

Fluoroquinolone resistance among *Neisseria gonorrhoeae* isolates from Shanghai, China: Detection of quinolone resistance-determining region mutations

Zhang Tiejun, Zhou Xiaoming¹, Zhang Jilun², Zhang Yinghu³, Ren Yanhua⁴, Chen Yue⁵, Gu Weiming⁶, Zhang Tao & Jiang Qingwu

Department of Epidemiology, School of Public Health, Fudan University, PR of China; Key Laboratory of Public Health Safety, Ministry of Education; ¹Shanghai Public Health Clinical Centre, Fudan University Affiliated, PR of China, ²Shanghai Entry-Exit Inspection & Quarantine Bureau; ³Shanghai Minghang Centre for Disease Control & Prevention; ⁴Shanghai Pudong Centre for Disease Control & Prevention, PR of China; ⁵Department of Epidemiology & Community Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Canada; ⁶Shanghai Skin Disease & Sexually Transmitted Disease Hospital, PR China

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Background & objectives: Fluoroquinolone has a broad spectrum of antimicrobial activity, and is widely used for gonorrhoea treatment. However, its efficacy can be compromised by the drug-resistance property of *Neisseria gonorrhoeae* isolates. Most resistant cases of *N. gonorrhoeae* are associated with mutations in the quinolone-resistance-determining-region (QRDR) within genes of *gyrA* and *parC*. This study was undertaken to describe resistance profile of *N. gonorrhoeae* to fluoroquinolones in Shanghai, P.R. of China, and also associated resistance mutations in *gyrA* and *parC*.

Methods: Eighty *N. gonorrhoeae* isolates were collected from Shanghai Skin Disease & Sexually Transmitted Disease Hospital or DongFang Hospital during April 2005 to April 2006 in Shanghai, P.R. of China. The minimum inhibitory concentrations (MIC) of fluoroquinolones for these isolates were determined by an agar dilution method. Mutation patterns within *gyrA* and *parC* were determined by direct sequencing or by using established restriction fragment length polymorphisms (RFLP) methods.

Results: Ninety five per cent (76 of 80) of isolates were resistant, 3.75 per cent (3 of 80) intermediate resistant, and 1.25 per cent (1 of 80) were sensitive to fluoroquinolone drug ciprofloxacin. Sequencing and RFLP analysis of *gyrA* and *parC* revealed that all resistant isolates had dual mutations of S91F and D95A/G/N in *gyrA*. Some isolates had an extra mutation within *parC* either of D86N, S87N or E91A/G. Mutation patterns for *gyrA* and *parC* were significantly ($P<0.05$) associated with MICs level.

Interpretation & conclusions: Mutations of S91F and D95A/G/N in *gyrA* combined with S87N in *parC* was the most prevalent mutation pattern of fluoroquinolone resistant *N. gonorrhoeae* isolates. This mutation pattern was associated with a high level of quinolone resistance (MIC >16.0 µg/ml) which can serve as a maker for quinolone-resistance prediction in Shanghai, P.R. of China.

Key words Fluoroquinolone - *gyrA* - *Neisseria gonorrhoeae* - *parC* - resistance

Gonorrhoea is a sexually transmitted disease due to infection by bacterium *Neisseria gonorrhoeae*. Patients are usually treated with antibiotics. In China, antibiotic-resistant *N. gonorrhoeae* isolates are common due to irregular prescription of antibiotics, and have become an important public health concern^{1,2}.

Fluoroquinolones are frequently used in treatment for gonorrhoea, with ciprofloxacin and ofloxacin being used as primary drugs in a number of countries. *N. gonorrhoeae* was highly susceptible to ciprofloxacin when this drug was first introduced in 1980s. In the last few years, a number of gonococci with decreased susceptibility or clinically significant resistance to fluoroquinolones including ciprofloxacin have been isolated all over the world³⁻⁸.

