

Plasmid content, auxotype and protein-I serovar of gonococci isolated in the Gambia

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SUMMARY

Twenty-nine strains of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and 30 non-penicillinase-producing strains, all isolated in the Gambia, were characterized in terms of their plasmid content, auxotype and protein-I serovar. Sixty-two per cent of the PPNG strains contained the 3.2 MDa penicillinase-encoding plasmid, and 38% had the 4.4 MDa plasmid. All the PPNG strains contained the 2.6 MDa cryptic plasmid but lacked the 24.4 MDa conjugative plasmid. In contrast, 46.7% of the non-PPNG strains harboured only the cryptic plasmid while 16.7% contained both the cryptic and conjugative plasmids. Seventeen per cent of the non-PPNG strains contained the conjugative plasmid only and 20% lacked plasmids.

The PPNG and non-PPNG strains also differed in terms of their protein-I serovar. Eighty-six per cent of the PPNG strains belonged to serogroup 1A, whereas the majority (60%) of non-PPNG strains belonged to serogroup 1B. There was no significant difference in the auxotypes of the PPNG and non-PPNG strains, with both groups consisting predominantly of prototrophic and proline-requiring strains, with a minority of strains requiring arginine. When the 59 strains were each characterized in terms of their combined plasmid profile, auxotype and serovar, 39 different combinations were noted, which indicates the heterogeneous nature of the gonococcal population found in the Gambia.

INTRODUCTION

Infections caused by *Neisseria gonorrhoeae* constitute a major public health problem in many parts of the world. In order to investigate the epidemiology of gonococcal infections it is necessary to be able to type clinical isolates of *N. gonorrhoeae*. In this way the distribution of individual strains of gonococci in defined patient populations may be monitored. Systems for typing gonococci for epidemiological studies include plasmid analysis (Perine *et al.* 1977), auxotyping (Catlin, 1973; Perine *et al.* 1977; Handsfield *et al.* 1982) and serotyping using either

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polyclonal (Handsfield *et al.* 1982) or, more recently, monoclonal antibodies directed against gonococcal protein-I (Knapp *et al.* 1984). In this paper we report the use of plasmid typing, auxotyping and serovar analysis with monoclonal antibodies to study 59 strains of *N. gonorrhoeae* isolated in the Gambia.

MATERIALS AND METHODS

Bacterial strains

Twenty-nine strains of penicillinase-producing *N. gonorrhoeae* (PPNG) and 130 non-penicillinase-producing strains isolated from consecutive patients presenting to the MRC Unit, Fajara, between January, 1983 and February, 1984 were collected originally. The organisms were identified as *N. gonorrhoeae* on the basis of Gram stain, oxidase test, and sugar fermentation tests. The production of penicillinase was determined using the nitrocefin test (O'Callaghan *et al.* 1972). All the PPNG strains and six non-PPNG strains were frozen in liquid nitrogen for storage and transportation to the United Kingdom. The remaining non-PPNG strains were lyophilized for storage and transportation. All the frozen cultures and 24 of the lyophilized cultures contained viable organisms after arrival in the UK.

Plasmid analysis

Plasmids were extracted from each strain using the method of Birnboim & Doly (1979). The extracted plasmids were electrophoresed in 0.8% agarose gels and after staining with ethidium bromide the DNA bands were visualized using long-wave ultraviolet light. The plasmid profile of each strain was assessed by comparison with gonococcal strains of known plasmid content, provided by Dr A. Jephcott, Public Health Laboratory, Bristol.

Auxotyping

Organisms of each strain were suspended in phosphate-buffered saline at a concentration of about 1×10^8 colony-forming units (c.f.u.)/ml. One microlitre volumes of each suspension were then inoculated on to chemically defined media described by Catlin (1973) using a multipoint inoculator (Denley). The plates were incubated for 24 h at 37 °C in an atmosphere of 5% CO₂ in air, after which the presence or absence of growth was noted. In addition to a complete medium, media lacking either proline, arginine, methionine, lysine, hypoxanthine or uracil, as well as medium lacking arginine but supplemented with ornithine, were used.

Protein-I serovar

The protein-I serovar of each strain was determined using a panel of monoclonal antibodies (Syva) as described by Knapp and colleagues (1984).

