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Antimicrobial susceptibilities and serotyping of *Neisseria gonorrhoeae* in southern Africa: influence of geographical source of infection

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SUMMARY

One hundred and ninety-two strains of *Neisseria gonorrhoeae* isolated from migrant mine-workers were tested for their susceptibility to antibiotics, auxotyped and serotyped. Of the total, 93 (48%) were acquired locally and 64 (33%) from different geographical locations. Plasmid-mediated resistance to penicillin was found in 28 (14·6%) strains and was associated predominantly with the presence of 5·0 kb penicillinase encoding plasmid (18/28, 64%). Chromosomal resistance to penicillin (MIC $\geqslant$ 1 mg/l) was detected in 14 (7·3%) strains. Resistance to tetracycline was chromosomally and not plasmid-mediated. Antibiotic resistance was encountered most commonly among strains acquired in Natal. The overall gonococcal population was sensitive to ceftriaxone, ciprofloxacin, spectinomycin and azithromycin. Nine auxotype/serovar (A/S) classes were encountered among penicillinase-producing *N. gonorrhoeae* (PPNG) compared to 24 A/S classes among non-PPNG strains. The most common A/S class was NR/IA-6 which accounted for 38% of PPNG and 15% of non-PPNG.

INTRODUCTION

Gonorrhoea remains the most frequent diagnosis made at clinics for sexually transmitted diseases in southern Africa, particularly among men who work in areas remote from home [1, 2]. These migrant workers could introduce strains of *Neisseria gonorrhoeae* from different geographical regions into the local community.

Until relatively recently penicillin remained the antibiotic of choice for the treatment of gonorrhoea in South Africa [3]. However, the emergence of a significant proportion of strains exhibiting plasmid-mediated penicillinase production has prompted the use of alternative treatments in some areas. In addition, infections caused by gonococcal strains refractory to treatment with penicillin but caused by non-penicillinase-producing strains have also become apparent [4].

In this study we report on the *in vitro* susceptibility of 192 strains of *N. gonorrhoeae* isolated from consecutive patients with uncomplicated gonococcal

urethritis attending a clinic at a mine hospital which serves migrant mineworkers. These susceptibility patterns together with auxotypes, serotypes and plasmid content have been correlated with geographical area of acquisition of disease.

MATERIALS AND METHODS

Source of gonococcal isolates

Strains of *N. gonorrhoeae* were isolated from consecutive migrant mineworkers attending a clinic for sexually transmitted diseases at the Leslie Williams' Memorial Hospital, Carltonville, Transvaal, South Africa between May 1989 and September 1989 (97 strains) and July 1990 and August 1990 (95 strains). These strains had been acquired either locally or in regions of the subcontinent remote from the clinic.

Isolation and identification

All strains were initially isolated on modified New York City medium [5]. Cultures were incubated in a candle extinction jar at 35 °C for up to 48 h and isolates identified as *N. gonorrhoeae* by Gram staining, oxidase reaction and by their ability to produce acid from glucose but not maltose, sucrose or lactose. Penicillinase production was detected by using the chromogenic cephalosporin (Nitrocefin, Oxoid, Basingstoke, Hants, UK) method [6] and all isolates were suspended in trypticase soy broth containing 20% glycerol and stored at −70 °C prior to shipment to London. All subcultures were made on GC agar base (Difco, West Molesey, Surrey, UK) containing 1% IsoVitaleX but no antibiotics.

Serotyping

Serotyping was performed using a coagglutination method employing a panel of 12 monoclonal antibodies raised to epitopes on the two types of the major outer membrane protein, PI, of *N. gonorrhoeae* bound to *Staphylococcus aureus* as described by Knapp and co-workers [7].

Auxotyping

Strains were tested for their nutritional requirements for growth for proline, arginine, hypoxanthine, uracil, methionine and histidine using the defined media and methods described by Copley and Egglestone [8]. Ability to use ornithine as an alternative substrate to arginine was also tested.

Antimicrobial susceptibility testing

In vitro susceptibilities of gonococcal isolates to penicillin (0·015–4·0 mg/l), ceftriaxone (0·001–1·0 mg/l), tetracycline (0·008–8·0 mg/l), spectinomycin (2–128 mg/l), ciprofloxacin (0·001–1·0 mg/l) and azithromycin (0·002–2·0 mg/l) were determined using an agar dilution technique described previously [9]. Briefly, one microlitre volumes containing 10^4 cfu were inoculated onto media containing appropriate dilutions of the antibiotic under test in Diagnostic Sensitivity Test (DST) Agar (Oxoid) containing 1% IsoVitaleX and 5% lysed horse blood (Tissue Culture Services, Slough, Berks, UK). The World Health Organization reference strains A–E were used as controls.

