ORIGINAL ARTICLE

# Susceptibilities of *Neisseria gonorrhoeae* to fluoroquinolones and other antimicrobial agents in Hyogo and Osaka, Japan

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**Objectives:** Decreasing susceptibility of *Neisseria gonorrhoeae* to fluoroquinolones has been reported in several countries. Knowledge of local *N gonorrhoeae* susceptibilities to various antimicrobials is important for establishing a rational treatment strategy in each region.

**Methods:** Isolates of *N gonorrhoeae* from male urethritis patients attending four urological clinics in Hyogo and Osaka prefectures in Japan were collected during 2002. The MICs for nine antimicrobials: penicillin G, tetracycline, cefixime, ceftriaxone, levofloxacin, gatifloxacin, ciprofloxacin, moxifloxacin, and spectinomycin were determined for each isolate. All isolates were also tested for β lactamase producing profiles.

**Results:** Among the 87 isolates obtained, only one isolate was revealed to produce β lactamase. $MIC_{90}$ values for ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin were over 8 μg/ml, over 8 μg/ml, 4 μg/ml, and 2 μg/ml, respectively. The proportion of isolates resistant to fluoroquinolones was over 60% (ciprofloxacin, 70.1%; levofloxacin, 65.5%; gatifloxacin, 70.1%). Chromosomally mediated penicillin and tetracycline resistance was identified in 12.6% and 33.3% of the isolates. $MIC_{90}$ values for cefixime and ceftriaxone and were 0.5 μg/ml and 0.0063 μg/ml. All isolates were sensitive to ceftriaxone and 90.8% of them were sensitive to cefixime. $MIC_{90}$ for spectinomycin was 32 μg/ml and all isolates were sensitive to it. Fluoroquinolone resistance correlated significantly with MICs for penicillin G but not tetracycline.

**Conclusion:** Ceftriaxone and spectinomycin demonstrated lower MICs and so are recommended for *N gonorrhoeae*. Susceptibilities of *N gonorrhoeae* should be monitored periodically by region.

*Neisseria gonorrhoeae* remains one of the most common sexually transmitted pathogens in developing and developed countries. Over the past decade, strains of *N gonorrhoeae* have been reported to develop high levels of resistance against several antimicrobial agents previously used for treatment of gonorrhoea.[1 2]

Until several years ago, fluoroquinolone regimens had been recommended for the treatment of gonorrhoea; however, the emergence of gonococcal isolates with reduced susceptibility or resistance to fluoroquinolones has been a significant concern in several countries, including Japan. The recommended therapy has been altered accordingly.[3]

Trends concerning resistance have varied from country to country and also between areas in a given country. We investigated MICs of *N gonorrhoeae* isolated from men with urethritis in an urban area of Japan to establish a rational treatment strategy appropriate to the area.

## METHODS

### Clinical specimens

Urine or urethral swab specimens were obtained from male patients with urethritis treated at four urological clinics in Hyogo and Osaka prefectures in Japan in 2002. Post-treatment isolates and other repeat isolates from the same patients were excluded from the study. All specimens were transferred to the clinical laboratory at our institution within 2 hours of collection.

### Antimicrobial susceptibility testing

MICs for all isolates were determined using the agar plate dilution method, with a GC agar base (Becton Dickinson, Cockeysville, MD, USA) containing 1% Iso VitaleX (Becton Dickinson) and serial twofold dilutions of antimicrobial agents. MICs at which 50% and 90% of the isolates tested were inhibited were defined as the $MIC_{50}$ and $MIC_{90}$. We used *N gonorrhoeae* ATCC 49226 as a quality control in susceptibility testing. β lactamase production was assayed using nitrocefin discs (BBL Cefinase; Becton Dickinson) with *Staphylococcus aureus* ATCC 25923 as a negative control.

The antimicrobial agents tested were penicillin G, tetracycline, cefixime, ceftriaxone, levofloxacin, gatifloxacin, ciprofloxacin, moxifloxacin, and spectinomycin.

Antimicrobial susceptibilities for strains were defined according to the MIC ranges listed in the National Committee for Clinical Laboratory Standards (NCCLS) 2002 guidelines (table 1).[4] The isolates were sequentially grouped into mutually exclusive categories according to the NCCLS guidelines as follows: penicillinase producing *N gonorrhoeae* (PPNG); plasmid mediated tetracycline resistant *N gonorrhoeae* (TRNG); chromosomally mediated penicillin resistant *N gonorrhoeae* (CMPR); chromosomally mediated tetracycline resistant *N gonorrhoeae* (CMTR); and chromosomally mediated penicillin and tetracycline resistant *N gonorrhoeae* (CMRNG) according to the paper by Tanaka *et al*.[3]

### Statistical analysis

Median MIC values were compared by the Mann-Whitney U test using a software package (StatView; Abacus Concepts, Berkeley, CA, USA). Statistical significance was set at 0.05.

