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Remarkable Increase in Central Japan in 2001-2002 of *Neisseria gonorrhoeae* Isolates with Decreased Susceptibility to Penicillin, Tetracycline, Oral Cephalosporins, and Fluoroquinolones

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Four hundred sixty-two clinical isolates of *Neisseria gonorrhoeae* recovered from 1999 through 2002 in central Japan were examined for MICs of antimicrobial agents. The majority was sensitive to ceftriaxone and spectinomycin, but a remarkable increase in isolates with decreased susceptibility to penicillin, tetracycline, oral cephalosporins, and fluoroquinolones was observed from 2001 through 2002.

Fluoroquinolones have been highly effective in curing gonococcal infections. However, treatment failure associated with the development of resistance to fluoroquinolones and clinical isolation of *Neisseria gonorrhoeae* strains with decreased susceptibility to these agents have been reported since the mid-1990s in Japan (6, 16). In place of fluoroquinolones, cephalosporins have been important potent antibiotics and are commonly used for the treatment of gonococcal infection in Japan. However, emergence of clinical isolates with decreased susceptibility to oral cephalosporins, including cefixime, has also been observed (1, 12).

We examined clinical isolates of *N. gonorrhoeae* recovered from Japanese men with gonorrhea from 1999 through 2002 for the MICs of antimicrobial agents. We attempted to determine the prevalence of drug resistance and assess the chronological changes in susceptibility to antimicrobial agents in *N. gonorrhoeae*.

N. gonorrhoeae isolates were recovered from a total 273 patients visiting Toyota Memorial Hospital, Toyota, Japan, or the Department of Urology, Gifu University Hospital, Gifu, Japan, from April 1999 through December 2000 (91 isolates), from January through December 2001 (86 isolates), and from January through December 2002 (96 isolates). In addition, isolates were collected from patients with gonorrhea who visited one of four other independent hospitals in 2001 (64 isolates) and 2002 (125 isolates). The six hospitals are located in the Gifu, Aichi, Shiga, and Shizuoka prefectures in central Japan. Patients had not received antibiotic treatment before visiting one of the clinics. MICs of penicillin G, tetracycline, cefixime, cefdinir, cefcapene pivoxil, cefodizime, ceftriaxone, levofloxacin, and spectinomycin were determined by the agar dilution method (13). β-Lactamase activities of the strains were tested with nitrocefin disks.

MIC distributions were analyzed statistically by the Wilcoxon rank sum test. Statistical significance was accepted at a P of <0.05.

Clinical strains were collected from Toyota Memorial Hospital and Gifu University Hospital from 1999 to 2002 but additionally from the other four hospitals from 2001 to 2002. The MIC distribution of each antimicrobial agent for the 86 and 96 isolates recovered in 2001 and 2002, respectively, from Toyota Memorial Hospital and Gifu University Hospital did not significantly differ from the MICs for 64 and 125 isolates collected during the corresponding periods from the other four hospitals. Because sampling bias was avoided, a total of 150 isolates collected in all six hospitals in 2001 and a total of 221 isolates collected in 2002 were compared with 91 isolates collected from 1999 to 2000 with respect to MIC distributions of antimicrobial agents and prevalence of drug resistance.

The antimicrobial susceptibilities of all 462 isolates are summarized in Table 1. MICs of penicillin G and tetracycline increased significantly over the 4 years. These antibiotics have not been commonly used for the treatment of gonococcal infection in Japan. The prevalence of penicillinase-producing *N. gonorrhoeae* or high-level tetracycline-resistant *N. gonorrhoeae* (MIC $\geq$ 16 μg/ml) was very low. However, the prevalence of *N. gonorrhoeae* with chromosomally mediated resistance to penicillin G (MIC $\geq$ 2 μg/ml) or tetracycline (MIC $\geq$ 2 μg/ml) increased markedly in 2001 and 2002.

MICs of levofloxacin for the clinical isolates rose significantly over the 4 years. The prevalence of levofloxacin resistance (levofloxacin MIC of $\geq$1 μg/ml equivalent to ofloxacin MIC of $\geq$2 μg/ml) increased from 27.5% in 1999-2000 to 78.3% in 2002. Since the late 1990s, an increase has occurred in the number of clinical strains with decreased susceptibility to fluoroquinolones; fluoroquinolone treatment of gonococcal infection has been excluded from the treatment guidelines in

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TABLE 1. MICs of antimicrobial agents for clinical strains of *N. gonorrhoeae* recovered in central Japan from 1999 to 2002

