Epidemiol. Infect. (1991), **107**, 253–262
Printed in Great Britain

Characterization of *Neisseria meningitidis* isolates by ribosomal RNA gene restriction patterns and restriction endonuclease digestion of chromosomal DNA

J. Z. JORDENS* AND T. H. PENNINGTON

Department of Medical Microbiology, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD, UK

(*Accepted 6 May 1991*)

SUMMARY

The use of ribosomal RNA (rRNA) gene restriction patterns to study the molecular epidemiology of *Neisseria meningitidis* was investigated. Ninety-four isolates of *Neisseria meningitidis* were characterized by their rRNA gene restriction patterns with 16+23 S rRNA from *Escherichia coli* as a probe. Thirteen rRNA gene restriction patterns were recognized; each of these patterns represented between 1 and 30 isolates. Isolates with the outbreak-associated phenotype B15P1.16 (sulphonamide resistant) all gave a single rRNA gene restriction pattern but this pattern also contained isolates with other phenotypes. Further discrimination between isolates was achieved by comparison of banding patterns resulting from restriction endonuclease digestion of chromosomal DNA with *Bgl* II. This gave a banding pattern consisting of about ten bands which was simple to interpret. Using this technique 94 isolates were classified in 54 patterns containing between 1 and 14 isolates. Restriction endonuclease analysis with *Bgl* II characterized outbreak-associated isolates with the phenotype B15P1.16 and enabled strains not typable by conventional methods to be identified as probable outbreak-associated isolates. The techniques should prove useful for epidemiological studies.

INTRODUCTION

Neisseria meningitidis is the commonest cause of bacterial meningitis in children in Britain. The disease is severe and has a mortality rate of about 10% [1]. Over the past 10 years there has been an increase in the incidence of meningococcal disease; this has been associated with serogroup B, serotype 15, sub-type 16, sulphonamide-resistant (B15P1.16R) strains which have caused major outbreaks in Gloucestershire [2].

Strains of *Neisseria meningitidis* are currently characterized by serogrouping and serotyping/subtyping techniques which examine the immunological specificity of capsular polysaccharides and outer membrane proteins respectively. However, not all isolates are typable and the technique requires absorbed sera or monoclonal reagents which are not generally available. Multi-locus enzyme

* Present address: Public Health Laboratory, Level 7, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.

electrophoresis has been used successfully to study the spread of particular strains [3, 4] but this technique is labour intensive and time consuming and is therefore impractical for the rapid identification of particular strains during an outbreak [5].

The above methods rely on phenotypic characteristics which are subject to variable expression; for a typing scheme to have a secure genetic basis it is preferable to analyse the bacterial genome itself. Two methods of chromosomal DNA analysis have been applied to the epidemiological study of bacteria [6]: comparison of banding patterns resulting from restriction endonuclease digestion (restriction endonuclease analysis, REA) or comparison of banding patterns after hybridization with a probe for a specific DNA sequence (probe-REA). Ribosomal RNA has been used as such a probe for different species of bacteria, both to distinguish between species [7, 8] and between strains within a species [9–11].

Previous studies [12, 13] have applied REA to the epidemiological study of *Neisseria meningitidis* but the patterns obtained consisted of a large number of fragments making analysis of large numbers of isolates impractical. A reduced number of bands would simplify the interpretation and enable larger numbers of isolates to be analysed. This can be achieved using gene probes such as rRNA. The present study assessed the discriminatory value of rRNA gene restriction patterns and REA for isolates of *Neisseria meningitidis* with a view to providing a technique applicable to routine epidemiological studies.

MATERIALS AND METHODS

Ninety-four clinical isolates of *Neisseria meningitidis* were obtained from Aberdeen Royal Infirmary, R. Fallon (Glasgow) and D. Jones (Manchester). All isolates had previously been serogrouped and serotyped; this data is given in Table 1. Isolates were freeze-dried or stored in beads at −70 °C (Protect vials, Technical Services Consultants, Bury, Lancs, UK). Bacteria were subcultured on chocolate blood agar and incubated for 18 h at 37 °C in the presence of 5% CO_2 immediately prior to use.

