

ORIGINAL ARTICLE

Antimicrobial susceptibility of *Neisseria gonorrhoeae* strains isolated in Guangzhou, China, 1996–2001

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Objective: To investigate the in vitro antimicrobial susceptibility and resistant trends of *Neisseria gonorrhoeae* strains isolated in Guangzhou, from 1996 to 2001.

Methods: The agar dilution method was used to determine the minimum inhibitory concentrations (MICs) to four antimicrobials, penicillin G, ciprofloxacin, ceftriaxone, and spectinomycin. The resistance of all strains to four antibiotics was interpreted according to criteria used in the project of surveillance of gonococcal antibiotic susceptibility in the WHO Western Pacific Region. Penicillinase producing *N gonorrhoeae* (PPNG) was analysed by the paper acidometric method.

Results: 793 consecutive *N gonorrhoeae* isolates collected in Guangzhou were studied from 1996 to 2001. A total of 55 strains of PPNG were identified and the prevalence rapidly spread from 2% to 21.8%. Of the four antibiotics examined, ceftriaxone and spectinomycin appeared to be the most effective agents although two spectinomycin resistant strains were isolated in 1996. Their MIC₅₀, MIC₉₀, and geometric mean MIC (MIC_{mean}) were all between the sensitive ranges of the interpretative criteria and remained stable over the years. However, resistance increased continuously to penicillin G and dramatically to ciprofloxacin. In 1996–2001, MIC₅₀, MIC₉₀, and MIC_{mean} of penicillin G increased from 1 µg/ml to 2 µg/ml, 4 µg/ml to 32 µg/ml, and 0.68 µg/ml to 2.35 µg/ml, respectively; those of ciprofloxacin steeply increased from 0.12 µg/ml to 4 µg/ml, 2 µg/ml to 32 µg/ml, and 0.14 µg/ml to 2.62 µg/ml in 1996–9, respectively, and then declined slightly in 2000–1. The prevalence of resistant isolates spread from 57.2% to 81.8% for penicillin G and from 17.6% to 72.7% for ciprofloxacin over the 6 years.

Conclusion: Resistance to penicillin and ciprofloxacin increased greatly during 1996–2001. Ceftriaxone and spectinomycin should be used as the first line agents in treating gonorrhoea. It is of great importance to continuously survey the susceptibilities of *N gonorrhoeae* to antibiotics in controlling the spread of gonococcal infections.

Neisseria gonorrhoeae infections represent one of the most widely disseminated sexually transmitted diseases in China. A total of 837 400 cases of gonorrhoea were reported in 1999, which accounted for 40.7% of all STDs and increased by 32.3% over 1998,¹ Guangdong, located in the far south of China, was one of the provinces with the highest prevalence. During 1996–2000, 131 738 cases were reported, with epidemiological links to Hong Kong and other south east Asian countries because of increasing travel to and from those destinations.²

The national guideline for therapy in 1993 recommends one of the following single doses regimens as first line therapy for uncomplicated gonorrhoea: ciprofloxacin 500 mg or ofloxacin 400–600 mg orally, ceftriaxone 250 mg or spectinomycin 2 g intramuscularly. Penicillin is only recommended if resistance is known to be rare and tetracycline is chosen if the patients are allergic to these antibiotics. With the emergence and spread of resistant strains, penicillin and tetracycline are no longer listed as first line agents for treatment of gonorrhoea in the new guideline since August 2000.

Prompt and appropriate antimicrobial treatment is important in eliminating the pathogen and limiting its transmission. However, the antibiotic resistance of *Neisseria gonorrhoeae* is constantly changing as a result of the extensive use and/or abuse of antibiotics for treatment of gonorrhoea and importation of resistant strains. An early survey showed that the rate of penicillin resistant strains increased from 47.8% in 1987 to 64.5% in 1992 in China.³ Recently, the strains with high level resistance to quinolones were found in Guangzhou.⁴ Therefore, the need for epidemiological study

of this organism and surveillance of its susceptibility to common antibiotics is reinforced. A project on the surveillance of gonococcal antibiotic susceptibility in the WHO Western Pacific Region has been carried out in China. As one of the members of the research group, we annually determine the in vitro susceptibility of *N gonorrhoeae* isolates in Guangzhou, the capital city of Guangdong province. This paper analysed the prevalence of penicillinase producing *N gonorrhoeae* (PPNG), the antimicrobial susceptibilities, and resistant trends of *N gonorrhoeae* isolated in Guangzhou from 1996 to 2001.