Antimicrobial agent	MICs[a] (μg/ml) for isolates collected in:								
	1999–2000 (n = 91)			2001 (n = 150)			2002 (n = 221)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Penicillin G	0.008–>32	0.25	1	0.06–>32	2	4	0.06–>32	2	8
Tetracycline	0.008–>32	0.5	2	0.125–>32	2	4	0.125–>32	2	4
Levofloxacin	≤0.004–8	0.125	4	≤0.004–32	1	8	0.008–64	8	16
Cefixime	≤0.004–0.125	0.008	0.06	≤0.004–1	0.06	0.5	≤0.004–2	0.06	0.5
Cefdinir	≤0.004–1	0.015	0.5	0.015–2	0.06	1	≤0.004–0.125	0.06	1
Cefcapene pivoxil	≤0.004–2	0.03	0.5	≤0.004–8	0.25	4	≤0.004–16	0.5	4
Ceftriaxone	≤0.004–0.06	0.008	0.03	≤0.004–0.25	0.03	0.125	≤0.004–0.5	0.06	0.125
Cefodizime	≤0.004–0.5	0.015	0.06	≤0.004–0.5	0.06	0.25	≤0.004–4	0.125	0.25
Spectinomycin	1–32	16	32	4–256	16	32	4–64	16	32

[a] 50%, MIC at which 50% of the isolates are inhibited; 90%, MIC at which 90% of the isolates are inhibited.

Japan (10). Surprisingly, the number of fluoroquinolone-resistant strains is still increasing in Japan.

MICs of cefixime, cefdinir, cefcapene pivoxil, ceftriaxone, and cefodizime for the isolates in 2001 were significantly higher than the corresponding MICs for the isolates in 1999-2000 (Table 1). The MICs of cefdinir and cefcapene pivoxil for the isolates in 2002 were significantly higher than the respective MICs for the isolates in 2001. However, there was not a significant difference in the susceptibility to cefixime (P = 0.1233), ceftriaxone (P = 0.2611), or cefodizime (P = 0.9010) between the isolates in 2001 and those in 2002. The cefixime MIC was ≥0.5 μg/ml for 26% of the isolates in 2001 and 30.3% in 2002. The ceftriaxone MIC was 0.5 μg/ml for two isolates (0.9%) recovered in 2002 (Table 2). The isolates had markedly reduced susceptibilities to other oral cephalosporins. The emergence of such isolates for which cefixime MICs were ≥0.5 μg/ml could threaten the efficacy of oral cephalosporin regimens recommended for the treatment of gonorrhea (3). Additionally, the emergence of isolates for which ceftriaxone MICs are 0.5 μg/ml is of serious concern.

There were no significant changes in the susceptibility to spectinomycin over the study period (Table 1). Only one spectinomycin-resistant strain for which the spectinomycin MIC was 256 μg/ml was isolated in 2001 (Table 2).

TABLE 2. Prevalence of resistance to antimicrobial agents in clinical strains of *N. gonorrhoeae* recovered in central Japan from 1999 to 2002

Strain characteristic	Prevalence (%)		
	1999–2000 (n = 91)	2001 (n = 150)	2002 (n = 221)
Penicillinase producing	1.1	0.7	0.5
High-level tetracycline resistance (MIC, ≥16 μg/ml)	2.2	0.7	0.5
Chromosomally mediated resistance to penicillin (MIC, ≥2 μg/ml)	2.2	59.3	73.3
Chromosomally mediated resistance to tetracycline (MIC, ≥2 μg/ml)	11.0	53.7	68.8
Levofloxacin resistance (MIC of ≥1 μg/ml equivalent to ofloxacin MIC of ≥2 μg/ml)	27.5	53.3	78.3
Decreased susceptibility to cefixime (MIC, ≥0.5 μg/ml)	0	26.0	30.3
Decreased susceptibility to ceftriaxone (MIC, ≥0.5 μg/ml)	0	0	0.9
Spectinomycin resistance (MIC, ≥128 μg/ml)	0	0.7	0

In Japan, longer and multiple dosing regimens of fluoroquinolones or oral cephalosporins, which result in exposure of *N. gonorrhoeae* clinical strains to low concentrations of the agents, have often been used as the primary treatment of gonorrhea. Continued heavy use of fluoroquinolones could not only alter the target enzymes but also induce changes in bacteria that would prevent drug access to the targets, leading to enhanced fluoroquinolone resistance and decreased susceptibility to structurally unrelated antibiotics (4, 5, 14, 15). Coincidentally, treatment with multiple doses of oral cephalosporins could bring about selection of clinical strains harboring altered penicillin-binding proteins, reduced porin permeability, and enhanced efflux pumps (7, 8, 9, 15). Such resistance mechanisms, which could not be directly associated with plasmid-mediated resistance to penicillin G or tetracycline, would not increase the number of *N. gonorrhoeae* isolates producing penicillinase or carrying the *tetM* gene (2, 11). As shown in this study, however, they appeared to increase the number of *N. gonorrhoeae* with chromosomally mediated resistance to penicillin G or tetracycline.

In conclusion, we observed a remarkable increase in *N. gonorrhoeae* clinical isolates with decreased susceptibility to penicillin, tetracycline, oral cephalosporins, and fluoroquinolones in central Japan. However, the majority of clinical *N. gonorrhoeae* isolates was still sensitive to ceftriaxone and spectinomycin. To prevent development and spread of *N. gonorrhoeae* resistant to these antimicrobial agents, it is particularly important that the agents not be used in lower-than-recommended doses. In addition, antimicrobial susceptibility of current gonococcal isolates must be monitored continually.

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