



Changing antimicrobial susceptibility and molecular characterisation of *Neisseria gonorrhoeae* isolates in Guangdong, China: in a background of rapidly rising epidemic

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ABSTRACT

The prevalence of *Neisseria gonorrhoeae* infections has increased rapidly since 2015 in China. Antimicrobial resistance and molecular mobilisation in *N. gonorrhoeae* are two important factors driving this increasing prevalence. This study explored changes in antimicrobial susceptibility and molecular characteristics of *N. gonorrhoeae* collected in Guangdong, China (2013–2017). A total of 704 isolates were collected in two cities in Guangdong. MICs of major antimicrobials were determined. Penicillinase-producing *N. gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG) were characterised, and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) was performed. High resistance to penicillin (68.2%), tetracycline (85.7%) and ciprofloxacin (98.2%) was observed. Spectinomycin, ceftriaxone and azithromycin appeared effective, with susceptibilities of 100%, 96.4% and 90.7%, respectively. Resistance to penicillin decreased significantly from 78.4% to 73.6% and to azithromycin from 11.9% to 3.7%. Total prevalence of PPNG, TRNG and PPNG/TRNG was 25.4%, 33.1% and 13.4%, respectively. Rates of PPNG decreased significantly from 37.3% to 23.9%, TRNG from 50.0% to 31.3%, and PPNG/TRNG from 23.5% to 11.7%. However, the ratio of African-type PPNG increased significantly (18.4% to 64.1%) compared with decreasing Asian-type PPNG (81.6% to 33.3%), and the ratio of American-type TRNG increased significantly (0% to 13.7%) compared with decreasing Dutch-type TRNG (100% to 86.3%). A total of 271 sequence types (STs) were identified by NG-MAST from 380 isolates collected in 2013, 2014 and 2017, with 145 novel STs. African-type PPNG is increasing and replacing Asian-type, and novel STs have emerged. Gonococcal isolates with new genotypes might contribute to the rising gonorrhoea epidemic in this area.

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1. Introduction

The re-emergence of *Neisseria gonorrhoeae* infections is a crucial public-health concern. The World Health Organization (WHO) esti-

mated that approximately 78 million cases of *N. gonorrhoeae* infection in adults were reported worldwide in 2012 [1]. The prevalence of gonorrhoea infections has increased rapidly. The US Centers for Disease Control and Prevention (CDC) reported that the number of gonorrhoea cases reached 555 608 in the USA in 2017. Specifically, the number of gonorrhoea cases in the USA in 2017 increased by 18.6% compared with 2016 and by 75.2% compared with the historic low in 2009 [2]. China has suffered a dramatic increase of 38.5% in *N. gonorrhoeae* infections, from 100 245 cases reported in 2015 to 138 855 cases in 2017, following over 10 years of sustained stable, or even slightly decreased, incidence with approximately 109 525 cases each year [3].

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Antimicrobial resistance is a potential reason for the re-emergence of *N. gonorrhoeae* infections. The widespread use and abuse of antibiotics have led to the emergence of resistant strains, and *N. gonorrhoeae* has become resistant to most antimicrobials, including sulfonamides, macrolides, penicillin, tetracycline, and first- and second-generation cephalosporins [4]. Resistance surveillance projects using the agar dilution method have been performed in numerous countries to monitor the antimicrobial resistance of *N. gonorrhoeae* isolates, and an improved microdilution method has been established to improve the stability and efficiency of resistance monitoring [5]. In 2014, the Gonococcal Isolate Surveillance Project (GISP) isolated *N. gonorrhoeae* strains demonstrating resistance to tetracycline (25.3%), ciprofloxacin (19.2%) and penicillin (16.2%) as well as decreased susceptibility to azithromycin (2.5%), cefixime (0.8%) and ceftriaxone (0.1%) from 27 sites in the USA [6]. In 2016, cases of *N. gonorrhoeae* with highly stable azithromycin resistance (7.5%), cefixime resistance (2.1%) and reduced ceftriaxone susceptibility (0.5%) were reported in 25 European countries [7]. From 1996 to 2016, strains with decreased susceptibility or resistance to ceftriaxone (from 2.5% to 4%) and cefixime (from 18% to 46%) were observed in Japan [8]. The first strain with ceftriaxone resistance, referred to as a 'superbug', was identified in Japan in 2011, with a ceftriaxone minimum inhibitory concentration (MIC) of 2 mg/L [9]. The discovery of this strain was followed by verified reports of treatment failures in Australia, France, Slovenia, Canada and Sweden [10]. Given the prevalence of ceftriaxone-resistant strains, the WHO believes that gonorrhoea may become untreatable again [10,11].

In addition to resistance, molecular characteristics are an important driving force of the re-emergence of *N. gonorrhoeae* infections. In Italy, 80 penicillinase-producing *N. gonorrhoeae* (PPNG) strains were characterised over the periods 2003–2004 and 2014–2015. These strains possessed four *bla*_{TEM} alleles, namely TEM-1, TEM-228, TEMP14s and TEM-135. The prevalence of the African-type strain with the TEM-1 allele increased from 42.5% to 55%, whereas that of the Toronto-type strain with the TEM-135 allele decreased from 57.5% to 35% [12]. Whole-genome sequencing data showed that the resistance of *N. gonorrhoeae* is closely related to genetic mutations, including in *ponA/penA* (β -lactams), *rpsJ* (tetracyclines), *gyrA/parC* (fluoroquinolones), *porB* (reduced membrane permeability) and *mtrR* (efflux pump overexpression) [13]. *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) results showed that the globally disseminated sequence type 1407 (ST1407) is related to resistant strains and that the genomes of the ST12556 and ST1407 strains from Russia are highly homologous [13]. In addition, ST12450, a phenotypically convergent strain with a genome that partially coincides with the genome of ST1407, has contributed to the emergence of new resistance determinants [13]. The current status and epidemic trends of local resistant *N. gonorrhoeae* strains can be determined through several methods to aid their treatment and control.

