Antimicrobial Susceptibilities of Neisseria gonorrhoeae Strains Isolated in Surabaya, Indonesia

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Until recently, the only common strains of antimicrobial agent-resistant Neisseria gonorrhoeae detected in Indonesia were penicillinase-producing N. gonorrhoeae (PPNG) strains. Despite the spread of resistance to other antimicrobial agents among N. gonorrhoeae in Southeast Asia, surveillance for such resistance in Indonesia has been limited. We evaluated the in vitro susceptibilities of 86 N. gonorrhoeae isolates from female sex workers in Surabaya, Indonesia, to 13 antimicrobial agents. Of the 86 isolates, 89% were resistant to penicillin (MIC, ≥2.0 μg/ml), 98% were resistant to tetracycline (MIC, ≥2.0 μg/ml), 18.1% were resistant to spectinomycin (MIC, ≥128.0 μg/ml), and 97.7% showed decreased susceptibility to thiamphenicol (MIC, 1 to 2 μg/ml). Thus, thiamphenicol and spectinomycin may be approaching the end of their usefulness as the drugs of choice for the treatment of gonococcal infections in Surabaya. While the susceptibilities of N. gonorrhoeae to cephalosporins (ceftriaxone, cefixime, and cefoxitin) and fluoroquinolones (ciprofloxacin and ofloxacin) are universal, these drugs have not been used because they are more expensive in Indonesia than thiamphenicol. We conclude that Surabaya had the highest reported rate of penicillin and tetracycline resistance among the Southeast Asian countries and that cephalosporins or fluoroquinolones should be reasonable alternatives for the treatment of gonorrhea in this locale.

Southeast Asia is suspected to be the origin of several strains of antimicrobial agent-resistant Neisseria gonorrhoeae. Penicillinase-producing N. gonorrhoeae (PPNG) strains were first isolated in 1976 in Southeast Asia (1, 16); PPNG remains prevalent there (5, 6, 12, 18, 25). Spectinomycin-resistant PPNG, first isolated in California in 1981, was also traced to Southeast Asia (2). A small number of N. gonorrhoeae strains that are resistant to spectinomycin and highly resistant to tetracycline have been isolated in Australia, but they are suspected to have been imported into Australia from Southeast Asia (23). The reasons for the frequent emergence in Southeast Asia of N. gonorrhoeae strains that are highly resistant to antimicrobial agents are not well understood.

Until recently, the only common strains of antimicrobial agent-resistant N. gonorrhoeae detected in Indonesia were PPNG. Despite the increase in resistance to other antimicrobial agents among N. gonorrhoeae in Southeast Asia, surveillance for such resistance in Indonesia has been limited because the methods to detect such resistance are expensive and technically complex.

Surabaya (population of approximately 2.7 million in 1992) is the busiest seaport in Indonesia. As the busiest seaport and a hub of the national road and rail network, Surabaya has a large commercial sex industry. A recent survey of the prevalence of PPNG among female sex workers in Surabaya found that PPNG rates were 17.8% among low-paid sex workers and 44.4% among high-paid sex workers (27). Because Southeast Asian isolates have generally been less susceptible to a number of antimicrobial agents than isolates from Western countries, knowledge of antimicrobial susceptibility is important for guiding the choice of antimicrobial therapy (24).

We report the in vitro susceptibilities to 13 antimicrobial agents of isolates obtained from female sex workers in Surabaya.

MATERIALS AND METHODS

Study population. This study was performed as part of a baseline survey for sexually transmitted diseases (STDs) among high-risk and other populations in Surabaya. The study protocol was approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board and by the local ethics panel. The female sex workers consented to participate in this survey.

The baseline survey among female sex workers started in December 1992, but we did not begin the study of antimicrobial resistance until March 1993. We recruited female sex workers from brothel complexes in the city of Surabaya (districts A, B, and C), from massage parlors, and among street walkers. Except for street walkers, sex workers were visited by a team consisting of a physician, nurses, and interviewers. We collected endocervical specimens for N. gonorrhoeae culture from female sex workers who participated in the survey from March to June 1993. During that period, 383 female sex workers (most of whom were asymptomatic) participated in the survey (11 women refused to participate). Of the 383 endocervical specimens, 92 (24%) were culture positive for N. gonorrhoeae.

Gonococcal isolates. Endocervical specimens were inoculated onto modified Thayer-Martin medium, and the plates were immediately placed in candle extinction jars. Within 4 h the jars were transferred to an incubator, where they were held at 35 to 36°C for 24 to 48 h. Isolates were presumptively
identified as *N. gonorrhoeae* on the basis of colony morphology, Gram stain appearance, and oxidase reaction (4). Pure *N. gonorrhoeae* isolates were obtained by subculture on enriched chocolate agar. For transport to CDC, Atlanta, Ga., a heavy suspension of 18- to 24-h growth of each pure *N. gonorrhoeae* subculture was placed into a tryptic soy broth freezing medium (Difco Laboratories, Detroit, Mich.) and stored at −70°C. At the end of June 1993, the frozen isolates were shipped as a group to CDC on dry ice.

