Plasmid patterns and antimicrobial susceptibilities of *Neisseria gonorrhoeae* in Bandung, Indonesia

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Abstract

Antimicrobial susceptibilities of *Neisseria gonorrhoeae* isolates from female sex workers and from men with urethritis in Bandung, Indonesia, were determined by an agar dilution technique. Typing of the Tet M plasmid in tetracycline-resistant isolates (TRNG) was performed using a polymerase chain reaction (PCR) technique and plasmid profiles of penicillinase-producing isolates (PPNG) were determined. All PPNG possessed the 4·4 MDa β-lactamase plasmid and all TRNG showed a PCR fragment characteristic of the 'Dutch' type Tet M plasmid. Of the 50 gonococci isolates tested, all were resistant to tetracycline; 47 were TRNG, 26 were PPNG, and 6 were resistant to thiamphenicol. Chromosomal resistance to penicillin was not detected. All isolates were susceptible to ceftriaxone, ciprofloxacin, norfloxacin, ofloxacin, kanamycin, spectinomycin, and trimethoprim/sulfamethoxazole. Spectinomycin and fluoroquinolones are useful primary drugs for treatment of gonococcal infection in Bandung. Continued surveillance of antimicrobial resistance should be part of gonorrhoea control in Indonesia.

Keywords: gonorrhoea, *Neisseria gonorrhoeae*, antimicrobial susceptibility, penicillinase production, tetracycline resistance, plasmid analysis, Indonesia

Introduction

Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) were first isolated in south-east Asia in 1976 and gonococcal isolates highly resistant to spectinomycin and to tetracycline (TRNG) have been reported since the 1980s (ASHFORD *et al.*, 1976, 1981; TAPSALL *et al.*, 1991). Fluoroquinolone resistant gonococci emerged in several countries of the Far East during the early 1990s and have spread in some regions to high levels (CLENDENNEN *et al.*, 1992a, 1992b; TAPSALL *et al.*, 1992; TANAKA *et al.*, 1994; KAM *et al.*, 1995; TAPSALL, 1995).

Monitoring of antimicrobial susceptibility patterns in gonococci is an essential activity to develop, evaluate and adapt treatment guidelines. Despite the rapid spread of resistance all over south-east Asia, surveillance for such resistance in Indonesia is limited. Until recently the only common resistant strains described in Indonesia were PPNG, documented in Jakarta and Surabaya (SOENDJOJO *et al.*, 1981; ROCKHILL *et al.*, 1982; SASTROWIDJOJO & IDAJADI, 1983; UTORO *et al.*, 1991). A more recent study in 1992–1993 in Surabaya documented gonococcal resistance to spectinomycin and extremely high rates of PPNG and TRNG (JOESOEF *et al.*, 1994).

Different β-lactamase plasmids of 4·4 MDa, 3·9 MDa, 3·2 MDa, and 3·05 MDa have been observed among gonococci from the Far East; the majority of PPNG possessed the 4·4 MDa plasmid (POH *et al.*, 1991; SARAFIAN *et al.*, 1991; KNAPP *et al.*, 1997a). TRNG carry a 25·2 MDa Tet M conjugative plasmid; 2 different restriction endonuclease patterns, designated 'American' and 'Dutch' type, of this plasmid have been described (GASCOYNE *et al.*, 1991). A limited number of TRNG from the Far East have been characterized by GASCOYNE-BINZI *et al.* (1994); all isolates belonged to the 'Dutch' type.

Bandung, Indonesia's third largest city with a population of approximately 2 million, is the capital city of West Java, and is an important centre of commerce and industry; it includes a number of 'localized' commercial sex areas.

The objectives of this study were to document the antimicrobial susceptibilities *in vitro* of gonococcal isolates obtained from female sex workers and from males with urethritis and to characterize plasmids in PPNG and TRNG.

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Materials and Methods

Gonococcal isolates

Fifty isolates of *N. gonorrhoeae* were obtained in 1994 from 23 consecutive men with symptoms of urethritis in the sexually transmitted diseases (STD) clinic of the Hassan Sadikin General Hospital in Bandung and from an unselected group of 27 female sex workers in Saritem, one of the largest areas for prostitution in Bandung, who participated in an STD prevalence study. Initial gonococcal isolations were made on modified Thayer–Martin agar and presumptively identified by colony morphology, Gram staining and oxidase activity. β-Lactamase production was tested by use of nitrocefin disks. Overnight subcultures on non-selective chocolate medium were suspended in skimmed milk, frozen at −70°C, and sent in dry ice to the Institute of Tropical Medicine, Antwerp, Belgium for further testing. Definite identification of gonococci was based on sugar acidification and reactivity with monoclonal antibody.