The mechanism of fluoroquinolone-resistance of *N. gonorrhoeae* has been a subject of investigation^{9,10}. One possibility is that the gonococcus involves mutations in the quinolone-resistance-determining-region (QRDR) of *gyrA* and the analogous of *parC* locus on the chromosome. These mutations result in altered *GyrA* and *ParC* proteins^{9,11,12}. Altered proteins can no longer be bound by fluoroquinolones, therefore, the drug is unable to inhibit DNA replication and bacterium becomes less susceptible. The level of drug susceptibility appears to correlate with the location and number of mutations presented¹². This mechanism is analogous to those observed in *Escherichia coli* or other bacteria⁹. This study was carried out to provide further evidence of the correlation of the pattern of mutation of *gyrA* and *parC* genes with the drug resistant of *N. gonorrhoeae* in isolates from Shanghai, P.R.China, where higher resistance to ciprofloxacin has been reported than those from other regions of the world⁶.

Material & Methods

Bacterial isolates: *N. gonorrhoeae* isolates were obtained from urethral and endocervical swabs, taken from gonorrhoea patients who had visited Shanghai Skin Disease & Sexually Transmitted Disease Hospital or DongFang Hospital during the period of April 2005 to April 2006. A total of 435 patients visited these hospitals for sexually transmitted disease treatment during the study period. Case exclusion criteria included: (i) co-infection with pathogens other than *N. gonorrhoeae*; and (ii) antibiotic treatment for the current episode of infection before enrollment. All eligible patients gave written consents for their enrollment. A total of 80 isolates were successfully

obtained from patients who met the inclusion requirements of this study. Reference strains WHO-A, B, C, D and E were provided by National Institute for Control of Pharmaceuticals and Biological Products (Beijing, P.R. of China).

Antibiotics: Ofloxacin (Daiichi Sankyo Ltd, Tokyo, Japan), lomefloxacin (Abbott Ltd, Illinois, United States) and ciprofloxacin (Bayer Ltd, Leverkusen, Germany) were provided by National Institute for Control of Pharmaceuticals and Biological Products (Beijing, P.R. of China).

Minimum inhibitory concentration (MIC): An agar dilution method recommended by the WHO Western Pacific Regional Resistance Surveillance Programme was used to determine minimum inhibitory concentration (MIC)¹³. MIC tests were performed on chocolate agar base (Oxiod Ltd, Basingstoke, United Kingdom) supplemented with 10 per cent defibrinated fresh sheep blood (Zhongqing Biotech Inc. Ltd, Shanghai, China) and 1 per cent Iso VitaleX (Oxiod Ltd, Basingstoke, United Kingdom). Agar plates were inoculated with 10⁸ cfu/ml bacteria, incubated at 36°C with 5 per cent CO₂ for 36 h. Antibiotics were diluted in agar at concentrations from 0.002 to 16.0 µg/ml for ciprofloxacin, 0.0078 to 16.0 µg/ml for ofloxacin and 0.0078 to 16.0 µg/ml for lomefloxacin. Reference strains (WHO A-E) with known MICs were co-tested with samples as control. MICs were determined as the lowest antibiotic concentration that had completely inhibited bacteria growth¹⁴.

Amplification of quinolone-resistance-determining-region: Oligo primers were synthesized by Sangon Biotech Co, Ltd. (Shanghai, P.R. of China). Primers for amplification of QRDR within *gyrA* were: Forward 5'-CGC GAT GCA CGA GCT GAA AAA-3', Reverse 5'-ATT TCG GTA TAG CGC ATG GCT G-3'; for QRDR within *parC* were: Forward 5'-GTT TCA GAC GGC CAA AAG CCC-3', Reverse 5'-GGA CAA CAG CAA TTC CGC AAT-3'. PCRs were carried on using 25 µl volume that consisted of 12.5 µl 2XPCR master mix (Fermentas UAB, Vilnius, Lithuania), 9.5 µl sterile water, 1 µl each of forward and reverse primers (0.2 µM) and 1 µl DNA template. Reaction condition was 35 cycles of denaturation for 60 sec at 94 °C, annealing for 50 sec at 52 °C, and extension for 50 sec at 72 °C.