DNA extraction

Bacterial DNA was extracted by a modification of the method of Pitcher and colleagues [14]. Briefly, bacteria from a single chocolate agar plate were harvested into a microfuge tube containing 0·3 ml 10 mM-Tris (pH 8·0), 1 mM-EDTA (TE8) and lysed by the addition of 0·3 ml of GES reagent (containing 5 M guanidium thiocyanate, 0·1 M-EDTA and 0·5% v/v Sarkosyl) for 10 min at room temperature. After the addition of 0·25 ml 7·5 M cold ammonium acetate the tubes were placed on ice for 10 min, 0·5 ml of chloroform-isoamyl alcohol (24:1) added and the suspensions mixed thoroughly and then centrifuged for 20 min at 12500 ***g***. DNA was recovered from the resulting aqueous phase by precipitation with 0·54 volumes cold isopropanol followed by centrifugation at 6500 ***g*** for 2·5 min. The pellet was air dried, dissolved in TE8 and DNA re-precipitated with 2·5 volumes of cold absolute ethanol. After brief centrifugation (2·5 min at 12500 ***g***) the DNA was dried and finally dissolved in 350 μl TE8. The process could be completed in about 2 h.

Table 1. *Numbers of isolates of given serogroup and serotype used in the present study*

	Serogroup									
Serotype	A	B	C	NG	D	X	Y	Z	W135	Totals
1	—	2	1	—	—	—	—	—	—	3
2a	—	1	10	—	—	—	—	—	—	11
2b	—	4	12	—	—	—	—	—	—	16
4	—	8	—	2	—	—	—	—	—	10
15	—	17	2	2	—	—	—	—	—	21
15w	—	1	—	—	—	—	—	—	—	1
16	—	1	—	—	—	—	—	—	—	1
NS	11	6	3	5	1	1	1	1	2	31
Totals	11	40	28	9	1	1	1	1	2	94

NG, not serogroupable; NS, not serotypable.

Restriction endonuclease digestion

Five micrograms of DNA was digested with 5–10 units of restriction endonuclease (RE) according to the manufacturers instructions (Boehringer-Mannheim, Lewes, Sussex). The REs examined included *Dra* I, *Hin*d III, *Eco*R I, *Sal* I, *Kpn* I and *Bgl* II. Fragments were separated on 0·8% horizontal agarose gels in 89 mM-Tris, 89 mM boric acid, 2 mM-EDTA (TBE buffer) and then stained with ethidium bromide. A 1 kb 'ladder' (Gibco-BRL) and *Hin*d III fragments of phage lambda were used as DNA size markers. For Southern blotting and probing with rRNA, *Serratia fonticola* 3965(7) (kindly supplied by F. Grimont, Institut Pasteur) digested with *Hin*d III was included as a size marker.

Probe labelling

Ribosomal 16+23 S rRNA from *Escherichia coli* (Boehringer-Mannheim) (10 μg) was end-labelled with 50 μCi [γ-^{32}P]ATP (Amersham International, Amersham, UK) using 10 units of T4 polynucleotide kinase in 50 mM-Tris-Cl (pH 7·6), 10 mM-$MgCl_2$, 5 mM-dithiothreitol, 0·1 mM spermidine, 0·1 mM-EDTA. After 60 min incubation at 37 °C the reaction was terminated by addition of 10 μl of 0·2% SDS, 20 mM-EDTA, and the probe used without further purification [15].

Southern blotting and DNA hybridization

Restriction endonuclease digests of chromosomal DNA, separated on agarose gels, were depurinated, denatured and transferred to nylon membranes (Hybond-N, Amersham International) by the method of Southern as described by Maniatis and colleagues [16]. The Southern blots were pre-hybridized for 3 h at 65 °C in 2 × SSC (1 × SSC is 0·15 M-NaCl, 0·015 M sodium citrate), 5 × FPG (1 × FPG is 0·02% Ficoll 400, 0·02% polyvinylpyrrolidone 350, 0·02% glycine), 0·5% SDS, 100 μg/ml denatured salmon sperm DNA and 1 μg/ml poly(A). The probe was added to this and the hybridization mix incubated at 65 °C overnight. Hybridized filters were then washed as previously described [7], dried and then exposed to X-ray film with an intensifying screen at −70 °C overnight, or longer if required.

