ORIGINAL ARTICLE

Incidence, epidemiology and evolution of reduced susceptibility to ciprofloxacin in *Neisseria gonorrhoeae* in Korea

Clin Microbiol Infect 1998; 4: 627–633

Kyungwon Lee[1], *Yunsop Chong*[1], *L. Erdenechemeg*[2], *Kyung Soon Song*[1] *and Kwang Hun Shin*[3]

[1]Department of Clinical Pathology, Yonsei University College of Medicine, Seoul, Korea;
[2]Department of Microbiology, National University of Mongolia, Ulaanbaatar, Mongolia; and
[3]National Institute of Health, Seoul, Korea

Objective: To verify the decrease of susceptibility to ciprofloxacin in *Neisseria gonorrhoeae*, determine the size of the recently reported new β-lactamase plasmid and explain the high prevalence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG).

Methods: Gonococci were isolated from prostitutes in Korea. Antimicrobial susceptibility was tested by NCCLS disk diffusion and agar dilution methods. Plasmid was isolated by an alkaline lysis method. Patterns of *Nhe*I-digested genomic DNA were compared after pulsed-field gel electrophoresis (PFGE).

Results: The minimum inhibitory concentration of ciprofloxacin for 50% of the isolates rose from 0.015 mg/L in 1993 to 0.12 mg/L in 1996. The proportion of PPNG remained at 70% or over during the 5-year period. The size of a novel β-lactamase plasmid, first reported in 1994, was determined to be approximately 3.2 MDa, and 48% of the PPNG isolates contained it. Twelve of 50 isolates had the same PFGE pattern and nine others another pattern.

Conclusion: The rapid decrease of fluoroquinolone-susceptible gonococci suggests that in the near future the drug may become less useful for gonorrhea treatment. The new 3.2-MDa plasmid may have been introduced as a result of the recent increase in overseas travel. The PFGE pattern suggests that high prevalence of PPNG may be due to dissemination of a few resistant clones among the high-risk groups.

Key words: *Neisseria gonorrhoeae*, β-lactamase, ciprofloxacin resistance, plasmid

INTRODUCTION

Neisseria gonorrhoeae remains a frequent cause of sexually transmitted disease worldwide. The antimicrobial resistance of gonococci is a major obstacle in the control of gonorrhea [1]. Gonococci are constitutively competent to uptake both plasmid and chromosomal DNA [2]. Therefore, we should expect to observe resistant gonococci when selective pressure of antimicrobial agents exists.

Resistant gonococci were very prevalent in Southeast Asia and Japan [3]. The failure of penicillin therapy of gonorrhea occurred in 1958 in Korea [3], when the resistance was not well recognized in other countries [4]. After the first strain of penicillinase-producing *N. gonorrhoeae* (PPNG) in Korea was isolated in 1979 [5], the proportion steadily increased to reach over 30% in the early 1980s among those strains isolated from patients in a general hospital [6] and those from

*Corresponding author and reprint requests:
Yunsop Chong, Department of Clinical Pathology, Yonsei University College of Medicine, CPO Box 8044, Seoul, Korea.
Tel: +82 02 361 5862 Fax: +82 02 313 0956
E-mail: whonetkor@yumc.yonsei.ac.kr
Accepted 13 May 1998

men in a sexually transmitted disease clinic [7]. The proportion of PPNG was also high among strains isolated from US Army personnel in Korea [8].

Recent surveillance of the susceptibility of the strains by the disk diffusion test showed that the resistance pattern of the strains from the prostitutes in Korea was somewhat different from that in the rest of the western Pacific region: continued high prevalence of PPNG strains and scarcity of high-level tetracycline-resistant strains [9]. As in other countries [1], a rapid decrease of ciprofloxacin-susceptible strains was noted, but the result has not yet been confirmed by dilution test.