Guangdong Province in South China has a population of more than 10 million people and a full one-quarter of the migrant population from domestic and overseas. In 2017, 29 946 cases of gonorrhoea were reported in Guangdong, accounting for 21.6% of national notifiable cases [14]. The close social and economic ties to Hong Kong, Macao, nearby Southeast Asian nations and, in recent years, African countries have often resulted in linked sexually transmitted infection (STI) epidemics. In order to continually survey *N. gonorrhoeae* resistance, the Guangdong Provincial Centre for Skin Diseases and STI 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 and has established a network including eight sentinel cities in 2008. Early surveillance results indicated increasing resistance and detected new features of PPNG African-type plasmid epidemic

strains [15–17]. In response to the rapid growth of gonorrhoea, the aim of this study was to analyse the change in antimicrobial resistance of *N. gonorrhoeae* isolates to six antibiotics as well as the molecular characterisation, i.e. determination of PPNG and tetracycline-resistant *N. gonorrhoeae* (TRNG) as well as NG-MAST, of *N. gonorrhoeae* in Guangdong in 2013–2017.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Medical Ethics Committee of Guangdong Provincial Center for Skin Diseases and STI Control. Isolates used in the study were all residual clinical specimens, an no personal information was collected.

2.2. *Neisseria gonorrhoeae* strains from the Gonococcal Antibiotic Resistance Monitoring Network

The Gonococcal Antibiotic Resistance Monitoring Network includes eight sentinel cities and has been established since 2008. Owing to the highest incidence of gonorrhoea in Guangzhou and Zhuhai, strains from these two cities were selected to systematically study the change in antimicrobial resistance and molecular epidemiological characterisation before and after sharp increases in Guangdong during 2013 and 2017.

A total of 704 *N. gonorrhoeae* isolates were collected in the two monitoring sites (441 in Guangzhou and 263 in Zhuhai). Isolates were consecutively obtained from outpatients with gonorrhoea who attended sexually transmitted disease (STD) clinics in Zhuhai Center for STD Control and Guangdong Provincial Center for Skin Diseases and STI Control in Guangdong Province over the period 2013–2017. No restrictions regarding age, gender, partner, and other behaviours of the patients were considered.

2.3. Isolation and culture of *Neisseria gonorrhoeae* strains

Neisseria gonorrhoeae was isolated and cultured in Thayer–Martin (TM) medium and gonococcal agar supplemented with 10% defibrinated sheep blood. Identification of *N. gonorrhoeae* was performed by Gram stain as well as oxidase, catalase and sugar fermentation tests (all sugars were supplied by Fluka, Japan) as recommended by the WHO [18]. Briefly, a bacterial suspension was distributed in buffered salt indication solution, which contains, respectively, filter-sterilised glucose, maltose, lactose or sucrose. Following incubation for 4 h in a 37 °C water-bath, *N. gonorrhoeae* shows only glucose fermentation. Isolates from Zhuhai identified as *N. gonorrhoeae* were preserved in freeze-dried skimmed milk and were transported to Guangdong Provincial Center for Skin Diseases and STI Control. All strains were lyophilised using 5% skimmed milk and were preserved in liquid nitrogen for further testing.

2.4. Antimicrobial susceptibility testing

The agar dilution method, as recommended by the WHO Western Pacific *N. gonorrhoeae* monitoring programme [18], was used to monitor the resistance of *N. gonorrhoeae*. In brief, a pure subculture from 18–24 h of growth under 5% CO₂ at 35 °C on GC agar was suspended in 0.85% saline and was adjusted to a 0.5 McFarland standard as inoculum. The suspension was then inoculated through multipoint inoculation onto antimicrobial agar plates containing penicillin, tetracycline, ciprofloxacin, spectinomycin, ceftriaxone or azithromycin. Following inverted culture at 35 °C in a 5% CO₂ incubator overnight, growth of the isolates was observed and the MIC was recorded. *Neisseria gonorrhoeae* WHO-D, G, J, L, K and

P (provided by Dr Yueping Yin, National Center for STD Control, Nanjing, China) were used as control strains.

Antimicrobial susceptibility was interpreted according to the criteria provided by the WHO WPR Resistance Surveillance Programme guidelines [18]. For penicillin and ciprofloxacin, isolates with MICs of ≥ 1.0 mg/L were classified as resistant, those with MICs of 0.06–0.50 mg/L as intermediate-susceptible and those with MICs of ≤ 0.03 mg/L as susceptible. For tetracycline, isolates with MICs of ≤ 0.5 mg/L were classified as susceptible and those with MICs of ≥ 1.0 mg/L as resistant. For spectinomycin, isolates with MICs of ≤ 64 mg/L were classified as susceptible and isolates with MICs of ≥ 128 mg/L as resistant. For ceftriaxone, isolates with MICs of ≥ 0.125 mg/L were classified as decreased susceptibility to ceftriaxone (DSC) and isolates with MICs of ≤ 0.06 mg/L as susceptible [18]. For azithromycin, isolates with MICs of ≥ 1.0 mg/L were classified as resistant and isolates with MICs of ≤ 0.5 mg/L as susceptible [19].

