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Emergence of In Vitro Resistance to Fluoroquinolones in *Neisseria gonorrhoeae* Isolated in Japan

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To investigate emerging fluoroquinolone resistance in *Neisseria gonorrhoeae* isolated in Japan, we compared the in vitro antimicrobial susceptibilities of 79 gonococcal isolates from 1992 through 1993 to 14 fluoroquinolones and 14 other antibiotics with those of 27 isolates from between 1981 and 1984. The MICs at which 90% of the isolates were inhibited by nine fluoroquinolones, including norfloxacin, enoxacin, ofloxacin, ciprofloxacin, tosufloxacin, lomefloxacin, fleroxacin, levofloxacin, and sparfloxacin, for isolates from 1992 to 1993 were 8- or 16-fold higher than those for isolates from 1981 to 1984. Furthermore, the MICs at which 90% of the isolates were inhibited by five fluoroquinolones, including OPC-17116, T-3761, DU-6859a, AM-1155, and Q-35, that have recently been synthesized but have not yet been introduced for clinical use in Japan for isolates from 1992 to 1993 were also 2- to 16-fold higher than those for isolates from 1981 to 1984. The gonococcal isolates from 1992 to 1993 showed no significant decreases in susceptibility to β-lactams, tetracyclines, macrolides, and spectinomycin, compared with those for isolates from 1981 to 1984. Our data indicate that the incidence of gonococcal strains with decreased susceptibilities to fluoroquinolones is increasing in Japan.

Fluoroquinolones demonstrate excellent in vitro activities against *Neisseria gonorrhoeae* strains, including penicillin- and tetracycline-resistant strains, and are highly effective in oral single-dose treatments of gonococcal infections (1, 17, 20). Treating gonorrhea with fluoroquinolones is one of the regimens recommended by the Centers for Disease Control and Prevention in the United States (4). However, decreased susceptibility of clinical gonococcal isolates to ciprofloxacin (9, 12) and treatment failure for gonococcal infection with this agent have recently been reported (9). In Japan, fluoroquinolones have frequently been used as first-line therapy for gonorrhea in the last few years. In a preliminary study (21), we reported a high prevalence of *N. gonorrhoeae* strains showing reduced susceptibilities to early fluoroquinolones, including norfloxacin, ofloxacin, and ciprofloxacin, which have been used frequently against gonococcal infections in Japan. In this study to investigate the emergence of gonococcal isolates with resistance to fluoroquinolones, we compared the antimicrobial susceptibilities to various antimicrobial agents, including not only early developed fluoroquinolones but also newer fluoroquinolones recently synthesized, of gonococcal strains isolated from 1992 through 1993 and those isolated from 1981 through 1984 in Japan.

A total of 79 *N. gonorrhoeae* strains obtained from men with acute urethritis who visited 15 sexually transmitted disease clinics from February 1992 through February 1993 were included in this study. None of these 79 strains were posttreatment isolates or repeat isolates from the same patients. The following clinical and epidemiologic information was recorded: patient's name, age, sexual preference, locality of contact, presence and duration of symptoms, prior antimicrobial exposure, and treatment throughout the study period. Specimens from each patient were inoculated directly onto Thayer-Martin selective agar (Becton Dickinson, Cockeysville, Md.), transported to the Mitsubishi Kagaku laboratory by using a commercially available transport system (Bio-Bag Environmental Chamber Type C; Becton Dickinson), and incubated for 24 to 48 h at 35°C in a 5% CO_2 atmosphere. *N. gonorrhoeae* isolates were identified as gram-negative diplococci by oxidase reaction and sugar utilization patterns. Isolates were stored at −80°C in GOD medium (Nissui, Tokyo, Japan) containing gelatin with 10% skim milk, 2% active charcoal, and 17% glucose prior to antibiotic susceptibility testing. For comparative purposes, 27 *N. gonorrhoeae* strains (kindly supplied by S. Yoshida, University of Occupational and Environmental Health, Kitakyushu, Japan) and 14 β-lactamase-producing (PPNG) and 13 non-PPNG strains isolated from 1981 through 1984 and stored at −80°C in our laboratory were also evaluated.

