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Genomic Fingerprinting in the Epidemiology of Gonorrhoea

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We investigated the usefulness of the restriction enzyme (RE) fingerprinting for epidemiological tracing in gonococcal disease. The RE patterns of three paired gonococcal isolates showed corresponding identical fingerprints. Within each of the three pairs of epidemiologically linked isolates the respective restriction patterns were completely identical. Also, the restriction patterns of 6 strains from a larger contact group were identical. Identical restriction patterns were also obtained in each of the two cases where isolates were recovered from both urethra and cervix. The serological findings were in perfect agreement with the genomic fingerprinting as to the identity between all strains of the same epidemiologic chain. Relapse of the original infection could be excluded in one case by the finding of a different RE pattern and also a different serovar pattern of the strain recovered 4 months later. *Key words: Genomic fingerprinting; Epidemiology; Gonorrhoea.* (Received September 17, 1984).

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Auxotyping and serological classification by co-agglutination (CoA) are at present the phenotypic markers most commonly used to study the epidemiology of gonorrhoea (1, 2, 3, 4, 5). Usually, the best information is achieved by the combined use of the two methods. Auxotyping is, however, complicated, laborious and time consuming, and serotyping requires well characterized and specific polyclonal or monoclonal anti gonococcal antibodies (6, 7).

We have recently described the use of genotypic markers to study the epidemiology of gonorrhoea (8). With the use of restriction enzymes (RE) the chromosomal DNA of various *Neisseria gonorrhoeae* strains gave different RE patterns, so called a genomic fingerprint, which was stable and reproducible for a particular strain, and a potential tool for epidemiological studies.

In the present study we show that the RE technique for genotypic characterization of gonococcal strains can be used to demonstrate the identity of strains from contact pairs. The results were compared with those of serogrouping by CoA with monoclonal antibodies.

MATERIAL AND METHODS

The study includes 18 gonococcal strains, selected on the basis of retrospective contact tracing from cases of uncomplicated urogenital gonorrhoea. Six strains were isolated from 3 contact pairs, 6 strains from a larger contact group, 4 from 2 patients providing isolates from both cervix and urethra, and finally 2 strains recovered at a 4 months interval from a patient with recurrent gonorrhoea. Culture and identification followed conventional procedures. The strains were stored at −70°C in brain heart infusion broth containing 10% glycerol.

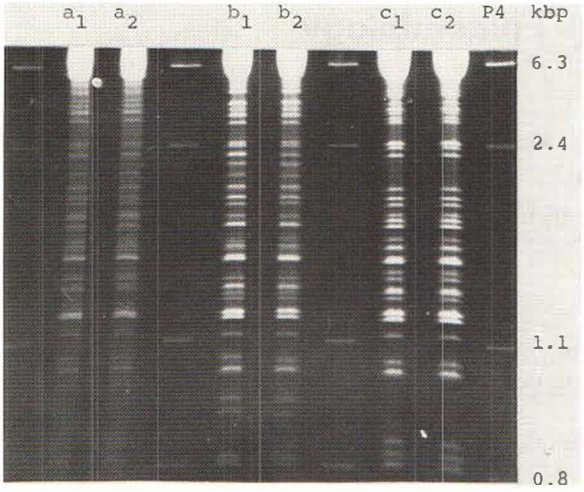

Fig. 1. Three pairs of epidemiologically linked gonococcal strains, each pair showing identical restriction patterns. (P4 size marker to the right) (Hind III digestion).

Restriction endonuclease fingerprinting

The procedure has been described previously (8). In short, lyophilized gonococci were thawed and grown overnight on gonococcal agar medium prepared from BBL GC agar medium base with the addition of Bacto hemoglobin and IsoVitalex without antibiotics. One colony was further subcultivated under the same conditions. Bacteria were then harvested and lysed by the addition of EDTA, lysozyme, RNase, pronase, and Triton X-100. DNA was extracted by repeated chloroform/phenol extractions and dialysed against a DNA buffer to the average concentration of 600–1 500 μg/ml. The DNA was then digested by the restriction endonuclease Hind III as described by the manufacturer (Amersham, UK). The resulting DNA fragments were separated electrophoretically in a 4% polyacrylamide gel which was run for 20 hours at 40 mA constant current at 10°C. DNA from the E. coli phage P4, MW 11.3 Kb (9) digested by Hind III was used as a fragment size marker. The gels were stained with ethidium bromide, washed and finally photographed in UV-light. The different band patterns (genomic fingerprints) were compared visually and grouped according to identity.