Sequencing of *gyrA* and *parC*: PCR products were separated by 1.5 per cent agarose gel electrophoresis, purified by PCR purification Kit (QIAGEN, Hilden, Germany) and sequenced by Bigdye Terminator

v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, United States). Mutations were identified by comparing translated amino acid sequences to reference *gyrA* sequence (Genbank accession no: U08817) or *parC* sequence (Genbank accession no: U08907).

Analysis of restriction fragment length polymorphism: Restriction fragment length polymorphism (RFLP) analysis was carried out as described earlier^{15,16}. Primers used for *gyrA* PCR amplification were: *gyrA*-F 5'-CGC GAT GCA CGA GCT GAA AAA-3' and *gyrA*-*Hinf*I 5'-CCG TCT ATC AGC ACA TAA CGC ATA GCG AAA TTT TGC GCC ATA CGG ACG ATG GAG-3'. Amplicon was further digested with endonuclease *Hinf*I (Fermentas UAB, Vilnius, Lithuania) for detection of mutations at nucleotides 272 and 284. Primers used for *parC* PCR amplification were: *parC*-F 5'-AAG CCG GTG AAA TCG GCG CGC-3', paired with *ParC*-*Sal*I 5'-GAG AAT TTG GGT AAA TAC CAT CCG CAC GTC-3', *ParC*-*Pst*I 5'-GGT AAA TAC CAT CCG CAC GGC TGC-3', *ParC*-*Hinf*I 5'-AAT CCT GAG CCA TGC GCA CCA TCG AC-3' or *parC*-R 5'-GTC GCC GTC GCG CGA ACC GAA-3'. Amplicons were each digested with *Sal*I, *Pst*I or *Hinf*I (Fermentas UAB, Vilnius, Lithuania) for detection of mutations at nucleotides 256, 260 and 272 of *parC* gene.

Statistical analysis: Association of mutation patterns with fluoroquinolone resistance was examined by Mann-Whitney test and Kruskal-Wallis test. Tests were performed using Statistic Packages for Social Science 10.0 (SPSS Inc., Chicago, IL, United States).

Results

Resistance to ciprofloxacin, ofloxacin, and lomefloxacin were observed in 95.0 (76 of 80), 95.0 (76 of 80) and 97.5 (78 of 80) isolates respectively (Table I). One isolate was susceptible to ciprofloxacin with an MIC less than 0.06 µg/ml.

Together with WHO-A, 14 isolates were randomly selected for a preliminary sequencing analysis. One sensitive isolate (MIC= 0.03 µg/ml), two intermediated

resistant isolates (MIC >0.125 µg/ml and <0.5 µg/ml) and 11 resistant (MIC= 1.0-16.0 µg/ml) were included. All intermediate and resistant isolates had a common mutation S91F in *gyrA*. Twelve out of 13 intermediate and resistant isolates had a mutation at codon 95 of *gyrA*, among these there were mutations D95G (5 isolates), D95A (6 isolates) and D95N (one isolate). The other isolate R302 demonstrated a mutation A92P, which was seldom reported in the mainland China. Sequence analysis of *parC* showed a variety of mutations at codons 86, 87 and 91, while some synonymous mutations were detected in other positions (Table II). Result of 14 selected isolates, analyzed with both sequencing and RFLP method were consistent with each other (Table II). It indicated that RFLP methods could be used to detect mutations in the QRDR in the *gyrA* and *parC*, except for the A92 mutation, a seldom reported mutation site.

