Epidemiol. Infect. (2000), **125**, 63–70. Printed in the United Kingdom

Interpretation of band differences to distinguish strains of *Serratia marcescens* by pulsed-field gel electrophoresis of *Xba*I DNA digests

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(*Accepted 6 March 2000*)

SUMMARY

The number of band differences in DNA macrorestriction profiles required to distinguish unrelated strains from an index strain varies in an outbreak with the species and restriction enzyme used. In order to define this difference for epidemiological studies of *Serratia marcescens*, we produced DNA fingerprints from 57 isolates of the organism using the restriction enzyme *Xba*I and pulsed-field gel electrophoresis (PFGE). The isolates were selected on the basis of their epidemiology, serotype and phage-typing patterns to include 28 unrelated strains and 29 representatives from 2 distinct outbreaks. One of the outbreaks was prolonged, lasting for several years. Electrophoretic profiles consisting of 20 or more clearly resolved bands were obtained for all isolates. Twenty-six of the unrelated strains had unique profiles with over 10 band differences from all other strains, while 27 of the outbreak representatives could be assigned to the appropriate outbreak with confidence. The majority of the outbreak isolates had none or 2 band differences from the index profile, although 3 isolates differed by 5–7 bands. The 2 exceptions among the unrelated strains differed by 4 bands, and 3 phage typing reactions, and were isolated from London and Berlin 3 years apart, while the 2 exceptions among the outbreak collection had clearly unique profiles with over 20 band differences from each other and the outbreak profiles. Cluster analysis using Dice coefficient and UPGMA gave cut-off values of 75–78% similarity overall for related isolates, while the closest similarity for unrelated strains was 70%. The results of this study together with those of the 6 previous reports of PFGE for *S. marcescens* (which used either enzymes *Xba*I or *Spe*I) confirm that this technique is of value for this species and that with *Xba*I at least, most epidemiologically related strains will only differ by 3–4 bands. However, on occasion up to 7 band differences can be found within an apparent outbreak, which may be suggestive of genetic drift.

INTRODUCTION

Serratia marcescens is a ubiquitous Gram-negative bacterium and can commonly be isolated from the environment. Over the last 40–50 years it has also been recognized as a successful opportunist pathogen, able to produce outbreaks of infection in immuno-compromised patients. It is a significant cause of nosocomial infection, with recently reported frequencies of 1% of all United States nosocomial infections and of all bacteraemias in the United Kingdom [1, 2]. To implement effective infection control measures, knowledge of individual strain identities is helpful to provide evidence of cross-infection to eliminate hospital environment sources or

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https://doi.org/10.1017/S0950268899004112 Published online by Cambridge University Press

patients from an outbreak. A major role of our laboratory is to operate a reference typing service to provide such information. To do this, we have developed O and K serotyping schemes that, in conjunction with phage typing, have proved highly satisfactory for epidemiological typing of large collections of *S. marcescens* isolates worldwide [3]. These methods require a considerable investment of time and resources and are thus most suited to a reference laboratory.

Among the genotypic methods described in the past two decades, DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has been the most successfully adapted for a wide range of microorganisms [4]. Criteria for the interpretation of PFGE profiles have been put forward based on the number of band differences allowed as evidence of strain distinguishability [4, 5]. However, it is clear that owing to differing evolutionary rates and population structure, i.e. clonal or panmictic [6, 7], the adoption of band difference criteria must be based on prior validation using appropriate isolates of the species under investigation.

We therefore designed a study to evaluate PFGE for DNA fingerprinting *S. marcescens*, using the rare cutting restriction endonuclease *Xba*I. We approached the analysis from the standpoint that related isolates should share banding profiles by which they can be distinguished from independent unrelated isolates and therefore panels of isolates were selected on the basis of their epidemiology, O and K serotypes and patterns of sensitivity to typing phages. For most of the isolates, strain identity was unambiguous but a few equivocal isolates were deliberately included.

