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Analysis of penicillinase-producing *Neisseria gonorrhoeae* isolates in Madrid (Spain) from 1983–85

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SUMMARY

Between April 1983 and December 1985, 576 strains of *Neisseria gonorrhoeae* were isolated in our laboratory from patients attending Sexually Transmitted Diseases (STD) clinics. Of these, 61 (10·6%) were penicillinase-producing. Studies on these strains by plasmid analysis, auxotyping and serogrouping showed that the predominant type strains harboured the Asian resistance plasmid, were prototrophic, and were of serogroup WII/WIII. About half of the strains, both of the African and Asian type, harboured the transfer plasmid. Strains of serogroup WII/WIII were less sensitive to tetracycline and cefoxitin than serogroup WI strains.

INTRODUCTION

A penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strain was first isolated in Spain in 1979 (Alomar, Gil Sanchez & Arteaga, 1979).

In Spain gonorrhoea was not a reportable disease until 1982, and the available data on the isolation of PPNG was both scarce and incomplete. In 1982 12·5% of strains in Barcelona were PPNG and during 1983 such strains constituted 9·7% of gonococcal isolates in Centro Nacional de Microbiología (C.N.M.V.I.S.) from the Madrid region (Vázquez *et al.* 1985).

In this study the PPNG strains isolated during the period from April 1983 to December 1985 from the Madrid region have been characterized by auxotyping, serogrouping and plasmid analysis.

Minimum inhibitory concentrations (MIC) to five antibiotics (benzylpenicillin, spectinomycin, tetracycline, cefoxitin and ceftazidime) were also determined in order to demonstrate possible relationships between antibiotic susceptibilities and other features of the organisms.

MATERIAL AND METHODS

Population studied

Between April 1983 and December 1985, specimens from 1648 males and 1294 females attending Sexually Transmitted Diseases (STD) clinics were examined for the presence of *N. gonorrhoeae*.

Isolation and identification

The transport of specimens to the laboratory, the isolation in Thayer–Martin medium and identification of the strains by means of the Phadebact test (Pharmacia Diagnostic, Piscataway, New Jersey) have been described previously (Vázquez, Berrón & Fenoll, 1985).

All isolates were tested for penicillinase production using the acidometric method ('beta-lactamase detection papers', Oxoid Ltd, Basingstoke, England).

Auxotyping

Gonococcal auxotyping was performed by the method of Catlin (Catlin, 1978). Strains of *N. gonorrhoeae* of known auxotype were used as controls: NCTC (National Culture Type Collection) 10928, prototrophic (wild type); NCTC 10930, Pro Hyx (proline, hypoxanthine); NCTC 10931, Pro Met Thi (proline, methionine, thiamine); NCTC 10932, Arg Met (arginine methionine); NCTC 10933 Arg Hyx Ura (arginine, hypoxanthine, uracil).

Serogrouping

Gonococcal serogrouping was performed using reagents against WI and WII/WIII antigens commercially available (Phadebact Monoclonal GC Test-Pharmacia Diagnostic, Uppsala, Sweden).

Plasmid determination

Plasmid DNA was extracted and purified according to the method of Birboim & Doly, (1979), with an additional incubation at 55 °C for 30 min to achieve complete lysis of the strains. Electrophoresis was carried out on 0·6 % agar (Meyers *et al.* 1976). *Escherichia coli* V517 (Macrina *et al.* 1978) containing eight plasmids of known molecular weight was used as control.

Antibiotics and susceptibility testing

Antibiotic susceptibility tests were performed by the agar dilution technique (Thornsberry, Gerlach & Sherris, 1977) with strains of *N. gonorrhoeae* with known antibiotic susceptibilities as controls (provided by Dr Knut Lincoln, University of Goteborg, Sweden).

Gonococci were inoculated using a multipoint inoculator (Microtiter AM 80) on 3·5 % chocolate agar (GC medium base, Difco) containing 5 % haemolysed human blood and 1 % Kellog supplement (Wite & Kellog, 1985) together with twofold dilutions of the drug to be tested. After incubation for 24 h at 37 °C in 5 % CO^2 the MIC was read.

The range of concentrations of the antibiotics tested was 0·007–128·0 mg/l benzyl penicillin G, 0·12–8·0 mg/l tetracycline, 0·12–4·0 mg/l cefoxitin, 2·0–64·0 mg/l spectinomycin and 0·007–0·12 mg/l ceftazidime.