Since pilot sequence analysis had shown that mutations of *gyrA* and *parC* mostly took place at *gyrA* 91 and 95, *parC* 86, 87 and 91 codons, so all other isolates were re-examined by using RFLP. Theoretically, a wide type of *gyrA* gene amplified with the primers *gyrA*-F and *gyrA*-*Hinf*I could yield a 165bp product, which contains a natural *Hinf*I cleavage site at Ser 91 codon and an artificially created cleavage site at Asp 95 codon. Consequently, *Hinf*I could digest the amplified fragment to produce three products with lengths of 96 bp, 54 bp and 15 bp, respectively. When this region had mutations, *Hinf*I cleavage site at Ser 91 and/or Asp 95 would be destroyed. Therefore, digestion of PCR products with *Hinf*I would produce restriction fragment length polymorphism to show the mutations of *gyrA* gene. Representative data from the experiments are shown in Fig. a. Digestion of the wild type WHO-A resulted in 96 bp and 54 bp fragments. Isolate R302 (lane 1) yielded 111bp and 45 bp fragments, indicating only a mutation at codon 91 was detected, while a mutation at Ala 92 could not be detected. All other isolates yielded a 165 bp fragment, indicated mutations at both codon 91 and 95. In other words, all isolates, including the sensitive isolate,

Table I. Minimum inhibitory concentration (MIC) of isolates (n=80) for ciprofloxacin, ofloxacin and lomefloxacin

Antibiotics	Distribution of MIC (µg/ml)															MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
	0.0078	0.0156	0.0312	0.0625	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0		
Ciprofloxacin	0	0	1	0	0	0	3	5	44	8	5	14	0	0	0	2.0	16.0
Ofloxacin	0	0	0	0	0	1	2	1	14	43	19	0	0	0	0	4.0	8.0
Lomefloxacin	0	0	0	0	1	0	0	1	78	0	0	0	0	0	0	2.0	2.0

Table II. Mutations in *gyrA* and *parC* revealed by both preliminary sequencing analysis and RFLP analysis

Isolates	MIC ($\mu\text{g/ml}$)	Sequencing results						RFLP results	
		<i>gyrA</i>			<i>parC</i>			<i>gyrA</i>	<i>parC</i>
		91	92	95	86	87	91	Mutation site	Mutation site
WHO-A	0.016							None	None
R345	0.03			Asp to Ala				Asp95	None
D347	0.5	Ser to Phe		Asp to Gly				Ser91, Asp95	None
R307	0.5	Ser to Phe		Asp to Gly		Ser to Asn		Ser91, Asp95	Ser87
D327	1	Ser to Phe		Asp to Asn				Ser91, Asp95	None
D339	1	Ser to Phe		Asp to Ala				Ser91, Asp95	None
G313	1	Ser to Phe		Asp to Ala			Glu to Ala	Ser91, Asp95	Glu91
R302	2	Ser to Phe	Ala to Pro					Ser91*	None
D301	2	Ser to Phe		Asp to Gly				Ser91, Asp95	None
D356	2	Ser to Phe		Asp to Ala				Ser91, Asp95	None
R341	4	Ser to Phe		Asp to Ala	Asp to Asn			Ser91, Asp95	Asp86
Q317	4	Ser to Phe		Asp to Gly			Glu to Gly	Ser91, Asp95	Glu91
R306	8	Ser to Phe		Asp to Ala			Glu to Ala	Ser91, Asp95	Glu91
R335	8	Ser to Phe		Asp to Ala				Ser91, Asp95	None
R316	16	Ser to Phe		Asp to Gly		Ser to Arg		Ser91, Asp95	Ser87

MIC: value for ciprofloxacin; MIC \leq 0.06, sensitive; MIC 0.125~0.5, intermediate resistant; MIC \geq 1.0, resistant;

* Ala92 could not be detected here, because of no endonuclease recognized cleavage site

had mutations in the *gyrA* gene. All isolates resistant or intermediately resistant to fluoroquinolones had a mutation at Ser 91, and a mutation at Asp 95 or Ala 92.