In contrast to the patterns in other Asian countries, only the 4.4-MDa β-lactamase plasmid was reported in Korea, until the presence of a smaller plasmid was reported in 1994 [10], requiring confirmation of the finding. In Germany, pulsed-field gel electrophoresis (PFGE) analysis of genomic DNA of PPNG suggested that a restricted number of clones was spread throughout the prostitute population [11]. In Korea, the main source of gonorrhea was considered to be prostitutes, but there has not been such molecular epidemiologic analysis of the isolates by the recently available PFGE technique.

The aim of this study was to verify, by the agar dilution test, the decreased susceptibility of gonococci to ciprofloxacin and the scarcity of high-level tetracycline-resistant *N. gonorrhoeae* (TRNG), to determine the size of the recently recognized β-lactamase plasmid, and to investigate, by the PFGE pattern of endonuclease-digested genomic DNA, the possibility that the high prevalence of PPNG strains is due to the spread of a limited number of related clones from prostitutes.

MATERIALS AND METHODS

Gonococcal isolates

The gonococci used for this study were isolated from prostitutes who attended the control program of sexually transmitted disease in Seoul, Korea, 1992–97. Modified Thayer-Martin medium was used for the isolation, and the species identification was based on morphology and conventional cultural and biochemical tests [12].

Susceptibility testing

The NCCLS disk diffusion susceptibility [13] was tested immediately after the isolation. GC II agar base supplemented with 1% IsoVitaleX (Becton Dickinson, Cockeyville, MD, USA) was used for susceptibility testing. Antimicrobial disks and β-lactamase disks were obtained from Becton Dickinson (Cockeyville, MD, USA).

The strains which were kept frozen in skimmed milk were used for agar dilution testing [14]. The antimicrobial agents used were: penicillin G (Sigma Chemical Co., Saint Louis, MO, USA), ceftriaxone (Hanmi Pharmaceutical, Seoul, Korea), spectinomycin (Upjohn Co., Kalamazoo, MI, USA), tetracycline (Pfizer Korea, Seoul, Korea), and ciprofloxacin (Miles Pharmaceutical, West Point, CT, USA). Inocula were prepared from overnight culture on chocolate agar and diluted to obtain approximately 10^4 CFU when inoculated with a Steers replicator (Craft Machine Inc., Woodline, PA, USA). The inoculated plates were placed in a 5% CO_2 incubator at 35°C for 24 h before the results were read. *N. gonorrhoeae* ATCC 49226 was used for quality control.

Plasmid isolation and PFGE

Plasmids were isolated by the alkaline lysis method [15] and the size was estimated by comparing the electrophoretic mobility with those of *Escherichia coli* V517. For PFGE, the method of Birren and Lai [16] was used with some modifications. Briefly, to prepare the plugs, one loopful of the cells grown overnight on chocolate agar was suspended in 1 mL of saline EDTA solution. The genomic DNA was digested with *Nhe*I (Takara, Tokyo, Japan) for about 18 h at 35°C. A CHEF DR II instrument (Bio-Rad, Hercules, CA, USA) was used to separate the fragments, with switch times of 0.5 s and 54 s and a running time of 20 h at 6 V/cm. The band patterns were compared according to the recommendations of Tenover et al [17].

RESULTS

Susceptibility

Among the 754 strains of gonococci isolated in 1992–96, 70–76% were PPNG (mean 71.4%), depending on the year of isolation (Table 1). The disk test showed that all of the isolates were susceptible to ceftriaxone and to spectinomycin, but none were susceptible to penicillin G and to low-level tetracycline. The proportion of TRNG strains was 2.8%. Over the years, the rates of susceptibility to these antimicrobial agents remained similar; however, a marked decrease of ciprofloxacin-susceptible isolates was noted, from 91% in 1992 to 46% in 1996.