Statistical analysis

The statistical significance of results was determined using the χ^2 test (Swinscow, 1976).

RESULTS

Plasmid content

The plasmid content of the 59 strains of gonococci is shown in Table 1. All 29 strains of PPNG contained the 2.6 MDa cryptic plasmid but lacked the 24.4 MDa conjugative plasmid. Eighteen strains (62%) of PPNG contained the 3.2 MDa penicillinase-coding plasmid and 11 strains (38%) had the 4.4 MDa penicillinase-coding plasmid.

Fourteen (47%) of the 30 strains of non-PPNG harboured only the cryptic plasmid and five strains (17%) contained both the cryptic and conjugative plasmids. Five strains (17%) of non-PPNG contained only the 24.4 MDa conjugative plasmid and six strains (20%) lacked plasmids.

Auxotype and serovar

Each strain was found to belong to one of only four auxotypes, namely wild type (or prototrophic), proline-requiring, arginine-requiring and proline- and arginine-requiring (Table 2). The PPNG consisted of 48% wild-type strains and 48% proline-requiring strains, with one strain (3.4%) requiring both proline and arginine. Forty per cent of the non-PPNG strains were wild type, with 47% of the strains requiring proline and 13% of the strains requiring arginine (Table 2). The difference between the distribution of auxotypes among the PPNG and non-PPNG was not significant ($P > 0.1$).

When the PPNG strains were typed on the basis of their protein I serovar, 86% of the strains belonged to serogroup 1A, 10.5% of the strains belonged to serogroup 1B and one strain (3.5%) was untypable. In contrast, 40% of the non-PPNG strains belonged to serogroup 1A and 60% belonged to serogroup 1B ($P < 0.001$). One strain in the latter group was, however, atypical in that it also reacted with monoclonal antibody 2F12. Among the PPNG strains, five different 1A serovars (1A-2, 1A-4, 1A-6, 1A-10 and 1A-16) and three different 1B serovars (1B-8, 1B-20 and 1B-22) were seen. The most prevalent serovar among the PPNG strains was serovar 1A-4 (34.5%), and it was of interest to note the absence of this serovar from the non-PPNG strains ($P < 0.001$). Among the non-PPNG strains, six 1A serovars (1A-2, 1A-3, 1A-6, 1A-10, 1A-16 and 1A-17) and five 1B serovars (1B-1, 1B-3, 1B-4, 1B-20 and 1B-22) were seen. The most prevalent non-PPNG serovar was 1B-3 (30%), which was not represented among the PPNG strains ($P < 0.001$).

Characterization of each strain on the basis of its combined plasmid profile, auxotype and serovar indicated that both the PPNG and non-PPNG strains were a heterogeneous collection, with 39 different plasmid-auxotype-serovar combinations being noted. It was of interest to note that 9 of the 10 strains of serovar 1A-4, the most prevalent serovar among the PPNG strains, harboured the 3.2 MDa plasmid. Five of these strains, however, were wild type while four were proline-requiring. Strains of serovar 1B-3, the most prevalent serovar among the non-PPNG strains, were also found to vary in their auxotype and also in their plasmid content. Wild-type, proline-requiring and arginine-requiring strains of serovar 1B-3 were all found. In addition, strains of this serovar were represented among each of the four plasmid-groups seen among the non-PPNG strains.

Table 1. *Plasmid content of strains of Neisseria gonorrhoeae isolated in the Gambia*

	No. strains	No. (%) of strains with plasmids of indicated molecular weight (MDa)					No plasmid
		2.6	2.6+3.2	2.6+4.4	2.6+24.4	24.4	
Gonococci PPNG	29	—	18 (62)	11 (38)	—	—	—
Non-PPNG	30	14 (47)	—	—	5 (17)	5 (17)	6 (20)

Table 2. *Distribution of auxotypes among strains of N. gonorrhoeae isolated in the Gambia*

	No. strains	No. (%) of strains of indicated auxotype			
		Wild type	Pro-	Arg-	Pro-/Arg-
PPNG	29	14 (48)	14 (48)	—	1 (3.4)
Non-PPNG	30	12 (40)	14 (47)	4 (13)	—

Despite the apparent heterogeneity of the gonococcal population studied, there was a similarity between the distribution of protein-I serovars among the different auxotypes. This was particularly marked with serovars of the 1A group, where 6 of the 7 serovars found were common to both wild-type and proline-requiring strains.