Three DNA fragments can be amplified from *parC* gene with primer pairs of *parC-sal I/parCR*, *parC-Pst I/parCR* or *parCF/parC-Hinf I*. Lengths of each fragment were 132 bp, 123 bp and 105 bp, respectively. When these isolates had any mutations at codons of *parC* 86, 87 or 91, such as D86N, S87N/D or E91A/G/R91, the cleavage sites would change and the restriction enzyme digested fragments could show some polymorphisms in length. Two isolates (lane 12 and 15) (Fig. b) could not be digested by *sal I*, indicating a mutation at Asp 86. Six isolates (lane 21, 24, 27, 28, 29, and 30) (Fig. c) could not be digested by *Pst I*, indicating a mutation at Ser 87. Three isolates (lane 32, 33, and 37) (Fig. d) could not be digested by *Hinf I*, indicating a mutation at Glu 91. Overall, 12 isolates had a mutation at *parC*86, 44 isolates had a mutation at *parC*87, 7 isolates had a mutation at *parC*91.

RFLP results were summarized in Table III. It had shown MICs and mutations of *gyrA* and *parC* for all resistant isolates in this study. A significant association ($P < 0.05$) was observed between mutation patterns with the level of MICs. Isolates with mutations in *gyrA* combined with *parC*87 mutation showed a significantly ($P < 0.01$) higher level of resistance to

ciprofloxacin (MIC $>$ 16.0 $\mu\text{g/ml}$, 31.8 per cent (14 of 44)) than just with *gyrA* only mutations.

Discussion

In the last decade, the third generation cephalosporin and fluoroquinolones were recommended for the treatment of gonococcal infections worldwide. In recent years, there have been many reports on increasing number of quinolone resistant strains in United States, as well as in other countries^{6-8,17-20}, which has compromised its utility. According to the WHO Gonococcal Antimicrobial Surveillance Programme (GASP) results, the proportion of resistance was less than 16 per cent before 1995. After 1997, quinolone resistance had been increased rapidly

Table III. Minimum inhibitory concentrations (MIC) and mutation patterns for isolates (n=80)

Mutations	MIC ($\mu\text{g/ml}$)						total
	0.5	1.0	2.0	4.0	8.0	16.0	
<i>gyrA</i>	2	3	10	2	2		19
<i>gyrA+parC86</i>			9	1	1	1	12
<i>gyrA+parC87</i>	1	1	23	4	1	14	44
<i>gyrA+parC91</i>		1	3	1	1	1	7

MIC, value for ciprofloxacin

Some isolates had both mutations in *parC*86 and *parC*87, so they had been calculated twice in this Table. The sensitive isolate was not included