The agar dilution test showed that the MIC range, MIC_{50} and MIC_{90} of penicillin G, ceftriaxone and tetracycline for the strains isolated in 1993 were similar to those in 1996 (Table 2). MIC ranges and MIC_{90} values of ciprofloxacin for both of the strains isolated

Table 1 Antimicrobial susceptibility of *N. gonorrhoeae* tested by disk diffusion test

Year (No. of isolates)	% of isolates with: Penicillin I	R	PPNG	Tetracycline I	R	TRNG	Ciprofloxacin S
1992 (43)	16	84	70	7	93	5	91
1993 (225)	10	90	71	<1	100	7	76
1994 (192)	12	88	70	<1	100	2	68
1995 (95)	10	90	76	0	100	0	58
1996 (199)	10	90	70	<1	100	1	46
Average	11.6	88.4	71.4	1.0	98.6	3.00	67.8

PPNG, penicillinase-producing *N. gonorrhoeae*; TRNG, high-level tetracycline-resistant *N. gonorrhoeae*; S, susceptible; I, intermediate; R, resistant.
All of the isolates were susceptible to ceftriaxone and all isolates tested for spectinomycin in 1995 and 1996 were susceptible. None of the isolates were susceptible to penicillin G, i.e. they were either β-lactamase producers or had altered penicillin-binding proteins.

Table 2 Antimicrobial susceptibility of *N. gonorrhoeae* isolated in 1993 and 1996 by agar dilution test

Antimicrobial agents	Year (No. of isolates)	MIC (mg/L)[a] Range	50%	90%	% with: S	I	R
Penicillin G	1993 Non-PPNG (24)	0.5–2	2	2	0	42	58
	PPNG (24)	64 to ≥128	≥128	≥128	0	0	100
	1996 Non-PPNG (13)	0.12–2	2	2	0	42	58
	PPNG (29)	16 to ≥128	≥128	≥128	0	0	100
Ceftriaxone	1993 (48)	≤0.008–0.25	0.03	0.12	100	NA	0
	1996 (43)	≤0.008–0.12	0.03	0.06	100	NA	0
Spectinomycin	1996 (43)	8–64	16	32	100	0	0
Tetracycline	1993 (48)	2–16	8	8	0	0	100
	1996 (43)	1–16	4	8	0	2	98
Ciprofloxacin	1993 (48)	≤0.008–1	0.015	0.5	73	23	4
	1996 (43)	≤0.008–1	0.12	0.5	39	59	2

[a] 50% and 90%, MICs at which 50% and 90% of isolates, respectively, were inhibited.
S, susceptible; I, intermediate; R, resistant; NA, not applicable; PPNG, penicillinase-producing *N. gonorrhoeae*.

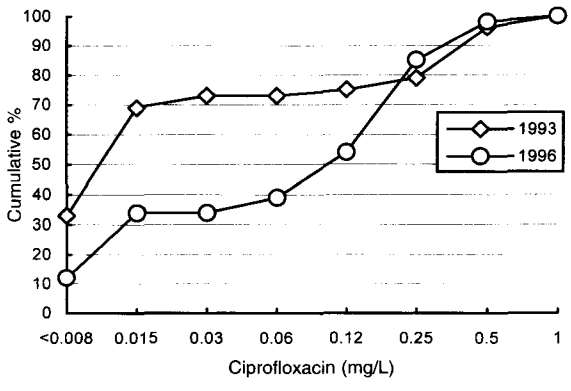

Figure 1 Cumulative percentage of *N. gonorrhoeae* isolates inhibited by ciprofloxacin. Although MIC ranges were the same for the isolates in 1993 and 1996, as the lowest concentration tested was 0.008 mg/L, an increase of MIC_{50} from 0.015 mg/L to 0.12 mg/L was noted, resulting in a decrease of susceptible isolates from 73% to 39%.

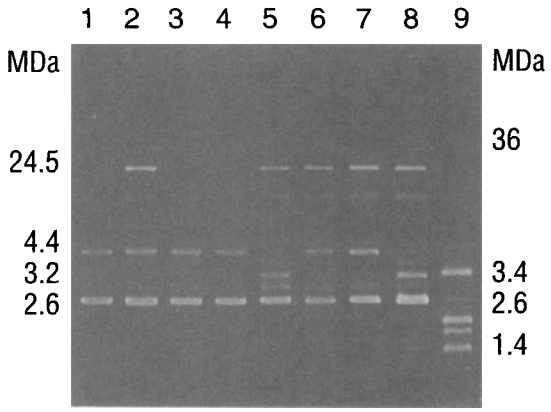

Figure 2 Plasmid profile of PPNG strains showing 4.4-MDa (lanes 1–4, 6, 7) and 3.2-MDa (lanes 5 and 8) β-lactamase plasmids and 24.5-MDa conjugative plasmid (lanes 2, 5–7). Lane 9 is *Escherichia coli* V517 plasmid size marker.

