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Relationship Between Plasmid Content and Auxotype in *Neisseria gonorrhoeae* Isolates

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One hundred and forty strains of *Neisseria gonorrhoeae*, representing 12 different auxotype groups, were examined for differences in plasmid content. Most auxotype groups harbored a phenotypically cryptic 2.6-megadalton plasmid; a few groups also carried a 24.5-megadalton plasmid which has been previously characterized as a transfer plasmid. However, isolates of the proline-, citrulline-, and uracil-requiring (PCU^-) auxotype were consistently free of plasmids. The correlation between auxotype and plasmid content is especially significant since, in Canada, PCU^- isolates have the second highest prevalence of all auxotypes.

Two distinct plasmids have been characterized in non-penicillinase-producing isolates of *Neisseria gonorrhoeae*. The larger 24.5-megadalton plasmid, first described by Stiffler et al. (19), has been phenotypically and genetically characterized as a transfer plasmid (14, 18). In addition, Norlander et al. (11) found that this plasmid affected gonococcal competence in transformation experiments. Roberts et al. (13) reported that approximately 15% of their gonococcal isolates carried the 24.5-megadalton plasmid. This plasmid was more prevalent in isolates from certain geographical areas (e.g., the Philippines).

No phenotype has been ascribed to the small 2.6-megadalton plasmid, first observed by Maness and Sparling (9) and subsequently characterized and genetically analyzed by several groups (1, 3-5, 10, 12). Roberts et al. (15) examined 261 isolates of *N. gonorrhoeae* for plasmid content and found that 96% harbored this plasmid.

Several groups (3, 13, 15) have documented the isolation and incidence of plasmid-free strains of *N. gonorrhoeae*. In the study by Roberts et al. (15), these strains comprised only 2% of 261 isolates examined; furthermore, they did not differ significantly from plasmid-containing isolates in auxotype, colonial morphology, or antimicrobial susceptibility (13, 15). In the present study, we have examined 140 non-penicillinase-producing *N. gonorrhoeae* isolates and report a relationship between plasmid content and auxotype for the proline-, citrulline-, and uracil-requiring (PCU^-) group.

The isolates for this study were selected so as to examine all major auxotype groups found in Canada and to ensure that the isolates came from diverse geographical origins. Ninety-five of the isolates were submitted as part of a national antibiotic sensitivity survey. Ten isolates were obtained from K. Givan, Women's College Hospital, Toronto, and 35 isolates were kindly supplied by I. O. Stewart, Hamilton General Hospital, Hamilton. Although all isolates were submitted to our laboratory as confirmed *N. gonorrhoeae*, they were reconfirmed by us with standard identification procedures (7). All isolates were stored at −70°C in brain heart infusion broth (Difco Laboratories) supplemented with 20% glycerol. Isolates were subcultured on GC medium base (Difco Laboratories) containing 1% defined supplement as described by Kellogg et al. (8). Auxotype media preparation and characterization were performed as described by Hendry and Stewart (6). Auxotype medium also served as a preliminary test for differentiating *N. gonorrhoeae* from *Neisseria meningitidis*. Meningococci grow on all auxotype media, including the negative control which lacks cystine and cysteine.

The recovery and agarose gel analysis of plasmid deoxyribonucleic acid (DNA) from cleared lysates were carried out as described previously (2). Isolates which appeared to be plasmid free in preliminary screenings were subjected to at least one additional extraction, using one of several different methods of plasmid DNA preparation (4, 16, 18).

The relationship between auxotype and plasmid content is shown in Table 1. The PCU^- auxotype was exceptional in that not one of the 85 isolates examined in this group harbored any plasmids. Only one other proline-, citrulline-, uracil-, and hypoxanthine ($PCUH^-$)-requiring isolate was plasmid free. All other auxotype

groups carried the 2.6-megadalton cryptic plasmid; some of these non-PCU$^-$ strains (9.1%) also harbored the 24.5-megadalton plasmid. The larger plasmid was characterized only in the nonrequiring (NR) or wild-type, proline-requiring (PRO$^-$), and isoleucine-requiring (ISO$^-$) groups. (The ISO$^-$ group can be considered as an NR subgroup since it is categorized (6) as a minor requirement.) It is also interesting to note that the 24.5-megadalton plasmid, found in 65.2% of all penicillinase-producing gonococci isolated in Canada (2), was isolated only from strains with the NR (including ISO$^-$) or PRO$^-$ auxotypes.