2.5. Detection of resistance plasmids

PPNG isolates were determined by the paper acidimetric method as recommended by the WHO WPR Resistance Surveillance Program [18]. WHO reference strains A and E were used as negative and positive controls, respectively. Isolates with a tetracycline MIC ≥ 16 mg/L were considered as TRNG isolates [20]. WHO reference strains A and G were used as negative and positive controls, respectively.

2.6. Extraction of plasmid DNA

Lyophilised strains were pure cultured on TM medium after resuscitation (5% CO₂ incubator at 35 °C for 18–24 h). Appropriate colonies were then selected, were eluted in centrifuge tubes with physiological saline, were centrifuged at 12 000 rpm for 3 min and were re-suspended in 50 μ L of 1% NP40 cell lysate (blow and mix). Finally, the suspension was immersed in a boiling water-bath for 10 min and was centrifuged at 12 000 rpm for 5 min. The supernatant was collected and was stored at -20 °C for further use.

2.7. Genotyping of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG)

Plasmid types of PPNG and TRNG were analysed as described previously by our team. Four pairs of PCR primers were used to amplify the *bla*_{TEM} gene of PPNG. The *tetM* gene of TRNG was amplified to identify American (AR) and Dutch (DR) variants. All primers were synthesised by Invitrogen Bio. Co. (Shanghai, China) [17]. PPNG control plasmids pJD4 (Asian type), pJD5 (African type) and pJD7 (Toronto type) were kindly provided by J.R. Dillon (University of Saskatchewan, Saskatoon, SK, Canada). In addition, WHO-G and WHO-97QA3 with AR- and DR-type plasmids, respectively, were used as TRNG control plasmids.

2.8. *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST)

The molecular characteristics of strains collected in 2013–2014 and 2017 were compared and NG-MAST analysis was performed to understand the rapid increase in prevalence of PPNG and TRNG [20,21]. DNA sequencing was conducted by Life Technologies (Carlsbad, CA, USA). Sequence types (STs) were defined using the NG-MAST database (<http://www.ng-mast.net>).

2.9. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics v.22.0 (IBM Corp., Armonk, NY, USA). A two-sided *P*-value of

<0.05 was considered statistically significant. Descriptive analyses were performed to depict the distribution of resistance characteristics and genotypes. In addition, χ^2 analysis was performed to test the trends exhibited by the resistance characteristics and PPNG and TRNG distribution in the study areas over the period 2013–2017.

3. Results

3.1. Antimicrobial susceptibility testing

The susceptibility of the *N. gonorrhoeae* isolates to six antibiotics was analysed (Table 1). All 704 *N. gonorrhoeae* isolates were highly resistant to previously recommended first-line antibiotics, including penicillin (68.2%), tetracycline (85.7%) and ciprofloxacin (98.2%). Ceftriaxone and spectinomycin appeared to be effective agents against *N. gonorrhoeae*, with susceptibility rates of 96.4% and 100%, respectively. The susceptibility rate to azithromycin was 90.7%. The penicillin resistance rate of isolates from the two cities decreased significantly from 78.4% (80/102) to 73.6% (120/163) ($P=0.001$) during the study period. The azithromycin resistance rate of isolates from the two cities decreased significantly from 11.9% (10/84) to 3.7% (6/163) ($P=0.004$) during the study period. The penicillin resistance rate of isolates collected in Zhuhai decreased significantly from 83.3% (15/18) in 2013 to 67.9% (53/78) in 2017 ($P=0.008$) and the azithromycin resistance rate of isolates collected from Guangzhou decreased significantly from 11.9% (10/84) in 2013 to 3.5% (3/85) in 2017 ($P=0.02$). However, the resistance rates of the isolates to other antibiotics remained stable during the study period. The MIC₅₀ and MIC₉₀ values (MICs required to inhibit 50% and 90% of the isolates, respectively) of spectinomycin against isolates from the two cities increased from 16 mg/L in 2013 to 32 mg/L and 64 mg/L in 2017, respectively (Table 2). Currently, ceftriaxone is widely used as a first-line treatment for gonorrhoea. The rate of DSC (MIC ≥ 0.125 mg/L) increased from 1.0% (1/102) to 4.9% (8/163) ($P=0.525$) during the study period, which requires further monitoring.

3.2. Prevalence and genotypes of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG)

Among 649 *N. gonorrhoeae* isolates, 165 (25.4%) were PPNG type and 215 (33.1%) were TRNG type, among which 87 (13.4%) were PPNG/TRNG type. The prevalence of PPNG type decreased significantly from 37.3% (38/102) in 2013 to 23.9% (39/163) in 2017 ($P=0.002$). The prevalence of TRNG types decreased significantly from 50.0% (51/102) in 2013 to 31.3% (51/163) in 2017 ($P=0.004$). The prevalence of PPNG/TRNG type decreased significantly from 23.5% (24/102) in 2013 to 11.7% (19/163) in 2017 ($P=0.017$) (Fig. 1; Table 3).

