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Short Communication

Surveillance of Antibiotic Susceptibility Patterns of *Neisseria gonorrhoeae* from 2013 to 2015 in Guangxi Province, China

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SUMMARY: *Neisseria gonorrhoeae* is a sexually transmitted pathogen highly prevalent worldwide with an increasing trend of resistance to antimicrobial treatment. We conducted this study to trace the susceptibility of *N. gonorrhoeae* to penicillin (PC), spectinomycin (SPCM), ciprofloxacin (CPFX), azithromycin (AZM), cefixime (CFIX), and ceftriaxone (CTRX) in Guangxi province. In total, 303 *N. gonorrhoeae* isolates were obtained from patients infected with *N. gonorrhoeae* in 6 cities in Guangxi during 2013–2015, and the antibiotic susceptibility patterns were analyzed by an agar dilution assay. The results showed that *N. gonorrhoeae* was susceptible to treatment with cephalosporins, including CTRX (99.7% of isolates), CFIX (99%), SPCM (100%), and AZM (96.4%), and this is the first report of antibiotic susceptibility for AZM surveillance of *N. gonorrhoeae* in Guangxi. Penicillinase-producing *N. gonorrhoeae* (PPNG) isolates increased in prevalence from 37% in 2013 to 64% in 2015 ($P = 0.068$), and tetracycline-resistant *N. gonorrhoeae* (TRNG) prevalence increased from 23% in 2013 to 44% in 2015 ($P = 0.071$). High resistance of *N. gonorrhoeae* to PC was associated with infection in patients at ages 25 to 30 years ($P < 0.05$), whereas PPNG positivity ($P < 0.01$), and TRNG positivity were risk factors for CPFX resistance ($P = 0.0407$). Our study provides plausible evidence for therapeutic strategies and *N. gonorrhoeae* infection control and prevention in Guangxi, China.

Neisseria gonorrhoeae is a sexually transmitted pathogen that infects more than 100 million sexually active individuals every year worldwide (1). Urethritis, epididymitis, prostatitis, endometritis, and salpingitis are common complications of *N. gonorrhoeae* infection, and an increase in the risk of HIV infection was also reported in relation to this infection (2). Effective antimicrobial agents are the first-line treatments of *N. gonorrhoeae* owing to a lack of a vaccine. Nevertheless, in 2012, Goire et al. reported that some *N. gonorrhoeae* strains are resistant to ceftriaxone (CTRX) (3), which is the most effective antibiotic for isolates resistant to tetracycline (TC), penicillin (PC), and quinolones, and treatment failures were reported in recent years. Based on the widespread prevalence of *N. gonorrhoeae* and increasing resistance to treatment, it is necessary to monitor the antibiotic susceptibility of *N. gonorrhoeae*, especially in the Chinese, among whom antimicrobial overuse is reported to be highly frequent.

We conducted this study to trace the patterns of susceptibility of *N. gonorrhoeae* to PC, spectinomycin (SPCM), ciprofloxacin (CPFX), azithromycin (AZM), cefixime (CFIX), and CTRX from 2013 to 2015 in Guangxi province and to identify the prevalence (%) of plasmid-mediated penicillinase-producing *N. gonorrhoeae* (PPNG) and TC-resistant *N. gonorrhoeae* (TRNG), which can explain high-level resistance of *N. gonorrhoeae* to PC and TC, respectively. Another aim was to assess potential risk factors of *N. gonorrhoeae* resistance to CPFX and PC.

A total of 303 *N. gonorrhoeae* isolates were collected from patients with urethritis or cervicitis and with a diagnosis of uncomplicated gonorrhea who visited the sexually transmitted disease clinics in 6 cities of Guangxi Province including Nanning, Chongzuo, Beihai, Qinzhou, Yulin, and Guigang between January 1, 2013 and December 31, 2015. Among these samples, 286 (94.4%) were collected from men with urethritis and 17 were from females with urethritis or cervicitis. The mean age of the patients was 32.2 years (range, 15–64 years). Collection and storage of samples were strictly compliant with the National Supervision Programme for *Neisseria gonorrhoeae* from the National Supervision Programme for Sexually Transmitted Diseases (Trial Implementation) released by the Chinese Center for Disease Control and Prevention (China CDC) in 2007.