Susceptibility testing

Minimum inhibitory concentrations (MICs) of ceftriaxone, ciprofloxacin, kanamycin, norfloxacin, ofloxacin, penicillin, spectinomycin, tetracycline, thiamphenicol and trimethoprim/sulfamethoxazole (1/19) were determined with an agar dilution technique using inocula of 10^4 colony forming units. World Health Organization (WHO) gonococcal reference strains A–E and American Type Culture Collection (ATCC) GC strain 49226 were included. Trimethoprim/sulfamethoxazole was tested on diagnostic sensitivity test agar with 5% lysed horse blood and 1% Kellogg's supplement. All other antimicrobial compounds were tested on gonococcal agar base supplemented with 1% IsoVitaleX™. Inoculated plates were incubated at 36°C in 5% carbon dioxide with high humidity. The MICs were determined after 20 h.

Plasmid profiles

Plasmids were detected according to procedures described by KADO & LIU (1981) with minor modifications. Gonococci were cultured overnight on blood agar. One loopful (10 μL) of growth was suspended in Eppendorf microcentrifuge tubes containing 250 μL of lysing solution. After vigorous vortexing, the suspensions were heated at 80°C for 20 min. After cooling on ice the lysed suspensions were mixed with 250 μL of phenol–chloroform (50:50, v/v) and vortexed. After

centrifugation at 12000 rev/min for 10 min at 4°C, the upper aqueous phase was used for gel electrophoresis. A panel of 6 GC strains (collected from various laboratories) showing different plasmid patterns was used as control.

Typing of 25·2 MDa plasmid by PCR

For the detection of 25·2 MDa Tet M plasmid types, primers described by XIA *et al.* (1995) were used and their PCR method was slightly modified. Cells of TRNG, grown overnight on blood agar, were suspended in distilled water to a density of one McFarland unit ($2–3\times10^8$ bacteria per mL). Five µL of 1N NaOH were added to 95 µL of bacterial suspension and the mixture was heated at 95°C for 15 min. After cooling to room temperature, 10 µL of 1 M Tris–HCl (pH 8·0) were added. Five µL of bacterial lysate were added to 20 µL of PCR mixture containing 200 µM of each deoxynucleoside triphosphate, 1×PCR buffer (50 mM KCl plus 10 mM Tris–HCl, pH 8·3), 2·0 mM $MgCl_2$, 0·3 µM of each primer, and 2 units of *Taq* polymerase (Ampli Taq™, Perkin Elmer Cetus, Norwalk, Connecticut, USA). The amplification was performed in a thermal cycler (Gene Amp™ 9600, Perkin Elmer). After an initial denaturation step at 94°C for 3 min, the temperature was cycled at 94°C for 30 sec, 44°C for 30 sec, and 72°C for 1 min, for a total of 30 cycles, with a final extension step at 72°C for 7 min. PCR was followed by gel electrophoresis. Three GC control strains (Tet M negative, 'Dutch' type Tet M, and 'American' type Tet M; supplied by Dr Gascoyne-Binzi [University of Leeds, UK] and Dr Van Klingeren [RIV, Bilthoven, The Netherlands]) were included.

Results

Antimicrobial susceptibilities

All gonococcal isolates were susceptible to ceftriaxone (MICs 0·001–0·015 mg/L), ciprofloxacin (MICs 0·002–0·008 mg/L), norfloxacin (MICs 0·015–0·060 mg/L), ofloxacin (MICs 0·004–0·030 mg/L), kanamycin (MICs 8·0–32 mg/L), spectinomyciun (MICs 8·0–32 mg/L), and trimethoprim/sulfamethoxazole (MICs 0·125/2·375–2·0/38 mg/L). The MICs of thiamphenicol varied from 0·125 mg/L to 4·0 mg/L; 6 isolates were resistant, with MICs ≥2·0 mg/L, 21 showed decreased susceptibility (MIC1·0 mg/L) and 23 were fully susceptible (MICs 0·125–0·5 mg/L). Of the 50 isolates tested, 48 showed plasmid-mediated resistance: 26 were PPNG/TRNG, 21 were TRNG, and 1 was PPNG. All 3 non-TRNG isolates were chromosomally resistant to tetracycline (MICs 2·0–4·0 mg/L) and to thiamphenicol (MICs 2·0–4·0 mg/L), and all 23 non-PPNG isolates were susceptible or moderately susceptible to penicillin (MICs 0·030–1·0 mg/L).