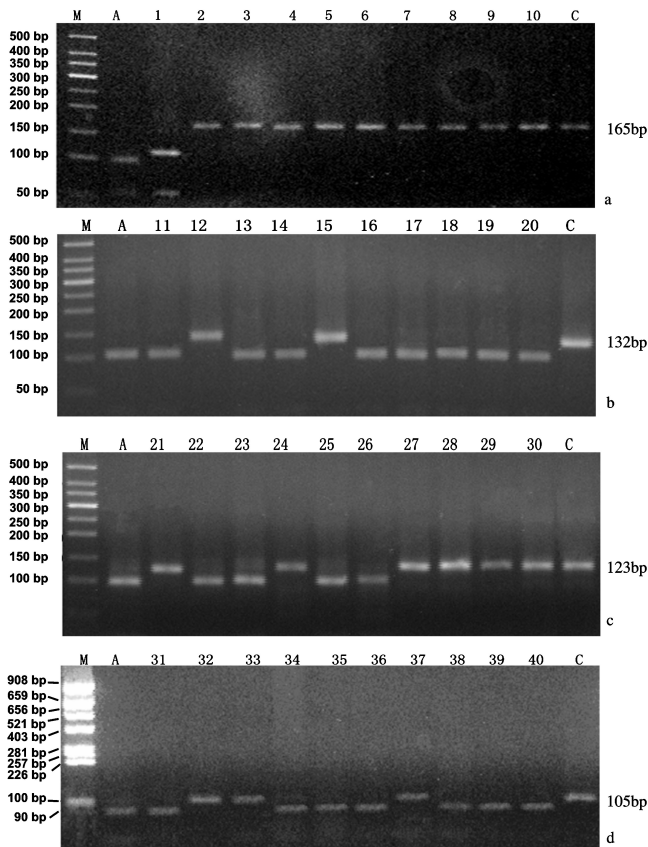


Fig. Restriction fragment length polymorphisms analysis of selected isolates. M: DNA Marker, A: RFLP for WHO-A strain, a positive digestible control. **(a)** 165bp *gyrA* digested by *Hinf* I. Lane 1: mutation in codon 92, others: mutations in codons 91 and 95. **(b)** 132bp *parC* digested by *Sal* I. Lanes 12 and 15: mutation in codon 86, others: wild type. **(c)** 123bp *parC* digested by *Pst* I. Lane 21, 24 and 27-30: mutation in codon 87, others: wild type. **(d)** 105bp *parC* digested by *Hinf* I. Lane 32, 33 and 37: mutation in codon 91, others: wild type.

and the resistance proportion reached to 80 per cent. It had been reported as high as 94.3 per cent in 2003²¹⁻²². Our results (96% resistant isolates) also showed that fluoroquinolones are no longer suitable for clinical treatment of gonorrhoea in Shanghai.

Quinolones have a bactericidal effect when these bind with two target enzymes, DNA gyrase and topoisomerase IV, which are essential for DNA replication within the cell. The lethal effect of quinolone occurs when an intermediary complex of drug and enzymes blocks its replication, and *gyrA* and *parC* genes encode two key target enzymes. The important mechanism for the fluoroquinolone resistance in the gonococcus involves mutations in the analogous region of QRDR of *gyrA* and *parC* in *Escherichia coli*, which was first reported by Belland

*et al*⁹. The pattern of mutations within *gyrA* and *parC* varied in different regions. Mutations at *gyrA* codon 91 and 95 were more often reported than mutation at *gyrA* codon 92. Compared with *gyrA*, mutations within *parC* were more variable all over the world. Mutations at *parC* codon 86, 87, and 91 were often reported²³. An earlier study in mainland China had shown that the main mutations were S91F and D95G in *gyrA*, S87N and D86N in *parC*²⁴. A study conducted in Hong Kong had shown that the most prevalent mutation pattern was S91F and D95G in *gyrA* and S87R in *parC*, which accounted for about 25 per cent of total mutations; the second most frequent pattern was S91F and D95G in *gyrA*, which accounted for 21 per cent²⁵. In this study, the major mutation pattern was S91F and D95G/A in *gyrA* and S87R/N in *parC*. Mutations in *gyrA* that lead to substitutions of phenylalanine for serine at position 91 were consistent in all isolates in this study, while Asp95 had more mutation patterns that could also be detected in the sensitive strain. Results from this study and other studies in China had suggested that Ser91 mutation might play an important role in mediating quinolone resistance in gonococci^{24,26-27}. DNA gyrase had two subunits, GyrA and GyrB, which were encoded by *gyrA* and *gyrB*, while topoisomerase IV was encoded by *parC* and *parE* genes²⁸. It may be a primary target of fluoroquinolones. Mutations in *parC* could be detected simultaneously with a mutation in *gyrA*, suggesting that this mutation may play a compensatory role in the resistance mechanism. Most mutation patterns, which had been reported in other countries, have also been detected in Shanghai^{10,23,29}.

RFLP analysis had indicated that all intermediate and resistant isolates had mutations in QRDR of *gyrA* and/or *parC*. The resistance level of isolates with a mutation in *gyrA* combined with *parC* was higher than that in those isolates with only *gyrA* mutation. It suggests that mutations in *gyrA* might determine the main resistance ability to quinolone, while additional *parC* mutation might mediate a higher level of resistance to quinolones.

