MOLECULAR CHARACTERISATION OF BACTERIA

Utility of ribotyping, restriction endonuclease analysis and pulsed-field gel electrophoresis to discriminate between isolates of *Neisseria gonorrhoeae* of serovar IA-2 which require arginine, hypoxanthine or uracil for growth

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Summary. *Neisseria gonorrhoeae* isolates that require arginine—i.e., either citrulline (C), or ornithine (O)—uracil (U) and hypoxanthine (H) have generally been considered to be similar when characterised by auxotype, serovar and plasmid content. The MICs of penicillin, tetracycline, erythromycin, spectinomycin, cefoxitin and ceftriaxone for 552 isolates belonging to serovar IA-2 with these phenotypes were found to be similar. Therefore, restriction fragment length polymorphism analysis of rRNA genes (ribotyping), restriction enzyme (RE) analysis of chromosomal DNA, and pulsed-field gel electrophoresis (PFGE) were evaluated to determine whether these isolates could be distinguished by molecular methods. A subset of 27 isolates of *N. gonorrhoeae* that were OUH-requiring, CUH-requiring or OH-requiring, belonged to serovar IA-2 and carried a 2.6-MDa plasmid, were selected for further study. Based on the RE analysis of *Sma*I-digested genomic DNA, the 27 isolates fell into a single RE pattern, five ribotypes and 17 PFGE profiles which did not correlate with the specific arginine-requiring subtypes of these isolates. Each ribotype varied by the presence of only a single fragment, which was of a different size in each pattern, and 17 (63 %) of the 27 isolates belonged to ribotype I. PFGE yielded the highest level of discrimination with 17 different profiles.

Introduction

Neisseria gonorrhoeae isolates require various compounds for growth in chemically defined media.¹ Arginine auxotrophy is one of the most frequently encountered nutritional defects in gonococcal isolates in Canada, and is found in 40–60 % of clinical isolates.² Arginine-requiring (A) isolates can be subtyped by auxotype into citrulline-requiring (C) or ornithine-requiring (O) isolates.³ Isolates that require arginine (i.e., require ornithine or citrulline) as well as hypoxanthine and uracil (AHU⁻) have increased sensitivity to penicillin and are resistant to the bactericidal action of normal human serum; they have also been associated with disseminated gonococcal infection.^{4,5} Most AHU-requiring strains belonging to serovar IA-2 have been previously considered to be

clonal since they generally cannot be distinguished by antibiogram or plasmid content.⁶

With a homogeneous group of isolates, the discriminatory capacity of classical gonococcal typing schemes—such as auxotyping, serotyping and plasmid content analysis—is often not powerful enough to distinguish between isolates of the same class.⁷ Several molecular methods have been used to subtype gonococcal isolates, including restriction endonuclease (RE) analysis,^{8,9} ribotyping,^{10,11} multilocus enzyme electrophoresis^{10,12} and pulsed-field gel electrophoresis (PFGE),^{10,13,14} although few of these studies have specifically included the AHU-requiring class of isolates.^{10,14}

In the present study, 552 AHU-requiring isolates of serovar IA-2 were typed by antimicrobial susceptibility and plasmid content to provide the relevant context for molecular subtyping. CUH⁻ and OUH⁻ isolates of serovar IA-2 were analysed, as well as OH⁻ isolates which were similar to CUH⁻ and OUH⁻ isolates by antibiogram and plasmid content. These epidemiologically unrelated isolates—which were pheno-

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typically identical with respect to antibiogram, plasmid content (each carried a plasmid of 2.6 MDa) and their serovar (IA-2)—were further characterised by ribotyping, RE analysis and PFGE to determine whether they were identical.