METHODS

Bacterial isolates

In all, 793 consecutive isolates of *N gonorrhoeae* were collected from outpatients with gonorrhoea attending STD clinics of Guangdong Provincial Centers for STIs and Skin Diseases Control and Prevention, and Guangzhou Center for STD Control and Prevention from 1996 to 2001 in Guangzhou, China.

Isolation of *N gonorrhoeae*

Urethral and endocervical specimens were directly inoculated on Thayer-Martin medium (GC agar base + haemoglobin + growth supplement + VCNT) and GC agar supplemented with 10% defibrinated sheep blood. The plates were incubated at 35°C in a humid candle extinction jar for 36–48 hours. The isolates were identified as *N gonorrhoeae* on the basis of colony morphology, Gram staining, oxidase test, and carbohydrate

Table 1 Interpretation of susceptibility ($\mu\text{g/ml}$)

Antibiotic	Sensitive	Intermediate	Resistant
Penicillin	0.03	0.06–0.5	≥ 1
Spectinomycin	≤ 32	–	≥ 64
Ceftriaxone	≤ 0.03	0.06–0.5	≥ 1
Ciprofloxacin	≤ 0.03	0.06–0.5	≥ 1

degradation tests. All stains were preserved in skimmed milk and stored at -70°C before use.

Antibiotic susceptibility

All the isolates were examined for susceptibility to penicillin G, ciprofloxacin, ceftriaxone, and spectinomycin by the agar plate dilution method for minimum inhibitory concentrations (MICs).⁵ The ranges of concentration of antibiotics were as follows: penicillin G, 0.31–32 $\mu\text{g/ml}$; tetracycline, 0.5–32 $\mu\text{g/ml}$; spectinomycin, 4–128 $\mu\text{g/ml}$; ciprofloxacin, 0.15–32 $\mu\text{g/ml}$ and ceftriaxone, 0.002–1 $\mu\text{g/ml}$. The sensitivity of isolates was determined according to agar plate dilution methods similar to those in use by the Western Pacific WHO GASP programme as follows. The medium used was GC agar (Oxoid) supplemented with 10% defibrinated sheep blood. Pure subculture from 18 to 24 hours' growth on GC agar was suspended in saline and adjusted to contain approximately 10^8 cfu/ml as inoculum. A multi-point inoculator was used to inoculate the suspension onto antibiotic containing media. After overnight incubation at 35°C in a carbon dioxide incubator, the MICs were read.

Interpretation of susceptibility

Two methods were used in AHPWPR, one is the American method and other the non-American method. We apply the latter method, modified by using sheep blood instead of lysed horse blood. The laboratory obtained satisfactory results in the China GASP QA programme external quality assurance programme conducted by the Nanjing National Reference STD centre. The American method uses MIC ≥ 2 mg/l as penicillin resistance, while the non-American method standard is 1 mg/l. The criteria for resistance in this test system are shown in table 1.

WHO *N gonorrhoeae* reference strains A–E, kindly provided by Dr Ye Shunzhang, the National Center for STD control, Nanjing, were used as controls in each antimicrobial assay. Because these reference strains are all quinolone sensitive, we also reference the external QA results as control.

A β lactamase assay was performed by the paper acidometric method.⁶ WHO reference strains A and E were used as negative and positive control, respectively.

Statistics

Geometric MICs were determined using the base₂ logarithms. The Mann-Whitney U test was used for evaluating the trends of the resistant rates.

RESULTS

Among the 793 gonococcal isolates examined, 55 PPNG strains were identified by the paper acidometric method. The prevalence dramatically increased from 2% to 21.8% in 1996–2001 ($U = 6.38$, $p < 0.01$) (table 2).

The distribution of MIC₅₀, MIC₉₀, and MICmean of the four antibiotics to 793 consecutive strains collected in Guangzhou during these 6 years is summarised in table 3. Out of the four antibiotics tested, ceftriaxone and spectinomycin appeared to be the most effective agents and the MICs remained stable over the years, while MICs of penicillin G and ciprofloxacin enlarged dramatically. MIC₅₀, MIC₉₀, and MICmean of penicillin G multiplied by 2, 8, and 3.5 times from 1996 to 2001, respectively; those of ciprofloxacin by 33, 16, and 18.7 times higher in 1996–9, respectively, and declined slightly in 2000–1.