3.3. Molecular epidemiology using *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST)

The prevalence of PPNG and TRNG types in 2013–2014 were compared with those in 2017 and NG-MAST analysis was performed (Fig. 2). A total of 271 STs were identified among the 380 *N. gonorrhoeae* isolates from the two cities of Guangdong Province in 2013–2014 and 2017. Among these isolates, 145 (38.2%) new STs were genotyped for the first time in the present study, and 51 STs were shared by at least two isolates. The most prevalent STs were ST5308 ($n=10$), ST5061 ($n=7$), ST3741 ($n=6$), ST5161 ($n=6$), ST10346 ($n=6$) and ST-new4 (*porB*, 99% allele 2978; *thpB*, 21; $n=6$). The STs with the highest prevalence in 2013 were ST5161 ($n=5$) and ST2841 ($n=5$). The STs with the highest prevalence in 2014 were ST10346 ($n=6$), ST5061 ($n=4$) and ST4676 ($n=4$). The ST with the highest prevalence in 2017 was ST-new4

Table 1
Susceptibility [n (%)] to six antibiotics of *Neisseria gonorrhoeae* isolates collected in Guangzhou and Zhuhai, Guangdong Province, China, 2013–2017

Region	Year	No. of isolates	Penicillin			Tetracycline		Ciprofloxacin			Spectinomycin		Ceftriaxone		Azithromycin	
			S	I	R	S	R	S	I	R	S	R	S	DS	S	R
Guangzhou	2013	84	0 (0)	19 (22.6)	65 (77.4)	10 (11.9)	74 (88.1)	0 (0)	2 (2.4)	82 (97.6)	84 (100.0)	0 (0)	83 (98.8)	1 (1.2)	74 (88.1)	10 (11.9)
	2014	84	1 (1.2)	19 (22.6)	64 (76.2)	13 (15.5)	71 (84.5)	0 (0)	4 (4.8)	80 (95.2)	84 (100.0)	0 (0)	77 (91.7)	7 (8.3)	65 (77.4)	19 (22.6)
	2015	100	1 (1.0)	19 (19.0)	80 (80.0)	14 (14.0)	86 (86.0)	0 (0)	5 (5.0)	95 (95.0)	100 (100.0)	0 (0)	97 (97.0)	3 (3.0)	93 (93.0)	7 (7.0)
	2016	88	2 (2.3)	41 (46.6)	45 (51.1)	18 (20.5)	70 (79.5)	0 (0)	0 (0)	88 (100.0)	88 (100.0)	0 (0)	84 (95.5)	4 (4.5)	76 (86.4)	12 (13.6)
	2017	85	0 (0)	18 (21.2)	67 (78.8)	9 (10.6)	76 (89.4)	0 (0)	0 (0)	85 (100.0)	85 (100.0)	0 (0)	79 (92.9)	6 (7.1)	82 (96.5)	3 (3.5) †
Total	441	4 (0.9)	116 (26.3)	321 (72.8)	64 (14.5)	377 (85.5)	0 (0)	11 (2.5)	430 (97.5)	441 (100.0)	0 (0)	420 (95.2)	21 (4.8)	390 (88.4)	51 (11.6)	
Zhuhai	2013	18	0 (0)	3 (16.7)	15 (83.3)	1 (5.6)	17 (94.4)	0 (0)	0 (0)	18 (100.0)	18 (100.0)	0 (0)	18 (100.0)	0 (0)	– ^a	– ^a
	2014	31	0 (0)	1 (3.2)	30 (96.8)	5 (16.1)	26 (83.9)	0	0 (0)	31 (100.0)	31 (100.0)	0 (0)	31 (100.0)	0 (0)	31 (100.0)	0 (0)
	2015	41	0 (0)	20 (48.8)	21 (51.2)	0 (0)	41 (100.0)	0 (0)	0 (0)	41 (100.0)	41 (100.0)	0 (0)	39 (95.1)	2 (4.9)	38 (92.7)	3 (7.3)
	2016	95	7 (7.4)	48 (50.5)	40 (42.1)	23 (24.2)	72 (75.8)	0 (0)	0 (0)	95 (100.0)	95 (100.0)	0 (0)	95 (100.0)	0 (0)	88 (92.6)	7 (7.4)
	2017	78	0 (0)	25 (32.1)	53 (67.9) *	8 (10.3)	70 (89.7)	1 (1.3)	1 (1.3)	76 (97.4)	78 (100.0)	0 (0)	76 (97.4)	2 (2.6)	75 (96.2)	3 (3.8)
	Total	263	7 (2.7)	97 (36.9)	159 (60.5)	37 (14.1)	226 (85.9)	1 (0.4)	1 (0.4)	261 (99.2)	263 (100.0)	0 (0)	259 (98.5)	4 (1.5)	232 (94.7)	13 (5.3)
	Total	2013	102	0 (0)	22 (21.6)	80 (78.4)	11 (10.8)	91 (89.2)	0 (0)	2 (2.0)	100 (98.0)	102 (100.0)	0 (0)	101 (99.0)	1 (1.0)	74 (88.1)
2014	115	1 (0.9)	20 (17.4)	94 (81.7)	18 (15.7)	97 (84.3)	0 (0)	4 (3.5)	111 (96.5)	115 (100.0)	0 (0)	108 (93.9)	7 (6.1)	96 (83.5)	19 (16.5)	
2015	141	1 (0.7)	39 (27.7)	101 (71.6)	14 (9.9)	127 (90.1)	0 (0)	5 (3.5)	136 (96.5)	141 (100.0)	0 (0)	136 (96.5)	5 (3.5)	131 (92.9)	10 (7.1)	
2016	183	9 (4.9)	89 (48.6)	85 (46.4)	41 (22.4)	142 (77.6)	0 (0)	0 (0)	183 (100.0)	183 (100.0)	0 (0)	179 (97.8)	4 (2.2)	164 (89.6)	19 (10.4)	
2017	163	0 (0)	43 (26.4)	120 (73.6) **	17 (10.4)	146 (89.6)	1 (0.6)	1 (0.6)	161 (98.8)	163 (100.0)	0 (0)	155 (95.1)	8 (4.9)	157 (96.3)	6 (3.7) ‡	
Total	704	11 (1.6)	213 (30.3)	480 (68.2)	101 (14.3)	603 (85.7)	1 (0.1)	12 (1.7)	691 (98.2)	704 (100.0)	0 (0)	679 (96.4)	25 (3.6)	622 (90.7)	64 (9.3)	