MATERIALS AND METHODS

Bacterial isolates

All isolates were maintained on nutrient agar and stored on glass beads in 20% (v/v) glycerol broth at −20 °C. Species identity was confirmed with a short set of biochemical tests [8]. Serotyping was carried out using an ELISA with adsorbed sera [3], and sensitivity to typing phages was determined as previously described [9]. Both typing methods were performed when we received the isolates, but phage typing was repeated on a single occasion after selection to maximize reproducibility. All lytic phage reactions of 20 or more plaques were recorded as contributing to the phage pattern. Isolates from the same epidemiological incident of the same serotype and phage pattern (allowing a single phage-reaction difference) were considered to represent the same strain. Isolates of different serotype, or of the same serotype but with four or more phage reaction differences, were considered to represent clearly unrelated strains, while the strain relationships of isolates of the same serotype but with phage patterns differing by two or three reactions were considered equivocal.

Two categories of isolates were selected. Category I contained 29 isolates from 2 independent outbreaks (O14:K14 outbreak A and O27:K14 outbreak B) in the same hospital, identified by serotype and either indivisible or equivocal by phage pattern. Category II contained 28 unrelated isolates including 8 from sporadic infections in the same hospital and 20 distinct strains from 15 other hospitals.

Pulsed-field gel electrophoresis (PFGE)

Briefly, DNA was prepared in agarose blocks using lysis buffer containing 0·5 M EDTA and 1% (w/v) *N*-lauryl sarcosine followed by a proteinase K digestion step to remove cellular proteins and DNase. Following copious washing to remove proteinase K, digestion was carried out at 37 °C overnight with the restriction enzyme *Xba*I (Gibco, Paisley, UK) and the resulting fragments separated using a CHEF DRII electrophoresis system (BioRad, Hemel Hempstead, UK) with a 5–35 s ramp and 22 h run time [10]. The agarose gels were stained with ethidium bromide and viewed under UV illumination.

The isolates were run in four gels, with a group A representative (3927) in every gel to facilitate gel-to-gel comparison. The resulting DNA profiles were interpreted visually by two different operators and all band positions recorded as 0 or 1. These data were used both to determine the number of band differences and for computer-assisted analysis to produce dendrograms showing unweighted pair group matching average (UPGMA) cluster analysis of Dice similarity coefficients produced from pair-wise comparisons of the coded profiles [11].

RESULTS

The results of the visual interpretation of the CHEF profiles are summarized in Tables 1 and 2, and representative profiles are shown in Figure 1. Bands sizes varied from approximately 50–1000 kb. Twenty-

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Table 1. *Origin, serotype, phage sensitivities and PFGE patterns for* S. marcescens *isolates from category I*