Table 3 Plasmid profile of penicillinase-producing *N. gonorrhoeae* isolated in 1993 and 1997

Year of isolation	No. of isolates tested	% of isolates with indicated plasmid (MDa)			
		24.5	4.4	3.2	2.6
1993	114	75.4	49.1	50.9	100
1997	16	75.0	75.0	25.0	100
Average	130	75.2	62.1	38.0	100

Table 4 Predominant PFGE patterns of endonuclease-digested genomic DNA of *N. gonorrhoeae*

Organism (No. of isolates)	No. (%) of isolates with PFGE pattern		
	C1–C4	G1–G4	Others
PPNG (44)	7 (16)	22 (50)	15 (34)
Non-PPNG (13)	6 (46)	4 (31)	3 (23)
Total (57)	13 (23)	26 (46)	18 (32)

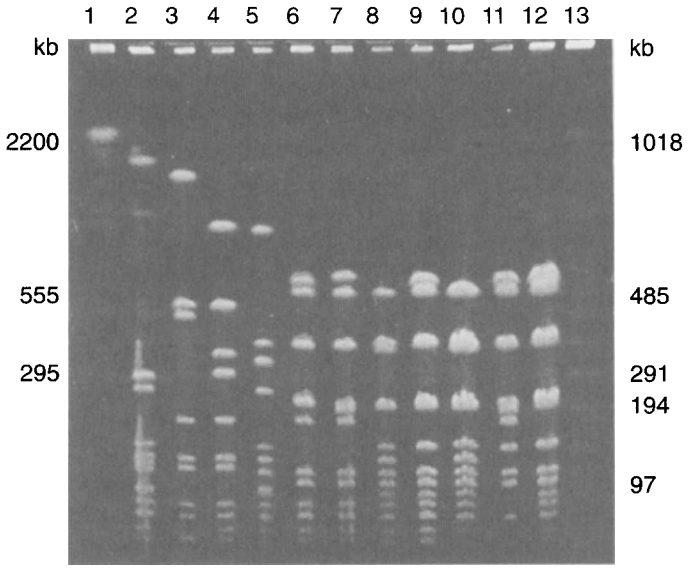

Figure 3 PFGE separation of *Nhe*I-digested chromosomal DNA of *N. gonorrhoeae*. Patterns for only 11 strains are shown. Molecular markers are chromosome of *Saccharomyces cerevisiae* (lane 1) and concatemers of λ phage DNA (lane 13).

in 1993 and 1996 were equal, ≤0.008–1 mg/L and 0.5 mg/L, but MIC_{50} values were 0.015 mg/L and 0.12 mg/L, respectively. The ciprofloxacin-susceptible isolates decreased from 73% in 1993 to 39% in 1996, but resistance rates remained similar, 4% and 2%, respectively (Table 2; Figure 1).

Plasmid profile

β-Lactamase plasmids of 4.4 MDa were detected from 68 (52.3%) of the 130 PPNG strains isolated in 1993 and 1997. The smaller β-lactamase plasmid found in the rest of the PPNG strains (62; 47.7%) was estimated to be 3.2 MDa. Among the PPNG strains, 98 (75.4%) also carried 24.5-MDa plasmids (Table 3; Figure 2).

PFGE

Fifty-seven isolates were tested for PFGE patterns of *Nhe*I-digested genomic DNA (Figures 3 and 4). Twelve isolates (lane 13; G1; 10 PPNG and two non-PPNG) showed one pattern, and nine isolates (lane 9; C1; four PPNG and five non-PPNG) the other pattern (Table 4). When subtypes were included together, 26 (46%; lanes G1-G4) and 13 (23%; lanes C1-C4) of the strains were considered to be closely related.