TABLE 1. *Plasmid content of 12 N. gonorrhoeae auxotypes*

Auxotype[a]	No. tested	No. with plasmids having mol wt of[b]		
		No plasmids	2.6	2.6 + 24.5
NR	12		10	2
PCU$^-$	85	85	0	
OUH$^-$	14		14	
CUH$^-$	4		4	
ORN$^-$	2		2	
PRO$^-$	5		3	2
PRO$^-$ ORN$^-$	1		1	
ISO$^-$	6		5	1
No growth[c]	2		2	
PCUH$^-$	3	1	2	
POUH$^-$	5		5	
POH$^-$	1		1	

[a] The auxotype designations follow the designations of Hendry and Stewart (6). NR means nonrequiring or wild-type; PCU$^-$ requires proline, citrulline, uracil; OUH$^-$ requires ornithine, uracil, hypoxanthine; CUH$^-$ requires citrulline, uracil, hypoxanthine; ORN$^-$ requires ornithine; PRO$^-$ requires proline; ISO$^-$ requires isoleucine.

[b] Molecular weight in megadaltons as determined by agarose gel electrophoresis.

[c] The no growth group comprises strains which fail to grow on auxotype medium. This category is the sixth most prevalent group of strains in Canada (Dillon, unpublished data).

The plasmid content of the non-PCU$^-$ isolates, as revealed by agarose gel electrophoresis, is shown in Fig. 1. They all carried a 2.6-megadalton plasmid, and the isolates in lanes 6 and 11 also harbored a 24.5-megadalton plasmid. The plasmid-free strain reported in lane 7 was kindly supplied by S. Falkow and has an NR auxotype. Lane 12 is an extract of a penicillinase-producing reference strain. It contains both 2.6- and 24.5-megadalton plasmids, as well as a 4.5-megadalton penicillinase-producing plasmid. Figure 2 typifies the banding patterns obtained with extracts of PCU$^-$ isolates (lanes 3 to 7). All are plasmid free. The extraction procedures employed to confirm these results included a phenol extraction (16), the preparation of plasmid DNA, using cesium chloride-ethidium bromide ultracentrifugation (2, 4) and the method of Sox et al. (18). It should also be mentioned that to reduce the possibility of testing a single clone of gonococci, the isolates in this study came from 9 of Canada's 10 provinces. For example, 24 of the 85 PCU$^-$ isolates originated in British Columbia, 9 in Alberta, 45 in Ontario, 3 in Newfoundland, 2 in Nova Scotia, and 1 from both Manitoba and Prince Edward Island.