In conclusion, mutation patterns revealed in this study, showed that S91F in *gyrA* could serve as a quinolone resistance marker for isolates from Shanghai, while S87R/N in *parC* could serve as a high-level quinolone resistance marker.

References

1. Ye SZ. Antibiotics treatment of *Neisseria gonorrhoeae*. *Chin J Dermatol* 2003; 36 : 296-300.

2. Ye S, Su X, Wang Q, Yin Y, Dai X, Sun H. Surveillance of antibiotic resistance of *Neisseria gonorrhoeae* isolates in China, 1993-1998. *Sex Transm Dis* 2002; 29 : 242-5.
3. World Health Organization. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific Region, 2006. *Commun Dis Intell* 2008; 32 : 48-51.
4. Goold PC, Bignell CJ. No way back for quinolones in the treatment of gonorrhoea. *Sex Transm Infect* 2006; 82 : 225-6.
5. Borgen K, van Loo I, Koedijk F, van de Laar M. Increase of gonococcal quinolone resistance in the Netherlands from 2002-2004. *Euro Surveill* 2005; 10 : E05 117.4.
6. Yang Y, Liao M, Gu WM, Bell K, Wu L, Eng NF, et al. Antimicrobial susceptibility and molecular determinants of quinolone resistance in *Neisseria gonorrhoeae* isolates from Shanghai. *J Antimicrob Chemother* 2006; 58 : 868-72.
7. Centers for Disease Control and Prevention (CDC). Increases in fluoroquinolone-resistant *Neisseria gonorrhoeae* among men who have sex with men - United States, 2003 and revised recommendations for gonorrhoea treatment, 2004. *MMWR Morb Mortal Wkly Rep* 2004; 53 : 335-8.
8. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Changing epidemiologic profile of quinolone-resistant *Neisseria gonorrhoeae* in London. *J Infect Dis* 2005; 192 : 1191-5.
9. Belland RJ, Morrison SG, Ison C, Huang WM. *Neisseria gonorrhoeae* acquires mutations in analogous regions of *gyrA* and *parC* in fluoroquinolone-resistant isolates. *Mol Microbiol* 1994; 14 : 371-80.
10. Unemo M, Sjöstrand A, Akhras M, Gharizadeh B, Lindbäck E, Pourmand N, et al. Molecular characterization of *Neisseria gonorrhoeae* identifies transmission and resistance of one ciprofloxacin-resistant strain. *APMIS Acta Pathol Microbiol Immunol* 2007; 115 : 231-41.
11. Uthman A, Heller-Vitouch C, Stary A, Bilina A, Kuchinka-Koch A, Soltz-Szots J, et al. High-frequency of quinolone-resistant *Neisseria gonorrhoeae* in Austria with a common pattern of triple mutations in *GyrA* and *ParC* genes. *Sex Transm Dis* 2004; 31 : 616-8.
12. Deguchi T, Yasuda M, Nakano M, Ozeki S, Ezaki T, Saito I, et al. Quinolone-resistant *Neisseria gonorrhoeae*: correlation of alterations in the *GyrA* subunit of DNA gyrase and the *ParC* subunit of topoisomerase IV with antimicrobial susceptibility profiles. *Antimicrob Agents Chemother* 1996; 40 : 1020-3.
13. World Health Organization. Sensitivity testing of *Neisseria gonorrhoeae*: methodologies for use by participations in the WHO Western Pacific Regional Resistance Surveillance Programme. In: *WHO/WPR Regional Antimicrobial Surveillance Working Group Meeting Proceedings*. 1992; 1 : 33-5.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. *CLSI* 2008; M100-S19.
15. Deguchi T, Yasuda M, Asano M, Tada K, Iwata H, Komeda H, et al. DNA gyrase mutations in quinolone-resistant clinical isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1995; 39 : 561-3.