DISCUSSION

None of the strains tested in this study showed susceptibility to penicillin G disks; that is, they were either intermediate or resistant (Table 1). Among the penicillin-non-susceptible isolates, 71.4% were β-lactamase producers. Therefore, the resistance of the remaining strains was probably due to altered penicillin-binding proteins. Gonococci were extremely susceptible to various antimicrobial agents, including penicillin G. In Korea, treatment failure of gonorrhea began to

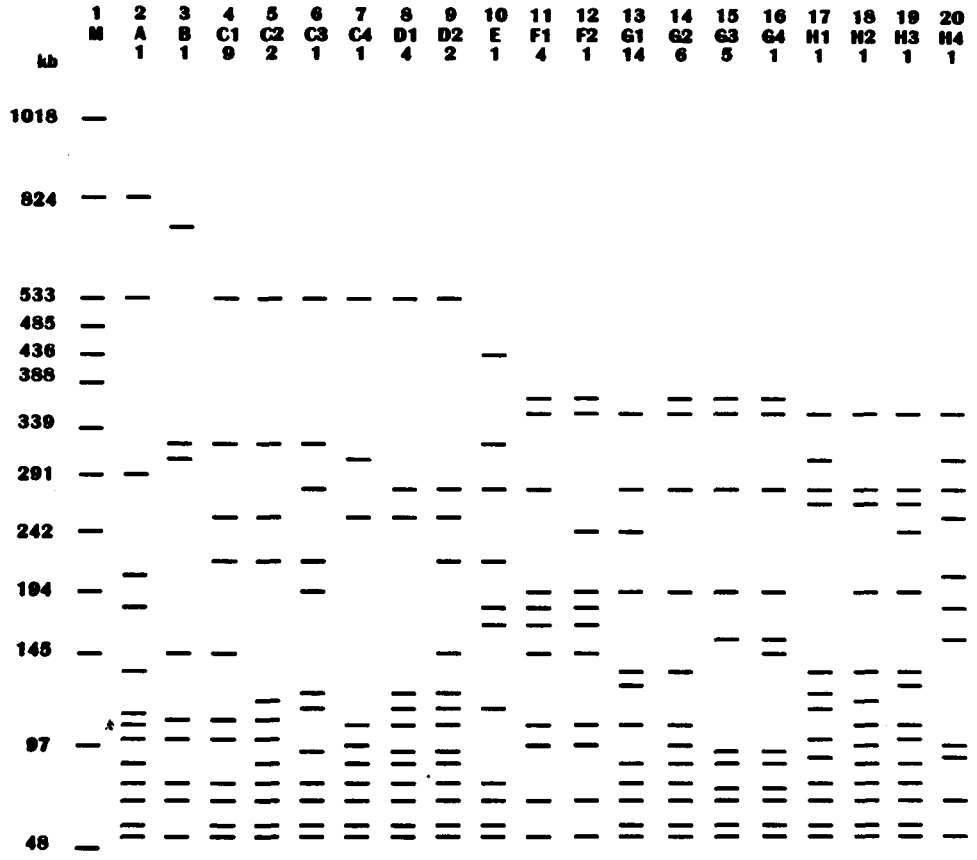

Figure 4 A schematic presentation of PFGE patterns of *Nhe*I-digested genomic DNA of *N. gonorrhoeae*. Prevalent patterns were G1 (lane 13; 14 isolates) and C1 (lane 4; nine isolates). When subtypes were included together, 26 (46%; G1–G4) and 13 (23%; C1–C4) of the strains were interpreted to be closely related clones.

appear in the late 1950s [3], but such reports were not well accepted [4]. In 1966–67, only 7.7% of the strains isolated from prostitutes were inhibited by 0.06 mg/L penicillin G [18]. PPNG, which began to be isolated in 1979, became very prevalent. Among the strains that were mostly from women patients in a general hospital, the proportion became 34% in 1982 [6], while among those from male patients in a sexually transmitted disease clinic, the rate rose to 43% in 1985 [7]. Among the isolates from US Army personnel in Korea, a rate of 46% was reported in 1982 [8]. In Korea, the main source of male gonorrhea was considered to be prostitutes. Therefore, it may not be unusual that the proportions of PPNG strains from the three sources were similar.