Materials and methods

Bacterial strains and culture conditions

In total, 552 serovar IA-2 isolates—subtyped as auxotypes OUH⁻ (459; 83.2%), CUH⁻ (61; 11.1%) and OH⁻ (32; 5.8%) isolates—collected between 1988 and 1992, were obtained from the culture collection of the National Laboratory for Sexually Transmitted Diseases (NLSTD), Laboratory Centre for Disease Control, Ottawa, Canada. Twenty-seven of these isolates of diverse geographical origins in Canada and different isolation dates were selected for further study. The 12 OUH-requiring isolates (nos. 880375, 880420, 881035, 882208, 882245, 882306, 882398, 882521, 883122, 883171, 883342 and 883463) were from eight provinces; the eight CUH-requiring isolates (nos. 880270, 880379, 880454, 881540, 882132, 882610, 883099 and 883729) were from four provinces; and the seven OH-requiring isolates (nos. 881097, 881342, 881506, 882376, 882642, 882794 and 883212) were from four provinces in Canada.

N. gonorrhoeae isolates were either frozen previously at -70°C in Brain Heart Infusion Broth (Difco) plus glycerol 15% v/v, or were lyophilised in skimmed milk 2% w/v¹⁵ or horse serum plus inositol 5% w/v. Gonococci were subcultured on GC Medium Base (GCMB; Difco), supplemented with modified defined supplement 1% v/v¹⁶ and were incubated for 18–24 h at 35°C in a humid atmosphere supplemented with CO₂ 5% v/v.

Escherichia coli strain C600 carrying plasmid pKK3535 (supplied by Dr J. Brosius, Columbia University, New York, NY, USA), containing the *rrnB* operon,^{10,17} was grown on Tryptic Soy Agar (TSA; Difco) supplemented with ampicillin (Sigma) 100 mg/L and incubated at 37°C overnight.

Auxotype, serovar and plasmid content determination

The auxotype of the *N. gonorrhoeae* isolates was determined by a modification¹⁶ of the method of Hendry and Stewart.¹⁸ Serovar was determined as described by Knapp *et al.*¹⁹ Monoclonal antibodies for serovar determination were provided by Syva (Palo Alto, CA, USA). Isolates were analysed for plasmid content as described previously.²⁰

Antimicrobial susceptibility testing

The MICs of penicillin, tetracycline, erythromycin, cefoxitin, ceftriaxone and spectinomycin for the isolates were determined by an agar dilution method.²¹ The media contained Kellogg's Defined Supplement

1% v/v¹⁵ and the bacterial inoculum was prepared in auxotyping minimal medium.⁶

RE analysis and ribotyping

To isolate genomic DNA, *N. gonorrhoeae* cells were lysed by the method of Dillon *et al.*²² Chromosomal DNA (c. 10 µg) was digested with *Sma*I, *Ava*II or *Hind*II according to the manufacturer's instructions (Boehringer Mannheim), followed by electrophoresis in NA agarose (Pharmacia) 0.7% w/v gels for 40 h at constant voltage (25 V) in Tris-acetate (TA) buffer (400 mM Tris-base 200 mM sodium acetate and 18 mM EDTA, pH 7.8). Molecular size markers included lambda DNA (BRL), lambda DNA digested with *Kpn*I, and a 1-kb ladder (BRL). Gels were stained and photographed as described previously.¹⁰ Fragment sizes from at least three gels were averaged after measuring distances of band migration in comparison to DNA standards.

The riboprobe, comprising the 7.2-kb *Bam*HI-*Pst*I fragment from plasmid pKK3535, was prepared as described previously¹⁰ and labelled with ³²P-dCTP (Dupont) by the random primer-Klenow polymerase method²³ with a Megaprime Labelling Kit (Amersham) in accordance with the manufacturer's instructions.

DNA was transferred from agarose gels to a positively-charged nylon membrane (BMC) by the alkaline transfer method.²⁴ Hybridisation with the radioactive-labelled probe was performed under high stringency conditions (68°C) by the method of Sambrook *et al.*²⁵

The fragment sizes from at least three blots were averaged after measuring distances of band migration in comparison with molecular size markers. The reproducibility of ribotypes generated by *Sma*I was examined by analysis of the same bacterial strain on three separate occasions.