16. Trees DL, Sandul AL, Whittington WL, Knapp JS. Identification of novel mutation patterns in the *parC* gene of ciprofloxacin-resistant isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1998; 42 : 2103-5.
17. Centers for Disease Control and Prevention. Increases in fluoroquinolone-resistant *Neisseria gonorrhoeae*-Hawaii and California, 2001. *MMWR Morb Mortal Wkly Rep* 2002; 51 : 1041-4.
18. Sethi S, Sharma D, Mehta SD, Singh B, Smriti M, Kumar B, et al. Emergence of ciprofloxacin resistant *Neisseria gonorrhoeae* in north India. *Indian J Med Res*. 2006; 123 : 707-10.
19. Ray K, Bala M, Kumar J, Misra RS. Trend of antimicrobial resistance in *Neisseria gonorrhoeae* at New Delhi, India. *Int J STD AIDS* 2000; 11 : 115-8.
20. Farhi D, Gerhardt P, Falissard B, Poupet H, Poyart C, Dupin N. Increasing rates of quinolone-resistant *Neisseria gonorrhoeae* in Paris, France. *J Eur Acad Dermatol Venereol*. 2007; 21 : 818-21.
21. (No author Listed). The Gonococcal Antimicrobial Surveillance Programme (GASP). WHO western Pacific region, 1995. *Wkly Epidemiol Rec* 1997; 72 : 25-7.
22. WHO Western Pacific Gonococcal Antimicrobial Surveillance Programme. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific Region, 2003. *Commun Dis Intell* 2005; 29 : 62-4.
23. Giles JA, Falconio J, Yuenger JD, Zenilman JM, Dan M, Bosh MC. Quinolone resistance-determining region mutations and por type of *Neisseria gonorrhoeae* isolates: resistance surveillance and typing by molecular methodologies. *J Infect Dis* 2004; 189: 2085-93.
24. Wang SC, Liu YF, Su MQ, Wang Q. Correlation of *in vitro* susceptibilities to quinolones of naturally occurring quinolone-resistant *Neisseria gonorrhoeae* strains isolated with changes in *gyrA* and *parC* in China. *Di4 Jun Yi Da Xue Bao* 2001; 22 : 2257-61.
25. Kam KM, Kam SS, Cheung DT, Tung VW, Au WF, Cheung MM. Molecular characterization of quinolone-resistant *Neisseria gonorrhoeae* in Hong Kong. *Antimicrob Agents Chemother* 2003; 47 : 436-9.
26. Zou MX, Xia ZD, Chen SZ, Tang Y, Liu HL, Zhang GQ. Correlation between fluoroquinolone resistance and mutations of *Neisseria gonorrhoeae gyrA* and *parC* genes. *Chin J Dermatol* 2002; 35 : 199-202.
27. Wang B, Xu JS, Wang CX, Mi ZH, Pu YP, Hui M, et al. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolated in Jiangsu Province, China, with a focus on fluoroquinolone resistance. *J Med Microbiol*. 2006; 55 : 1251-5.
28. Lindback E, Rahman M, Jalal S, Wretling B. Mutations in *gyrA*, *gyrB*, *parC* and *parE* in quinolone-resistant strains of *Neisseria gonorrhoeae*. *Acta Pathol Microbiol et Immunol* 2002; 110 : 651-7.
29. Shigemura K, Shirakawa T, Okada H, Hinata N, Acharya B, Kinoshita S, et al. Mutations in the *gyrA* and *parC* genes and *in vitro* activities of fluoroquinolones in 91 clinical isolates of *Neisseria gonorrhoeae* in Japan. *Sex Transm Dis* 2004; 31 : 180-4.

Reprint requests: Dr Zhou Xiaoming, Department of Epidemiology, Shanghai Public Health Clinical Centre
CaoLang Road 2901, Shanghai 201508, P.R. of China
e-mail: xmzhou@shmu.edu.cn, ztj1127@yahoo.com.cn