The MICs for all isolates were determined by an agar dilution technique with a GC agar base (Difco Laboratories, Detroit, Mich.) containing 1% IsoVitaleX (Becton Dickinson) and twofold dilutions of antibiotic (18). Plates were inoculated with 5 μl of 10^6 CFU of each isolate per ml by a multipoint inoculator. World Health Organization *N. gonorrhoeae* reference strains A through E (kindly supplied by J. W. Tapsall, The Prince of Wales Hospital, Randwick, Australia) were included as quality standards. The plates were incubated for 24 h at 35°C in a 5% CO_2 atmosphere. Each MIC was read as the lowest concentration of antibiotic that inhibited bacterial growth. β-Lactamase production was tested by an acidometric assay (β-check; Pfizer Pharmaceuticals, Inc., Tokyo, Japan). The fluoroquinolones tested were norfloxacin, enoxacin, ofloxacin, ciprofloxacin, tosufloxacin, lomefloxacin, fleroxacin, levofloxacin, sparfloxacin, OPC-17116, T-3761, DU-6859a, AM-1155, and Q-35. The antimicrobial agents tested other than fluoroquinolones were β-lactams (penicillin G, piperacillin, imipenem, aztreonam, cefotiam, cefotaxime, ceftriaxone, cefixime, and cefteram), tetracyclines (tetracycline and minocycline), macrolides (erythromycin and azithromycin), and spectinomycin. All antibiotics were obtained as powders of stated potency from their manufacturers.

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TABLE 1. Susceptibilities of *N. gonorrhoeae* isolates from 1992 to 1993 and those from 1981 to 1984 to fluoroquinolones

Antibiotic	MIC (μg/ml)[a]						MIC ratio[b]		P[c]
	1992 to 1993 isolates (n = 79)			1981 to 1984 isolates (n = 27)					
	50%	90%	Range	50%	90%	Range	50%	90%	
Norfloxacin	0.25	4.0	0.004–8.0	0.063	0.25	0.004–2.0	4	16	
Enoxacin	0.25	2.0	0.008–8.0	0.063	0.125	0.008–2.0	4	16	
Ofloxacin	0.125	1.0	0.002–2.0	0.063	0.125	0.004–1.0	2	8	<0.001
Ciprofloxacin	0.031	0.5	≤0.001–1.0	0.008	0.063	≤0.001–0.25	4	8	<0.001
Tosufloxacin	0.016	0.25	≤0.001–1.0	0.008	0.016	≤0.001–0.063	2	16	
Lomefloxacin	0.125	1.0	0.004–2.0	0.031	0.063	0.004–1.0	4	16	
Fleroxacin	0.25	1.0	0.004–2.0	0.031	0.125	0.008–0.25	8	8	
Levofloxacin	0.031	0.25	≤0.001–0.5	0.016	0.031	0.002–0.125	2	8	<0.001
Sparfloxacin	0.016	0.125	≤0.001–0.5	0.004	0.016	≤0.001–0.063	4	8	<0.001
OPC-17116	0.016	0.125	≤0.001–0.5	0.004	0.008	≤0.001–0.063	4	16	<0.001
T-3761	0.125	0.5	0.004–2.0	0.016	0.063	0.002–0.25	8	8	
DU-6859a	0.004	0.016	≤0.001–0.031	0.002	0.004	≤0.001–0.031	2	4	<0.001
AM-1155	0.016	0.063	≤0.001–0.125	0.008	0.031	≤0.001–0.25	2	2	<0.005
Q-35	0.063	0.5	0.004–0.5	0.031	0.125	0.002–0.5	2	4	

[a] 50% and 90%, MICs at which 50 and 90% of the isolates were inhibited.
[b] Each ratio was calculated by dividing the MIC_{50} (or MIC_{90}) for isolates from 1992 to 1993 by the MIC_{50} (or MIC_{90}) for isolates from 1981 to 1984.
[c] The proportions of strains from 1992 to 1993 with decreased susceptibilities to ofloxacin, ciprofloxacin, levofloxacin, sparfloxacin, OPC-17116, DU-6859a, and AM-1155 were compared with those of strains from 1981 to 1984 by the chi-square test.