Serological classification

The gonococcal strains were serogrouped by CoA with the use of monoclonal antibodies against protein IA (WI) and protein IB (WII/WIII) antigens (1, 7, 10). The monoclonal antibodies were a gift from Dr M. Tam, Genetic Systems Corp., Seattle, USA, and CoA reagents from Dr E. Sandström, Södersjukhuset at Karolinska Institute, Stockholm, Sweden. Each strain was tested by CoA against a set of 6 anti-protein IA specific antibodies designated 4G5=e, 2F12=d, 6D9=g, 5C2=k, 5G9=i & 5D1=h (11), and 7 protein IB specific antibodies, designated 3C8=a, 2D6=c, 2H7=e, 2G2=g, 2D4=h, 3B10=j & 2H1=k. The serogroup and serovar of a particular strain was written according to reactivity, for example IA/edih, IB/acjk etc. as proposed by Sandström et al. (11).

RESULTS

Fig. 1 shows the RE patterns of the 3 paired isolates. The corresponding fingerprints for each pair (a_1–a_2, b_1–b_2, and c_1–c_2, respectively) were identical but as can be seen the three pairs had different RE patterns. Two of them (a_1–a_2 and c_1–c_2), however, had identical serovar patterns (IA/edgkih), whereas the third (b_1–b_2) had a different pattern (IB/acejk). The stability of the RE patterns during transmission of gonorrhoea is further evidenced by the identity of the patterns of the larger contact group (Fig. 2). Here, the intervals between primary and secondary infections averaged 2–3 weeks. All strains (d_1–d_6) in this contact group also had the same serovar pattern (IB/acejk) (Table I). Moreover, the same RE pattern and serovar pattern (IB/acejk) were found in pair b_1–b_2 (Fig. 1) and in the larger contact group d_1–d_6 (Fig. 2). However, no data were available which could confirm an

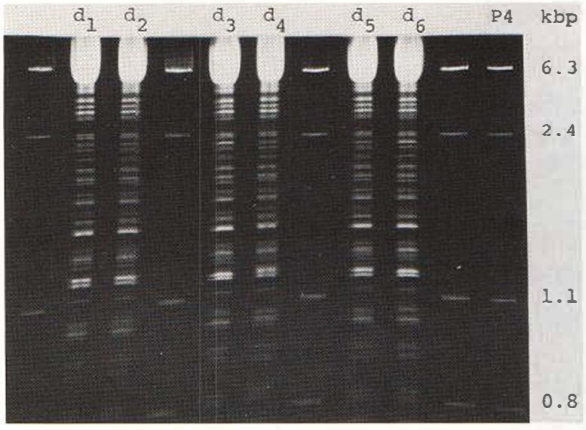

Fig. 2. Identical restriction patterns in 6 epidemiologically linked gonococcal strains. (P4 size marker to the right) (Hind III digestion).

epidemiological linkage between the strains b_1–b_2 and d_1–d_6. Identical fingerprints and serovar patterns were also obtained in the two patients (f_1–f_2 and g_1–g_2) providing isolates from 2 different locations (cervix and urethra) (Fig. 3 and Table I). Identical RE and serovar patterns were found in strains a_1–a_2 (Fig. 1) and g_1–g_2 (Fig. 3) without any known epidemiological linkage. The RE patterns of strains f_1–f_2 and d_1–d_6 differed only with regard to 2 or 3 bands, but had quite different serovar patterns (IA/edgk and IB/acejk, respectively). In the patient of recurrent gonorrhoea 4 months after the first infection (e_1 and e_2), relapse could be excluded by the findings of clearly different fingerprints (Fig. 3) and of different serovar patterns (IB/ak and IB/ajk, respectively).

