consistency between two methods was conducted using 100 isolates and 6 reference strains. The MIC of each antibiotic was read by the double-blind method for the microdilution and agar methods. The performance of the microdilution method was determined by using the essential agreement (EA), categorical agreement (CA), very major error (VME), major error (ME), and minor error (MIE), which were compared with those of the agar method. EA refers to the MIC result of the microdilution method which is within the ±1 doubling dilution of the MIC result of the reference method. CA is the agreement of intermediate and resistant results between a breakpoint test or an MIC test and the reference method. VME indicates the test result by the reference method interpreted as resistant and the microdilution method device result of susceptible. ME indicates the test result by the reference method interpreted as susceptible and the microdilution method device result of resistant. MIE indicates the test result by the reference method interpreted as resistant or susceptible and the microdilution method device result of intermediate, or the reference result interpreted as intermediate and the microdilution method device result of resistant or susceptible (ISO 20776–2, 2007). Acceptable performance rates were measured as follows: ≥80% for EA or CA, ≤3% for VME or ME, and ≤7% for MIE (Clark et al., 2009). We performed the Wilcoxon signed-rank test to determine trends regarding the discrepancies between the MICs obtained by the agar reference method and those obtained by the microdilution method. A P value of ≤0.05 is defined as a statistically significant association.

2.7. Statistical analysis

Statistical analysis was performed on SPSS 20.0 software. MICs difference between 2 methods was determined by the Wilcoxon signed-rank test and percentage comparison by the chi square (χ²).

3. Results

3.1. Establishment of microdilution method for testing _N. gonorrhoeae_ antibiotic susceptibility

_N. gonorrhoeae_ was inoculated in the liquid medium and distributed into each well of the ELISA plate. After incubation at 35 °C under 5% CO2 for 24 h, _N. gonorrhoeae_ grew well both in the 10-mL bottle and wells of the plate. The MIC breakpoints were clear and easy to interpret (Fig. 1).

3.2. Comparison between the microdilution and agar dilution methods

The consistency rate between the two methods was determined by using 100 isolates and 6 reference strains. The MICs of the 7 antibiotics against the reference strains were determined through the 2 methods. All the values obtained were within the reference ranges, and no more than ±1 of the dilution difference was observed (Table 1). The results of the microdilution method were consistent with those of the agar dilution method (Table 2). The EA, CA, ME, VME, and MIE were 96%–100%, 94%–100%, 0%–3%, 0%–2%, and 0%–6%, respectively. The EA between the 2 methods was 96%–100%, indicating good consistency (P > 0.05) by the Wilcoxon signed-rank test.

3.3. Susceptibility of _N. gonorrhoeae _isolates from 8 cities in Guangdong Province

The MICs of the 7 antibiotics against 634 _N. gonorrhoeae_ isolated from the 8 cities in 2016 were determined by the microdilution method. The distributions of MIC90 and MIC50 of each antibiotic are summarized in Table 3. Of the antibiotics tested, ceftriaxone and spectinomycin appeared to be the most effective agents. However, the MIC90 of ceftriaxone indicated that the antibiotic had decreased susceptibility for the isolates, with an MIC of 0.06 mg/L. Meanwhile, the MIC90 of cefixime, as the third generation of cephalosporin, had reached the critical point. The MIC50 of penicillin G, tetracycline, and ciprofloxacin were 32-fold higher than the resistant cutoff point.

The antimicrobial susceptibilities of the 634 strains were interpreted according to the WHO standards. No strain isolated from the 8 cities was resistant to spectinomycin, cefixime, and ceftriaxone. However, the percentages of the isolates with decreased susceptibility to ceftriaxone and cefixime were 2.1% and 12%, respectively. The resistant rates of the isolates to ciprofloxacin, tetracycline, penicillin, and azithromycin were 99.8%, 88.3%, 53.8%, and 11%, respectively (Table 4).