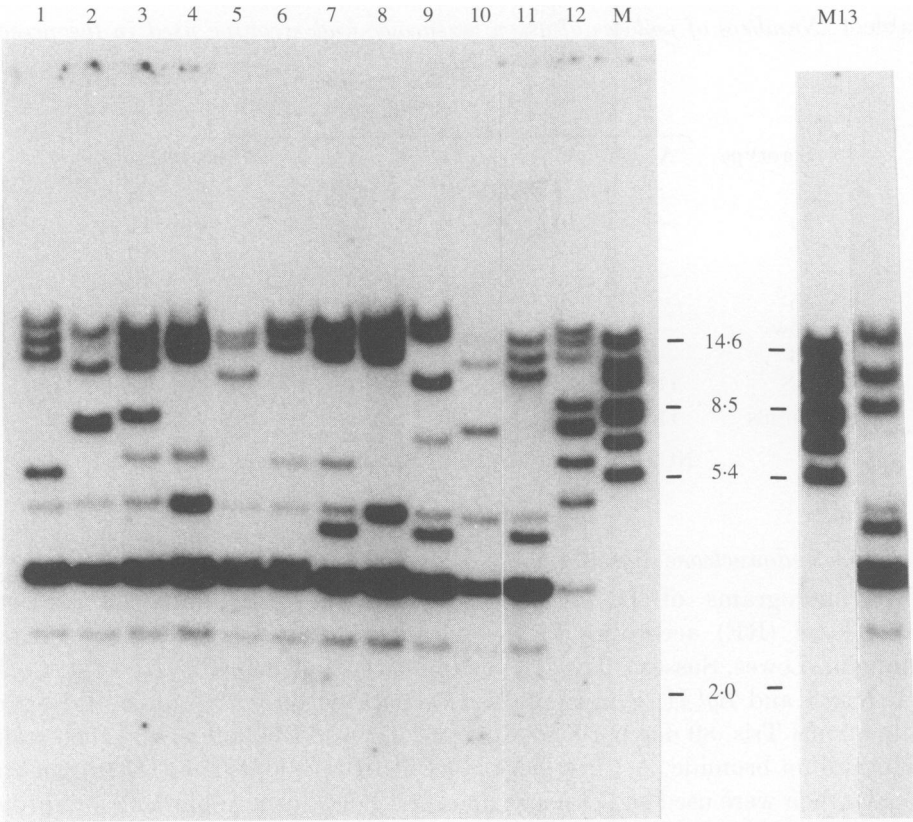

Fig. 1. Ribosomal RNA gene restriction patterns (1–13) of *Neisseria meningitidis* isolates. Southern blot of *Eco*R I digests of *Neisseria meningitidis* DNA probed with rRNA from *E. coli*. M, *Hin*d III digest of *Serratia fonticola* 3965 used as size marker. Fragment sizes in kb.

RESULTS

Ribosomal RNA gene restriction pat erns

The DNA extraction technique yielded 0·3–0·4 mg of high-molecular-weight DNA, from the bacterial growth on a single agar plate.

Of the REs tested, Southern blots of *Eco*R I digests resulted in the most discriminatory banding patterns of rRNA genes, giving between 5 and 8 bands greater than 1 kb in size. All 94 isolates were characterized by their rRNA gene restriction patterns after digestion with *Eco*R I. 13 patterns were identified (Fig. 1). The majority of isolates (57/94) gave patterns 1 (27 isolates) or 2 (30 isolates). Patterns 3–9 contained 10, 7, 6, 5, 1, 2 and 2 isolates respectively and the remaining patterns (10–13) represented single isolates.

The groups of isolates formed on the basis of rRNA gene restriction patterns did not correlate well with serogroup except for serogroup A isolates which all gave patterns 5 or 6 (Table 2). Serotype 2a and 2b isolates, irrespective of serogroup, all gave pattern 1 with the exception of one isolate. No other correlations between rRNA gene restriction pattern and serotype were detected (Table 3).