Table I. *DNA restriction enzyme patterns by Hind III digestion (provisional designations) and protein I serogroup/serovar patterns of gonococcal strains with known and unknown epidemiological linkage*

Strain	Serogroup/serovar	RE Hind III pattern	Provisional designation
a_1	IA/edgkih	Identical	I
a_2	IA/edgkih		
g_1 Cx[a]	IA/edgkih	Similar to I	I
g_2 U	IA/edgkih		
c_1	IA/edgkih	Dissimilar to I	II
c_2	IA/edgkih		
f_1 Cx	IA/edgk	Dissimilar to I and II	III
f_2 U	IA/edgk		
b_1	IB/acejk	Dissimilar to I, II and III	IV
b_2	IB/acejk		
d_1	IB/acejk		
d_2	IB/acejk		
d_3	IB/acejk	Similar to IV	IV
d_4	IB/acejk		
d_5	IB/acejk		
d_6	IB/acejk		
e_1	IB/ak	Dissimilar to all	V
e_2	IB/ajk	Dissimilar to all	VI

[a] Cx = cervix; U = urethra

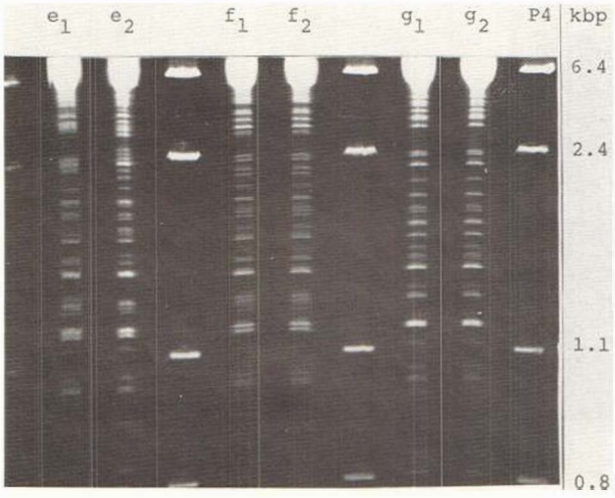

Fig. 3. Different restriction patterns of two isolates (e_1 and e_2) recovered with 4 months interval from the same patient. f_1–f_2 and g_1–g_2 illustrate identical restriction patterns of strains isolated from two different locations (urethra and cervix) of two patients. (P4 size marker to the right) (Hind III digestion).

DISCUSSION

Genomic fingerprinting by restriction enzymes has recently been applied by various research groups for epidemiological studies of viral and bacterial infections (12, 13, 14). The results of the present and two previous investigations (8, 15) show that this technique, alone or in combination with methods for the demonstration of phenotypic markers, can be a valuable tool to study the epidemiology of gonorrhoea. The reproducibility and reliability of the method was demonstrated in a previous work (8) and was further confirmed in the present study.

Gonococci are known to harbor one or more plasmids, i.e. extrachromosomal DNA, which in fact has been used by several research groups for epidemiological studies of penicillinase producing strains (16, 17, 18, 19, 20) but the appearance of steadily new combinations of plasmids makes these markers less suitable for such studies. It should also be pointed out that plasmids, which are demonstrated by agarose gel electrophoresis, do not interfere with the chromosomal DNA RE patterns (8, 15) that are demonstrated by polyacrylamide gel electrophoresis.

The serological findings were in perfect agreement with the genomic fingerprinting as to the identity between strains of the same epidemiological chain, although the strains were often recovered with several weeks' intervals (Table I and Fig. 1 & 2). It was of interest to note, however, that the RE method differentiated between each of the involved clones (a_1–a_2; b_1–b_2; c_1–c_2) whereas serogrouping by CoA with monoclonal antibodies differentiated between only 2 of them (a_1–a_2/c_1–c_2 and b_1–b_2; Table I). Interestingly, isolates from one contact pair (b_1–b_2) both had the same RE pattern and serovar pattern (IB/acejk) as the strains d_1–d_6 from the larger contact group. Moreover, strains a_1–a_2 and g_1–g_2 had identical RE patterns and were of the same serovar (IA/edgkih) although no epidemiological linkage was known.

The potential use of the RE method for epidemiological tracing in gonococcal infections is further illustrated by the considerable number of individual and easily differentiated fingerprints among gonococci within a fairly restricted geographical area. In this respect the RE method was even more sensitive than CoA with monoclonal antibodies. Both methods can also be used to characterize strains from patients where relapse or reinfection are questioned.

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