Isolate ref.	City	Year	Serotype	Phage pattern	Phage differences	PFGE pattern	Band differences from 3927 or 2533	Lane nos. in Fig. 1
3927	Dublin	1989	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	3, 7, 11, 16, 20
5345	Dublin	1987	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	—
3799	Dublin	1988	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	4
3804	Dublin	1988	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	5
3866	Dublin	1988	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	6
3904	Dublin	1989	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	—
4062	Dublin	1989	O14:K14	1/4/10	0 from 3927	A1	0 from 3927	—
3716	Dublin	1988	O14:K14	1/4/10	0 from 3927	A2	2 from 3927	1
3795	Dublin	1988	O14:K14	1/4/10	0 from 3927	A3	2 from 3927	2
4053	Dublin	1989	O14:K14	1/4/10	0 from 3927	A4	2 from 3927	—
5293	Dublin	1993	O14:K14	1/4/10	0 from 3927	A5	2 from 3927	—
5327	Dublin	1993	O14:K14	1/4/10	0 from 3927	A6	3 from 3927	—
5142	Dublin	1993	O14:K14	1/4/10	0 from 3927	A7	4 from 3927	—
4749	Dublin	1991	O14:K14	1/4/9/10	1 from 3927	A8	2from 3927	9
3645	Dublin	1987	O14:K14	1/3/10	2 from 3927	A1	0 from 3927	—
5307	Dublin	1993	O14:K14	10	2 from 3927	A9	2 from 3927	—
5138	Dublin	1993	O14:K14	1/9/10	2 from 3927	A10	4 from 3927	10
5030	Dublin	1992	O14:K14	1/4/9	2 from 3927	A11	7 from 3927	8
4762	Dublin	1991	O14:K14	4	2 from 3927	Unique	> 20 from 3927	—
4848	Dublin	1992	O14:K14	4/9	3 from 3927	A12	6 from 3927	—
2525	Dublin	1983	O14:K14	non-typable	3 from 3927	Unique	> 20 from 3927	—
2533	Dublin	1983	O27:K14	4/11	0 from 2533	B1	0 from 2533	—
2541	Dublin	1983	O27:K14	4/11	0 from 2533	B1	0 from 2533	—
2716	Dublin	1983	O27:K14	4/11	0 from 2533	B2	1 from 2533	—
2701	Dublin	1983	O27:K14	4/11	0 from 2533	B3	2 from 2533	—
2532	Dublin	1983	O27:K14	4/11	0 from 2533	B4	4 from 2533	—
2536	Dublin	1983	O27:K14	2/4/11	1 from 2533	B1	0 from 2533	—
2526	Dublin	1983	O27:K14	4	1 from 2533	B1	0 from 2533	—
2520	Dublin	1983	O27:K14	4	1 from 2533	B5	5 from 2533	—

seven of the 29 isolates in category I could be assigned readily to the correct outbreak on the basis of DNA fingerprint (Table 1). There were 8 O14:K14 isolates with indistinguishable fingerprints representing the index profile of outbreak A (coded A1), while 11 further O14:K14 isolates produced recognizable outbreak A profiles, although these differed from the index strain profile by 1–7 bands (coded A1–A12). Four O27:K14 isolates with indistinguishable fingerprints represented the index profile of outbreak B (B1) and another 4 O27:K14 isolates produced profiles which differed from this by 1–5 bands (B2–B5). The profiles of the outbreak strains A and B differed from each other by well over 20 bands. The two exceptions which could not be assigned to either outbreak were O14:K14 isolates with DNA fingerprints which were clearly unrelated to either of the outbreak profiles, differing from both as well as each other, by more than 20 bands. Both these isolates were among the 7 whose strain identities were equivocal because their phage patterns differed from the outbreak A pattern by 2 or 3 reactions.

In contrast, 26 of the 28 category II isolates (Table 2) produced unique DNA fingerprints, which differed by over 20 bands from all other profiles, with the exception of 5 O27:K14 isolates which showed 10–12 differences from the O27:K14 Dublin outbreak B profile. The two isolates which did not produce unique profiles were serotype O14:K14 isolates. Their DNA patterns differed by only 4 bands from each other (C1 and C2) and their phage patterns by 3 reactions, although one originated from a hospital in London and the other from Berlin.

These findings were mirrored by UPGMA cluster analysis using Dice coefficients. Figure 2 shows the dendrograms of the O14:K14 isolates from each group. The 19 O14:K14 group I isolates with the outbreak. A profile clustered at 78% while the 8 O27:K14 outbreak B isolates clustered at 84%. The similarity between the two outbreaks was 46%. The

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Table 2. *Origin, serotype, phage sensitivities and PFGE patterns for* S. marcescens *isolates from category II*