Antimicrobial susceptibilities of 793 strains, interpreted according to WHO standards, were shown in table 2. It appeared that the strains were more sensitive to ceftriaxone and spectinomycin. Only two spectinomycin resistant gonococcal strains were isolates during the 6 years. The proportion of sensitive strains to penicillin decreased from 8.4% to 0.9%, while the resistant rate increased from 57.2% to 81.8% ($U = 4.39$, $p < 0.01$). A significant trend for increasing resistance to ciprofloxacin developed dramatically from 1996 to 2001. The proportion of sensitive organisms dropped from 18.6% to 1.8%, while the resistant rate rose steeply from 17.6% to 72.7% ($U = 9.67$, $p < 0.01$).

DISCUSSION

The antibiotic resistant profile changes constantly over time. Since the emergence of PPNG in 1976 and the chromosomally mediated penicillin resistant isolate in 1983, the resistant gonococci have spread worldwide and the prevalence increased rapidly. In south east Asian, very high proportions of penicillin resistance were recorded—Korea (91%), the Philippines (89%), China (80%), Singapore (58%), Hong Kong (54%), and Vietnam (48%) in 2000.⁷ We identified 546 penicillin resistant strains out of 793 consecutive isolates in Guangzhou, in which 55 were PPNG and 491 chromosomally mediated penicillin resistant. The prevalence of PPNG were lower during the first 4 years but rose steeply from 5.4% to 21.8% ($U = 4.2$, $p < 0.01$) in the past 2 years, indicating that Guangzhou had become a highly prevalent region. The penicillin resistant rates increased from 57.2% to 72.7% in 1996–2001 and were higher than the report (66.7%) by the National Center for STD Control.³ The resistant trend was statistically significant ($U = 4.39$, $p < 0.01$). The MIC₅₀, MIC₉₀ and MICmean in 2001 were 2, 8, and 3.5 times higher than those in 1996, respectively. A high

Table 2 Comparison of susceptibility and PPNG of *N gonorrhoeae* isolated in Guangzhou during 1996–2001

Year	No	Penicillin			Spectinomycin			Ceftriaxone			Ciprofloxacin			PPNG (%)
		S (%)	I (%)	R (%)	S (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)		
1996	201*	17 (8.4)	69 (34.3)	115 (57.2)	199 (99)	2 (1)	120 (62.5)	72 (37.5)	0 (0)	37 (18.6)	127 (63.8)	35 (17.6)	4 (2)	
1997	51	2 (3.9)	13 (25.5)	36 (70.6)	51 (100)	0 (0)	40 (78.4)	11 (21.6)	0 (0)	2 (3.9)	18 (35.3)	31 (60.8)	1 (2)	
1998	203	1 (0.5)	81 (39.9)	121 (59.6)	203 (100)	0 (0)	122 (60.1)	81 (39.9)	0 (0)	16 (7.9)	64 (31.5)	123 (60.6)	7 (3.4)	
1999	167	3 (1.6)	24 (14.4)	140 (83.8)	167 (100)	0 (0)	153 (91.6)	14 (8.4)	0 (0)	5 (3.0)	31 (18.6)	131 (78.4)	9 (5.4)	
2000	61	0 (0)	17 (27.9)	44 (72.1)	61 (100)	0 (0)	50 (81.9)	11 (18.0)	0 (0)	0 (0)	15 (24.6)	46 (75.4)	10 (16.4)	
2001	110	1 (0.9)	19 (17.3)	90 (81.8)	110 (100)	0 (0)	90 (81.8)	20 (18.2)	0 (0)	2 (1.8)	28 (25.5)	80 (72.7)	24 (21.8)	

*192 strains were tested for ceftriaxone and 199 for ciprofloxacin.