**Antimicrobial susceptibility testing.** At CDC, the frozen isolates were thawed and recultured on modified Thayer-Martin medium and chocolate agar medium. Of the 92 isolates, 86 (93%) were successfully recovered.

The minimum MICs of the 13 antimicrobial agents were determined by an agar plate dilution technique (20). All susceptibility test runs were quality controlled with *N. gonorrhoeae* ATCC 49226, which met the acceptable control ranges recommended by the National Committee for Clinical Laboratory Standards (11), and with three additional *N. gonorrhoeae* strains: F-28 (spectinomycin resistant), F-45 (a strain with chromosomally mediated resistance to penicillin and tetracycline), and either 76.061782 (PPNG) or P681E (a strain with plasmid-mediated resistance to penicillin and tetracycline). β-Lactamase activity of PPNG was tested by using nitrocefin disks with *Haemophilus influenzae* as a negative control.

**Antimicrobial agents.** We used the following standard antimicrobial reference powders provided by the U.S. manufacturers or importers: penicillin G, tetracycline, erythromycin, gentamicin, kanamycin, spectinomycin, ceftriaxone, cefotixin, ciprofloxacin, cefixime, ofloxacin, chloramphenicol, and thiamphenicol.

**Statistical analyses.** On the basis of MICs of penicillin and tetracycline and the results of assays for β-lactamase, the 86 *N. gonorrhoeae* isolates were divided into four categories: (i) PPNG, β-lactamase positive and MIC of <16 μg of tetracycline per ml; (ii) plasmid-mediated tetracycline-resistant *N. gonorrhoeae* (TRNG), MIC of ≥16 μg of tetracycline per ml and β-lactamase negative; (iii) PPNG/TRNG, β-lactamase positive and MIC of ≥16 μg of tetracycline per ml; and (iv) susceptible, MICs of ≤0.06 μg of penicillin and ≤0.25 μg of tetracycline per ml. We computed the geometric mean of the MICs of each antimicrobial agent for the four categories of isolates.

### RESULTS

Of the 86 isolates recovered, only 1 (1%) was susceptible, 72 (84%) were PPNG/TRNG, 12 (14%) were TRNG, and 1 (1%) was PPNG. Most of these isolates belonged to a single auxotype/serovar class (Pro/1A-8). On the basis of susceptibility criteria established by the National Committee for Clinical Laboratory Standards, 89% of the isolates were resistant to penicillin (MIC, ≥2.0 μg/ml) and 98% were resistant to tetracycline (MIC, ≥2.0 μg/ml) (11). Among PPNG/TRNG strains, 79.2% were highly resistant to penicillin (MIC, ≥16.0 μg/ml) and 100% were highly resistant to tetracycline (MIC, ≥16.0 μg/ml) (Table 1). Among TRNG strains, 100% were highly resistant to tetracycline (MIC, ≥16.0 μg/ml) (Table 2).

All strains were susceptible to erythromycin (MIC, ≤0.5 μg/ml), gentamicin (MIC, ≤8.0 μg/ml), ceftriaxone (MIC, ≤0.25 μg/ml), cefoxitin (MIC, ≤2.0 μg/ml), cefixime (MIC, ≤0.25 μg/ml), ofloxacin (MIC, ≤0.25 μg/ml), ciprofloxacin (MIC, ≤0.06 μg/ml), and chloramphenicol (MIC, ≤8.0 μg/ml) (Tables 1 and 2) (11, 15). A total of 94.2 and 91.7% of the strains had decreased susceptibilities to kanamycin (MIC, ≥16.0 μg/ml) and thiamphenicol (MIC, ≥1.0 μg/ml), respectively (11, 13, 26). A total of 18.1% were resistant to spectinomycin (MIC, ≥128.0 μg/ml) (11). These resistant strains were found only among PPNG/TRNG strains, not among TRNG strains.

Because the numbers of PPNG and susceptible strains were small (one for each category), we did not compare those strains with other strains (PPNG/TRNG and TRNG). The geometric mean MIC of penicillin was significantly different for PPNG/TRNG and TRNG strains (15.85 versus 0.35 μg/ml; *P* < 0.01). For other antimicrobial agents, the geometric mean MICs were similar.

### DISCUSSION

The data demonstrate convincingly that the *N. gonorrhoeae* strains from Surabaya, Indonesia, are frequently resistant to multiple antibiotics and exhibit the highest rate of penicillin and tetracycline resistance in Southeast Asia. Although the number of *N. gonorrhoeae* isolates collected in this study was small, they were isolated from female sex workers with different levels of socioeconomic status. The sex workers from the massage parlor have the highest socioeconomic status (mean price per sexual encounter is $16.00), followed by those from the brothel complexes (mean price per sexual encounter
ranges from $8.60 in district C to $3.20 in district B) and street walkers (mean price per sexual encounter is $2.70). The distributions of PPNG/TRNG and TRNG isolates among these different types of sex workers were similar. In addition, the success rate for reculture of shipped specimens in this study (93%) is much higher than those in other studies, for which success rates ranging from 34 to 72% were reported (3, 10, 14).