Isolate ref.	City	Year	Serotype	Phage pattern	Phage differences	PFGE pattern	Band differences	Lane nos. in Fig. 1
1790	Dublin	1982	O14:K14	1/9/11	4 from 3927	Unique	> 20 from 3927	—
5161	Dublin	1993	O14:K14	1/2/3/4/6/7/8/11	7 from 3927	Unique	> 20 from 3927	17
5168	Dublin	1993	O14:K14	2/7	5 from 3927	Unique	> 20 from 3927	18
1787	Dublin	1982	O27:K14	1/3/4/5/8/11	4 from 2533	Unique	> 20 from 2533	—
1797	Dublin	1982	O16:K28	1/4	na	Unique	> 20 from all	—
5271	Dublin	1993	O16:K2	11	na	Unique	> 20 from all	—
3022	Dublin	1984	O21:K3	1/4	na	Unique	> 20 from all	—
3876	Dublin	1988	O8:K14	1/3/5/7	na	Unique	> 20 from all	15
4776	Leicester	1991	O14:K14	4	2 from 3927	Unique	> 20 from 3927	13
4357	Madrid	1990	O14:K14	4	2 from 3927	Unique	> 20 from 3927	14
4382	Madrid	1990	O14:K14	10	2 from 3927	Unique	> 20 from 3927	—
5364	Barcelona	1993	O14:K14	1/4/8/11	3 from 3927	Unique	> 20 from 3927	12
5112	Bristol	1993	O14:K14	1/3/4/9/11	4 from 3927	Unique	> 20 from 3927	21
4532	Berlin	1990	O14:K14	2/7	5 from 3927	Unique	> 20 from 3927	—
5178	Ashford	1993	O14:K14	1/2/3/4/5/7/8/9/10	6 from 3927	Unique	> 20 from 3927	19
5415	Madrid	1993	O14:K14	1/2/4/5/6/7/9/11	7 from 3927	Unique	> 20 from 3927	—
5350	London	1993	O14:K14	1/2/3/5/6/7/10/11	7 from 3927	C1	0 from 5350	—
4584	Berlin	1990	O14:K14	1/2/3/4/5/6/7/8/9/10/11	8 from 3927	C2	4 from 5350	—
1461	Oxford	1980	O27:K14	4/11	0 from 2533	Unique	10 from 2533	—
1879	London	1982	O27:K14	4/11	0 from 2533	Unique	11 from 2533	—
4113	Leuven	1990	O27:K14	4/11	0 from 2533	Unique	12 from 2533	—
2857	London	1984	O27:K14	4/11	0 from 2533	Unique	12 from 2533	—
1027	Leeds	1980	O27:K14	4/5/11	1 from 2533	Unique	12 from 2533	—
4614	Berlin	1990	O27:K14	4	1 from 2533	Unique	> 20 from 2533	—
1254	Plymouth	1980	O27:K14	NT	2 from 2533	Unique	> 20 from 2533	—
4006	London	1989	O27:K14	3	3 from 2533	Unique	> 20 from 2533	—
4618	Madrid	1990	O27:K14	4/5/9	3 from 2533	Unique	> 20 from 2533	—
4585	Berlin	1990	O27:K14	1/2/3/7/8	7 from 2533	Unique	> 20 from 2533	—