## RESULTS

### Susceptibilities for fluoroquinolones

*N gonorrhoeae* was isolated from urine or urethral swab specimens of 87 male patients with urethritis; 67 strains (77%) were obtained in Hyogo prefecture, and 20 (23%) in

**Table 1** Antimicrobial susceptibility and prevalence of *N gonorrhoeae* susceptible to each drug

| Antimicrobial agents | $MIC_{50}$ (μg/ml) | $MIC_{90}$ (μg/ml) | MIC range (μg/ml) | MIC range (μg/ml) of interpretive standard (No of isolates) | | |
|---|---|---|---|---|---|---|
| | | | | Sensitive* | Intermediate* | Resistant* |
| Penicillin G | 0.5 | 2 | <0.031–64 | ⩽0.06 (9/87) | 0.12–1 (61/87) | ⩾2 (17/87) |
| Tetracycline | 1 | 8 | <0.031–64 | ⩽0.25 (16/87) | 0.5–1 (34–87) | ⩾2 (17/87) |
| Cefixime | 0.125 | 0.5 | <0.004–0.5 | ⩽0.25 (79/87) | | ⩾0.5 (8/87) |
| Ceftriaxone | 0.016 | 0.063 | <0.004–0.063 | ⩽0.25 (87/87) | | ⩾0.5 (0/87) |
| Ciprofloxacin | 4 | >8 | <0.002->8 | ⩽0.06 (9/87) | 0.12–0.5 (18/87) | ⩾1 (60/87) |
| Levofloxacin | 4 | >8 | <0.002->8 | ⩽0.25 (24/87) | 0.15–1 (5/87) | ⩾2 (58/87) |
| Gatifloxacin | 1 | 4 | <0.002->8 | ⩽0.125 (19/87) | 0.25 (7/87) | ⩾0.5 (61/87) |
| Spectinomycin | 32 | 32 | <4–32 | ⩽32 (87/87) | 64 (0/87) | ⩾128 (0/87) |

*Sensitive, intermediate, and resistant are classified according to the NCCLS guidelines, 2002.

Osaka prefecture. The two cities adjoin one another and share the same cultural and economic background.

The proportion of isolates sensitive to ciprofloxacin, levofloxacin, and gatifloxacin was 10.3%, 27.6%, and 21.8%, respectively. The $MIC_{90}$ of ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin against the isolates was over 8 μg/ml, over 8 μg/ml, 4 μg/ml, and 2 μg/ml, respectively (table 1). Median MICs of penicillin G were significantly higher for the isolates with resistance or reduced susceptibility to fluoroquinolones (ciprofloxacin, levofloxacin, and gatifloxacin) than fluoroquinolone susceptible isolates ($p<0.001$, $p = 0.0078$ and $p = 0.0079$, respectively). Median MICs of tetracycline for isolates with resistance or reduced susceptibility to fluoroquinolones (ciprofloxacin, levofloxacin, and gatifloxacin) were not significantly different from those for isolates with fluoroquinolone susceptible isolates (table 2).

### Susceptibilities to penicillin and tetracycline

The proportion of isolates sensitive to penicillin G and tetracycline was 10.3% and 18.4%, respectively (table 1). $MIC_{90}$ values of penicillin G and tetracycline against isolates were 2 μg/ml and 8 μg/ml. One isolate each was categorised as PPNG and as TRNG. No isolate was classified as PPNG-TRNG. Proportions of isolates with chromosomally mediated penicillin resistance (CMPR) and chromosomally mediated tetracycline resistance (CMTR) were 12.6% and 33.3%. Seven isolates (8.0%) were categorised to CMRNG.

### Susceptibilities to cephems and spectinomycin

$MIC_{90}$ values of cefixime, ceftriaxone, and spectinomycin against *N gonorrhoeae* were 0.5 μg/ml, 0.063 μg/ml, and 32 μg/ml, respectively. Proportions of isolates susceptible to cefixime, ceftriaxone, and spectinomycin were 90.8%, 100%, and 100%, respectively (table 1).