The proportion of PPNG was high in many Asian countries compared to that in the USA and some other regions of the world [9,19]. However, decreases of PPNG have been reported in some countries: from 71% in 1983 to 28% in 1990 in Bangkok, Thailand [20], a dramatic decrease from 25.5% in 1993 to 4.3% in 1994 in Hong Kong [21], and a decrease to 2.5% in Japan [22]. In the USA the proportion of PPNG decreased from 11% in 1991 to 2.1% in 1994 [19]. The decrease was considered to be due to the use of drugs other than penicillin for the treatment of gonorrhea. Also, it was suggested that use of fluoroquinolone possibly cured β-lactamase plasmids [19]. Among the isolates with ciprofloxacin MICs ≥0.125 mg/L, 9% had plasmid-mediated resistance to penicillin or tetracycline, compared to 16.2% of the ciprofloxacin-susceptible isolates [19]. In Korea, fluoroquinolone became widely used for various infections, but the proportion of PPNG remained high, as was the case in the Philippines and Singapore, where the rates were 76% and 54%, respectively, in 1996 [9]. Self-medication among sex industry employees in the Philippines was cited as a significant factor in fostering antibiotic resist-

ance [23]. The situation should be similar in Korea. It may be interesting to observe future trends of prevalence of PPNG and fluoroquinolone-resistant strains.

Spectinomycin was first used on a wide scale in Korea and in the Philippines in 1981 as the primary drug for treatment of gonorrhea [23]. In this study, all of the gonococci isolated during 1992–96 were susceptible to spectinomycin, while none of them were susceptible to tetracycline. In the USA, Fox et al [19] reported that ceftriaxone MIC_{50}, MIC_{90} and MIC range did not change over time, although strains for which the MIC was >0.25 mg/L were noted. In our study, the MIC range of ceftriaxone was similar to those in the USA, but isolates for which the MIC was >0.25 mg/L were not found. Therefore, in Korea, either spectinomycin or ceftriaxone may be used when the susceptibility of the isolate to other antimicrobial agents is unknown.

Fluoroquinolone is a very active drug against gonococci and is the recommended drug for the treatment of uncomplicated gonorrhea in the USA [24]. However, reports of failure of ciprofloxacin therapy in male urethral gonorrhea began to appear [1]. When 250 mg ciprofloxacin was used to treat gonorrhoea, strains with MICs of 0.06–0.25 mg/L were isolated from infections that did not respond to treatment [1]. Rapid increases of strains with intermediate resistance have been reported from many countries. In 1994–95, the proportions of strains exhibiting decreased susceptibility to ciprofloxacin were approximately 36%, 54% and 22% in Hong Kong, the Philippines, and Thailand, respectively [1].

The disk test has been used for the surveillance of ciprofloxacin-resistant gonococci in the Western Pacific region [9]. In our study too, a rapid decrease of susceptible isolates from 91% in 1992 to 46% in 1996 was noted. However, as the resistance breakpoint was not decided until 1995 [25], and as the disk test may not discriminate resistant and intermediate strains, in this study the susceptibilities of some of the isolates were tested by the agar dilution method. Recent reports showed that, in Hong Kong, the strains resistant to 0.1 mg/L fluoroquinolone increased to 85.2% in 1994 [21], while in Japan the MIC_{90} has recently increased 8–16-fold [22]. In our present study, the susceptible strains decreased from 73% in 1993 to 39% in 1996, which is not significantly different from results obtained by disk test. However, most of the non-susceptible strains were intermediate, and only 4% and 2% of the isolates, respectively, were resistant, in contrast to the rate of 16% obtained by the disk test [9]. In our study, all of the isolates were inhibited by ≤1 mg/L ciprofloxacin, while in Japan and in other countries an MIC of up to 8 mg/L has been reported [1].