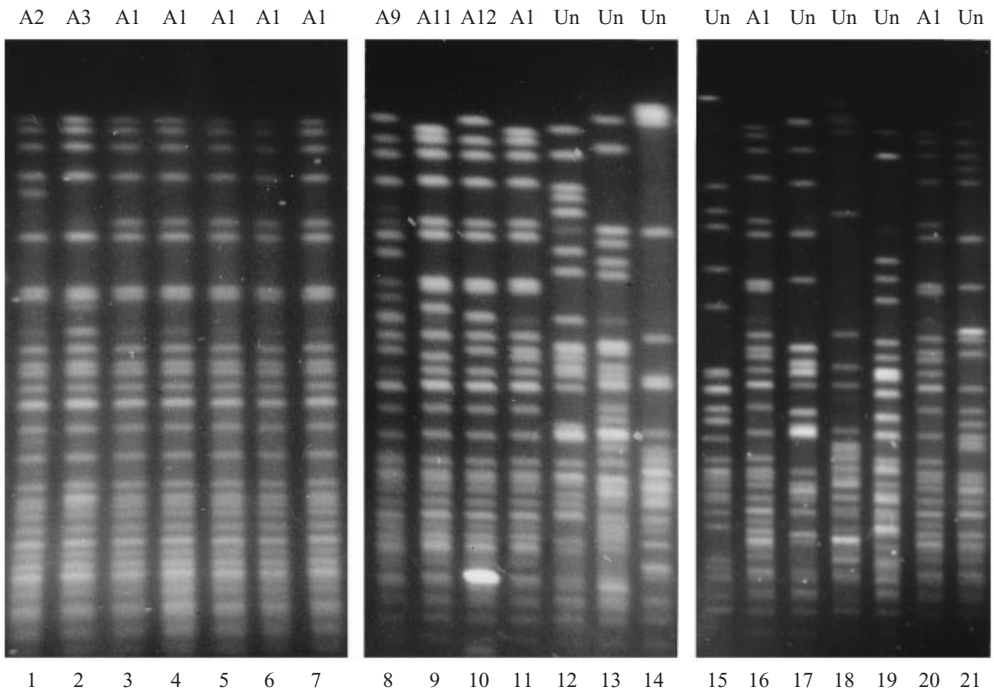

Fig. 1. PFGE patterns of DNA from *S. marcescens*, digested with *Xba*I, with isolate 3927 as the O14:K14 outbreak A representative in lanes 3, 7, 11, 16 and 20. Lanes 1–11, O14:K14 outbreak isolates from Dublin; lanes 12–15, 17–19, 21, non-outbreak isolates from category II.

two O14:K14 isolates in category I (2525 and 4762) with unique profiles were linked to each other at 56% similarity and to outbreak A at 50% whereas the two O14:K14 isolates of category II with similar DNA profiles grouped at 93%, although they were clearly unrelated to outbreak A at only 28% similarity. The closest relationships to outbreaks A and B were seen at 69% (isolate 5112) and 72% similarity (isolate 1027) respectively. All the group II isolates showed less similarity to each other than this, except for two O27:K14 isolates (4584 and 4618) which clustered at 75%.

DISCUSSION

In our experience, a common difficulty in determining strain relationships among ubiquitous Gram-negative nosocomial species, such as *S. marcescens*, is that outbreaks may occur either explosively or persist for months or even years as a result of strains persisting in environmental niches and giving rise to intermittent infections. Thus bacterial populations may alter in genetic structure due to the stress of survival in hostile environments. This is reflected in the appearance of genetic rearrangements and in the variability of phenotypic markers. To allow for this, reaction rules have been proposed for many phenotypic typing systems based on pattern reactions [12]. It is worth noting that due to the structures of bacterial populations, typing systems can only be used with confidence to identify distinct strains. They do not allow total confirmation of a single strain descendent from a common ancestor, and therefore such rules should ideally be used to confirm strain differences and not strain identity. However, in practice it is useful to have criteria which can provide a high level of probability that certain isolates are related within the time-scale of a nosocomial outbreaks. To this end, we have examined the stability of PFGE patterns for *S. marcescens*, using populations in which epidemiological data together with results from phenotypic typing schemes with well-established reaction difference rules, clearly differentiate related strains from sporadic ones.

There have been six previous reports on the use of PFGE for epidemiological typing of *S. marcescens* with *Xba*I and/or *Spe*I [13–18]. Four describe outbreak investigations (three among human patients, the fourth in a dairy herd) while two compare typing results obtained by a variety of methods for isolates collected over a period of time. Although none set out to do so directly, when taken together with this report, they address most of the performance criteria for evaluation of typing systems suggested by the Euro-