Plasmid profiles

Plasmid profiles of all 50 gonococcal isolates were determined. As shown in the Table, all PPNG isolates possessed the 4·4 MDa β-lactamase plasmid. All 47 TRNG carried the 25·2 MDa Tet M plasmid and the 3 non-TRNG possessed the 24·5 MDa conjugative plasmid. All gonococcal isolates were subjected to PCR of the downstream region of the incomplete Tet M transposon: all 47 TRNG produced a 700 base pair PCR fragment, corresponding to the 'Dutch' restriction plasmid type, and the 3 non-TRNG remained negative.

Table. Plasmid profiles of 50 *Neisseria gonorrhoeae* isolates

Resistance phenotype[a]	No.	Plasmid profiles (MDa)
PPNG/TRNG	26	2·6,4·4,25·2
TRNG	21	2·6,25·2
PPNG/Tet R	1	2·6,4·4,24·5
Non-PPNG/non-TRNG/Tet R	2	2·6,24·5

[a]PPNG=penicillin-producing *N. gonorrhoeae*, TRNG=tetracycline-resistant *N. gonorrhoeae*.

Discussion

The data provided by this study should be useful for local and national prospective studies and for monitoring gonococcal infection.

All TRNG isolates analysed in this study possessed the 'Dutch' type Tet M 25·2 MDa plasmid; information from other south-east Asian countries is not available. Recently characterized PPNG isolates from Singapore all carried the 4·4 MDa β-lactamase plasmid (POH *et al.*, 1991; SARAFIAN *et al.*, 1991), similar to our findings in this study for PPNG from Bandung. SARAFIAN *et al.* (1991) and KNAPP *et al.* (1997a) detected different β-lactamase plasmids in south-east Asia: 3·05 and 4·4 MDa in Japan and Taiwan; 3·05, 3·2, 3·9 and 4·4 MDa in the Philippines.

Although the number of gonococcal isolates analysed in this study was relatively small, our data clearly demonstrate that plasmid-mediated resistance to penicillin and to tetracycline is very common in Bandung, no difference in prevalences of PPNG and TRNG being observed between isolates from male and female subjects. TRNG prevalences seem to be similar in Bandung (94%) and Surabaya (98%), while PPNG are less frequent in Bandung (54%) than in Surabaya (85%) (JOESOEF *et al.*, 1994).

Antimicrobial susceptibility patterns observed in isolates from male and female subjects were similar except for thiamphenicol, all 6 isolates resistant to that compound being obtained from female sex workers. Thiamphenicol is still one of the primary drugs for treatment of gonorrhoea in Indonesia. The relatively high rates of less susceptible and resistant isolates from Bandung and Surabaya (JOESOEF *et al.*, 1994) make its efficacy for treatment of gonorrhoea in Indonesia questionable (NSANAZE *et al.*, 1984; TUPASI *et al.*, 1984). The absence of chromosomal resistance to penicillin in Bandung is remarkable since regular administration of 1·2 megaunits of benzathine penicillin for prevention of syphilis was still common practice in the brothels where the sex workers were recruited.

Other primary drugs for treatment of gonorrhoea in Indonesia are spectinomycin, ofloxacin and ciprofloxacin. Resistance to spectinomycin was not detected in this study, in contrast to a prevalence of 18% resistance observed in Surabaya (JOESOEF *et al.*, 1994); this underlines the importance of regional decentralized surveillance in large countries. Fluoroquinolones have been introduced in Indonesia for treatment of gonorrhoea quite recently, and are now the nationally recommended treatment. No resistance *in vitro* was detected either in this study or in Surabaya (JOESOEF *et al.*, 1994). The use of fluoroquinolones, however, has resulted in rapid stepwise development of resistance due to different mutations in the gonococcus (BELLAND *et al.*, 1994). The alarming spread of fluoroquinolone resistance in different countries in south-east Asia (KAM *et al.*, 1993, 1995; KNAPP *et al.*, 1997a, 1997b) clearly illustrates that periodic and careful surveillance of antimicrobial susceptibility remains highly recommended for effective control of gonococcal infection.

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