DISCUSSION

In this study strains of PPNG and non-PPNG isolated in the Gambia have been characterized in terms of their plasmid content, auxotype and protein-I serovar. Although the majority of PPNG strains harboured the so-called 'African' penicillinase-coding plasmid (3.2 MDa), 38% of the PPNG strains also harboured the 4.4 MDa plasmid, which was originally linked epidemiologically with the Far East (Perine *et al.* 1977). This confirms the findings of Bello *et al.* (1985), who described strains of both plasmid types in the Gambia in an earlier study. Strains containing the 4.4 MDa plasmid have also been found in other countries in Africa (Anderson, Odugbemi & Johnson, 1982; Perine *et al.* 1983; Bogaerts *et al.* 1986), so that it is clear that it is no longer valid to label strains of PPNG as 'African' or 'Asian'.

A noteworthy finding in the present study was the absence of the 24.4 MDa conjugative plasmid from the PPNG strains, although this plasmid was found in one-third of the non-PPNG strains. The absence of the conjugative plasmid from the PPNG strains may be of epidemiological importance since this plasmid acts to promote the transfer of penicillinase-coding plasmids to non-PPNG strains (Roberts & Falkow, 1977). It will clearly be of importance to monitor whether PPNG strains harbouring the conjugative plasmid emerge in the Gambia, either by the importation of such strains, or as a result of the transfer of the conjugative plasmid from non-PPNG.

A further finding of interest in the present study was that 11 strains (18%) lacked the 2.6 MDa cryptic plasmid. Previous studies have shown that more than 96% of strains of *N. gonorrhoeae*, from various geographical regions, contain this

plasmid (Roberts, Piot & Falkow, 1979; Perine *et al.* 1977, 1983; Plummer *et al.* 1985). An exception to this trend, however, was reported by workers in Canada, who found that gonococci of the proline-citrulline-uracil-requiring (P.C.U.) auxotype, the second most prevalent auxotype in Canada, consistently lacked plasmids (Dillon & Pauze, 1981). Interestingly, these workers found such plasmid-free isolates only among non-PPNG strains, and not among PPNG strains (Dillon & Pauze, 1981; Dillon, Duck & Thomas, 1981). While our results resemble those reported by Dillon and colleagues in that only non-PPNG strains lacking the cryptic plasmid were found in the Gambia, 4 of the 11 Gambian strains lacking the cryptic plasmid were wild type, 6 were proline-requiring and 1 required arginine. Although Totten *et al.* (1983) also found an association between absence of plasmids and the P.C.U. auxotype, they additionally found prototrophic and arginine-requiring strains that were plasmid-free as reported here. Besides its epidemiological interest, the absence of the cryptic plasmid among gonococcal strains might have diagnostic importance, since this plasmid has been used as a DNA probe in DNA hybridization tests to diagnose gonococcal infection (Totten *et al.* 1983). Clearly, strains lacking the cryptic plasmid might be expected to give false negative results.

When all the strains were characterized on the basis of their combined plasmid composition, auxotype and serovar, 39 different combinations were noted. Although these results indicate the heterogeneous nature of the gonococcal population in the Gambia, further useful analysis is limited by the small number of strains available for study. Another problem that potentially confounds analysis is the question of whether the small proportion of non-PPNG strains which survived transportation are representative of the original population. As there is no information concerning the relative ability of different strains of gonococci to survive freeze-drying, it has been assumed for the purposes of the present study that the non-PPNG strains available for examination are representative of the original population. Clearly, this supposition would need to be revised should relevant data on the survival of different types of gonococci undergoing lyophilization become available. It should be mentioned, however, that this problem is not unique to this study. For example, in four recent studies in which gonococci isolated in Africa were transported (either lyophilized or frozen) to laboratories in Belgium, England or the USA (two studies), the proportions of strains which survived were 34% (Bogaerts *et al.* 1986), 55% (Osoba *et al.* 1987), 62% (Bello *et al.* 1985) and 63% (Perine *et al.* 1983) respectively. The loss of about 40% or more of strains during transport in each of these studies serves to highlight one of the many problems facing workers investigating gonorrhoea and other sexually transmitted infections in Africa.

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