Plasmid analysis

Strains of PPNG were grown overnight on GC agar base (Difco) supplemented with 1% IsoVitaleX and 1 mg/l penicillin to enhance plasmid expression. Plasmids were extracted using the method of Birnboim and Doly [10], electrophoresed on 1% agarose gels in 20 mM sodium acetate buffer, pH 7·8 and visualized by staining with ethidium bromide.

Statistics

Differences between numbers of strains acquired in different geographical areas were determined by use of the chi-squared test. Differences in susceptibility to antibiotics detected between strains from different geographical areas were determined using the Mann–Whitney test for non-parametric means.

RESULTS

Of 192 gonococcal strains available for susceptibility testing, auxotyping and serotyping, 93 (48%) were acquired locally and 64 (33%) were known to be acquired in various distinct geographical areas remote from the study centre. The geographical source of infection was unknown in 35 cases. The relative prevalence of penicillin-resistant strains of *N. gonorrhoeae* analysed by geographical source of acquisition is shown in Table 1. Overall, 28 (14.6%) strains were PPNG and a further 14 (7·3%) were designated as chromosomally-mediated resistant *N. gonorrhoeae* (CMRNG, MIC $\geqslant$ 1·0 mg/l). Resistance to penicillin, either plasmid or chromosomally mediated, was found to be significantly associated with acquisition in Natal but not with other geographical areas (PPNG, $P = 0{\cdot}02$; CMRNG, $P = 0{\cdot}01$).

Gonococcal strains containing both the 5·0 kb penicillinase-encoding plasmid and 38 kb conjugative plasmid constituted 17 of 28 (61%) PPNG strains. Only one strain carried the 5·0 kb plasmid alone. Seven of the remaining ten PPNG strains carried the 6·8 kb penicillinase-encoding plasmid alone and three strains carried the 6·8 kb and 38 kb plasmid (Table 1). All PPNG strains were found to carry the 4·0 kb cryptic plasmid.

The results of antimicrobial susceptibility testing of both PPNG and non-PPNG strains to a variety of antibiotics are shown in Table 2. Overall, isolates remained sensitive to ceftriaxone, ciprofloxacin, spectinomycin and azithromycin, while resistance to penicillin and tetracycline was commonly encountered. Examination of susceptibilities of non-PPNG strains by geographical area of acquisition indicated that strains acquired in Natal were significantly more resistant to penicillin ($P = 0{\cdot}01$), ceftriaxone ($P = 0{\cdot}001$) and tetracycline ($P = 0{\cdot}03$) but not to ciprofloxacin ($P = 0{\cdot}12$) (Fig. 1, A–D) and azithromycin ($P = 0{\cdot}59$) when compared to strains acquired elsewhere (Fig. 1, A–D). Differences for spectinomycin were found to be of borderline significance ($P = 0{\cdot}05$).

In the total gonococcal population 24 serovars, 7 auxotypes and 50 A/S classes were found. However, among PPNG strains only 7 serovars, 2 auxotypes and 9 A/S classes were identified (Table 3). The A/S class NR/IA-6 was the most

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Table 1. *Relative prevalence of penicillin-resistant* N. gonorrhoeae *by geographical source of acquisition*

Source of infection	Total no. strains	No. CMRNG (%)	No. PPNG (%)	Plasmid content (kb)* (no.)			
				5·0	5·0+38·0	6·8	6·8+38·0
Local	93	6 (6·5)	10 (10·8)	0	6	3	1
Lesotho	21	0	2 (9·5)	1	1	0	0
Natal	23	5 (21·7)	7 (30·4)	0	6	0	1
Botswana	16	0	2 (12·5)	0	1	1	0
Transkei	4	0	2 (50·0)	0	1	1	0
Unknown	35	3 (8·5)	5 (14·3)	0	2	2	1
Total	192	14 (7·3)	28 (14·6)	1	17	7	3

* All strains carried 4·0 kb cryptic plasmid.