In 1982 a study conducted in an STD clinic in Jakarta, Indonesia, reported an 8% prevalence of PPNG strains (all with MICs of $\geq 128 \mu$g/ml) (18). Among PPNG isolates, 100% were resistant to tetracycline (MIC, $\geq 2.0 \mu$g/ml). Among non-PPNG isolates, 54% were resistant to penicillin (MIC, $\geq 2.0 \mu$g/ml) and 62% were resistant to tetracycline (MIC, $\geq 2.0 \mu$g/ml). In Surabaya, the first PPNG strains were isolated in 1980 (22). By 1982, Sastrowidjoyo and Ijudari reported a rate of PPNG of 13.5% among the Surabaya sex workers (19). A study in 1991 in Surabaya documented a high rate of PPNG strains (17.8% in low-paid sex workers and 44.4% in high-paid sex workers) (27). Thus, the findings of the present study showed a substantial increase in resistance to penicillin (89%) and to tetracycline (98%) in Surabaya. This is the highest reported rate of resistance among the Southeast Asian countries. In the Philippines in 1989, the penicillin resistance rate (MIC, $\geq 2.0 \mu$g/ml) was 78%, and the tetracycline resistance rate (MIC, $\geq 2.0 \mu$g/ml) was 47% (6). In Thailand in 1990, the penicillin resistance rate (MIC, $\geq 2.0 \mu$g/ml) was 64%, and the tetracycline resistance rate (MIC, $\geq 2.0 \mu$g/ml) was 70% (5).

The high rates of plasmid-mediated penicillin and tetracycline resistance in Surabaya could be related to widespread self-medication by sex workers who take penicillin or tetracycline for STD prevention and treatment or for other illnesses. Both antimicrobial agents are easily obtained from sidewalk vendors. In addition, the weekly administration of 1.2 million units of benzathine penicillin for syphilis prevention in several brothel complexes may contribute to the penicillin resistance. Self-medication among sex workers in the Philippines and Thailand has been cited as contributing to the emergence of antibiotic resistance (7, 21).

As far as we know, this study is the first to report the presence of N. gonorrhoeae strains resistant to spectinomycin in Indonesia. In 1982, a study conducted in an STD clinic in Jakarta reported no resistance to spectinomycin (MIC, $\geq 128.0 \mu$g/ml) (18). Although spectinomycin was not a drug of choice for treatment of N. gonorrhoeae in Surabaya, some clinicians reported using spectinomycin as the first-line drug to treat gonorrhea infections. Because Surabaya received a large number of travellers (mostly businessmen and sailors) from South Korea, Thailand, and the Philippines, spectinomycin-resistant strains from those countries could have been easily introduced. Spectinomycin-resistant strains were first isolated in South Korea and the Philippines in 1981 and in Thailand in 1990 (5, 6, 8, 17). The prevalence of spectinomycin resistance in Surabaya (18.1%) was similar to that in the Philippines (22% in 1988) but higher than that in Thailand (8.9% in 1990) (5, 6).

The efficacy of thiamphenicol as the primary drug for N. gonorrhoeae in Surabaya is now in question. A total of 100% of the PPNG/TRNG isolates and 91.7% of the TRNG isolates showed decreased susceptibility to thiamphenicol (MIC, 1 to 2 \mu$g/ml). In 1982 in the city of Medan (northern Indonesia), 82% of isolates collected from the sex workers showed a decreased susceptibility to thiamphenicol (MIC, $\geq 2 \mu$g/ml) (9). In general, patients experienced treatment failure rates ranging from 3.1 to 8.3% with a MIC of 1 \mu$g/ml and treatment failure rates ranging from 14.3 to 18.7% with a MIC of 2 \mu$g/ml (13, 26).

We documented a substantial increase in resistance to penicillin and tetracycline and a decreased susceptibility to thiamphenicol and spectinomycin in Surabaya, Indonesia. Because of a high rate of decreased susceptibility to thiamphenicol and a rate of resistance to spectinomycin of 18.1%, thiamphenicol and spectinomycin may be approaching the end of their usefulness as the primary drugs for the treatment of N. gonorrhoeae in Surabaya. Although susceptibilities to cefalosporins (ceftriaxone, cefixime, and cefoxitin) and fluoroquinolones (ciprofloxacin and ofloxacin) are universal, those drugs are more expensive than thiamphenicol. Cefalosporins or fluoroquinolones should be reasonable alternatives for the treatment of gonorrhea in Surabaya, especially for female sex workers and their sexual contacts. Continued surveillance (such as systematic testing of in vitro antimicrobial susceptibilities and careful investigation of suspected treatment failures) for cefalosporin- or fluoroquinolone-resistant strains in the busy seaport of Surabaya should be used to guide the choice of antimicrobial treatment for gonorrhea.

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REFERENCES