Pulsed-field gel electrophoresis

Chromosomal DNA was prepared by a modification of the procedure of Chang and Taylor²⁶ in which the cell suspensions were adjusted to 1.5 × 10⁹ bacteria/ml. The agarose (Low-Melting-Point Agarose; Gibco-BRL) block containing genomic DNA was equilibrated at room temperature for 4 h in 100 µl of *Spe*I buffer (BMC). Digestion with *Spe*I—which was chosen because it had been used previously to construct a physical and genetic map of the gonococcal chromosome of strains MS11-N198 and FA1090^{27,28}—was performed at 37°C for 24 h in 100 µl of fresh buffer with 5 U of restriction endonuclease according to the manufacturer's instructions (BMC). After digestion, the agarose block was washed once in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and loaded into the wells of an agarose (BioRad) 1% w/v gel, which was then sealed with the same concentration agarose in gel buffer. Electrophoresis was performed with the contour-clamped homo-

geneous electric field (CHEF-DRII) system (BioRad), with agarose 1% w/v gels at 12°C in 0.5 × Tris-borate-EDTA (TBE) buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.5). A constant voltage of 175 V was applied, with ramped pulse times of 0.2–54.2 s over a period of 24 h. Under these conditions, fragments of 5–600 kb were separated and sized in comparison with lambda DNA concatemers (Promega) as molecular size markers. The presence of standard DNA mol. wt markers on each gel enabled comparisons to be made between gels. Fragment sizes and averages were determined from two-to-four replicates. Gels were stained with ethidium bromide, visualised with a UV-transilluminator, and photographed as described previously.¹⁰

Results

Antimicrobial susceptibility of AHU⁻ isolates of serovar IA-2

Five hundred and fifty-two arginine-requiring gonococcal isolates of serovar IA-2 which carried a cryptic plasmid were further subtyped on the basis of requiring either ornithine or citrulline as well as uracil and hypoxanthine (OUH⁻: 459, 83%; CUH⁻: 61, 11%) for growth; isolates which required only O and H (i.e., OH⁻: 32, 6%) were also included since they belonged to serovar IA-2 and carried the 2.6-MDa plasmid. None carried β -lactamase-producing plasmids or transfer plasmids. The MIC range, MIC₅₀ and MIC₉₀ values for these isolates of penicillin, tetracycline, erythromycin, spectinomycin, cefoxitin, and ceftriaxone (table I) indicated that the isolates rarely differed beyond a two-fold dilution difference, a difference not considered to be clinically significant. The most notable difference was seen with spectino-

mycin MICs; OUH⁻ and CUH⁻ isolates were more susceptible (MIC ranges of 2.0 and 4.0–32.0 mg/L, respectively) than OH⁻ isolates (MIC range of 16.0–32.0 mg/L).

Ribotyping of gonococci

Twenty-seven isolates were selected for further study to determine whether they could be differentiated by molecular methods. Ten of 27 gonococcal chromosomal DNA preparations were analysed in preliminary experiments with restriction endonucleases *Ava*II, *Hind*II and *Sma*I to determine which enzymes would provide the greater discrimination for ribotyping. Hybridisation of *Ava*II fragments with the riboprobe yielded two ribotype patterns with four bands (either 17.0, 13.2, 6.6 and 3.6 kb, or 15.2, 6.6, 5.1 and 3.6 kb) for most strains (data not shown). Hybridisation of *Hind*II fragments with the riboprobe produced a single ribotype pattern (data not shown). Analysis with *Sma*I produced highly resolved fragments suitable for analysis, and the 27 OUH⁻ or CUH⁻ or OH⁻/IA-2/2.6 isolates were subtyped into five ribotypes designated I, II, III, IV and V. Fragment sizes ranged from 2.8 to 29.0 kb, and five fragments (23.1, 12.0, 8.2, 4.8 and 2.8 kb) were common across all types (fig. 1). These ribotypes did not correlate with a specific auxotype, and each ribotype differed from the other by the presence of only a single different sized fragment. The 12 OUH⁻/IA-2 isolates included all five ribotypes; the eight CUH⁻/IA-2 isolates were subdivided into ribotype patterns I and V; and the seven OH⁻/IA-2 isolates were subdivided into ribotype patterns I, II and V. Ribotype I dominated, representing 17 (65%) of the 27 isolates.