The resistant rates of _N. gonorrhoeae _isolated from Shenzhen against penicillin and ceftriaxone were 77.8% and 5.6%, respectively, which were higher than the overall level in the present study (χ² = 17.31, P < 0.01 and χ² = 3.98, P > 0.05). The deceased susceptibility of _N. gonorrhoeae _isolated from Zhuhai to cefixime was 30.1%, which was higher than the overall level in the present study (χ² = 21.72, P < 0.01). Jiangmen City had the highest prevalence of azithromycin-resistant isolates (16.8%).
4. Discussion

The WHO recommends the agar dilution method for gonococcal susceptibility testing (WHO, 1992). However, its cumbersome procedure limits its laboratory application. In this study, we established and improved the microdilution method based on the microdilution method previously reported (Geers and Donabedian, 1989; Shapiro et al., 1984). The modified method is more convenient than the agar dilution method. N. gonorrhoeae is a fastidious organism that is usually grown in solid media with nutritious additives and in environments containing carbon dioxide. Notably, N. gonorrhoeae exhibits poor growth in liquid media, although the liquid medium possesses higher nutrient requirements than solid media (Hendry, 1983; Norrod and Morse, 1982; Shockley et al., 1980). Cartwright reported that the pigments of hemoglobin in liquid media for N. gonorrhoeae susceptibility testing affect the formation of bacterial colonies or turbidity (Cartwright et al., 1994). In our study, the formulation was modified with appropriate additives suitable for the optimal growth of organisms. After the formation of colony suspension, the MIC breakpoints were easily observed by eye or ELISA reader.

To identify the performance of the microdilution method, we detected 100 clinical N. gonorrhoeae isolates and compared it with the WHO-recommended agar dilution method. The results suggested that the two methods are highly consistent when used for the antibiotic susceptibility testing of N. gonorrhoeae, showing 96%–100% EA, 94%–100% CA, 0%–3% ME, 0%–2% VME, and 0%–6% MIE, indicating good consistency (P > 0.05) by the Wilcoxon signed-rank test.

The susceptibility rates of 634 N. gonorrhoeae isolates from the central, eastern, western, and northern parts of Guangdong Province were determined through the microdilution method. The antimicrobial resistance rates of ciprofloxacin, tetracycline, and penicillin were 99.8%, 88.3%, and 53.8%, respectively, which were higher than those of ciprofloxacin (42.1%–36.2%), penicillin (31%–35%), and tetracycline (21.8%–22.6%) used in Latin America by found in Caribbean (Thakur et al., 2017). The resistant rates in 6 southeast Asian countries increased from 25% to 100% for penicillin, 10% to 100% for tetracycline, and 38% to 100% for ciprofloxacin from 2009 to 2012 (Bala M et al., 2013), thereby showing the same trend as that reported in Guangzhou from 1996 to 2001 (Zheng HP et al., 2003). Here, we found that Shenzhen had the highest rates of resistance to penicillin (95.7%) and tetracycline (76.8%) among the 8 cities ($\chi^2 = 17.31, P < 0.01$; $\chi^2 = 4.85, P < 0.05$). Azithromycin has been used for the treatment of STD pathogens, especially Chlamydia trachomatis and Mycoplasma. It is an effective antibiotic for treating adult pharyngeal gonococcal infections (Furuya et al., 2006; Sathia et al., 2007). WHO recommends the use of azithromycin along with spectinomycin or gentamicin as alternative therapy (azithromycin 2 g orally and gentamicin 240 mg intramuscularly or azithromycin 2 g orally and spectinomycin 2 g intramuscularly). However, the widespread use of azithromycin in the treatment of STDs led to the emergence of resistant isolates. In 2004, the first N. gonorrhoeae isolate resistant to azithromycin was detected in Scotland (Palmer et al., 2008). Since then, drug-resistant isolates have been reported worldwide (Galarza et al., 2009; Starnino and Stefanelli, 2009). N. gonorrhoeae isolates with high-level azithromycin resistance