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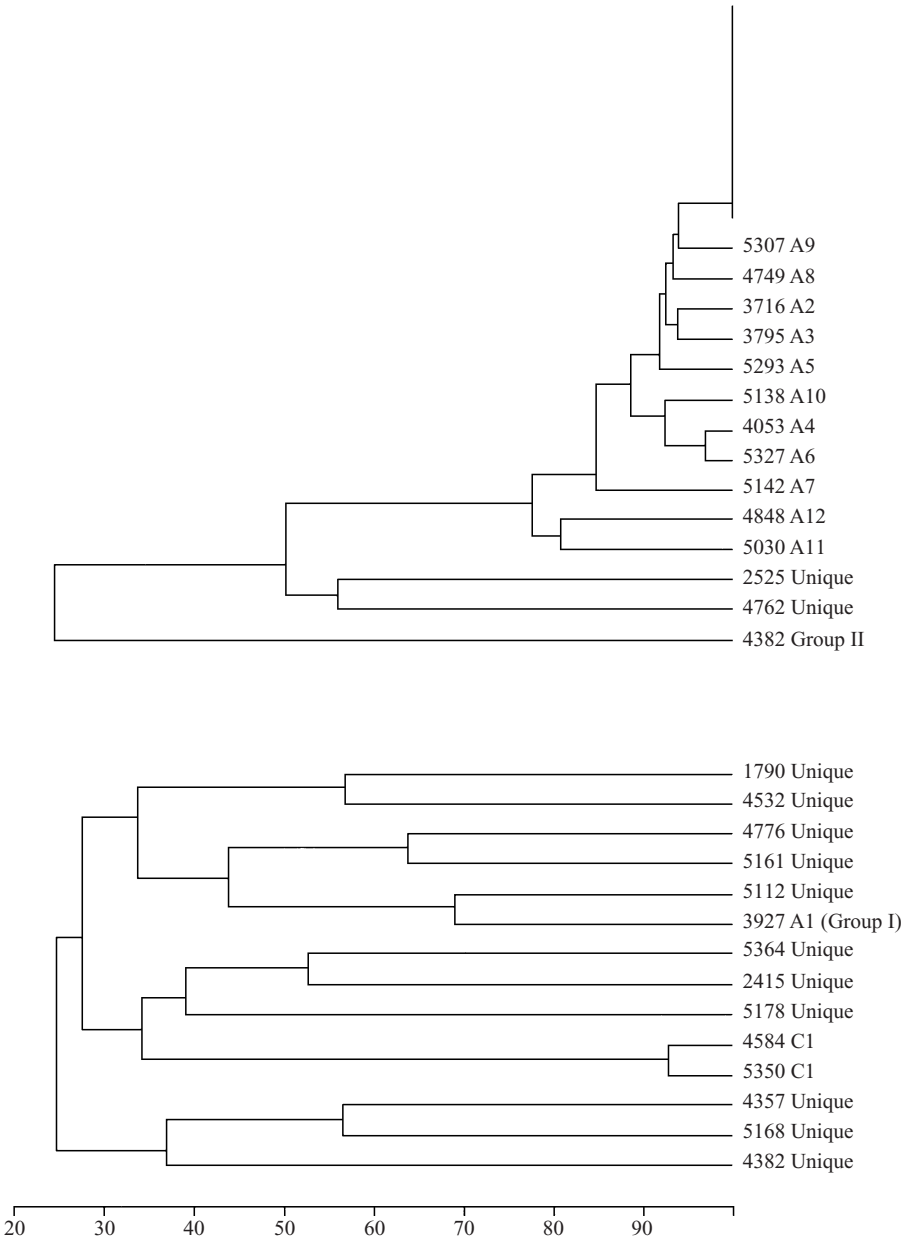

Fig. 2. Dendrogram showing UPGMA cluster analysis of pair-wise comparisons of similarity between the PFGE patterns of selected O14:K14 isolates from Dublin and elsewhere representing categories I and II.

pean Study Group on Epidemiological Markers [5]. None of the papers report failure to produce profiles so typability can be considered as 100% . However, in our experience, the occasional isolate does produce only a smear of unresolvable DNA fragments even on repeat testing. Absolute reproducibility was reported by Shi and colleagues [17] who repeated the testing of 45 isolates on three different occasions, and stability of profile is inferred from the fact that several of the papers report identical profiles from isolates obtained from outbreaks of several months' duration. This is reinforced by our own study, which found isolates from 1987, 1988 and 1989 with identical profiles, although isolates from 1993, while clearly related, showed 2–4 band differences. Discrimination and its converse, epidemiological concordance, have also not been studied formally, although again they appear to be satisfactory. Those studies involving unrelated strains reported highly variable patterns, while those involving outbreak investigations [13, 14, 17] or multiple isolates from individual patients [18] showed highly similar if not identical profiles for these isolates. Our own study found only 2 of the 30 distinct strains tested could not be distinguished from each other.