S, susceptible; I, intermediate-susceptible; R, resistant; DS, decreased susceptibility.

^a Azithromycin was not tested against isolates from Zhuhai in 2013.

* $P=0.008$;

** $P=0.001$;

† $P=0.02$;

‡ $P=0.004$.

Table 2
Minimum inhibitory concentrations (MICs, in mg/L) of six antibiotics against *Neisseria gonorrhoeae* isolates collected in Guangzhou and Zhuhai, Guangdong Province, China, 2013–2017

Region	Year	Penicillin				Tetracycline				Ciprofloxacin			
		MIC ₅₀	MIC ₉₀	Range	R (%)	MIC ₅₀	MIC ₉₀	Range	R (%)	MIC ₅₀	MIC ₉₀	Range	R (%)
Guangzhou	2013	1	≥8	0.06 to ≥8	77.4	2	≥32	≤0.25 to ≥32	88.1	8	≥16	0.5 to ≥16	97.6
	2014	2	≥8	0.015 to ≥8	76.2	4	≥32	≤0.2 to ≥32	84.5	4	≥16	0.125 to ≥16	95.2
	2015	2	≥32	0.03 to ≥32	80.0	4	≥32	≤0.25 to ≥32	86.0	4	≥32	0.25 to ≥32	95.0
	2016	0.5	≥32	≤0.0125 to ≥32	51.1	1	≥32	≤0.25 to ≥32	79.5	8	≥32	1 to ≥32	100.0
	2017	1	≥32	0.125 to ≥32	78.8	2	≥32	≤0.25 to ≥32	89.4	4	8	1–16	100.0
Zhuhai	2013	1	≥32	0.5 to ≥32	83.3	4	≥32	≤0.25 to ≥32	94.4	4	16	2 to ≥32	100.0
	2014	2	≥32	0.5 to ≥32	96.8	1	≥32	≤0.25 to ≥32	83.9	16	16	4 to ≥32	100.0
	2015	1	≥32	0.125 to ≥32	51.2	≥32	≥32	1 to ≥32	100.0	16	≥32	4 to ≥32	100.0
	2016	0.5	≥32	≤0.0125 to ≥32	42.1	1	≥32	0.5 to ≥32	75.8	16	≥32	2 to ≥32	100.0
	2017	1	16	0.06 to ≥32	67.9	2	≥32	≤0.25 to ≥32	89.7	8	16	≤0.0125 to ≥32	97.4
		Spectinomycin				Ceftriaxone				Azithromycin			
		MIC ₅₀	MIC ₉₀	Range	R (%)	MIC ₅₀	MIC ₉₀	Range	DS (%)	MIC ₅₀	MIC ₉₀	Range	R (%)
Guangzhou	2013	16	16	≤8–32	0	0.03	0.06	≤0.008–0.125	1.2	0.25	1	≤0.03 to ≥8	11.9
	2014	16	32	1–64	0	0.03	0.06	0.002–0.125	8.3	0.5	1	≤0.03 to ≥8	22.6
	2015	16	16	8–32	0	0.03	0.06	0.004–0.06	3.0	0.25	0.5	0.06 to ≥8	7.0
	2016	16	16	8–32	0	0.03	0.06	0.004–0.125	4.5	0.125	2	≤0.03 to ≥8	13.6
	2017	32	64	32–64	0	0.03	0.06	≤0.002–0.5	7.1	0.125	0.25	≤0.03 to ≥8	3.5
Zhuhai	2013	16	16	8–16	0	0.015	0.06	0.008–0.06	0	– ^a	– ^a	– ^a	– ^a
	2014	16	16	4–16	0	0.03	0.06	0.008–0.06	0	0.25	0.25	0.05–0.5	0
	2015	16	32	≤4–32	0	0.03	0.06	0.004–0.125	4.9	0.5	0.5	0.06–1	7.3
	2016	16	16	8–32	0	0.015	0.03	0.002–0.06	0	0.25	0.25	0.003 to ≥8	7.4
	2017	32	64	16–64	0	0.03	0.06	≤0.002–0.25	2.6	0.25	0.5	0.06–1	3.8

MIC_{50/90}, MICs required to inhibit 50% and 90% of the isolates, respectively; R, resistant; DS, decreased susceptibility.

