Journal of General Microbiology (1979), **114**, 491–494. *Printed in Great Britain*

SHORT COMMUNICATION

The Ecology of Gonococcal Plasmids

By MARILYN ROBERTS[1], PETER PIOT[2] AND STANLEY FALKOW[1]

[1] *University of Washington, School of Medicine, Department of Microbiology and Immunology, Seattle, Washington* 98195, *U.S.A.*

[2] *Institute of Tropical Medicine, Antwerp, Belgium*

(*Received* 9 *May* 1979)

Of 261 strains of *Neisseria gonorrhoeae* examined for plasmids, 6 were plasmid-free, 217 contained only a small multicopy $2{\cdot}6 \times 10^6$ dalton plasmid and 38 carried a large $24{\cdot}5 \times 10^6$ dalton plasmid. Restriction enzyme digests and DNA–DNA hybridization studies revealed that the large plasmids isolated between 1940 and 1978 share a common core of DNA sequences (70 to 100%) and represent a group of closely related molecules.

INTRODUCTION

Two plasmids have been described in *Neisseria gonorrhoeae*, a small $2{\cdot}6 \times 10^6$ dalton (2·6 Mdal) multicopy plasmid and a large $24{\cdot}5 \times 10^6$ dalton (24·5 Mdal) plasmid (Mayer *et al.*, 1974). No phenotype could be correlated with the presence of either plasmid (Elwell & Falkow, 1977). Recently, the 24·5 Mdal plasmid has been shown to have sex factor activity (Baron *et al.*, 1977; Eisenstein *et al.*, 1977; Kirven & Thornesberry, 1977; Roberts & Falkow, 1977, 1978; Roberts *et al.*, 1977) and can mediate transfer of itself, R plasmids and chromosomal genes. This paper describes the incidence of plasmids in *N. gonorrhoeae* isolated from different geographic sources. Earlier studies of a small number of strains (108) had shown that the 24·5 Mdal plasmid was common in only a limited number of geographic areas (Roberts *et al.*, 1978). We have now studied an additional 153 strains and have examined the molecular relationships between the various large gonococcal plasmids.

METHODS

Bacterial strains. The gonococcal strains were all clinical isolates (Table 1). The Seattle strains, collected at the United States Public Health Hospital, were provided by Dr Joan Knapp. The Danish strains were provided by Dr Alice Reyn, the Far East strains and the penicillin-resistant strains by Drs C. Thornesberry, W. Ashford, A. Percival and P. Piot, the Dutch strains by Dr Ernest Stolz and the Kenyan strains by Dr Peter Perrine. The African and Belgian strains were collected by one of the authors (P. Piot).

Media. The solid and liquid media used for the growth of *N. gonorrhoeae* have been described previously (Roberts *et al.*, 1977).

Agarose gel electrophoresis of DNA. Cleared lysates of strains of *N. gonorrhoeae* were prepared and samples were subjected to electrophoresis through a 0·7% (w/v) agarose gel (Meyers *et al.*, 1976). Strains that appeared to be plasmid-free were examined at least twice.

Preparation of unlabelled plasmid DNA. Unlabelled plasmid DNA was prepared from gonococcal strains as described previously (Roberts *et al.*, 1977). Residual chromosomal DNA and small molecular weight plasmid DNA were removed by running the preparation through two 5 to 20% (w/v) neutral sucrose gradients for 4 h at 35000 rev. min^{-1} (Crosa *et al.*, 1975).

Preparation of [^3H]*thymidine-labelled plasmid DNA.* Purified plasmid DNA that had been subjected to two caesium chloride–ethidium bromide gradients followed by two sucrose gradients was labelled *in vitro* according to the procedure described by Maniatis *et al.* (1975).

0022-1287/79/0000-8749 $02.00

Table 1. *Plasmids in Neisseria gonorrhoeae*

Geographic area	Date isolated	Total no. of strains	Plasmid complement of strains: 2·6 Mdal only	2·6 and 24·5 Mdal	24·5 Mdal only	No plasmids
Denmark	1940–5	15	10	5	—	—
Denmark	1950	14	8	6	—	—
Denmark	1963	12	9	1	1	1
Denmark	1974	14	14	—	—	—
Belgium	1974	9	8	—	—	1
Belgium	1978	12	10	1	1	—
Holland	1978	8	6	1	—	1
Seattle, U.S.A.	1973	28	28	—	—	—
Seattle, U.S.A.	1977–8	15	13	—	—	2
Ethiopia	1973	15	13	—	2	—
Rwanda	1975	12	12	—	—	—
Rwanda	1978	12	12	—	—	—
Zaïre	1977	4	4	—	—	—
Swaziland	1978	12	11	1	—	—
Kenya	1978	11	10	—	1	—
Philippines	1973	17	11	5	—	1
Philippines	1977	10	7	3	—	—
Hong Kong	1977	4	2	2	—	—
Taiwan	1977	5	5	—	—	—
Far East (Pc^r) (4·4 Mdal R plasmid)	1976–7	20	12	8	—	—
Great Britain (Pc^r) (3·2 Mdal R plasmid)	1976	6	6	—	—	—
West Africa (Pc^r) (3·2 Mdal R plasmid)	1978	6	6	—	—	—
Total		261	217 (83%)	33 (13%)	5 (2%)	6 (2%)