The MIC at which 50% of the isolates were inhibited (MIC_{50}) and MIC_{90} ratios of each agent for isolates from 1992 to 1993 and those from 1981 to 1984 were compared. The MIC ratio was calculated by dividing the MIC_{50} (or MIC_{90}) for isolates from 1992 to 1993 by the MIC_{50} (or MIC_{90}) for isolates from 1981 to 1984. The proportions of strains from 1992 to 1993 with decreased susceptibilities to seven fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, OPC-17116, DU-6859a, and AM-1155) were also compared with those of strains from 1981 to 1984 by the chi-square test. There are no established criteria that define gonococcal resistance to fluoroquinolones, although the recommendations of the National Committee for Clinical Laboratory Standards define gonococcal isolates for which the MICs of ciprofloxacin are ≤0.06 μg/ml as susceptible to ciprofloxacin (18). Therefore, we set the criteria for decreased susceptibility as follows: ≥0.125 μg/ml for ciprofloxacin; ≥0.25 μg/ml for ofloxacin; ≥0.063 μg/ml for levofloxacin; ≥0.031 μg/ml for sparfloxacin; ≥0.016 μg/ml for OPC-17116; ≥0.008 μg/ml for DU-6859a; and ≥0.063 μg/ml for AM-1155.

The antimicrobial susceptibilities of the 79 isolates from 1992 to 1993 and the 27 isolates from 1981 to 1984 to the 14 fluoroquinolones tested are summarized in Table 1. The MIC_{50} ratios, calculated by dividing the MIC_{50} for isolates from 1992 to 1993 by the MIC_{50} for isolates from 1981 to 1984, ranged from a twofold to an eightfold difference. The MIC_{90} ratios ranged from a 2-fold to a 16-fold difference. The fluoroquinolones showing 16-fold-different MIC_{90} ratios were norfloxacin, enoxacin, tosufloxacin, lomefloxacin, and OPC-17116. The fluoroquinolones with eightfold-different MIC_{90} ratios were ofloxacin, ciprofloxacin, fleroxacin, levofloxacin, sparfloxacin, and T-3761. The MIC_{90} ratio differences for AM-1155, DU-6859a, and Q-35 were two-, four-, and fourfold, respectively. These results demonstrate significant decreases in the susceptibilities of *N. gonorrhoeae* isolates from 1992 to 1993 to not only the early developed fluoroquinolones, such as norfloxacin, ofloxacin, and ciprofloxacin, but also to recently synthesized fluoroquinolones, such as OPC-17116 and T-3761. Of the 79 strains from 1992 to 1993, 37 (46.8%) showed reduced susceptibility to ciprofloxacin (MIC, ≥0.125 μg/ml), while only 1 (3.7%) of the 27 strains from 1981 to 1984 showed reduced susceptibility to ciprofloxacin. The difference in the incidences of strains showing reduced susceptibility to ciprofloxacin between isolates from 1992 to 1993 and those from 1981 to 1984 was statistically significant ($P < 0.001$). The incidences of the 1992 to 1993 and 1981 to 1984 strains showing reduced susceptibilities to ofloxacin (MIC, ≥0.25 μg/ml), levofloxacin (MIC, ≥0.063 μg/ml), sparfloxacin (MIC, ≥0.031 μg/ml), OPC-17116 (MIC, ≥0.016 μg/ml), DU-6859a (MIC, ≥0.008 μg/ml), and AM-1155 (MIC, ≥0.063 μg/ml) were 48.1 and 3.7, 48.1 and 3.7, 46.8 and 3.7, 59.5 and 3.7, 40.5 and 3.7, and 32.9 and 3.7%, respectively. The differences in the incidences of strains with reduced susceptibilities to ofloxacin, levofloxacin, sparfloxacin, OPC-17116, DU-6859a, and AM-1155 between strains from 1992 to 1993 and those from 1981 to 1984 were also statistically significant ($P < 0.001$ or $P < 0.005$).

The antimicrobial susceptibilities of the 79 isolates from 1992 to 1993 and the 27 isolates from 1981 to 1984 to the β-lactams, tetracyclines, macrolides, and spectinomycin tested are summarized in Table 2. Of the 79 strains from 1992 to 1993, only 2 (2.5%) were PPNG strains. Therefore, for the penicillins studied only the results with non-PPNG strains were compared. For penicillin G and piperacillin, the MIC_{50} ratios were four- and twofold lower, respectively. The MIC_{90} ratios of penicillin G and piperacillin were both twofold lower. For β-lactams other than penicillins, tetracyclines, macrolides, and spectinomycin, the MIC_{50} ratios ranged from a twofold-lower difference to no change and the MIC_{90} ratios ranged from a twofold-lower difference to a twofold-higher difference. These results indicate that no significant decreases in the susceptibilities of strains from 1992 to 1993 to β-lactams, tetracyclines, macrolides, and spectinomycin have occurred and that the strains from 1992 to 1993 show relatively increased susceptibilities to penicillins, compared with those of strains from 1981 to 1984.