^a Azithromycin was not tested against isolates from Zhuhai in 2013.



Fig. 1. Distribution of types of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG) isolated in 2013–2017 in Guangzhou and Zhuhai, Guangdong Province, China.

($n=6$). These results show that the most prevalent STs changed on a yearly basis. Moreover, the STs were also different between Guangzhou and Zhuhai; all ST5161 and ST10346 isolates were collected in Guangzhou, and all of the ST-new4 isolates were collected in Zhuhai. All ST4676 strains isolated in Guangzhou in 2014 showed DSC (MIC ≥ 0.125 mg/L). ST1407, a widespread clone that has caused numerous cases of cephalosporin and azithromycin treatment failure, emerged in Guangzhou in 2017. The MICs of cef-

triaxone and azithromycin against this strain were 0.06 mg/L and 0.25 mg/L, respectively.

4. Discussion

The increasing antimicrobial resistance of *N. gonorrhoeae* is an important cause of rapidly growing gonorrhoea epidemics. The current findings indicate that antimicrobial resistance of gono-

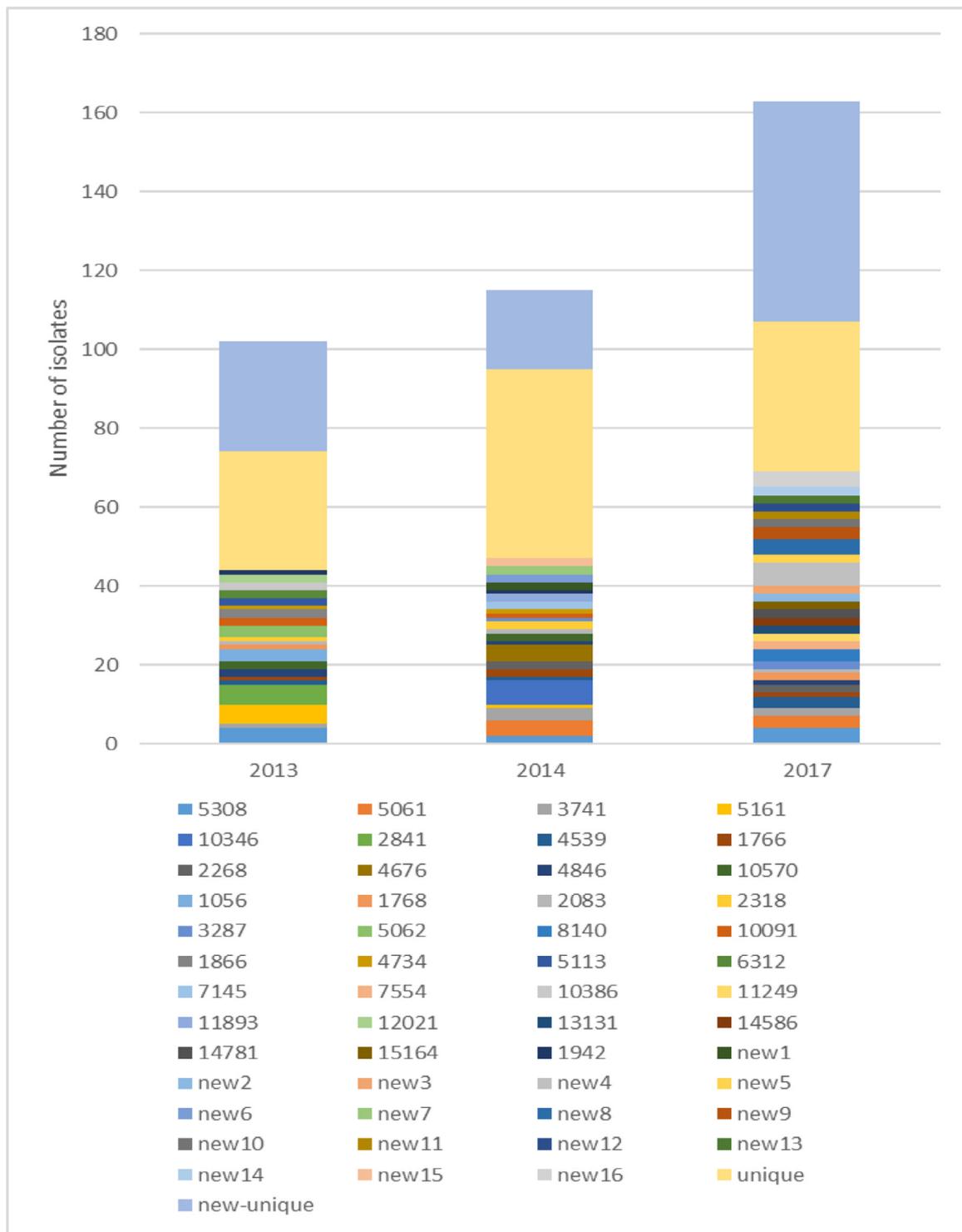


Fig. 2. Number of isolates of each sequence type (ST) according to *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) submitted in 2013–2014 and 2017 in Guangzhou and Zhuhai, Guangdong Province, China. Colour coding for the major NG-MAST STs in the data set is shown at the bottom. Isolates of unique and unique-new NG-MAST STs are grouped together in the histogram.