Isolates with the phenotype B15P1.16R, the phenotype associated with prolonged outbreaks of meningitis in Gloucester and other areas [2], all gave

Table 2. *Relationship between serogroup and rRNA gene restriction pattern*

Serogroup	No. isolates	rRNA gene restriction pattern 1	2	3	4	5	6	Other
A	11	0	0	0	0	6	5	0
B	40	4	21	9	3	0	0	3
C	28	23	1	0	2	0	0	2
NG	9	0	6	0	1	0	0	2

NG, not serogroupable.

Table 3. *Relationship between serotype and rRNA gene restriction pattern and restriction endonuclease analysis with* Bgl *II*

Serotype	No. isolates	No. rRNA patterns	rRNA pattern – (and nos. in pattern)	No. *Bgl* II patterns
1	3	3	3, 4, 8	3
2a	11	2	1 (10), 4	4
2b	16	1	1	4
4	10	2	2 (7), 3 (3)	7
15	21	5	2 (14), 3 (4), 4, 8, 12	14
15w	1	1	3	1
16	1	1	9	1
NS	14	6	1, 2 (7), 4 (3), 11, 7, 13	12*

NS, not serotypable (serogroup B, C or NG).
* Plus one isolate which was not typable with *Bgl* II.

Table 4. *Specimen type and source of isolates giving rRNA gene restriction pattern 2*

Isolate	Phenotype	Age*	Specimen†	Location	DNA pattern rRNA	*Bgl* II
1	B15P1.16R	20	CSF	Liverpool	2	1
2	B15P1.16R	X	CSF	Gloucester	2	1
3	B15P1.16R	4	BC	Taunton	2	1
4	B15P1.16R	X	BC	Gloucester	2	1
5	B15P1.16R	X	TS	Stonehouse	2	1
6	NG15P1.16R	X	TS	Stonehouse	2	1
7	NG15P1.16R	X	TS	Stonehouse	2	2
8	B15P1.15R	3	BC	Nottingham	2	3
9	BNSP1.15R	17 m	BC	Edgware	2	4
10	BNSR	8	CSF	Walsall	2	1
11	BNSP1.15R	5	CSF	Bishops Stortford	2	5
12	BNSP1.15R	15	CSF	Chertsey	2	1
13	CNSP1.15R	10 m	BC	Sheffield	2	6

* Age in years, except m, months; X, age not known.
† CSF, cerebrospinal fluid; BC, blood culture; TS, throat swab.