The characteristic isolate banding patterns with *Sma*I remained unchanged when DNA was prepared

Table I. Susceptibility of 552 serovar IA-2 isolates of three auxotypes to six antimicrobial agents

Antimicrobial agent		MIC (mg/L) data for auxotype		
		OUH ⁻ (n = 459)	CUH ⁻ (n = 61)	OH ⁻ (n = 32)
Penicillin	Range	0.004–0.25	0.004–0.032	0.004–0.25
	MIC ₅₀	0.016	0.016	0.016
	MIC ₉₀	0.016	0.016	0.032
Tetracycline	Range	0.063–1.0	0.063–0.5	0.125–0.5
	MIC ₅₀	0.25	0.25	0.25
	MIC ₉₀	0.5	0.5	0.5
Erythromycin	Range	0.032–2.0	0.063–1.0	0.125–0.5
	MIC ₅₀	0.5	0.5	0.5
	MIC ₉₀	1.0	1.0	1.0
Spectinomycin	Range	4.0–32.0	2.0–32.0	16.0–32.0
	MIC ₅₀	32.0	32.0	16.0
	MIC ₉₀	32.0	32.0	32.0
Cefoxitin	Range	0.004–0.5	0.125–0.25	0.004–1.0
	MIC ₅₀	0.25	0.25	0.25
	MIC ₉₀	0.25	0.25	0.25
Ceftriaxone	Range	≤ 0.00025–0.002	≤ 0.00025–0.002	≤ 0.00025–0.002
	MIC ₅₀	0.00025	0.00025	0.00025
	MIC ₉₀	0.0005	0.0005	0.0005

Fig. 1. Examples of ribotype patterns (designated I-V) obtained after hybridisation of the riboprobe with *Sma*I-digested chromosomal DNA. Strains shown are (left to right): nos. 882610, 880454 (ribotype I), 880420, 882208 (ribotype II), 882398 (ribotype III), 881035 (ribotype IV), 881540 (ribotype V).

from randomly selected strains grown on separate occasions, and the ribotype did not change after an individual strain was subcultured on at least six occasions.

Restriction endonuclease analysis

Gels of chromosomal DNA digested with *Sma*I and run under conditions for optimum ribotype discrimination were analysed retrospectively to evaluate RE analysis as a subtyping method (data not shown). Under the conditions described in the methods, *Sma*I digestion produced 80–100 visible bands. The fragment size range 9.7–27 kb was reproducible and stable, and was selected for band analysis, while DNA fragments of < 9.7 kb generally contained low in-

tensity stained bands and fragments of > 27.0 kb were not well-resolved. Only one RE pattern, comprising 28 bands varying from 27.0–9.7 kb (data not shown), was observed for the 27 isolates.