coccal isolates collected from Guangdong to six tested antibiotics remained high before and after dramatically increasing in 2015 (Table 1). Isolates resistant to ciprofloxacin (98.2%), tetracycline (85.7%) and penicillin (68.2%) were highly prevalent. This result is in accordance with the findings for Latin America [22], six Southeast Asian countries [23] and regions in China [16]. The MIC₅₀ and MIC₉₀ values of spectinomycin, the recommended first-line treatment for gonorrhoea, against isolates collected from

the two cities investigated in the present study increased from 16 mg/L in 2013 to 32 mg/L and 64 mg/L, respectively, in 2017. By contrast, the MIC₅₀ and MIC₉₀ values of ceftriaxone against gonococcal isolates were stable. Spectinomycin and ceftriaxone remained effective against gonococcal isolates, with susceptibility rates of 100% and 96.4%, respectively. In addition, gonococcal isolates showed increased susceptibility to penicillin and azithromycin during the study period. The prevalence of penicillin-resistant

Table 3
Distribution of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG) isolates collected in Guangzhou and Zhuhai, Guangdong Province, China, 2013–2017

Region	Year	PPNG [n (%)]			TRNG [n (%)]			PPNG/TRNG [n (%)]			
		No. of isolates	Type of TEM	No. of isolates	Type of tetM	No. of isolates	Asian/Dutch	African/Dutch	Toronto/Dutch	African/American	
			Asian	African	Toronto	Dutch	American				
Guangzhou	2013	84	23 (27.3)	6 (20.7)	0	41 (100.0)	0	16 (88.9)	2 (11.1)	0	
	2014	84	17 (68.0)	8 (32.0)	0	26 (96.3)	1 (3.7)	3 (33.3)	5 (55.6)	0	
	2015	45 ^a	7 (70.0)	2 (20.0)	1 (10.0)	12 (92.3)	1 (7.7)	5 (71.4)	0	1 (14.3)	
	2016	88	5 (62.5)	2 (25.0)	1 (12.5)	21 (91.3)	2 (8.7)	5 (62.5)	0	1 (12.5)	
	2017	85	10 (45.5)	11 (50.0)	1 (4.5)	19 (82.6)	4 (17.4)	8 (9.1)	2 (25.0)	0	
	Total	386	62 (66.0)	29 (30.9)	3 (3.2)	119 (93.7)	8 (6.3)	50 (13.0)	31 (62.0)	9 (18.0)	2 (4.0)
	Zhuhai	2013	18	8 (88.9)	1 (11.1)	0	10 (100.0)	0	6 (33.3)	0	0
		2014	31	7 (70.0)	3 (30.0)	0	6 (100.0)	0	3 (9.7)	0	0
		2015	41	10 (24.4)	9 (90.0)	0	20 (100.0)	0	7 (17.1)	0	0
		2016	95	14 (56.0)	11 (44.0)	0	23 (95.8)	1 (4.2)	9 (90.0)	0	1 (10.0)
2017		78	3 (17.6)	14 (82.4)	0	25 (89.3)	3 (10.7)	2 (18.2)	9 (81.8)	0	
Total		263	41 (57.7)	30 (42.3)	0	88 (95.5)	4 (4.5)	37 (14.1)	27 (73.0)	9 (24.3)	0
Total		2013	102	31 (81.6)	7 (18.4)	0	51 (100.0)	0	24 (23.5)	2 (8.3)	0
		2014	115	35 (30.4)	11 (31.4)	0	33 (28.7)	1 (3.0)	12 (10.4)	6 (50.0)	5 (41.7)
		2015	86	20 (23.3)	3 (15.0)	1 (5.0)	32 (97.0)	1 (3.0)	14 (16.3)	12 (85.7)	0
		2016	183	33 (18.0)	13 (39.4)	1 (3.0)	44 (93.6)	3 (6.4)	18 (9.8)	14 (77.8)	0
	2017	163	39 (23.9) [*]	25 (64.1)	1 (2.6)	44 (86.3)	7 (13.7)	19 (11.7) [†]	4 (21.1)	11 (57.9)	
	Total	649	165 (25.4)	103 (62.4)	3 (1.8)	203 (94.4)	12 (5.6)	87 (13.4)	58 (66.7)	18 (20.7)	2 (2.3)

^a Only 45 isolates in 2015 from Guangzhou were detected as PPNG and TRNG.

^{*} $P = 0.002$;

^{**} $P = 0.004$;

[†] $P = 0.017$.

isolates from Guangdong continuously decreased to 73.6% until 2017 ($P = 0.001$) as well as decreasing from 91.1% to 81.9% between 2002 and 2011 [16]. These findings correspond with those reported by the China–Gonorrhoea Resistance Surveillance Programme (China–GRSP) [24] as well as the results for Australia [25], Japan [26] and South Africa [27]. Azithromycin treatment failure was reported in 2016 in the UK [28]. Moreover, *N. gonorrhoeae* isolates with high-level azithromycin resistance were identified in England between 2014 and 2018 [29]. However, the prevalence of azithromycin-resistant isolates in Guangzhou in the current study decreased from 11.9% to 3.7%. The azithromycin resistance of isolates from Guangzhou was lower than that of isolates from Japan [26] and European and American countries [30–32]. It was reported that consumption of antibiotics in Chinese public general tertiary hospitals significantly decreased from 75.86 defined daily doses (DDD)/100 inpatient days (ID) in 2011 to 47.03 DDD/100 ID in 2014 [33], in which use of narrow-spectrum penicillins, third-generation cephalosporins and ciprofloxacin was reduced from 1.94 to 1.14, from 15.94 to 12.72, and from 0.52 to 0.28 DDD/100 ID, respectively. One of the potential reasons for decreasing antimicrobial resistance in the current study may be related to the reduction of antibiotic consumption in our region.