Evolution of β-lactamase genes carrying plasmids has been known [26]. It was considered that the 4.4-MDa plasmid is the Asian type and the 3.2-MDa plasmid the African type. Interestingly, in Korea, only the 4.4-MDa plasmid had been reported [27] until another plasmid was reported in 1994 [10]. To confirm the presence of the new plasmid, we tested more strains and found that the size was approximately 3.2 MDa, rather than 3.02 MDa. A significant proportion of the PPNG strains carried the plasmid. In the Philippines, the 3.2-MDa plasmid was known to be prevalent, while in Taiwan the 3.05-MDa plasmid was reported [28]. The presence of the 3.2-MDa plasmid may suggest that the type was introduced as a result of the frequent traveling of Korean people, especially to Southeast Asia, since the early 1990s.

In Frankfurt, Germany, PFGE analysis suggested that a restricted number of resistant gonococcal strains were spread throughout the prostitute populations [11]. Our PFGE pattern of the endonuclease-digested genomic DNA of gonococci from prostitutes showed that two clones were predominating. This may suggest that the continued high prevalence of PPNG among the strains isolated from a male sexually transmitted disease clinic [7] and those isolated mainly from female patients in a general hospital [6] is due mostly to the spread of a few resistant clones from the prostitutes.

In conclusion, in Korea, the continued prevalence of PPNG and recent increases in strains with intermediate resistance to fluoroquinolones may suggest difficulties in the treatment of gonorrhea in the future with both of these drugs. The recently found PPNG strain with the 3.2-MDa plasmid may have been introduced by travelers, and the prevalence of strains with related PFGE patterns suggests the dissemination of a few resistant clones among high-risk patients and prostitutes.

Acknowledgments

Part of this study was reported at the 6th International Congress for Infectious Diseases, Prague, Czech Republic, 1994. We would like to thank Professor J. K. Davies, Department of Microbiology, Monash University, Clayton, Victoria, Australia for the confirming the size of the 3.2-MDa plasmid, and Dr John Tapsall, Prince of Wales Hospital, Sydney, Australia for providing quality control strains. We also like to thank Miss Young Hee Seo for her technical assistance.

References

1. Knapp JS, Fox KK, Trees DL, Whittington WL. Fluoroquinolone resistance in *Neisseria gonorrhoeae*. Emerg Infect Dis 1997; 3: 33–9.
2. Biwas GD, Thomson SA, Sparling PF. Gene transfer in *Neisseria gonorrhoeae*. Clin Microbiol Rev 1989; 2(suppl): S24–8.