Analysis of 27 arginine-requiring gonococcal isolates by PFGE

The 27 isolates were analysed further by PFGE. *Spe*I digestion of chromosomal DNA produced 10–15 fragments, ranging from 38–500 kb, which were resolved into 17 patterns (fig. 2); the average molecular size of the bands in each pattern is shown in table II. The PFGE patterns observed for each isolate were reproducible (data not shown). All isolates had eight bands in common (410.0, 107.0, 84.0, 78.0, 73.0, 63.0, 41.0 and 38.0 kb). Twenty-five of the 27 isolates also had a 176.0-kb fragment; the exceptions were isolates 880397 and 880420. The 12 OUH⁻/IA-2 isolates produced 10 distinct PFGE patterns, the eight CUH⁻/IA-2 isolates produced five PFGE patterns, and the seven OH⁻/IA-2 isolates produced five PFGE patterns (table III). P15 was found in both the CUH⁻/IA-2 group and the OUH⁻/IA-2 group, P1 was found in the OUH⁻/IA-2 and OH⁻/IA-2 group, and P3 was found in the CUH⁻/IA-2 and OH⁻/IA-2 group. The 17 isolates belonging to ribotype I were subtyped further into 10 PFGE patterns; four isolates belonging to ribotype II were subtyped into four PFGE patterns; two isolates belonging to ribotype III were subtyped into two PFGE patterns; and three isolates belonging to ribotype V were subtyped into three PFGE patterns.

In conclusion, the 27 IA-2 isolates were represented by three arginine-requiring auxotypes, one RE pattern, five ribotype patterns and 17 *Spe*I PFGE patterns (table III).

Discussion

Several typing systems have been developed to characterise individual strains of *N. gonorrhoeae*, including auxotype, serotype, plasmid content and antimicrobial susceptibility determination. In previous studies, the combination of antimicrobial susceptibility testing with plasmid content analysis, serotyping and auxotyping has been shown to provide greater discrimination between isolates than the use of only one of these techniques.^{7,29} However, these methods have not been successful in distinguishing OUH⁻, CUH⁻ or OH⁻ isolates (i.e., AHU⁻) of serovar IA-2.

Although several molecular fingerprinting techniques, such as RE analysis, ribotyping and PFGE analysis, have been used for typing *N. gonorrhoeae*,^{8,10,11,13} none of the auxotype/serotype classes described in this study has been analysed previously with these methods. Previous studies on RE pattern analysis of *N. gonorrhoeae*^{8,30,31} used polyacrylamide 4% w/v gels to study fragments of

Fig. 2. Examples of PFGE patterns obtained following digestion of *N. gonorrhoeae* chromosomal DNA digested with *SpeI*. M, lambda concatemers; P (pattern) 1, strain no. 881506; P2, 883171; P3, 880270; P4, 881035; P5, 882642; P6, 882208; P7, 882521; P8, 882398; P9, 882610; P10, 882132; P11, 882306; P12, 881342; P13, 880375; P14, 883212; P15, 881540; P16, 880379; P17, 880420.

HindIII-digested DNA of < 2 kb. However, other studies found that this enzyme was not useful for discriminating *N. gonorrhoeae* isolates by ribotyping when an agarose gel system was used.¹⁰ For this reason, an agarose gel system which resolved fragments of *SmaI*-digested DNA in the 9.0–27.0-kb range was used. Previous studies have also indicated that RE analysis can distinguish isolates belonging to the same serovar,^{8,30,31} but this was not achieved by the conditions and enzyme used for RE analysis in the present study. The limitations of RE analysis, including the number of bands produced and difficulties in interpretation, have been noted previously,³² and for these reasons, it is not used routinely as a subtyping method.

In general, RE analysis has been more successful when combined with hybridisation to a gene probe, such as a ribosomal nucleic acid gene, to select a small number of fragments.³³ In the present study, the number of bands to interpret by ribotyping was reduced to between six and seven, compared to ≥ 80 for RE typing. The 27 isolates were classified into five ribotype patterns, and no isolate was non-typable. This suggested that there may be sufficient heterogeneity of rRNA patterns within *N. gonorrhoeae* for

them to be useful epidemiological markers. It should be noted that the five ribotypes did not recognise exclusively the OUH⁺, CUH⁺ or OH⁺/IA-2/2.6 isolates in the present study; other isolates of different auxotype/serotype classes also had these ribotypes.³⁴ Previous studies^{10,11} have also shown that common DNA bands are present in the ribotype patterns of all *N. gonorrhoeae* isolates, indicating the conserved nature of the rRNA genes within the species. There was no absolute correlation between rRNA gene restriction patterns and auxotype/serotype class, as has also been observed previously.^{10,11}