Table 3 Comparison of MIC₅₀, MIC₉₀, MICmean of the four antibiotics to *N gonorrhoeae* isolated in Guangzhou during 1996–2001 ($\mu\text{g/ml}$)

Year	No	Penicillin			Spectinomycin			Ceftriaxone			Ciprofloxacin						
		Range	MIC ₅₀	MIC ₉₀	MICmean	Range	MIC ₅₀	MIC ₉₀	MICmean	Range	MIC ₅₀	MIC ₉₀	MICmean				
1996	201*	16–0.016	1	4	0.68	64–1	8	16	6.80	0.5–0.002	0.031	0.25	0.03	2–0.002	0.1	2	0.14
1997	51	16–0.008	1	4	1.01	32–8	16	16	15.36	0.5–0.004	0.031	0.06	0.02	16–0.008	1	8	1.19
1998	203	16–0.125	2	4	1.59	64–4	16	32	16.67	0.25–0.002	0.031	0.06	0.04	16–0.016	4	16	1.30
1999	167	32–0.004	2	16	2.09	32–2	16	16	13.27	0.25–0.002	0.016	0.02	0.02	32–0.002	4	32	2.62
2000	61	>32–0.06	1	32	1.74	16–4	8	16	8.96	0.25–0.002	0.031	0.06	0.021	32–0.06	1	4	1.24
2001	110	>32–0.03	2	32	2.35	16–4	8	16	8.79	0.25–0.002	0.015	0.06	0.018	32–0.03	2	8	1.44

*192 strains were tested for ceftriaxone and 199 for ciprofloxacin, *mean = geometric mean MIC.

rate (21.8%) of PPNGs in 2001 might cause the MIC increase. The high prevalence of PPNGs may cause an increase of penicillin MIC and, in addition, the widespread use of penicillin for treatment might also have contributed to the findings of a steep increase in syphilis infection in Guangzhou in recent years.

Quinolones had been recommended by the Centers For Disease Control and Prevention (CDC) for treatment of gonorrhoea since 1989.⁸ With widely use of these agents, resistance has sharply increased in many districts of south east Asia and the resistant strains have spread recently. In Hong Kong, quinolone resistant *N gonorrhoeae* (QRNG) increased from 0.5% in 1992 to 10.4% in 1994⁹ and from 60% in 1998 to 80% in 2000.⁷ Other information identified an increasing number of QRNG in Japan.¹⁰ A report from Sydney, Australia, represented 3% QRNG from 1991 to 1994, and in the first 6 months of 1995, the proportion (8%) of QRNG rose substantially.¹¹ It was considered that the appearance of QRNG in Sydney has been caused by the continuing importation of resistant strains by travellers entering or returning to Australia from Asian destinations. In Guangzhou, our finding was similar. There had been a significant increase in the rate of ciprofloxacin resistant *N gonorrhoeae* from 17.6% in 1996 to 72.7% ($U = 9.67$, $p < 0.01$) in 2001. A WHO document stated that an agent should be withdrawn from treatment regimens if the rate of 5% resistance to the agent occurs.¹² The MIC₅₀, MIC₉₀, and MICmean multiplied by 33, 16, and 18.7 times in 1996–9, respectively, and then declined slightly in 2000–1. A recent study on the correlation between the in vitro susceptibility to ciprofloxacin and treatment outcome showed that 96% isolates from patients who had treatment failure were MICs 1–32 $\mu\text{g/ml}$.¹³ The emergence and wide spread of the highly ciprofloxacin resistant strains (MIC ≥ 32 $\mu\text{g/ml}$) might mean no dose of this agent (or other quinolones) would effect a cure.

Spectinomycin and ceftriaxone are highly effective as single dose treatment for gonorrhoea. Susceptibility to ceftriaxone was proved to have risen slightly in recent years.¹⁴ In Guangzhou, the susceptibilities to both of the antibiotics remained unchanged from 1996 to 2001. The MIC₅₀, MIC₉₀, and MICmean all fell in the sensitive range of the interpretative criteria although two spectinomycin resistant strains were isolated in 1996. The results showed that spectinomycin and ceftriaxone should be the first line treatment for gonorrhoea in Guangzhou, China.

In summary, the results of this study clearly demonstrate that penicillin/ciprofloxacin resistant gonococcal strains are frequently found in Guangzhou. Local susceptibility data of periodic surveillance are useful in guiding clinicians in the use of appropriate antibiotics for the treatment of gonorrhoea.

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