DNA–DNA hybridization studies. Sheared and denatured labelled plasmid DNA was reannealed with unlabelled whole-cell DNA extracted from representative strains of *N. gonorrhoeae* carrying a large plasmid. The hybridization was performed as described previously (Roberts *et al.*, 1977).

Restriction enzyme digestions. The restriction enzymes *Eco*RI and *Bam*HI were obtained from Bethesda Research Labs, and plasmid restriction digests were prepared according to the manufacturer's directions. The digested plasmids were subjected to electrophoresis on agarose gels.

RESULTS AND DISCUSSION

Table 1 summarizes the plasmid complement of 261 strains of *Neisseria gonorrhoeae*. Of the 255 strains found to harbour plasmids, 217 contained only a 2·6 Mdal plasmid and 38 possessed a 24·5 Mdal plasmid, either alone (5 strains) or in association with the 2·6 Mdal plasmid (33 strains). The six plasmid-free strains did not exhibit any unusual phenotypic characteristics, including colonial morphology, antimicrobial susceptibility, auxotype or IgA protease activity (Plaut, 1978).

The isolates examined were used because of their availability and probably do not represent random samples. In addition, the number of strains available for a particular year or area were limited. Nevertheless, among the 163 strains isolated after 1973 from Western Europe, Africa, Great Britain and Seattle (U.S.A.), only seven (4·2%) carried a 24·5 Mdal plasmid. In contrast, among 56 strains isolated or acquired in the Far East, 18 (34%) carried a 24·5 Mdal plasmid. Therefore, the 24·5 Mdal plasmid appears to be more common in some geographic areas, such as the Philippines, than in others.

For the determination of the polynucleotide sequence relationship between the various large plasmids, two well-characterized strains were chosen as references: strain CDC67

Table 2. *Hybridization between* ^{3}*H-labelled plasmid* pLE2450 *DNA or* ^{3}*H-labelled plasmid* pLE2451 *DNA and unlabelled whole-cell DNA of Neisseria gonorrhoeae*

Strain	Geographic area	Date isolated	Plasmid complement (Mdal)	Relative DNA sequence homology with pLE2451* (%)	Relative DNA sequence homology with pLE2450† (%)
KH45	Denmark	1963	24·5	100	83
CDC67	Philippines	1976	24·5, 4·4, 2·6	75	100
KH4314	Philippines	1973	24·5, 2·6	90	—
78–13901	Kenya	1978	24·5	100	96
KH7907	Denmark	1940	24·5, 2·6	75	69
F62	U.S.A.	1960s	2·6	0	3

—, Not tested.

* Binding between homologous ^{3}H-labelled pLE2451 and unlabelled KH45 was 75%; this value was set at 100% and other values were normalized to give relative homologies.

† Binding between ^{3}H-labelled pLE2450 and unlabelled CDC67 was 75%.

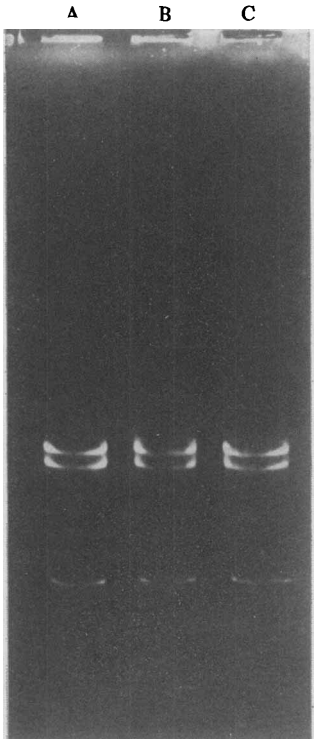

Fig. 1. Agarose gel electrophoresis of *Eco*RI digests of 24·5 Mdal plasmids; A, plasmid pLE2450 from strain CDC67 isolated in 1976; B, plasmid pLE2451 from strain KH45 isolated in 1963; C, plasmid from strain KH7907 isolated in 1940.