Table 1

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MICs</th>
<th>WHO D</th>
<th>WHO G</th>
<th>WHO J</th>
<th>WHO L</th>
<th>WHO K</th>
<th>WHO P</th>
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<td>Reference MICs</td>
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<td></td>
<td>MIC by MM</td>
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<td>≥2</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>MIC by AM</td>
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<td>1</td>
<td>≥32</td>
<td>4</td>
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<td>-</td>
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<tr>
<td>Tetracycline</td>
<td>Reference MICs</td>
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<td>16–64</td>
<td>≤8</td>
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<tr>
<td></td>
<td>MIC by MM</td>
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<td>32</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MIC by AM</td>
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<td>32</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>8–32</td>
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<td>0.125</td>
<td>0.5</td>
<td>4</td>
<td>32</td>
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<td>≤0.04</td>
<td>≤0.5</td>
<td>≤0.125</td>
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<td>16</td>
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<td>16</td>
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<tr>
<td>Ceftriaxone</td>
<td>Reference MICs</td>
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<td></td>
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<td>0.008</td>
<td>0.03</td>
<td>0.125</td>
<td>0.03</td>
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<td>0.008</td>
<td>0.125</td>
<td>0.03</td>
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<tr>
<td>Cefixime</td>
<td>Reference MICs</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>0.25–1</td>
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<tr>
<td></td>
<td>MIC by MM</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
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<td>-</td>
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</table>

Fig. 1. A: Growth of N. gonorrhoeae in bottle of the liquid culture medium (A1. negative; A2. N. gonorrhoeae) B: Results of N. gonorrhoeae antibiotic susceptibility in microplate.
(MIC of 4096 μg/mL) have emerged in England and Wales (Chisholm et al., 2009). In Canada, the resistance rate of azithromycin increased from 0.4% to 3.3% (P < 0.001) in 2010–2014 (Martin et al., 2016), whereas that in Latin America was 1.0%–1.7% (Thakur et al., 2017). WHO recommends that the selection of antibiotic according to the results of susceptibility test or modification of treatment regimen for bacteria with resistance rates exceeding 5% (WHO, 2012). Our results showed that the resistance rate to azithromycin was between 4% and 20% in all cities, with an average of 11.04%. Therefore, monitoring the susceptibility of isolates to azithromycin is of clinical importance.

Third-generation cephalosporins are the recommended antibiotics for the treatment of gonorrhea. However, the susceptibility of the disease to ceftriaxone (injectable) and cefixime (oral) has decreased globally (Barry and Klausner, 2009; Kirkcaldy et al., 2011; Tapsall et al., 2009). The GISP reported that the proportion of strains with decreased susceptibility to ceftriaxone (MIC ≤0.125 μg/mL) increased from 0.05% to 0.3% between 2006 and 2012 (Kirkcaldy et al., 2013). Moreover, the GASP reported that the strains with decreased susceptibility increased from 5.3% to 9.4% in India in 1995–2011 (Bala et al., 2013). In Hanoi, Vietnam, 5% of N. gonorrhoeae strains showed a decrease in ceftriaxone susceptibility in 2011 (MIC ≤0.125 μg/mL; Olsen et al., 2013). Tapsall JW et al. tested more than 36,000 N. gonorrhoeae strains collected in Australia between 1997 and 2006 and found that 134 strains had decreased susceptibility to ceftriaxone (Tapsall et al., 2008). Recently, the first high-level ceftriaxone resistant gonococcal strain (H041) with MIC of ≥2 μg/mL was isolated from the pharynx of a female in Japan in 2011 (Ohnishi et al., 2011). In 2012, a second strain (F89) with high-level cefixime and ceftriaxone resistance was confirmed and characterized in France (Unemo et al., 2012). Although no cefixime-resistant N. gonorrhoeae was found in Guangzhou, the emergence and spread of strains with decreased susceptibility (MIC ≤0.06 μg/mL) increased from 37.5% in 1996 to 46.5% in 2011 (Zheng et al., 2003; Zheng et al., 2014). In this study, the gonococcal isolates with MIC of ≥0.125 μg/mL for ceftriaxone were 2.1% in Guangdong Province, and 5.6% of these isolates was found in Shenzhen (χ² = 3.98, P < 0.05). The isolates with decreased susceptibility (MIC ≥0.25 μg/mL) to cefixime were 12% in Guangdong Province, and 30.1% of these isolates were found in Zhubai (χ² = 21.72, P < 0.01). To overcome or delay the problem of increasing ceftriaxone and cefixime resistance, a higher dose of intramuscular ceftriaxone (500 mg) is recommended as the first-line treatment in UK (www.bashh.org/documents/3920.pdf), while China and the US CDC continue to recommend ceftriaxone at 250 mg. Considering our findings, treating gonococcal infections with high doses of ceftriaxone is reasonable.