Urethral or cervical swab samples were directly inoculated onto Thayer-Martin agar plates, which were cultured in candle jars at 36°C for 24–48 hours. Cultured isolates were identified according to the results of Gram staining, colony morphology analysis, and an oxidase

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reaction assay. We used the sugar fermentation method to confirm *N. gonorrhoeae* strains. The isolates meeting inclusion criteria were selected and cultured on the GC Agar Base medium (Difco, Detroit, MI, USA) supplemented with 10% defibrinated sheep blood, and the resulting colonies were collected by swabbing, resuspended in skimmed milk, and stored at −85°C for antibiotic susceptibility tests.

CPFX (Bayer Leverkusen, Leverkusen, Germany), SPCM (Upjohn, Kalamazoo, MI, USA), PC (SmithKline Beecham, Philadelphia, PA, USA), CTRX (Roche, Basel, Switzerland), CFIX (Sinopharm, Shanghai, China), AZM (Sinopharm), and TC (Sigma, St. Louis, MO, USA) were all provided by Dr. Tapsall from the Prince of Wales Hospital, the Cooperation Center of World Health Organization (WHO) Western Pacific Region Resistance Surveillance Programme. Concentration ranges of CTRX, CFIX, SPCM, CPFX, PC, and AZM were 0.008–0.500 mg/L, 0.008–0.500 mg/L, 4–64 mg/L, 0.015–8.000 mg/L, 0.015–4.000 mg/L, and 0.03–4.00 mg/L, respectively. The concentration of TC was only 8 mg/L, which was used to detect *N. gonorrhoeae* with plasmid-mediated strong TC resistance (4). GC Agar Base (Difco, Oxoid, UK) supplemented with 10% defibrinated sheep blood was used for cultivation.

Paper acidometric testing was applied to detect β -lactamase. An agar dilution test was carried out to evaluate the antibiotic susceptibility. WHO reference strains G (TRNG), J (PPNG), and L (strongly CPFX-resistant strain with decreased CTRX resistance) and international reference strain ATCC49266 were provided by the Chinese Academy of Medical Sciences and served as controls (4). Antibiotic susceptibility was confirmed according to the WHO Western Pacific Region Resistance Surveillance Programme guidelines (5).

The surveillance results indicated that *N. gonorrhoeae* isolated in this study was very susceptible to treatment with cephalosporins including both CTRX (99.7%) and CFIX (99%), SPCM (100%), and AZM (96.4%). Among the 303 *N. gonorrhoeae* isolates, only one with decreased susceptibility to CTRX was found in 2013, and 3 isolates with decreased susceptibility to CFIX were identified: 2 in 2013 and one in 2014.

Table 1 shows the changes in MIC_{50} and MIC_{90} of CTRX, CFIX, SPCM, AZM, PC, and CPFX from 2013 to 2015. Of the 303 isolates, 141 were diagnosed as TRNG with a prevalence increase from 37% in 2013 to 64% in 2015 ($P = 0.068$). Ninety-three isolates were found to be PPNG, and their prevalence rate increased from 23% in 2013 to 44% in 2015 ($P = 0.071$). A total of 293 isolates were diagnosed as CPFX resistant, and their prevalence slightly increased from 95% in 2013 to 96.2% in 2014 ($P = 0.81$) and kept increasing, to 98.4% in 2015 ($P = 0.34$).

We studied the risk factors of *N. gonorrhoeae* resistance to CPFX and PC by univariate analysis, including age, gender, year of isolation, course, PPNG positivity, TRNG positivity or negativity, a history of gonorrhea infection or no such history, previous antibiotic exposure or no exposure, localized or not localized infection, and sample location. The results showed that infection age from 25 to 30 years ($P < 0.05$) and PPNG positivity ($P < 0.01$) significantly correlated with *N. gonorrhoeae* isolates with high resistance to PC, and TRNG positivity was a risk factor of CPFX resistance ($P = 0.0407$).

The surveillance of antibiotic susceptibility patterns of *N. gonorrhoeae* began in the 1980s, and the records could be tracked to 2000 in Nanning, Guangxi (6). This study is the first report of *N. gonorrhoeae* susceptibility to CFIX and AZM in Guangxi, and the study is important as these antibiotics have been included in the surveillance system since 2013 in China.

Extended-spectrum cephalosporins (ESCs) are the last remaining options for first-line monotherapy of gonorrhea, and treatment failures with ESCs have been reported in several regions, including the USA (7), Canada (8), and Europe (9). Fortunately, we found no resistant strains and only 3 strains with decreased susceptibility to CFIX in Guangxi from 2013 to 2015. Global transmission is likely to be the main reason for initial emergence of ESC resistance (10); therefore, we may conclude that temporarily, there are no imported *N. gonorrhoeae* strains resistant to CFIX in Guangxi.