Table 2. *Antimicrobial susceptibilities of 164 non-PPNG and 28 PPNG strains*

Antibiotic		(mg/l)		
		MIC_{50}	MIC_{90}	Range
Penicillin	non-PPNG	0·12	0·5	0·008–2
	PPNG	⩾4	⩾4	⩾4
Ceftriaxone	non-PPNG	0·002	0·008	0·002–0·03
	PPNG	0·004	0·008	0·002–0·015
Ciprofloxacin	non-PPNG	0·004	0·008	0·002–0·015
	PPNG	0·004	0·008	0·004–0·015
Spectinomycin	non-PPNG	16	32	2–32
	PPNG	32	32	16–32
Tetracycline	non-PPNG	1·0	4·0	0·25–8
	PPNG	1·0	2·0	1–4
Azithromycin	non-PPNG	0·12	0·5	0·03–1
	PPNG	0·25	0·5	0·06–1

common detected, accounting for 10 of 28 PPNG strains (36%) and was found only in strains carrying both 5·0 and 38 kb plasmids. PPNG (7 strains) carrying the 6·8 kb plasmid alone belonged to only 2 A/S classes, pro/IA-6 and pro/IB-5.

Twenty-four serovars were identified among non-PPNG strains of which 3 serovars accounted for 86 of 164 strains (Table 4). Seven auxotypes were found of which non-requiring (NR) and proline requiring (pro) accounted for 129 of 164 strains (79%). No arginine, hypoxanthine and uracil (AHU) requiring strains were encountered. Of the 48 A/S classes identified among non-PPNG strains, NR/IA-6 (25/164, 15·2%), NR/IB-3 (11/164, 6·7%) and Pro/IA-6 (10/164, 6·1%) were the most common (Table 4).

DISCUSSION

The results of this study are part of an on-going programme of surveillance of antibiotic susceptibility. The total gonococcal population from migrant mine-workers isolated over the past 8 years, including the strains collected for this study, shows an increase in the relative prevalence of PPNG strains (Table 5). In addition, while CMRNG accounted for 7·3% of non-PPNG isolates in the present study (1989–90) in 1985 CMRNG were not detected among 100 non-PPNG isolates obtained from the same clinic [11].

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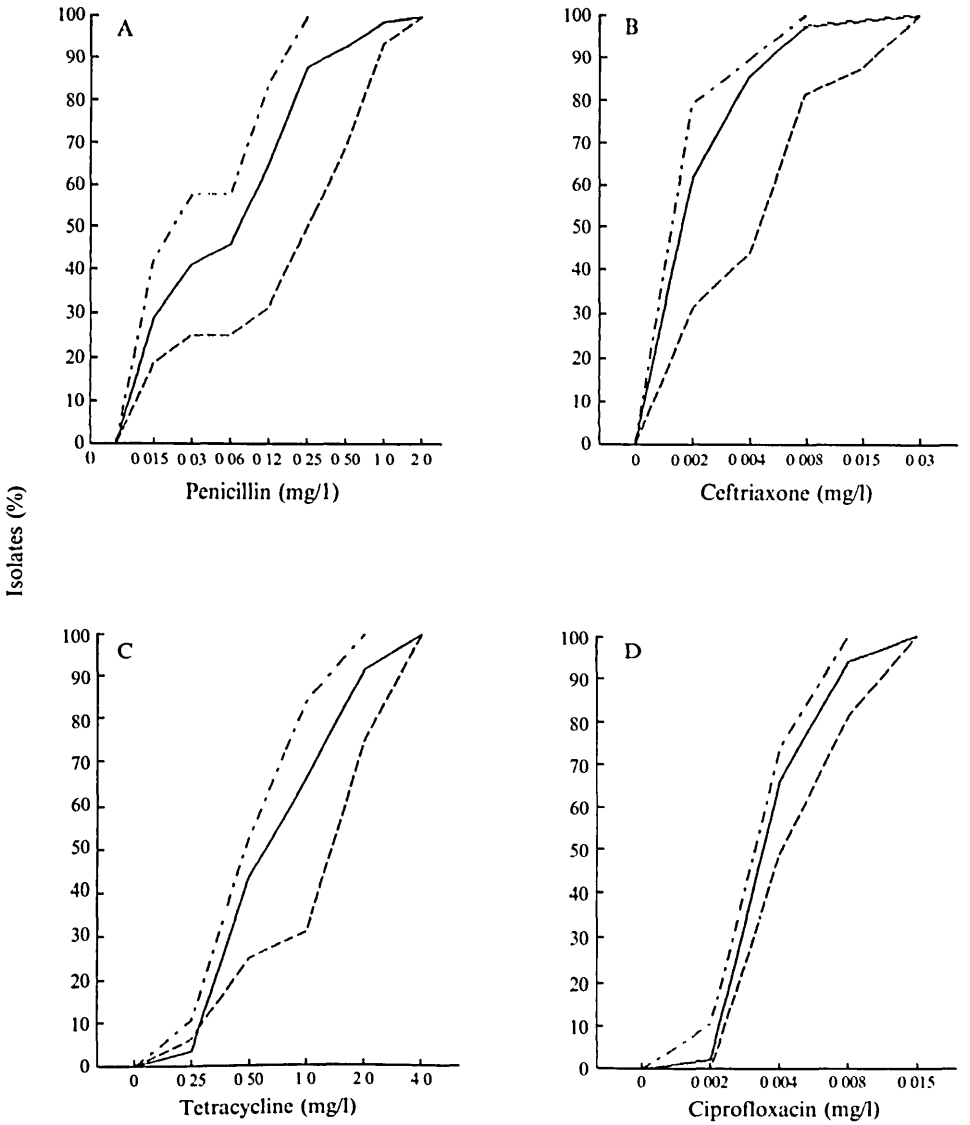