3. Epstein E. Failure of penicillin in treatment of acute gonorrhea in American troops in Korea. JAMA 1959; 169: 1055–9.
4. Gangrosa EJ. Validity of reports of penicillin-resistant gonococci. JAMA 1960; 173: 1808–10.
5. Chong Y, Park HJ, Lee SY, Ahn DW. Isolation of beta-lactamase-producing *Neisseria gonorrhoeae*. Yonsei Med J 1979; 20: 133–7.
6. Kwon Y-J, Chong Y, Lee SY. Isolation of *Neisseria gonorrhoeae* at Yonsei Medical Center in 1975–84 and penicillinase production and antimicrobial susceptibility of the isolates. Yonsei Med J 1985; 20: 163–71.
7. Kim JH, Yoon JY, Hur H, Yang IS, Kim JH. Prevalence of PPNG in Seoul (1985). J Kor Soc Chemother 1987; 5: 5–7.
8. Piziak MV, Woodbury C, Berlinger D, et al. Resistance trends of *Neisseria gonorrhoeae* in the Republic of Korea. Antimicrob Agents Chemother 1984; 25: 7–9.
9. WHO Regional Office for Western Pacific. Antibiotic susceptibility of *Neisseria gonorrhoeae* 1992–1995. Report of WHO Regional Office for the Western Pacific, Manila, Philippines, 1997.
10. Lee K, Chong Y, Kwon OH, Shin KH. Antimicrobial susceptibility and plasmid profile of *Neisseria gonorrhoeae* isolated in Korea [abstract 832]. In: Program and abstracts of the 6th International Congress for Infectious Diseases, Prague, Czech Republic. Boston, MA: International Society for Infectious Diseases, 1994: 267.
11. Schaefer V, Enzensberger R, Schnider C, Rickmann J, Nitschke-Oezbay H, Brade V. Epidemiology of penicillin-resistant *Neisseria gonorrhoeae* in Frankfurt, Germany. Eur J Clin Microbiol Infect Dis 1995; 14: 914–18.
12. Morrelo JA, Janda WM, Doern GV. *Neisseria* and *Branhamella*. In Balows A, Hausler WJ Jr, Hermann KL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology. Washington, DC: American Society for Microbiology, 1991: 258–76.
13. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard. M2-A5. Villanova, PA: NCCLS, 1993.
14. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. M7–A3. Villanova, PA: NCCLS, 1993.
15. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual, 2nd edn. Plain View, NY: Cold Spring Harbor Laboratory Press, 1989: 1.25–1.28.
16. Birren B, Lai E. Pulsed-field gel electrophoresis. A practical guide. San Diego: Academic Press, 1993: 25–74.
17. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strains typing. J Clin Microbiol 1995; 33: 2233–9.
18. Cho SD, Cho SY, Chong Y. Penicillin sensitivity of *Neisseria gonorrhoeae* strains isolated in Chinhae, Korea. J Rep Kor Naval Med Corps 1967; 12: 75–8.
19. Fox KK, Knapp JS, Holmes KK, et al. Antimicrobial resistance in *Neisseria gonorrhoeae* in the United States, 1988–1994: the emergence of decreased susceptibility to fluoroquinolones. J Infect Dis 1997; 175: 1396–403.
20. Clendnnen TE, Echeverria P, Saenguer S, Kees ES, Boslego JW, Wignall FS. Antibiotic susceptibility survey of *Neisseria gonorrhoeae* in Thailand. Antimicrob Agents Chemother 1992; 36: 1682–7.
21. Kam KM, Lo KK, Ho NKY, Cheung MM. Rapid decline in penicillinase-producing *Neisseria gonorrhoeae* in Hong Kong associated with emerging 4-fluoroquinolone resistance. Genito-urin Med 1995; 71: 141–4.
22. Tanaka M, Matsumoto T, Kobayashi I, Uchino U, Kumazawa J. Emergence of in vitro resistance to fluoroquinolones in *Neisseria gonorrhoeae* isolated in Japan. Antimicrob Agents Chemother 1995; 39: 2367–70.
23. Harrison WO, Wignall FS, Kerbs SBJ, Berg SW. Oral rosoxacin for treatment of penicillin-resistant gonorrhoea. Lancet 1984; ii: 566.
24. Centers for Diseases Control. Sexually transmitted diseases treatment guidelines. MMWR 1993; 42: 4–5.
25. Knapp JS, Hale JA, Wintersheid K, Neal SW, Whittigtton WA. Proposed criteria for the interpretation of susceptibilities of strains of *Neisseria gonorrhoeae* to ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, and norfloxacin. Antimicrob Agents Chemother 1995; 39: 2442–5.
26. Roberts MC. Plasmids of *Neisseria gonorrhoeae* and other *Neisseria* species. Clin Microbiol Rev 1989; 2(suppl): S14–23.
27. Shin YH. Study on the biological type of *Neisseria gonorrhoeae* isolated in Korea and phagocytic effect of human monocyte. PhD Thesis, Dankook University, Seoul.
28. Chu M-L, Ho L-J, Lin H-C, Wu Y-C. Epidemiology of penicillin-resistant *Neisseria gonorrhoeae* isolated in Taiwan, 1960–1990. Clin Infect Dis 1992; 14: 450-7.