The possibility remained that the PCU$^-$ iso-

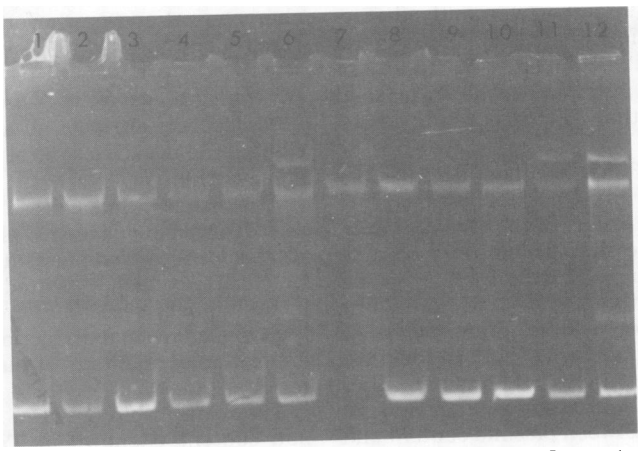

FIG. 1. *Plasmids isolated from strains with auxotypes other than PCU$^-$. Lanes 1 to 6 represent extracts from isolates of the NR, ISO$^-$, PRO$^-$ORN$^-$, CUH$^-$, OUH$^-$, and ISO$^-$ auxotypes, respectively. Lane 7 shows a plasmid-free (NR) isolate. Lanes 8 to 11 represent the plasmid profiles of the PRO$^-$, ORN$^-$, PRO$^-$, and NR auxotypes. Lane 12 shows a β-lactamase-positive isolate with a 4.5-megadalton penicillinase plasmid. Isolates in lanes 6, 11, and 12 harbor 24.5-megadalton plasmids. See Table 1 for explanation of auxotype designations.*

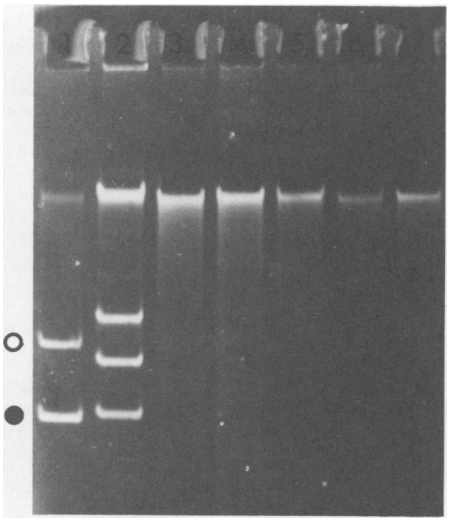

FIG. 2. *Plasmid content of typical PCU⁻ isolates. Lane 1 contains an extract of Kellogg strain F62, a PRO⁻ strain which harbors the 2.6-megadalton plasmid* (○, ●). *Lane 2 contains plasmid marker DNA having molecular weights of 2.6, 4.2, and 5.8. The remaining lanes carry extracts of PCU⁻ isolates which show the presence of chromosomal DNA only.*

lates did have plasmid DNA, but in copy numbers so low that they could not be detected in agarose gels. An analogous situation existed in some *Escherichia coli* cells transformed with the 3.2-megadalton penicillinase plasmid of *N. gonorrhoeae*. Although these cells became phenotypically ampicillin resistant, plasmid DNA could not be isolated from them by conventional means (J. R. Dillon and D. Y. Thomas, Abstr. Am. Soc. Microbiol. Conf. Genet. Mol. Biol. Ind. Microorganisms, 2nd, Bloomington, Ind., abstr. no. 7, 1980). In this case, the preparation of Southern blots (17), coupled with the hybridization of radioactively labeled 3.2-megadalton plasmid DNA, revealed the presence of this DNA as an extrachromosomal element. Southern transfers of DNA from 10 PCU⁻ strains, as well as one plasmid-bearing control strain, were prepared and hybridized with labeled 2.6-megadalton plasmid DNA. The PCU⁻ strains examined by this method, were found to be free of the 2.6-megadalton plasmid.

Although other groups (3, 13, 15) have noted a low incidence of plasmid-free strains in *Neisseria gonorrhoeae*, they have been unable to associate plasmid profile with other gonococcal phenotypes. All the PCU⁻ strains examined in this study were plasmid free. It should be further noted that these strains could be differentiated from several other auxotypes (e.g. PRO⁻, ORN⁻, NR) on the basis of colonial morphology by an experienced worker. The colonies are small, light in color, and grow poorly on GC medium base. In addition, PCU⁻ strains as a group differ significantly in their susceptibilities to antimicrobial agents as compared with all other auxotypes combined (Dillon, unpublished data). The correlation between plasmid content and auxotype has been useful in the laboratory diagnosis of mixed infections of *N. gonorrhoeae*.

In conclusion, we have found that the second most prevalent *N. gonorrhoeae* auxotype found in Canada, the PCU⁻ group, is consistently plasmid free. This result contrasts with previous publications (13, 15) in which plasmid-free strains were found at very low frequency and for which no correlation between plasmid content and auxotype was found.

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