The *N. gonorrhoeae* isolates in our study were found to be susceptible to CTRX (99.7% of isolates) with $MIC_{50} < 0.008$ mg/L. In our previous study on resistance surveillance from 2000 to 2012, there were 32.9% strains with decreased susceptibility to CTRX, and most of the strains had a MIC_{50} of > 0.032 mg/L (6). The prevalence of TRNG isolates in Guangxi was 46.5%, which was much lower than the prevalence detected in Nanning from 2003 to 2012. The above differences may be caused by dissimilarity of the sources of isolates. The samples collected in 2000–2012 were all from Nanning city, whereas almost 50% of the samples in 2013–2015 came from other 5 cities in Guangxi including Chongzuo, Beihai, Qinzhou, Yulin, and Guigang. The data indicated that Nanning had greater prevalence of *N. gonorrhoeae* strains resistant to CTRX and TC as compared to the other 5 cities.

Our study is the first report of *N. gonorrhoeae* susceptibility to AZM in Guangxi. The average prevalence rate of *N. gonorrhoeae* susceptibility to AZM is 96.4%, which is similar to the results of a few studies in China systematically reviewed by Yu et al. in 2015 (average prevalence of resistance of *N. gonorrhoeae* to AZM after meta-analysis was 6.3%; 95% confidence interval: 3.3%–11.6%) but is better than the situation in Guangdong, Nanjing, and Chongqing (11). Only 11 isolates in our study were resistant strains, which were defined by an MIC > 1.0 g/L (12). Among the 11 *N. gonorrhoeae* strains resistant to AZM, 3 strains had an MIC > 8.0, one strain had an MIC > 4.0, 2 strains had an MIC > 2.0, and 5 strains had an MIC > 1.0. Neither moderate (MICs of 16–32 mg/L) nor high-level (MICs of 256 mg/L) AZM resistance was detected, as reported in Europe by Jacobsson et al. (13).

The average percentage of CPFX-resistant *N. gonorrhoeae* isolates in Guangxi from 2013 to 2015 (96.6%) was consistent with that from 2006 to 2008 (98%) and from 2009 to 2012 (98.3%), and a little higher than that before 2005 (89.9% in 2000–2002 and 88.2% in 2003–2005). The multiple analyses showed that the only positive TRNG result was the risk factor of CPFX resistance in *N. gonorrhoeae* isolates in Guangxi. Age, gender, marital status, clinical course, isolation

Table 1. Susceptibilities of *N. gonorrhoeae* strains to penicillin, ciprofloxacin, spectinomycin, azithromycin, ceftriaxone, and cefixime in Guangxi, China