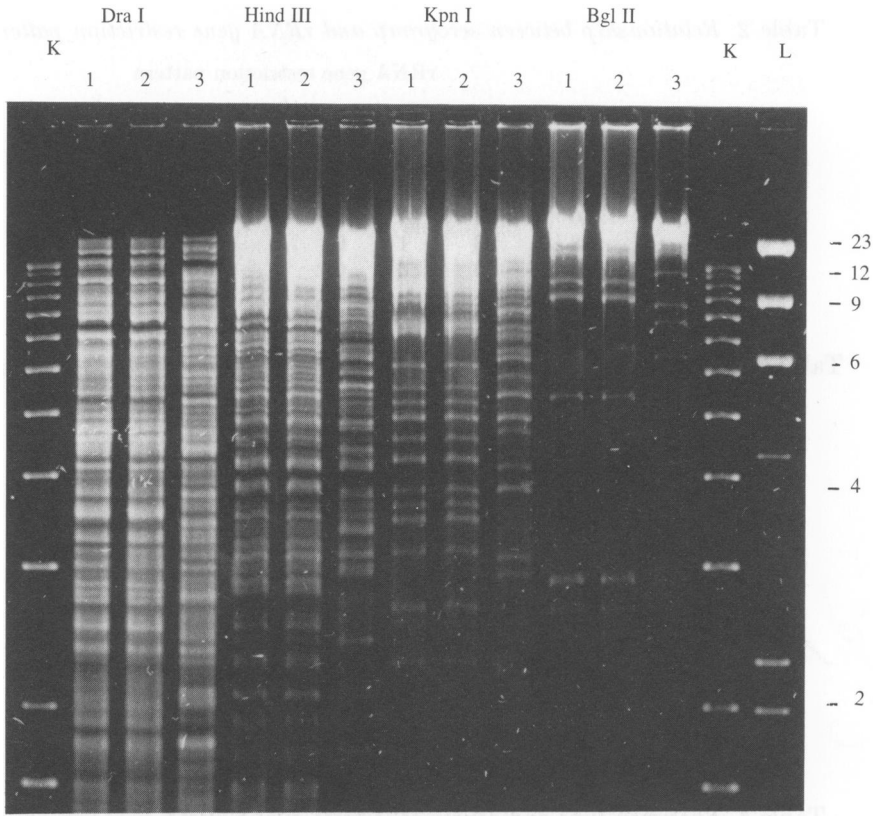

Fig. 2. Example of restriction endonuclease analysis of 3 isolates of *Neisseria meningitidis* (1, 2, 3, indistinguishable by serogroup, serotype and subtype, all B15P1.16) using different REs, as indicated. Note simple but highly discriminatory banding patterns obtained with *Kpn* I and *Bgl* II. K, 1 kb DNA ladder; L, *Hind* III digest of phage lambda DNA. Fragment sizes in kb.

rRNA pattern 2. This pattern also included isolates with other phenotypes (Tables 3 and 4). A single isolate of B15P1.16 (which was sulphonamide sensitive) gave pattern 4. The geographical source of 13/31 isolates which gave rRNA gene restriction pattern 2 is given in Table 4.

Restriction endonuclease analysis

DNA from *Neisseria meningitidis* was digested with six different REs. *Bgl* II gave the simplest banding pattern whilst retaining a high degree of discrimination. This pattern typically consisted of about 10 bands in the 2–20 kb size range. Figure 2 shows an example of the patterns for 3 isolates (indistinguishable by serotyping, all B15P1.16) obtained with 4 different REs.

All 94 isolates were characterized by digestion with *Bgl* II. An example of the resulting patterns is given in Fig. 3. On the basis of these patterns the 94 isolates could be classified in 54 patterns with only 1 isolate being non-typable. Forty-eight isolates were represented by 9 patterns containing 2–14 isolates and 45 isolates were represented by unique patterns. All isolates which were distinguishable by rRNA gene restriction patterns were also distinguishable by REA (Table 3).