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These isolates showed 3 phage reaction differences and 4 PFGE band differences, despite having originated from Germany and London 2 years apart. Without thorough investigation into a possible epidemiological link and further genetic analysis, it cannot be determined whether these isolates should be considered a single strain as typing suggests, or whether this is a genuinely anomalous result. Because the outbreaks investigated here were long-term, other typing data had to be taken into account during isolate selection and the results show that typing system concordance is high. The only two anomalies (putative outbreak A strains, 4762 and 2525, with unique profiles) were two isolates deliberately included as having equivocal phage typing results. No such uncertainty occurred during interpretation of the PFGE profiles.

The results of this study suggest that using PFGE with *Xba*I, *S. marcescens* isolates from the same incident of infection with seven or fewer band differences can be considered as representing a single strain. Isolates with 10 or more band differences represent distinct strains. Cut-off points for UPGMA cluster analysis of Dice similarity coefficients were between 75 and 78% similarity, which is slightly lower than the 80–85% normally suggested for this type of analysis [19]. However, this may well be due to the fact that the outbreaks included here were of lengthy duration. The windows between the cut-offs may seem small but the selection procedure was deliberately biased so that isolates with equivocal phage typing results were included to provide a more rigorous test of the method. If these isolates had not been included, the cut-off values would have been $> 85\%$ similarity or < 5 band differences and $< 73\%$ similarity or > 20 band differences. Such values merely serve as the PFGE equivalent of the well-known phage reaction difference rule [12] and cannot be taken as an indicator of genetic distance since band positions show some degree of interdependence [5].

The criteria published by Tenover and colleagues [4] for the interpretation of molecular fingerprints are based on the number of genetic differences shown by any one isolate in a putative outbreak compared with the most common profile, taken as representing the outbreak strain. One genetic event can result in up to three band differences and such an isolate would be considered to be closely related to the outbreak strain and therefore part of the outbreak. Two genetic events can produce up to six band differences and such isolates may possibly be part of the outbreak: this represents the 'grey' area of equivocal results where other factors must be taken into account. Three or more independent genetic events would produce seven or more band differences, which would typically correspond to fewer than 50% of bands in common. Such isolates are considered different and therefore would not be included as part of the outbreak. These authors also add the caveat that these criteria are proposed for 'analysing discrete sets of isolates obtained during epidemiological studies of potential outbreaks in hospitals or communities spanning relatively short periods of time'. This clearly does not apply to this study, but even so the results presented here support these criteria given that the isolate with seven band differences from O14:K14 outbreak A index profile shared 13 bands with it, thus more than 50% of bands were common. Such a degree of genetic variation is not surprising in this case as the O14:K14 outbreak continued for several years (unpublished data) and this isolate was cultured in 1992 while those with the outbreak A index profile were isolated in 1987–9. Furthermore, Goering [20] has shown that, in theory at least, it is possible for a single genetic event to result in four band differences, and therefore two genetic events could account for as many as eight band differences.

The PFGE results presented here allowed us to assign strain designations with confidence, particularly to isolates with equivocal phenotypic typing data, and together with other data from the literature, show that PFGE following *Xba*I restriction digestion is a valid method for determining strain identities of *S. marcescens* isolates. They also serve to illustrate the need to interpret typing data from all isolates in the light of their epidemiology.