Statistical analysis

χ^2 test with Yate's correction was used (Colton, 1974).

Table 1. *Incidence of* N. gonorrhoeae *isolations during 1983–85 in STD clinics in Madrid region*

Year	No. cases	M (males)	F (females)
		Total cases	
1983	354	178	176
1984	927	568	359
1985	1661	902	759
Total	2942	1648	1294
		Isolation of *N. gonorrhoeae* (%)	
1983	82 (23·2)	55 (30·9)	27 (15·3)
1984	224 (24·2)	163 (28·7)	61 (17·0)
1985	270 (16·2)	194 (21·5)	76 (10·0)
Total	576 (19·6)	412 (25·0)	164 (12·7)
		PPNG (%)	
1983	10 (12·9)	6 (10·9)	4 (14·8)
1984	23 (10·3)	16 (9·8)	7 (11·4)
1985	28 (10·4)	19 (9·8)	9 (11·8)
Total	61 (10·6)	41 (10·0)	20 (12·2)

RESULTS

Gonococci were isolated from 412 males and 164 females. Of the 576 isolates, 61 (10·6%) were PPNGs (Table 1). During 1983, 1984 and 1985 the prevalence of PPNG strains was about the same.

Of a total of 43 (70·5%) PPNG strains which had no special nutritional requirements (wild type), 6 (14%) belonged to the WII/WIII serogroup. Of the 15 (24·5%) strains requiring proline, 9 (60%) belonged to the WI serogroup and 6 (40%) to the WII/WIII serogroup (Fig. 1). Three strains (5%) belonged to other auxotypes.

The relationships between serogroups and *in vitro* susceptibility of the PPNG strains to tetracycline, cefoxitin and ceftazidime are presented in Table 2. For tetracycline, 5/16 strains in serogroup WI had an MIC $\geqslant$ 1 mg/l compared with 31/45 in the WII/WIII serogroup ($P < 0{\cdot}025$). For cefoxitin, 3/16 strains in the WI serogroup had an MIC $\geqslant$ 1 mg/l as did 30/45 in the WII/WIII serogroup ($P < 0{\cdot}005$). All strains in both serogroups were sensitive to ceftazidime and to spectinomycin.

All strains harboured the 2·6 MDa plasmid, 8 (13·1%) the 3·2 MDa plasmid (African) and 53 (86·9%) the 4·5 plasmid (Asian). In the 28 strains which harboured the 24·5 MDa plasmid as well, 4 were African type and 24 Asian

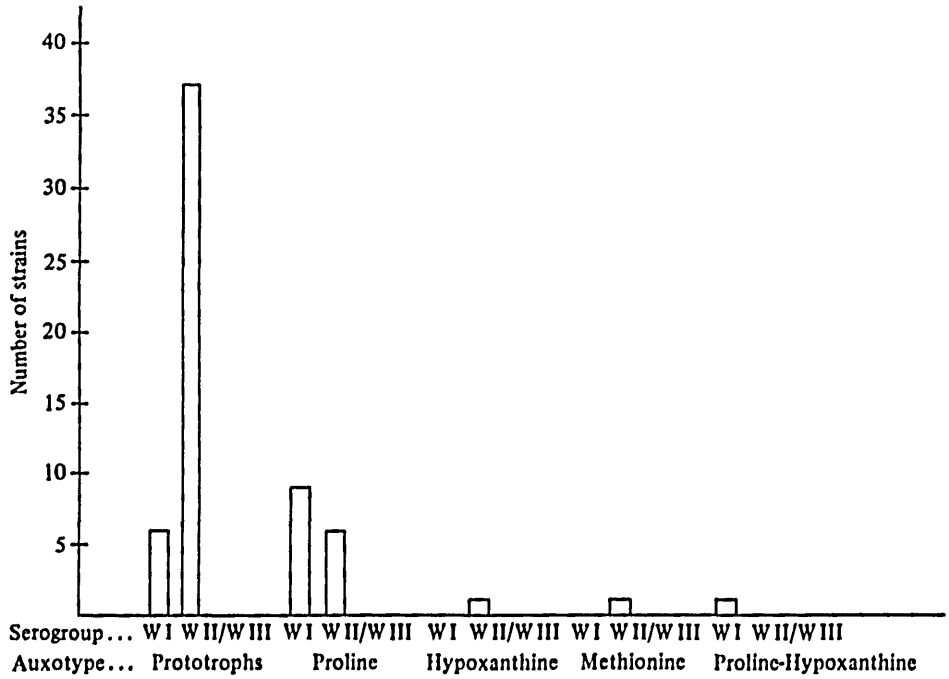

Fig. 1. Incidence of combinations of auxotypes and serogroups in 61 PPNG strains.