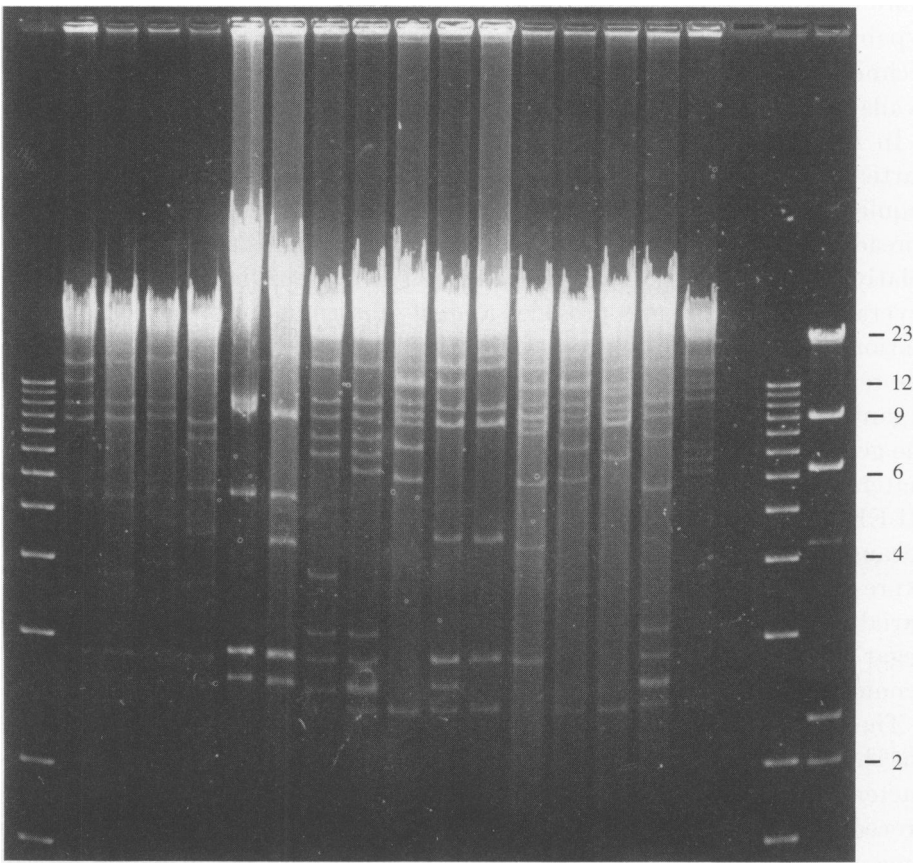

Fig. 3. Example of restriction endonuclease analysis of 17 isolates of *Neisseria meningitidis* using *Bgl* II. Size markers are 1 kb ladder (first and penultimate tracks) and *Hin*d III digest of phage lambda DNA (last track). Fragment sizes in kb.

Isolates with the phenotype B15P1.16R, including isolates from the outbreak in Gloucestershire, were associated with a single REA pattern indicating a correlation between REA patterns and epidemiology. In addition, two NG15P1.16R isolates from carriers in the outbreak area (isolates 6 and 7 in Table 4) were differentiated by this technique; only one of the isolates had the outbreak-associated pattern. One isolate which was not serotypable (isolate 10) and one BNSP1.15 isolate (isolate 12) also had the same pattern as the outbreak-associated isolates. This pattern was also given by isolates with the phenotype B4P1.15.

DISCUSSION

Outbreaks of meningococcal infection in recent years have highlighted our inability to use current epidemiological markers to predict the behaviour of this organism and to explain the patterns of spread of infection within and between communities [2]. Conventional typing methods for *Neisseria meningitidis* involve serogrouping and serotyping/subtyping which examine the immunological specificity of capsular polysaccharides and outer membrane proteins respectively. Serogrouping is of limited value in epidemiological studies because one or two groups usually predominate at any one time or place. Serotyping/subtyping is

more discriminatory, but suffers from the disadvantage that not all isolates are typable, in particular, isolates from healthy carriers are often not typable, and the techniques require absorbed sera or monoclonal antibodies which are not generally available.

In order to follow the course of an outbreak of infection, and to identify particular strains, more discriminatory methods of isolate characterization are required. Multi-locus enzyme electrophoresis (MLEE) has been used to study the spread of strains of meningococci [3, 4, 17, 18]. Strains are characterized by the relative electrophoretic mobilities of cytoplasmic enzymes. The combination of several different enzymes provides a highly discriminatory method of characterization [19, 20]. In addition, the multi-digit numerical data generated also enables the relationship between isolates to be estimated and hence the evolution of the organism to be followed [20, 21]. Although the enzymes utilized in MLEE reflect the genotype of the bacteria they are at least one step removed from the genetic material. The use of protein-based methods of isolate characterization, such as MLEE and those described above, is being increasingly recognized as unreliable because proteins are susceptible to variable expression. For example, pilin expression in *Neisseria meningitidis* has been shown to be variable [22] and variable serotyping reactions have been reported for single strains of coagulase-negative staphylococci [23]. It is therefore preferable to analyse the bacterial genome which is not subject to such variation.

The use of restriction endonuclease analysis (REA), or 'DNA fingerprinting', has shown considerable promise as a method of isolate characterization for several bacterial species [6] but the majority of techniques have involved DNA extraction procedures which require large volumes of bacterial culture, and are therefore time-consuming, and use REs which give large numbers of bands. In order to reduce the number of bands, and hence simplify the interpretation and application of the technique, specific gene probes have been used. Probes for exotoxin A genes, for example, enabled isolates of *Pseudomonas aeruginosa* to be characterized [24]. However, the application of such probes is obviously limited to organisms carrying that particular gene. Ribosomal RNA (rRNA) has been shown to be potentially useful as a 'broad spectrum' probe for taxonomic studies of many bacterial genera as species-specific patterns result [7, 8]. In addition, some bacterial species, for example *Haemophilus influenzae*, show considerable heterogeneity in their rRNA gene restriction patterns, enabling these patterns to be used as an epidemiological tool [9, 10, 25, 26].