Fig. 1. Susceptibility of non-PPNG acquired locally . in Lesotho – – – and in Natal ––––– to (A) penicillin, (B) ceftriaxone, (C) tetracycline and (D) ciprofloxacin.

During the same period resistance to tetracycline has also emerged as a significant problem with the MIC_{90} increasing from 0·5 mg/l in 1985 (range 0·015–0·5 mg/l) [11] to 4·0 mg/l (range 0·25–8 mg/l) in the present study. We have also recently detected high rates of tetracycline resistance among gonococcal isolates from a clinic in Johannesburg [4] which is the nearest city to the study site. In both these recent studies no evidence of plasmid-mediated (*tetM*) resistance was detected. It is believed that these strains may have emerged as a result of the routine practice of treating gonorrhoea in some clinics with tetracycline alone.

Although resistance to penicillin and tetracycline was found to be widespread it would appear that there are distinct regional differences in antimicrobial

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Table 3. *Association of auxotype/serovar classes and plasmid content of strains of PPNG*

A/S Class*	Total no.	Plasmid content (kb)† (no. of strains)			
		5·0	5·0+38·0	6·8	6·8+38·0
NR/IA-6	10	0	10	0	0
Pro/IA-6	5	0	1	3	1
NR/IA-2	1	0	1	0	0
NR/IA-8	2	1	1	0	0
NR/IB-5	1	0	1	0	0
Pro/IB-5	4	0	0	4	0
NR/IB-7	3	0	3	0	0
Pro/IB-18	1	0	0	0	1
Pro/IB-26	1	0	0	0	1
Total	28	1	17	7	3

* NR, non-requiring; Pro, proline-requiring.
† All strains carried a 4·0 kb cryptic plasmid.

Table 4. *Distribution of serovars and auxotypes of non-PPNG (164 strains)*

Serovar	Number (%)	Auxotype* (no. of strains)				
		NR	Pro	Arg	Pro, Arg	Others†
IA-6	46 (28)	25	10	3	8	0
IB-3	23 (14)	11	8	0	3	1
IB-6	17 (10)	6	9	1	0	1
IB-1	12 (7)	5	6	0	1	0
IB-7	8 (5)	6	2	0	0	0
IA-2	7 (4)	4	1	1	1	0
IA-8	7 (4)	7	0	0	0	0
IB-4	6 (4)	5	1	0	0	0
IA-4	5 (3)	5	0	0	0	0
IA-9	5 (3)	2	1	0	0	2
IB-5	5 (3)	0	5	0	0	0
IB-13	5 (3)	2	2	0	1	0
IA-5	4 (3)	2	1	1	0	0
Others‡	13 (8)	7	6	0	0	0
Total	163	87	52	6	14	4