### Table 2

Comparison between the microdilution and agar method by determining MICs of the 100 N. gonorrhoeae strains against 7 antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Methods</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MIC mean</th>
<th>%EA</th>
<th>%CA</th>
<th>%VME</th>
<th>%ME</th>
<th>%MIE</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>≥32</td>
<td>1.37</td>
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<td>≥32</td>
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<td>0.06</td>
<td>0.019</td>
<td>96</td>
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<td>0.33</td>
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</table>

MM = microdilution method; AM = agar dilution method.

<sup>a</sup> The essential agreement by the Wilcoxon signed-rank test.
Table 4

<table>
<thead>
<tr>
<th>City</th>
<th>No. of Strain</th>
<th>Tetracycline</th>
<th>Penicillin</th>
<th>Ceftriaxone</th>
<th>Azithromycin</th>
<th>Ciprofloxacin</th>
<th>Spectinomycin</th>
<th>Ceftaxime</th>
<th>DS</th>
<th>S</th>
<th>R</th>
<th>SD</th>
<th>S</th>
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<td>Guangzhou</td>
<td>127</td>
<td>1 (0.8)</td>
<td>66 (52)</td>
<td>60 (47.2)</td>
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<td>110 (86.6)</td>
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<td>127 (100)</td>
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<tr>
<td>Shenzhen</td>
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<td>0 (0)</td>
<td>20 (22.2)</td>
<td>70 (77.8)</td>
<td>4 (4.4)</td>
<td>86 (95.6)</td>
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<td>90 (100)</td>
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<tr>
<td>Zhuhai</td>
<td>93</td>
<td>7 (7.5)</td>
<td>48 (51.6)</td>
<td>38 (40.9)</td>
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<tr>
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<td>28 (42.4)</td>
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<td>66 (100)</td>
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</tbody>
</table>

S = susceptible; I = intermediate; R = resistant; DS = decreased susceptibility.

In summary, the data presented here indicated the levels of resistance of *N. gonorrhoeae* isolated from the 8 cities of Guangdong. However, a small number of isolates collected from Maoming and Shaoguan inadequately represent the real situation of *N. gonorrhoeae* resistance in western and northern Guangdong. The microdilution method developed was confirmed to be highly consistent with the agar dilution method recommended by the WHO. We expect this method to be useful in the timely detection of *N. gonorrhoeae* resistance in clinical laboratories.

Acknowledgments

The authors would like to thank Shujie Huang, Lei Chen, and Peizhen Zhao from the STD control department, especially Peizhen Zhao, who participated in statistical work. Funding: This work was supported by the grants from the Medical Science and Technology Research Foundation of Guangdong Province (No. A2015221). The authors declare no conflicts of interest.

References


In summary, the data presented here indicated the levels of resistance of *N. gonorrhoeae* isolated from the 8 cities in Guangdong Province through an improved … *Diagn Microbiol Infect Dis* (2018), https://doi.org/10.1016/j.diagmicrobio.2018.06.004