The present study classified 94 isolates in 13 groups based on rRNA gene restriction patterns suggesting that there may be sufficient heterogeneity of rRNA patterns within *Neisseria meningitidis* to make them useful epidemiological markers. However, the majority of isolates examined in the present study (61%) fell into only two groups. No correlation between serogroup and rRNA gene patterns was discerned, except for serogroup A isolates. In contrast, there was good correlation between rRNA pattern and serotypes 2a and 2b, irrespective of serogroup.

Although possibly useful for epidemiological studies, the expense and time involved in probing make the determination of rRNA gene restriction patterns less amenable to the busy laboratory than REA. REA provides a much quicker result, and involves less expense, than techniques involving probing. The present

study therefore tried to develop a method of REA which could be applied to large numbers of isolates of *Neisseria meningitidis*, by choosing an RE which gave a few bands as possible whilst retaining a high degree of discrimination. Previous studies have illustrated the use of REs giving about 20 discernible fragments [27], which is about the maximum number that can be compared visually across non-adjacent tracks on a gel. The use of *Kpn* I fulfilled this criteria for *Neisseria meningitidis* and is useful for comparing small numbers of isolates. The use of *Bgl* II, as demonstrated in the present study, takes this approach one step further as even fewer bands result, considerably simplifying the interpretation. The patterns obtained with this technique for B15P1.16R isolates from the Gloucestershire outbreak correlated well with epidemiology and enabled characterization of otherwise non-typable isolates from carriers. However, this pattern was also given by isolates with the phenotype B4P1.15; the phenotype associated with an outbreak of infection at a school in Scotland during 1988 [28]. This suggests that there is a relationship between these two outbreak-associated phenotypes which warrants further investigation. The technique could therefore be used in addition to serotyping and subtyping to identify particular strains during outbreaks enabling the spread of the organism to be studied.

The simple, rapid DNA extraction procedure used in the present study, together with the use of horizontal agarose gels, in contrast to polyacrylamide gels, for the separation of DNA fragments, considerably simplifies the technical complexity often associated with DNA fingerprinting techniques. The technique described here could therefore be readily adopted by most routine diagnostic laboratories.

In conclusion, rRNA gene restriction patterns may provide useful information about major groupings of isolates but probably give insufficient data (bands) to enable clonal groupings to be made. The clear discrimination offered by restriction endonuclease analysis, with restriction endonucleases such as *Bgl* II, should be appropriate for use in routine laboratories.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Medical Research Council. The authors are grateful to Drs Fallon and Jones for providing isolates.

REFERENCES

1. Duerden BI, editor. Meningococcal infection. J Med Microbiol 1988; **26**: 161–87.
2. Cartwright KAV, Stuart JM, Noah ND. An outbreak of meningococcal disease in Gloucestershire. Lancet 1986; **2**: 558–61.
3. Crowe BA, Wall RA, Kusecek, B, Neumann B, Olyhoek T, Abdillahi H, et al. Clonal and variable properties of *Neisseria meningitidis* isolated from cases and carriers during and after an epidemic in The Gambia, West Africa. J Infect Dis 1989; **159**: 686–700.
4. Moore PS, Reeves MW, Schwartz B, Gellin BG, Broome CV. Intercontinental spread of an epidemic group A *Neisseria meningitidis* strain. Lancet 1989; **2**: 260–3.
5. Achtman M. Molecular epidemiology of epidemic bacterial meningitis. Rev Med Microbiol 1990; **12**: 29–38.
6. Owen RJ. Chromosomal DNA fingerprinting – a new method of species and strain identification applicable to microbial pathogens. J Med Microbiol 1989; **30**: 89–99.
7. Grimont F, Grimont PAD. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur Microbiol 1986; **137B**: 165–75.
8. Thomson-Carter FM, Carter PE, Pennington TH. Differentiation of Staphylococcal species

and strains by ribosomal ribonucleic acid gene restriction patterns. J Gen Microbiol 1989; **135**: 2093–7.