Table 2. In vitro *susceptibility of PPNG strains to tetracycline, cefoxitin and ceftazidime in relation to serogroups*

Serogroup	Minimum inhibitory concentrations, mg/l: 0·007–0·03	0·06–0·125	0·25–0·50	1·0–4·0	Total
Tetracycline					
W I	—	1	10	5	16
W II/W III	—	—	14	31	45
Total	—	1	24	36	61
Cefoxitin					
W I	—	1	12	3	16
W II/W III	—	—	15	30	45
Total	—	1	27	33	61
Ceftazidime					
W I	16	—	—	—	16
W II/W III	41	4	—	—	45
Total	57	4	—	—	61

type (Table 3). A gel loaded with representative DNA preparations is shown in Fig. 2.

All African strains were of the prototrophic auxotype whereas the 53 Asian PPNG were distributed among five different auxotypes (Table 3).

Table 4 shows the MIC for the five antibiotics tested on strains with the African plasmid and with the Asian plasmid. The only difference nearing significance between the two groups was with tetracycline ($P < 0·05$).

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Table 3. *Plasmid content, auxotype and serogroup of 61 PPNG strains*

Plasmids (MDa)	No. of strains	Auxotype: Prototrophic	Proline	Hypoxanthine	Methionine	Proline-Hypoxanthine
			African type			
2·6–3·2	4	4/0*	—	—	—	—
2·6–3·2–24·5	4	0/4	—	—	—	—
Total	8	4/4	—	—	—	—
			Asian type			
2·6–4·5	29	1/16	5/5	—	0/1	1/0
2·6–4·5–24·5	24	1/17	3/2	0/1	—	—
Total	53	2/33	8/7	0/1	0/1	1/0

* No. in serogroup WI/no. in serogroup WII/WIII.

DISCUSSION

The high incidence of PPNG strains found by us during 1983 in Madrid (Vazquez *et al.* 1985) has changed little over the 3 years of this study and is similar in males and females. The incidence of PPNG strains resembles that described in the Netherlands (Ansink-Schipper *et al.* 1984), but is higher than reported from most European countries (Anon, 1983). It is not possible to assess whether the situation in the Madrid region is similar to that in other regions of Spain since data is both sparse and incomplete. The Spanish Weekly Microbiological Bulletin reported an incidence of PPNG strains of 7·3% during 1985 (Anon, 1986) but the number of isolates included was small.

Some information about the epidemiology of PPNG was obtained through the application of a number of typing techniques. The major typing patterns comprised strains of serogroup WII/WIII which were not nutritionally exacting (protoprophs) followed by strains of WI serogroup, proline dependent. Thereafter strains of WI serogroup, prototrophs and of WII/WIII serogroup, proline dependent were identified in similar numbers. Other auxotypes were occasionally found (Fig. 1). This dominant incidence of prototrophic strains followed by proline dependent ones is much the same as that reported from other countries (Ansink-Schipper *et al.* 1984; Yvert *et al.* 1985). In our study a greater proportion of proline dependent strains were of serogroup WI than were prototrophic strains ($P < 0{\cdot}001$).

Strains of serogroup WII/WIII were less sensitive to tetracycline and cefoxitin than strains of serogroup WI, a correlation that has been described before (Danielson, Bygdeman & Kallings, 1983; Bygdeman *et al.* 1982). Thus high incidence of serogroup WII/WIII in Spain could well be of clinical importance. Though spectinomycin-resistant PPNG strains have been reported in other countries (Anon, 1983; Pond *et al.* 1986), none were found in this study.

About 87% of the PPNG isolates possessed the Asian plasmid and half of those harboured the conjugative plasmid (24·5 MDa) as well. The African type was present in only 13% of PPNG strains. Comparable results have been reported from

