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Plasmid content and protein I serovar of non-pencillinase-producing gonococci isolated in Munich

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SUMMARY

One hundred and twenty-four strains of non-penicillinase-producing gonococci isolated in Munich in 1986 were characterized in terms of their plasmid content and protein I serovar. Eighty-two per cent of the strains belonged to serogroup 1-B with over half belonging to either serovar 1B-2 or 1B-3. Half of the 22 serogroup 1A strains belonged to serovar 1A-2. Nineteen strains (15·3%) were found to lack the 2·6 Md cryptic plasmid although seven of these strains contained the 24·4 Md conjugative plasmid. Nine of the 105 strains which harboured the cryptic plasmid also contained the conjugative plasmid. The 19 strains which lacked the cryptic plasmid comprised 10 different serovars, indicating the heterogeneous nature of this group of organisms.

INTRODUCTION

In order to investigate the epidemiology of gonococcal infections, it is necessary to be able to differentiate between strains of *Neisseria gonorrhoeae*. Systems currently used for typing gonococci include plasmid analysis (Perine *et al.* 1977), auxotyping (Catlin, 1973; Perine *et al.* 1977; Handsfield *et al.* 1982), and serotyping using either polyclonal (Handsfield *et al.* 1982) or monoclonal antibodies (Knapp *et al.* 1984) directed against gonococcal protein I. The value of these typing schemes for epidemiological studies has been demonstrated by their successful use in the investigation of both microepidemics (Ramstedt *et al.* 1985) and the inter-country and inter-continental spread of infection (Perine *et al.* 1977).

A fuller understanding of the epidemiology of gonorrhoea on a global basis requires the collection and characterization of strains from many different parts of the world. In this study, we have analysed the plasmid content and protein I serovar of 124 strains of non-penicillinase-producing gonococci isolated at the Ludwig-Maximilians University in Munich during 1986.

MATERIALS AND METHODS

Bacteria

One hundred and thirty-seven strains of *N. gonorrhoeae* (including two penicillinase-producing strains) were isolated at the Department of Dermatology, Ludwig-Maximilians-Universität, Munich, during 1986 (Abeck, Johnson & Korting, 1987). One hundred and twenty-four of the non-penicillinase-producing strains were transported (frozen) to the Clinical Research Centre for determination of their plasmid content and protein I serovar. The organisms were cultured on solid medium consisting of GC agar base (Difco) containing 1% (v/v) IsoVitalex (BBL) in an atmosphere of 5% CO_2 in air at 37 °C. Stock cultures were stored in skimmed milk at −70 °C.

Plasmid analysis

The plasmids were extracted from each strain using the method of Birnboim & Doly (1979). Extracted nucleic acids were subjected to electrophoresis in 0·8% agarose gels and after staining with ethidium bromide were visualized using ultraviolet light.

Protein I serovar

The protein I serovar of each strain was determined by a co-agglutination reaction using a panel of 12 monoclonal antibodies (Syva) as described by Knapp and colleagues (1984). Each co-agglutination was performed by mixing one drop of a suspension of boiled gonococci with one drop of a suspension of sensitized staphylococci, rotating the mixture for 2 min and then observing for agglutination under oblique transmitted light.

RESULTS

Plasmid content

The plasmid content of the 124 strains examined is shown in Table 1. One hundred and five strains (84·7%) contained the 2·6 Md cryptic plasmid, with nine of these strains also harbouring the 24·4 Md conjugative plasmid. Nineteen strains (15·3%) lacked the 2·6 Md plasmid, with 7 of these strains harbouring the 24·4 Md plasmid and the remaining 12 strains being plasmid-free.