## DISCUSSION

*N gonorrhoeae* shows unique region specific susceptibility profiles. Moreover, a longitudinal survey in the same geographic area showed that susceptibilities changed over time.[3 5–8]

In our present study the proportion of isolates susceptible to penicillin G and tetracycline was 10.3% and 18.4%, with a susceptibility profile fairly similar to those previously reported in Japan[9]; 90.8% of isolates were sensitive to cefixime, again in agreement with previous reports from other parts of Japan.[6 9 10] All isolates were found to show low MICs and susceptibility to ceftriaxone, which is used intravenously. This indicates that ceftriaxone can be a good choice of agent against *N gonorrhoeae* in our area.

Proportions of *N gonorrhoeae* resistant to fluoroquinolones differ from area to area worldwide. Prevalence of ciprofloxacin, levofloxacin, and gatifloxacin resistant *N gonorrhoeae* in the present study proved to be 69.0%, 66.7%, and 70.1%, respectively. Since fluoroquinolones had been used as first line antimicrobial agents for *N gonorrhoeae* infections in Japan, this heavy use created a selective advantage for microorganisms resistant to these drugs.[3] We found these mutations in over 90% of isolates (Shigemura *et al*, unpublished data), they also can account readily for cross resistance among fluoroquinolones. For example, a new fluoroquinolone not yet commercially available in Japan, moxifloxacin, already showed relatively high $MIC_{90}$ values (2 μg/ml) (data not shown).

MICs of structurally unrelated penicillin, tetracycline, and cephems for the isolates with reduced susceptibility to ciprofloxacin have been reported to be higher than those for isolates susceptible to ciprofloxacin. Our study also demonstrated that MICs of penicillin G and cephems for isolates with reduced susceptibility to fluoroquinolones were almost 4–16 times higher than those for fluoroquinolone susceptible isolates. However, in the present study, MICs of tetracycline for isolates with reduced susceptibility to fluoroquinolones were the same as those for fluoroquinolone susceptible isolates. The reason for this discrepancy from previous reports is not evident from our present data,[3] but is partly that tetracycline had not been used as often for gonococci treatment in Japan (table 2). Currently only one isolate each was categorised as PPNG or TRNG, and no isolate

**Table 2** $MIC_{50}$ values of structurally unrelated antimicrobial agents for fluoroquinolone sensitive and fluoroquinolone less sensitive *N gonorrhoeae*

| Antimicrobial agents | Ciprofloxacin | | | Levofloxacin | | | Gatifloxacin | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sensitive | Less sensitive* | p Value | Sensitive | Less sensitive* | p Value | Sensitive | Less sensitive* | p Value |
| Penicillin G | 0.031 | 0.5 | <0.001 | 0.375 | 0.5 | 0.0078 | 0.125 | 0.5 | 0.0079 |
| Tetracycline | 0.25 | 1 | 0.508 | 1 | 1 | 0.2152 | 4 | 1 | 0.7423 |
| Cefixime | 0.008 | 0.125 | <0.001 | 0.078 | 0.125 | 0.1599 | 0.016 | 0.125 | 0.0541 |
| Ceftriaxone | 0.004 | 0.016 | 0.0006 | 0.01 | 0.016 | 0.0477 | 0.004 | 0.016 | 0.0384 |
| Spectinomycin | 32 | 32 | 0.2022 | 32 | 32 | 0.4734 | 32 | 32 | 0.8326 |

*Less sensitive includes the category of intermediate and resistant according to the NCCLS guidelines, 2002.

was categorised as PPNG-TRNG; 12.6%, 33.3%, and 8% of isolates were categorised as CMPR, CMTR, and CMRNG respectively. A previous report from another part of Japan showed less prevalence of CMPR, CMTR, and CMRNG, even though the first line treatment was the same in both areas.[3]

In Japan, currently recommended therapy against *N gonorrhoeae* infection would be spectinomycin (2.0 g intramuscularly, given once) or a 3 day oral course of cefixime (200 mg twice a day).[11] Yet in recent reports from Japan, susceptibility of *N gonorrhoeae* to cefixime is decreasing.[9 10] Taking this, together with our results, spectinomycin and ceftriaxone are the current drugs of choice for treatment of *N gonorrhoeae* infection in our region, meaning that we have no optimal oral regimen for treatment of *N gonorrhoeae*. To establish a rational treatment strategy against *N gonorrhoeae* infection, periodic surveys of MICs of *N gonorrhoeae* isolated in particular areas are necessary, even when data have been reported from another part of the country. Moreover, specialists in treatment of STD should inform the other physicians who also treat patients with these infections of the current situation concerning resistance patterns and consequent adjustments in therapeutic strategy.


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