A family of six R-plasmids has been found to be associated with plasmid-mediated penicillin resistance. This family of R-plasmids, which confers resistance to penicillin via the production of a β -lactamase, includes three epidemic types, namely Asian (4.4 MDa, 7426 bp), African (3.2 MDa, 5599 bp) and Toronto (3.05 MDa, 5154 bp), and three sporadic types, namely Rio (2.9 MDa, 5154 bp), Nîmes (3.8 MDa, 6798 bp) and New Zealand (6.5 MDa, 9309 bp). High-level tetracycline resistance ($MIC > 8$ mg/L) in gonococci is mediated by a *tetM* determinant carried by a 25.2-MDa conjugative plasmid. The restriction endonuclease map of the 25.2-MDa conjugative plasmid from a TRNG strain originating from the USA differs from that of the 24.5-MDa conjugative plasmid from a TRNG strain isolated in the Netherlands [34]. These two types of *tetM*-carrying conjugative plasmids were designated as American and Dutch types. We previously reported that strains from Guangzhou were dominated by *N. gonorrhoeae* isolates with Asian-type plasmids [17]. Strains with African-type plasmids emerged in 2008. The prevalence of these strains then quickly increased to 14.0% in 2012 ($P < 0.001$). By contrast, all 639 TRNGs isolated over the period 2002–2012 carried Dutch-type plasmids [17]. Moreover, the African-type PPNG predominated in isolates collected from Australia (72.5%) in 2012 [35], from Argentina in 2008 (61.2%) and 2012 (73.4%) [36] and from France between 2010 and 2012 (89.2%) [37]. Moreover, 24.0% (37/154) and 76.0% (117/154) of TRNG isolates transmitted in South Africa in 2008 were identified as Dutch and American types, respectively [38]. In Bangladesh, 98.7% (377/382) and 1.3% (5/382) of TRNG isolates were identified as American and Dutch types, respectively [39]. In the present study, we found that the prevalence of African-type PPNG increased from 18.4% (7/38) to 64.1% (25/39) ($P < 0.001$), whereas that of Asian-type PPNG decreased from 81.6% (31/38) to 33.3% (13/39) ($P < 0.001$). The ratio of American-type TRNG increased from 0% (0/51) to 13.7% (7/51), whereas that of Dutch-type TRNG decreased from 100% (51/51) to 86.3% (44/51) ($P = 0.003$). These results indicate that the prevalence of PPNG and TRNG types in Guangzhou exhibited different trends over the period 2013–2017. The change in the prevalence of PPNG and TRNG types over time may be attributed to the increasing number of migrants from Africa and eastern Asian who are conducting commercial business in Guangdong.

NG-MAST revealed that the most prevalent STs were ST5308 ($n = 10$), ST5061 ($n = 7$). In addition, 145 (38.2%) novel STs were genotyped for the first time, and the most prevalent STs during 2013–2014 differed from those that were the most prevalent in

2017. The most prevalent STs in the study area were different from those in other counties. Specifically, the predominant STs in Belarus in 2010–2013 were ST1993, ST807, ST285 and ST9735, with numerous novel STs (41%) also identified [40]. Notably, the emergence of novel STs may have a considerable effect on the prevalence of gonorrhoea. Interestingly, all ST4676 strains ($n=4$) were previously unreported DSC types ($\text{MIC} \geq 0.125$ mg/L). The annual increase in the prevalence of DSC types has become an issue of global concern [41,42]. ST1407, a widespread clone that has caused numerous cases of cephalosporin and azithromycin treatment failures, was first isolated in Guangzhou in 2017. ST1407 is the most prevalent clone in Europe. It has been disseminated worldwide and has also become the predominant clone in cases from the UK and Japan and among cases reported in the USA in men who have sex with men (MSM) [43–47]. These results suggest that new genotypes of epidemic gonococcal strains have emerged in Guangdong and that partial types are closely related to strains with antimicrobial resistance. These results indicate that the increase in new STs may be the cause of gonorrhoea epidemics.

This study has several potential limitations. First, isolates were collected from economically developed areas in Guangzhou and Zhuhai during periods of high incidences of gonorrhoea. Therefore, selection bias may inevitably exist. Second, the absence of clinical information prevented us from further investigating the association of sex, age and behaviour with the prevalence of common strains of *N. gonorrhoeae*. Third, only 45 isolates collected from Guangzhou in 2015 were identified as PPNG and TRNG. The missing 55 strains may have introduced several errors to the results.

In conclusion, the prevalence of gonococcal strains with resistance to penicillin, tetracycline and ciprofloxacin remains high. African-type PPNG is becoming increasingly prevalent and is replacing Asian-type PPNG as the dominant type, and additional novel STs strains have emerged in Guangdong. Gonococcal isolates with novel genotypes might contribute to the growing epidemics of gonorrhoea in the study area.

Declarations

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