Protein I serovars

One hundred and twenty-two of the 124 strains were tested with monoclonal antibodies to determine their serovar distribution. Twenty-two of these strains (18%) belonged to serogroup 1A, and 100 strains (82%) belonged to serogroup 1B (Table 1). Eleven (50%) of the 22 strains belonging to serogroup 1A were of serovar 1A-2, with 10 of the remaining 11 strains comprising 5 different serovars (1A-3, 1A-4, 1A-6, 1A-10 and 1A-20). The serovar of one serogroup 1A strain could not be determined as inconsistent results were obtained upon repeated testing. Over half of the 100 strains within serogroup 1B belonged to either of two serovars, namely 1B-2 (23%) or 1B-3 (30%). Forty-four of the remaining 47 strains of sero- group 1B were distributed among 12 different serovars, the most

Table 1. *Plasmid content and serogroups of gonococci isolated in Munich*

Plasmid content (Md)	No. of strains (%)	Serogroup 1A		Serogroup 1B	
		No. of strains	No. of serovars	No. of strains	No. of serovars
2·6	96*(77·4)	19	6	75	12
2·6+24·4	9 (7·3)	1	1	8	6
24·4	7 (5·6)	2	1	5	4
No plasmids	12 (9·7)	—	—	12	6
Total	124 (100)	22		100	

* Two strains harbouring the 2·6 Md plasmid were not serotyped.

Table 2. *Distribution of protein I serovars among 19 strains of gonococci lacking the cryptic plasmid*

Plasmid content (Md)	No. of strains	No. of strains of indicated serovar									
		1A-4	1B-2	1B-3	1B-4	1B-7	1B-8	1B-10	1B-16	1B-26	1B-?*
No plasmid	12	—	4	—	1	1	—	1	4	1	—
24·4	7	2	—	1	—	—	1	—	—	1	2

* 1B-?, these strains could not be allocated to previously described 1B serovars.

prevalent of these being serovars 1B-8 (8 strains), 1B-1 (7 strains) 1B-4 (7 strains) and 1B-16 (6 strains). The other serovars detected included 1B-7, 1B-10, 1B-14, 1B-17, 1B-18, 1B-20, 1B-22 and 1B-26. The coagglutination reactions observed with three strains belonging to serogroup 1B was such that they could not be assigned to any of the previously designated serovars. Two of these strains reacted with all the protein 1B-specific monoclonal antibodies except 1F5 and 2D4, while one strain reacted only with monoclonal antibody 2G2.

The distribution of protein I serovars among the 19 strains which lacked the cryptic plasmid is shown in Table 2. The 12 plasmid-free strains comprised 6 different serovars, while 5 of the 7 strains containing the conjugative plasmid comprised 4 different known serovars. The two other strains reacted with serogroup 1B specific monoclonal antibodies, but in a pattern which did not permit allocation to serovars described previously.

DISCUSSION

The results presented here show that the majority (82%) of strains of non-penicillinase producing gonococci isolated in Munich during 1986 belonged to serogroup 1B. This finding is similar to the earlier observation that about 70% of penicillinase-producing strains isolated at the same clinic from 1981 to 1986 also belonged to serogroup 1B (Abeck *et al.* 1987), although the significance of this is unclear.

A further finding of particular interest was that approximately 15% of the

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gonococcal strains examined lacked the 2·6 Md cryptic plasmid. This is in contrast to the situation reported in the late 1970s when only 4% of 261 strains of *N. gonorrhoeae* isolated from different parts of the world lacked this plasmid (Roberts, Piot & Falkow, 1979). Although recent studies have shown that a large proportion of gonococcal isolates in Canada are also plasmid free (Dillon, Bygdeman & Sandstrom, 1987), there is an obvious difference between the situation seen in Canada and that seen in Munich. In a recent Canadian study, 93% of 53 plasmid-free strains of gonococci belonged to the same serovar (designated Bacejk) with 79% of the strains also belonging to the same auxotype (P-C-U-) (Dillon Bygdemann & Sandstrom 1987). In contrast, the 19 Munich strains which lacked the 2·6 Md cryptic plasmid comprised 10 different serovars, indicating a much greater degree of heterogeneity among these strains.

The data provided here should prove useful for epidemiological purposes as it not only provides base-line data for a prospective study of gonococcal infection in Munich, but may also allow comparison with gonococcal infections which are being monitored both in other areas of West Germany (Kohl *et al.* 1986), as well as in other parts of the world. In particular, it will be of interest to determine if there is a change in the proportion of strains isolated in Munich which lack the 2·6 Md cryptic plasmid, and if so, whether the distribution of protein I serovars among such strains differs from that seen at present.

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