isolated in the Philippines in 1976 which carries the 24·5 Mdal plasmid pLE2450 (Roberts *et al.*, 1977) and strain KH45 isolated in Denmark in 1963 which carries the 24·5 Mdal plasmid pLE2451 (Elwell & Falkow, 1977). The degree of DNA–DNA duplex formation between these plasmids and whole-cell DNA from representative strains was analysed by the S1 endonuclease method (Table 2). The data show that strains carrying a 24·5 Mdal plasmid have a substantial number of DNA sequences in common (70 to 100%) with the

two reference plasmids. In contrast, F62, which carries only the 2·6 Mdal plasmid, has few or no sequences in common (0 to 3%) with either reference plasmid, indicating that the 24·5 Mdal plasmid shares no DNA sequences with the 2·6 Mdal plasmid. The five 24·5 Mdal plasmids were then digested with *Eco*RI followed by electrophoresis on agarose gels. The patterns were identical for each of the five plasmid species; three are illustrated in Fig. 1. Digestion patterns with *Bam*HI were also found to be similar. Sox *et al.* (1978) have examined three 24·5 Mdal plasmids and have reported similar findings. That the *Eco*RI restriction digest patterns are identical is not surprising because there are only a few restriction sites present. Generally, restriction pattern differences are more noticeable when there are numerous sites on the plasmid.

To summarize, the 24·5 Mdal plasmid appears to be common only in certain geographic areas. The results of the hybridization and restriction enzyme studies suggest that the large plasmids found between 1940 and 1978 represent a group of closely related molecules.

This work was supported by Public Health Service Grant AI100191-03 from the National Institute of Allergy and Infectious Diseases and by a grant from the Charles Merrill Trust. P. Piot was supported by a NATO fellowship.

REFERENCES

BARON, E. S., SAZ, A. K., KOPECKO, D. S. & WOLHIETER, S. A. (1977). Transfer of plasmid-borne beta-lactamase in *Neisseria gonorrhoeae*. *Antimicrobial Agents and Chemotherapy* **12**, 270–280.

CROSA, J., LUTTROPP, L. K. & FALKOW, S. (1975). Nature of R-factor replication in the presence of chloramphenicol. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 654–658.

EISENSTEIN, B. I., SOX, T., BISWAS, G., BLACKMAN, E. & SPARLING, P. F. (1977). Conjugal transfer of the gonococcal penicillinase plasmid. *Science* **195**, 998–1000.

ELWELL, L. P. & FALKOW, S. (1977). Plasmids of the genus *Neisseria*. In *The Gonococcus*, pp. 137–154. Edited by R. B. Roberts. New York: John Wiley.

KIRVEN, L. A. & THORNESBERRY, C. (1977). Transfer of beta-lactamase genes of *Neisseria gonorrhoeae* by conjugation. *Antimicrobial Agents and Chemotherapy* **11**, 1004–1006.

MANIATIS, T., JEFFREY, A. & KLEID, D. G. (1975). Nucleotide sequence of the rightward operator of phage λ. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 1184–1188.

MAYER, L. W., HOLMES, K. K. & FALKOW, S. (1974). Characterization of plasmid deoxyribonucleid acid from *Neisseria gonorrhoeae*. *Infection and Immunity* **10**, 712–717.

MEYERS, J., SANCHEZ, D., ELWELL, L. P. & FALKOW, S. (1976). A simple agarose gel electrophoretic method for identification and characterization of plasmid deoxyribonucleic acid. *Journal of Bacteriology* **127**, 1529–1537.

PLAUT, A. G. (1978). Microbial IgA proteases. *New England Journal of Medicine* **298**, 1459–1463.

ROBERTS, M. & FALKOW, S. (1977). Conjugal transfer of R plasmids in *Neisseria gonorrhoeae*. *Nature, London* **266**, 630–631.

ROBERTS, M. & FALKOW, S. (1978). Plasmid-mediated chromosomal gene transfer in *Neisseria gonorrhoeae*. *Journal of Bacteriology* **134**, 66–70.

ROBERTS, M., ELWELL, L. P. & FALKOW, S. (1977). Molecular characterization of two beta-lactamase-specifying plasmids isolated from *Neisseria gonorrhoeae*. *Journal of Bacteriology* **131**, 557–563.

ROBERTS, M., ELWELL, L. P. & FALKOW, S. (1978). Introduction to the mechanisms of genetic exchange in the gonococcus: plasmids and conjugation in *Neisseria gonorrhoeae*. In *Immunobiology of Neisseria gonorrhoeae*, pp. 38–43. Edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer & F. E. Young. Washington, D. C.: American Society for Microbiology.

SOX, T. E., MOHAMMED, W., BLACKMAN, E., BISWAS, G. & SPARLING, P. F. (1978). Conjugative plasmids in *Neisseria gonorrhoeae*. *Journal of Bacteriology* **134**, 278–286.