The resistance of gonococcal isolates to antimicrobial agents is an increasing problem in the treatment of gonococcal infections. A high prevalence of plasmid-mediated high-level or chromosomally mediated low-level resistance to penicillin or tetracycline in Southeast Asia (5–7, 13, 14) and African countries (2, 11, 23) has been reported.

In this study, we have examined the in vitro susceptibilities

TABLE 2. Susceptibilities of *N. gonorrhoeae* isolates from 1992 to 1993 and those from 1981 to 1984 to various antibiotics

Antibiotic	MIC (μg/ml)[a]						MIC ratio[b]	
	1992 to 1993 isolates (n = 79)			1981 to 1984 isolates (n = 27)				
	50%	90%	Range	50%	90%	Range	50%	90%
Penicillin G[c]	0.25	1.0	0.016–2.0	1.0	2.0	0.063–2.0	1/4	1/2
Piperacillin[c]	0.063	0.125	≤0.008–0.25	0.125	0.25	≤0.008–0.5	1/2	1/2
Imipenem	0.063	0.125	≤0.008–0.5	0.125	0.125	0.016–0.5	1/2	1
Aztreonam	0.125	0.5	0.031–1.0	0.125	0.25	0.031–0.5	1	2
Cefotiam	0.25	1.0	0.031–2.0	0.5	1.0	0.063–2.0	1/2	1
Cefotaxime	0.031	0.125	0.004–0.25	0.031	0.125	0.004–0.25	1	1
Ceftriaxone	0.016	0.063	0.004–0.063	0.016	0.063	0.002–0.063	1	1
Cefixime	0.016	0.031	0.002–0.063	0.016	0.063	0.004–0.063	1	1/2
Cefteram	0.031	0.063	0.004–0.5	0.031	0.125	0.004–0.25	1	1/2
Tetracycline	1.0	2.0	0.125–4.0	1.0	4.0	0.25–4.0	1	1/2
Minocycline	0.5	1.0	0.063–4.0	0.5	1.0	0.063–1.0	1	1
Erythromycin	0.5	2.0	0.031–4.0	0.5	1.0	0.063–2.0	1	2
Azithromycin	0.125	0.25	0.016–1.0	0.125	0.25	0.031–0.5	1	1
Spectinomycin	16.0	16.0	4.0–32.0	16.0	16.0	8.0–32.0	1	1

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] Non-PPNG isolates only.

of gonococcal isolates obtained from men with acute urethritis in Japan to various antimicrobial agents for the emergence of fluoroquinolone resistance. Our results demonstrate a significantly higher prevalence of gonococcal strains with reduced susceptibilities to fluoroquinolones among isolates from 1992 to 1993 than among isolates from 1981 to 1984. The increase in fluoroquinolone-resistant *N. gonorrhoeae* isolates will become more of a problem in the near future, because fluoroquinolones are now frequently used as an empirical first-line regimen for sexually transmitted male urethritis and female cervicitis in Japan.

A high prevalence of gonococcal isolates with reduced susceptibilities to fluoroquinolones has also been observed in the Philippines (7) and the United States (15, 16). Furthermore, in Rwanda decreased susceptibilities of gonococci to norfloxacin and ofloxacin have been observed (2). According to the other papers recently published, *N. gonorrhoeae* isolates with decreased susceptibilities to fluoroquinolones are not yet widespread in the United Kingdom (8, 10), Australia (22), New Zealand (3), Thailand (6), Indonesia (13), Hong Kong (14), Zaire (23), or Gambia (11). However, the increase in fluoroquinolone-resistant gonococcal isolates may be a major problem in those countries in the near future as well as in Japan.

There were no significant decreases in susceptibilities to β-lactams, tetracyclines, macrolides, and spectinomycin between our isolates from 1992 to 1993 and those from 1981 to 1984. The non-PPNG isolates from 1992 to 1993 showed relatively higher susceptibilities to penicillins than did those from 1981 to 1984. The incidence of PPNG isolates (2.5%) in this study was lower than that (10 to 24%) previously reported in Japan between 1983 and 1986 (19). The relatively increased susceptibilities to penicillins of non-PPNG isolates from 1992 to 1993 and the low prevalence of PPNG isolates among those from 1992 to 1993 may be associated with an increase in the use of fluoroquinolones and a decrease in the use of penicillins for gonococcal infections in Japan.

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