9. Irino K, Grimont F, Casin I, Grimont PAD, the Brazilian Purpuric Fever Study Group. rRNA gene restriction patterns of *Haemophilus influenzae* biogroup aegyptius strains associated with Brazilian purpuric fever. J Clin Microbiol 1988; **26**: 1535–8.
10. Lipuma JL, Stull TL, Dasen SE, Pidcock KA, Kaye D, Korzeniowski OM. DNA Polymorphisms among *Escherichia coli* isolated from bacteriuric women. J Infect Dis 1989; **159**: 526–32.
11. Owen RJ, Hernandez J, Bolton F. DNA restriction digest and ribosomal RNA gene patterns of *Campylobacter jejuni*: a comparison with bio-, sero-, and bacteriophage-types of United Kingdom outbreak strains. Epidemiol Infect 1990; **105**: 265–75.
12. Bjorvatn B, Lund V, Kristiansen B-E, Kornes L, Spanne O, Lindqvist B. Applications of restriction endonuclease fingerprinting of chromosomal DNA of *Neisseria meningitidis*. J Clin Microbiol 1984; **19**: 763–5.
13. Kristiansen B-E, Sorensen B, Bjorvatn B, Falk ES, Fosse E, Bryn K, et al. An outbreak of group B meningococcal disease: tracing the causative strain of *Neisseria meningitidis* by DNA fingerprinting. J Clin Microbiol 1986; **23**: 764–7.
14. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Letts Appl Microbiol 1989; **8**: 151–6.
15. Jordens JZ, Hall LMC. Chromosomally-encoded gentamicin resistance in 'epidemic' methicillin-resistant *Staphylococcus aureus*: detection with a synthetic oligonucleotide probe. J Antimicrob Chemother 1989; **23**: 327–34.
16. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning. A laboratory manual. New York: Cold Spring Harbor Laboratory, Cold Spring Harbor 1982.
17. Haimanot RT, Caugant DA, Fekadu D, Bjune G, Belete B, Froholm LO, et al. Characteristics of serogroup A *Neisseria meningitidis* responsible for an epidemic in Ethiopia, 1988–89. Scand J Infect Dis 1990; **22**: 171–4.
18. Mendelman PM, Caugant DA, Kalaitzoglou G, Wedege E, Chaffin DO, Campos J, et al. Genetic diversity of penicillin G-resistant *Neisseria meningitidis* from Spain. Infect Immun 1989; **57**: 1025–9.
19. Caugant DA, Bovre K, Gaustad P, Bryn K, Holten E, Hoiby EA, et al. Multilocus genotypes determined by enzyme electrophoresis of *Neisseria meningitidis* isolated from patients with systemic disease and from healthy carriers. J Gen Microbiol 1986; **132**: 641–52.
20. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; **51**: 873–84.
21. Olyhoek T, Crowe BA, Achtman M. Clonal population structure of *Neisseria meningitidis* serogroup A isolated from epidemics and pandemics between 1915 and 1983. Rev Infect Dis 1987; **9**: 665–92.
22. Tinsley CR, Heckels JE. Variation in the expression of pili and outer membrane protein by *Neisseria meningitidis* during the course of meningococcal infection. J Gen Microbiol 1986; **132**: 2483–90.
23. Etienne J, Renaud F, Bes M, Brun Y, Greenland TB, Freney J, Fleurette J. Instability of characteristics amongst coagulase-negative staphylococci causing endocarditis. J Med Microbiol 1990; **32**: 115–22.
24. Ogle JW, Janda JM, Woods DE, Vasil ML. Characterisation and use of a DNA probe as an epidemiological marker for *Pseudomonas aeruginosa*. J Infect Dis 1987; **155**: 119–26.
25. Stull TL, Lipuma JJ, Edlind TD. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J Infect Dis 1988; **157**: 280–6.
26. Bruce KD, Jordens JZ. Characterisation of non capsulate *Haemophilus influenzae* by whole-cell polypeptide profiles, restriction endonuclease analysis, and rRNA gene restriction patterns. J Clin Microbiol 1991; **29**: 291–6.
27. Jordens JZ, Hall LMC. Characterisation of methicillin-resistant *Staphylococcus aureus* isolates by restriction endonuclease digestion of chromosomal DNA. J Med Microbiol 1988; **27**: 117–23.
28. Blackwell CC, Weir DM, James VS et al. Secretor status, smoking and carriage of *Neisseria meningitidis*. Epidemiol Infect 1990; **104**: 203–9.