Yr	2013 (n = 100)	2014 (n = 80)	2015 (n = 123)	Total 303
Penicillin[1)]				
Susceptible (%)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased (%)	12 (12%)	13 (16.3%)	25 (20.3%)	50 (16.5%)
Resistant (%)	88 (88%)	67 (83.7%)	98 (79.7%)	253 (83.5%)
MIC_{50} (g/L)	2	2	1	2
MIC_{90} (g/L)	≥ 8	≥ 8	≥ 8	≥ 8
MIC range (g/L)	0.25–≥ 8	0.25–≥ 8	0.125–≥ 8	0.125–≥ 8
Ciprofloxacin[2)]				
Susceptible (%)	2 (2%)	3 (3.8%)	0 (0)	5 (1.7%)
Decreased (%)	3 (3%)	0 (0)	2 (1.6%)	5 (1.7%)
Resistant (%)	95 (95%)	77 (96.2%)	121 (98.4%)	293 (96.6%)
MIC_{50} (mg/L)	8	8	8	8
MIC_{90} (mg/L)	≥ 16	≥ 16	≥ 16	≥ 16
MIC range (mg/L)	≤ 0.015–≥ 16	≤ 0.015–≥ 16	0.25–≥ 16	≤ 0.015–≥ 16
Spectinomycin[3)]				
Susceptible (%)	100 (100%)	80 (100%)	123 (100%)	303 (100%)
Resistant (%)	0 (0)	0 (0)	0 (0)	0 (0)
MIC_{50} (mg/L)	8	8	16	8
MIC_{90} (mg/L)	16	16	16	16
MIC range(mg/L)	8–32	≤ 4–64	≤ 4–64	≤ 4–64
Azithromycin[4)]				
Susceptible (%)	94 (94%)	79 (98.8%)	119 (96.7%)	292 (96.4%)
Resistant (%)	6 (6%)	1 (1.2%)	4 (3.3%)	11 (3.6%)
MIC_{50} (g/L)	0.125	0.125	0.125	0.125
MIC_{90} (g/L)	0.25	0.25	0.5	0.5
MIC range (g/L)	≤ 0.03–≥ 8	≤ 0.03–≥ 8	≤ 0.03–≥ 8	≤ 0.03–≥ 8
Ceftriaxone[5)]				
Susceptible (%)	99 (99%)	80 (100%)	123 (100%)	302 (99.7%)
Decreased (%)	1 (1%)	0 (0)	0 (0)	1 (0.3%)
MIC_{50} (mg/L)	≤ 0.008	≤ 0.008	≤ 0.008	≤ 0.008
MIC_{90} (mg/L)	≤ 0.008	0.015	0.015	0.015
MIC range (mg/L)	≤ 0.008–0.5	≤ 0.008–0.06	≤ 0.008–0.06	≤ 0.008–0.5
Cefixime[6)]				
Susceptible (%)	98 (98%)	79 (98.8%)	123 (100%)	300 (99%)
Decreased (%)	2 (2%)	1 (1.2%)	0 (0)	3 (1%)
MIC_{50} (g/L)	≤ 0.008	≤ 0.008	≤ 0.008	≤ 0.008
MIC_{90} (g/L)	0.015	0.03	0.015	0.015
MIC range (g/L)	≤ 0.008–0.5	≤ 0.008–0.25	≤ 0.008–0.06	≤ 0.008–0.5
TRNG (%)[7)]	37 (37%)	40 (50%)	64 (52%)	141 (46.5%)
PPNG (%)[8)]	23 (23%)	26 (32.5%)	44 (35.8%)	93 (30.7%)

[1)]: Susceptible, isolates with MICs < 0.03mg/L; Decreased, isolates with MICs of 0.03 to 0.50 mg/L; Resistant, isolates with MICs > 1.0 mg/L.
[2)]: Susceptible, isolates with MICs ≤ 0.06 mg/L; Decreased, isolates with MICs > 0.125 mg/L.
[3)]: Susceptible, isolates with MICs ≤ 64 mg/L; Resistant, isolates with MICs > 128 mg/L.
[4)]: Susceptible, isolates with MICs < 0.5 g/L,Resistant, isolates with MICs > 1.0 g/L.
[5)]: Susceptible, isolates with MICs < 0.06 mg/L; Decreased, isolates with MICs of 0.06 - 0.50 mg/L; Resistant, isolates with MICs > 1.0 mg/L.
[6)]: Susceptible, isolates with MICs < 0.25 g/L, Decreased, isolates with MICs > 0.25 g/L.
[7)]: TRNG, isolates with MICs > 16 mg/L to tetracycline were identified as TRNG.
[8)]: PPNG, plasmid-mediated PPNG.

year, a history of gonorrhea infection, and PPNG positivity were not related to decreased susceptibility of *N. gonorrhoeae* isolates to CPFX.

The prevalence of PPNG isolates in Guangxi from 2013 to 2015 was 30.7%, which is consistent with that from 2009 to 2012 (31.8%) but much higher than that before 2008 (5.6% during 2000–2003, 13.3% during 2003–2005, and 13.7% during 2006–2008). PPNG prevalence is similar to that of neighboring Guangdong province (31.1%) (14). The multiple analyses revealed that the age older than 50 is a risk factor of resistance. In contrast to 93 PPNG strains, 160 strains had chromosomally mediated PC resistance, and the remaining 50 strains showed decreased susceptibility to PC. High prevalence of PC resistance was also detected in North Asia and the WHO South East Asia Region. By contrast, in the Pacific Island states of Fiji, New Caledonia, and Tonga, there were reports of low or no resistance in 2009; the significant difference is mainly caused by antibiotic standards used in different countries

and the overuse of antibiotics in developing countries, including China.

In conclusion, cephalosporins, including CPFX and CTRX, and SPCM as the first-line treatment of *N. gonorrhoeae* infection are effective, with high sensitivity of *N. gonorrhoeae*. AZM is currently effective against *N. gonorrhoeae* infection, with the first resistant case reported in 2013. Therefore, further surveillance is necessary for its susceptibility evaluation and clinical application.

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Conflict of interest None to declare.

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