Recently, DNA macrorestriction analysis by PFGE has been used to compare chromosomal restriction patterns in a number of genera.^{35–38} A previous study of PFGE patterns generated by *SpeI* or *NheI* digests of *N. gonorrhoeae* differentiated 48 isolates of different auxotype/serotype classes into 38 *SpeI* PFGE groups or 40 *NheI* groups.¹⁴ Since the classes of the isolates in the present study differed from those examined previously, and since fragment size was not indicated, we were unable to compare the data with those obtained previously.¹⁴ However, both studies demonstrated that PFGE could subtype isolates within the same

Table II. PFGE patterns of 27 arginine-requiring gonococcal isolates of serovar IA-2

Fragment size (kb)	PFGE pattern number																
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
410.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
367.0	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
339.5	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+
297.0	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
275.0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
242.5	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
228.0	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
207.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
200.0	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
193.0	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
183.0	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
176.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
168.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
167.0	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
159.0	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
149.0	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
137.0	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
117.0	-	+	+	-	-	-	-	+	-	-	-	+	+	+	-	-	-
107.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
95.0	+	+	-	+	+	+	+	-	-	-	+	-	-	+	+	-	+
84.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
78.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
73.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
63.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
59.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
41.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
38.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Strain nos. generating each individual pattern were as follows: P1, 881506, 883342; P2, 883171; P3, 880270, 880454, 881097, 883099; P4, 881035; P5, 882642; P6, 882208; P7, 882521; P8, 882398; P9, 882610, 883729; P10, 882132; P11, 882306, 883463; P12, 881342, 882376, 882794; P13, 880375; P14, 883212; P15, 881540, 882245, 883122; P16, 880379; and P17, 880420.

Table III. Relationship between auxotype/serotype, ribotype and PFGE pattern for 27 gonococcal isolates

Strain no.	Auxotype/serotype	Ribotype pattern	PFGE pattern
880375	OUH ⁻ /IA-2	I	P13
882306			P11
883122			P15
883171			P2
883342			P1
883463	OUH ⁻ /IA-2	V	P11
880420			P17
882208			P6
882245	OUH ⁻ /IA-2	II	P15
881035			P4
882398			P8
882521	OUH ⁻ /IA-2	III	P7
880270			P3
880379			P16
880454	CUH ⁻ /IA-2	I	P3
882132			P10
882610			P9
883099			P3
883729			P9
881540	CUH ⁻ /IA-2	V	P15
881097			P3
881342			P12
881506	OH ⁻ /IA-2	I	P1
882376			P12
882794			P12
882642	OH ⁻ /IA-2	V	P5
883212	OH ⁻ /IA-2	II	P14

auxotype/serotype classes. In this study, PFGE typing provided greater differentiation than auxotype/serotype typing, RE typing or ribotyping alone. Phenotypically similar but epidemiologically unrelated strains could also be differentiated, and this should be a powerful method for confirming the identity of epidemiologically related isolates.³⁹

The present results show that isolates which appear to be clonal by classical typing methods can be distinguished by ribotyping and PFGE. All of the ribotype groups, and most of the different PFGE types, showed only one or two band differences from a conserved core profile present in all isolates, differences which could be explained by a single genetic event, such as deletion, insertion, inversion or mutation. These results reflect the high recombination rates of *N. gonorrhoeae* and provide further evidence for its non-clonal population structure.¹²

One of the limitations of both ribotyping and PFGE typing in *N. gonorrhoeae* is the lack of standardised methods and banding pattern numeration for recording ribotypes and PFGE patterns between laboratories, thereby making it almost impossible to compare data. In addition, PFGE may provide too much discrimination for use in population studies. The strength of PFGE as a typing scheme may ultimately be in its ability to confirm identity—i.e., in nosocomial infections or well-characterised epidemiological outbreaks—rather than to provide broad discrimi-

nation in biologically similar, but epidemiologically distinct isolates.

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