REFERENCES

1. Anon. National nosocomial infections surveillance (NNIS) report, data summary from October 1986–April 1996. Am J Infect Control 1996; **24**: 380–8.
2. Bacteraemia, England and Wales: laboratory reports 1995, 1996 and 1997. CDR Wkly 1998; **8**: 22.
3. Aucken HM, Wilkinson SG, Pitt TL. Re-evaluation of the serotypes of *Serratia marcescens* and separation into two schemes based on lipopolysaccharide (O) and capsular (K) antigens. Microbiology 1998; **144**: 639–53.
4. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
5. Struelens MJ and the European Study Group on Epidemiological Markers. Consensus guidelines for

https://doi.org/10.1017/S0950268899004112 Published online by Cambridge University Press

appropriate use and evaluation of microbial epidemiologic typing systems. Clin Microbiol Infect 1996; **2**: 2–11.
6. Maynard Smith J, Smith NH, O'Rourke M, Spratt BG. How clonal are bacteria? Proc Natl Acad Sci USA 1993; **90**: 4384–8.
7. Blanc DS, Hauser PM, Fancioli P, Bille J. Molecular typing methods and their discriminatory power. Clin Microbiol Infect 1998; **4**: 61–3.
8. Farmer JJ III, Davis BR, Hickman-Brenner FW, et al. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J Clin Microbiol 1985; **21**: 46–76.
9. Pitt TL, Erdman YJ, Bucher C. The epidemiological type identification of *Serratia marcescens* from outbreaks of infection in hospitals. J Hyg 1980; **84**: 269–83.
10. Kaufmann ME, Pitt TL. Pulsed-field gel electrophoresis of bacterial DNA. In: Chart H, ed. Methods in practical laboratory bacteriology. Boca Raton: CRC Press, 1994: 83–92.
11. Jones D, Sackin MS. Numerical methods in the classification and identification of bacteria with special reference to the Enterobacteriaceae. In: Goodfellow M, Board RG, eds. Microbial classification and identification. London: Academic Press, 1980: 73–106.
12. Williams REO, Rippon JE. Bacteriophage typing of *Staphylococcus aureus*. J Hyg 1952; **50**: 320–53.
13. Kamarudin MI, Fox LK, Gaskins CT, Gay JM. Environmental reservoirs for *Serratia marcescens* intramammary infections in dairy cows. J Amer Vet Med Assoc 1996; **208**: 555–8.
14. Lehmacher A, Olszewski M, Wolter M, Kohler B, Pertschy A, Methke C, Falkenhagen U. Pulsed-field gel electrophoresis and typing of nosocomial *Serratia marcescens* isolates. Med Microbiol Lett 1996; **5**: 245–52.
15. Traub WH, Eiden A, Leonhard B, Bauer D. Typing of nosocomial strains of *Serratia marcescens*: comparison of restriction enzyme cleaved genomic DNA fragment (PFGE) analysis with bacteriocin typing, biochemical profiles and serotyping. Zbl Bakt I Abt Orig A 1996; **284**: 93–106.
16. Miranda G, Kelly C, Solorzano F, Leanos B, Coria R, Patterson JE. Use of pulsed-field gel electrophoresis typing to study an outbreak of infection due to *Serratia marcescens* in a neonatal intensive care unit. J Clin Microbiol 1996; **34**: 3138–41.
17. Shi Z-Y, Liu PY-F, Lau Y-J, Lin Y-H, Hu B-S. Use of pulsed-field gel electrophoresis to investigate an outbreak of *Serratia marcescens*. J Clin Microbiol 1997; **35**: 325–7.
18. Chetoui H, Delhalle E, Melin P, Struelens MJ, De Ryck R, Osterrieth P, De Mol P. Typing of nosocomial strains of *Serratia marcescens*: comparison of pulsed-field gel electrophoresis of macrorestriction fragments with biotyping, esterase typing and ribotyping. Res Microbiol 1998; **149**: 137–43.
19. Kaufmann ME. Pulsed-field gel electrophoresis. In: Woodford N, Johnson AP, eds. Molecular bacteriology: protocols and clinical applications. New Jersey: Humana Press, 1998; 33–50.
20. Goering RV. The molecular epidemiology of nosocomial infection: and overview of principles, applications and interpretation. In: Specter S, Bendinelli M, Friedman H, eds. Rapid detection of infectious agents. New York: Plenum Press, 1998: 131–57.