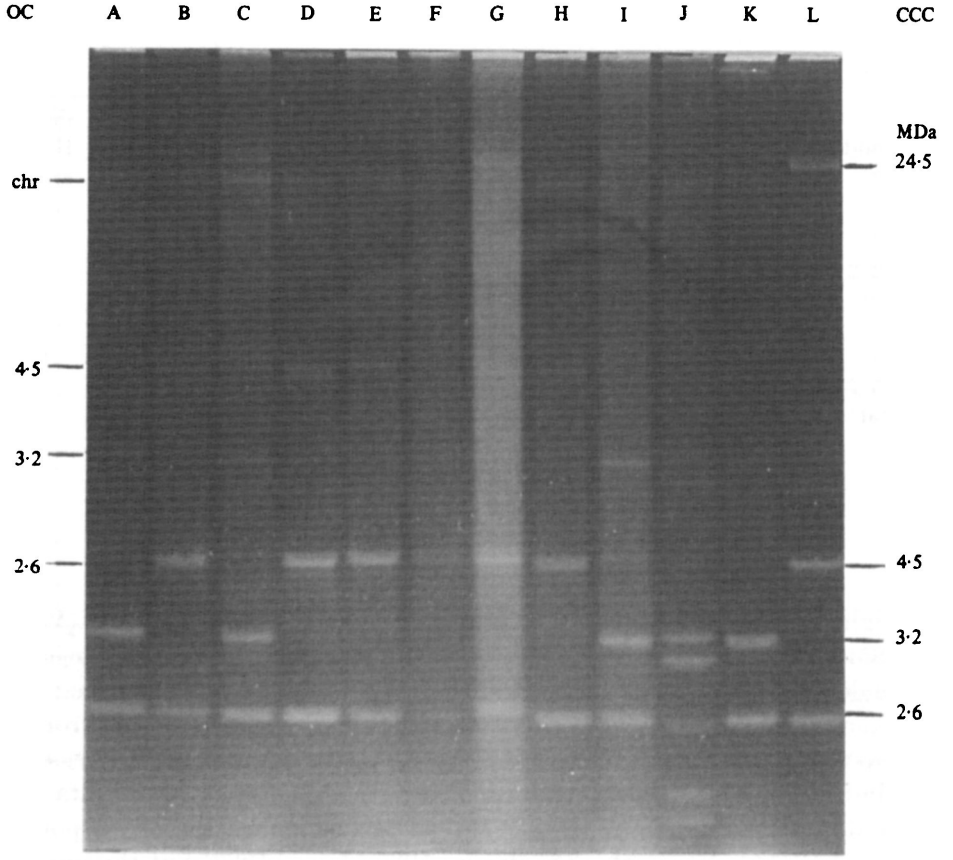

Fig. 2. Agarose gel electrophoresis of plasmid DNA from different PPNG strains containing: 2·6 and 3·2 MDa plasmid (A, K), 2·6, 3·2 and 24·5 MDa plasmid (C, I), 2·6 and 4·5 MDa plasmid (B, D, E, F, H) and 2·6, 4·5 and 24·5 MDa plasmid (G, L). Lane J shows plasmids from *E. coli* V 517.

Table 4. In vitro *susceptibility to antibiotic of PPNG strains harbouring the 'African' plasmid and PPNG strains carrying the 'Asian' plasmid*

Plasmid type	MIC$_{90}$ (mg/l)				
	Penicillin	Spectinomicin	Tetracycline	Cefoxitin	Ceftazidime
'African'	24·2	14·9	0·6	0·6	0·024
'Asian'	37·7	14·9	1·9	1·3	0·029

England and Switzerland (Goh *et al.* 1985; Arini *et al.* 1985) but in the Netherlands a higher incidence is recorded (Ansik-Schipper *et al.* 1984). Half of the isolates with the African plasmid harboured the transfer plasmid as well. The emergence of this combination was associated, in the Netherlands, with an increase in the incidence of PPNG infections (Ansik-Schipper *et al.* 1984). All African type strains in this study carrying the transfer plasmid were isolated during 1985 which may have a serious epidemiological impact.

Further features of note were a correlation demonstrable between the non-exacting auxotype and the African plasmid already noted elsewhere (Arini *et al.*

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1985; Ansik-Schipper *et al.* 1984), and the difference between African type and Asian type strains for *in vitro* susceptibility to tetracycline which has also been reported before (Goh *et al.* 1985; Perine *et al.* 1977).

Unfortunately, epidemiological data to assess whether the PPNG isolates were from locally acquired or imported infections is not available. However, from 20 PPNG strains isolated in women, 8 were found in prostitutes and 3 in promiscuous women not admitting to working in prostitution. Prostitutes have been described as an important reservoir of PPNG infections in some countries (Ansik-Schipper *et al.* 1984; Arya *et al.* 1984) and may well be so in Spain. Perhaps surprisingly no beta-lactamase producing strains were isolated from homosexual men.

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