* NR, non-requiring; Pro, proline-requiring; Arg, arginine-requiring; Pro, Arg, proline and arginine-requiring.
† Auxotypes: Pro, hyx; Pro, meth; Meth; Arg, his.
‡ Serovars: IB-2, IB-8 and IB-9 two strains each. IA-3, IA-10, IA-16, IB-11, IB-14, IB-19, IB-26, one strain.
NB One strain was unavailable for testing.

susceptibility patterns, with significantly higher rates of resistance to penicillin recorded among strains acquired in Natal when compared to acquisition in other regions. While resistance to ceftriaxone has not reached a level of clinical significance, it is interesting to note that a lower dose of ceftriaxone, 125 mg, than is recommended for therapy, 500 mg, [12] is currently used in Durban, Natal STD clinics [13]. The close relationship between chromosomal resistance to penicillin and reduced susceptibility to cephalosporins has previously been detected in Natal [3]. Chromosomal mutations responsible for penicillin resistance have been found

Table 5. *Relative prevalence of PPNG strains among mineworkers in Carltonville, South Africa*

Year	Total *N. gonorrhoeae* (%)	
	Total PPNG strains	Locally acquired PPNG strains
1983	12	3
1984	9	5
1985	13	9
1986	11	10
1987	12	13
1988	14	11
1989	15	13
1990	18	15

to be indistinguishable from those for reduced susceptibility to cephalosporins including ceftriaxone [14] and hence the use of low dosage may increase the selective pressure on strains which are already less sensitive to penicillin.

The majority of PPNG strains harboured the so called 'African' (5·0 kb) plasmid. However, 10 (36%) of the PPNG strains harboured the 6·8 kb plasmid which was originally linked epidemiologically with the Far East [15]. The presence of the strains harbouring the 'Asian' plasmid suggests that these PPNG or their ancestors were imported. Dissemination of penicillinase plasmids is dependent on the presence of the conjugative plasmid for transfer between strains of *N. gonorrhoeae* [16]. The conjugative plasmid is often found in combination with the Asian (6·8 kb) penicillinase plasmid and this could account for the spread of these PPNG worldwide including many countries in Africa [17–22]. In contrast PPNG harbouring both the 5·0 kb plasmid and the 38 kb conjugative plasmid have been isolated less frequently and its spread has been more limited. In this study the conjugative plasmid was carried by only 30% of PPNG which harboured the 6·8 kb penicillinase plasmid compared with 94% (17/18 strains) of those harbouring the 5·0 kb plasmid. If PPNG infections occur in this population by transfer of penicillinase plasmids between indigenous strains rather than importation of PPNG, the absence of the conjugative plasmid may result in a decrease in relative prevalence of PPNG strains harbouring the 6·8 kb plasmid in the future.

Antibodies have been used to serogroup strains of *N. gonorrhoeae* into IA (WI) and IB (WII/WIII) in various studies from countries in Africa. However, only a few studies have used the panel of monoclonal antibodies to determine the serotype [20, 23, 24]. In this study the gonococcal population was heterogeneous, although there were fewer serovars and A/S classes among the PPNG than the non-PPNG. We have encountered this phenomenon in other studies both in London, UK [25] and in The Gambia [26]. Serovar IA-6 strains were the most frequently detected in this study and have been reported as one of the predominant serovars in Kenya [24]. Serovar IA-2 strains are less common in this and other studies from countries in Africa compared with Europe [25] or the United States [7]. Serovar IA-2 strains are often AHU requiring. However, it is unusual to detect AHU strains in African studies. A possible explanation is that, in an environment

where antibiotics are readily available and resistance can be a major problem, the AHU/IA−2 strains which are hypersensitive to antibiotics [27] have been selected against. Only seven auxotypes were demonstrated in this study, of which non-requiring and proline-requiring were most prevalent.

We have identified a small cluster of PPNG strains (10/17, 59%) which belonged to A/S class NR/IA-6 and carried both the 5·0 and 38 kb plasmids. Five of these 10 PPNG were acquired locally.

The population seen in this clinic consisted of men who work in one area and migrate to their homes a few times during the year. Sexually transmitted diseases such as gonorrhoea will, therefore, be acquired and transmitted at either location. Strains of *N. gonorrhoeae* acquired by this population will be representative of many geographical areas and as seen in this study vary in their susceptibility to antibiotics and have the potential to be introduced and spread by the local population. Typing methods such as auxotyping and serotyping used in conjunction with plasmid analysis and susceptibility patterns are useful epidemiological tools to monitor such developments.

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