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Numerical analysis of SDS–PAGE protein patterns of *Serratia marcescens*: a comparison with other typing methods

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SUMMARY

Twenty-five cultures comprising 18 clinical isolates of *Serratia marcescens* from two hospitals, the type strain of *S. marcescens*, two reference strains of *S. marinorubra*, the type or a reference strain of three other *Serratia* species and a reference strain of undetermined species, were characterized by one-dimensional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of whole-cell proteins. The protein patterns were highly reproducible and were used as the basis of a numerical analysis which divided the clinical isolates into eight protein types. Comparison with O-serotyping indicated that the level of discrimination by SDS–PAGE was similar. As with O-serotyping, a secondary scheme, such as phage typing, is necessary to differentiate strains of the same protein type. We conclude that high-resolution SDS-PAGE of proteins provides an effective adjunct to other methods for typing isolates of *S. marcescens*.

INTRODUCTION

Although there are 10 species of *Serratia*, only 5 occur with some regularity in clinical specimens and among these *S. marcescens* is by far the most important. Reports of human infections by *S. marcescens* have become increasingly frequent in recent years. Most infections occur in immune compromised hospital patients and include sepsis of the respiratory and urinary tracts, of the meninges and of wounds [1]. These appear to be associated with manipulations of the urogenital tract, respiratory ventilation and previous exposure to broad spectrum antimicrobial agents which are largely inactive against this organism [2]. Some strains become endemic in hospitals and the urinary tract of both symptomatic and asymptomatic patients probably serves as a reservoir for nosocomial infection. Common-source outbreaks of sepsis due to *S. marcescens* arise because of the persistence and multiplication of the organism in equipment, such as respirators, or in solutions [2]. The clinical importance of *S. marcescens* and its prevalence have stimulated the development of effective typing schemes for investigation of the epidemiology of this species.

The most extensive schemes are based on serotyping by the somatic (O) and flagellar (H) antigens. Twenty-four O groups have been described; although sero-

group O14 predominates in clinical specimens (over 35% of all strains typed [2, 3]) not all strains share the same lipopolysaccharide antigens [4]. Discrimination of O-serotyping is poor as over 75% of strains fall into three serogroups: O14, O13 and O3 [5]. H-serogrouping was developed to increase discrimination between strains of the more common O groups. The original H-antigen scheme [6] comprised 13 types, but in France it was found necessary to introduce 12 new H types in order to identify strains not typable with the original scheme [5, 7]. Even with H-serogrouping certain types can predominate; for example, type H12 has accounted for over 40% of strains typed [8]. Furthermore, the combined serotype O14; H12 is widespread and can account for 16–50% of all strains [2]. The prevalence of certain types, plus the fact that up to 12% [9] and 16% [2] may be untypable by O- and H-serotyping respectively, limit the usefulness even when O- and H-serogrouping are combined as an epidemiological tool. Bacteriocin susceptibility is potentially useful in distinguishing strains of the same O-serotype from an outbreak of infection. However, small differences in pattern may occur after replicate typing [2]. The method appears to define only broad groups and has a lower discriminatory power than serotyping and phage typing [2]. Phage typing is also potentially useful in the subdivision of strains of the same O-serotype from an incident of infection. However, the 12 bacteriophages presently used are active on only about 75% of clinical strains. Despite the potential for a wide range of lytic patterns, only one or two phages lyse the great majority of strains [10]. Nineteen biotypes, based on the ability to utilise various substrates, have been described for *S. marcescens*; 98·6% of isolates were typable and the method does discriminate adequately between strains [11, 12]. Conventional tests, used for species identification, are generally unsuitable for biotyping due to the similarity of biochemical reactions within *S. marcescens*.

The commercial SDS–PAGE system (AMBIS) has been used to analyse [^{35}S]methionine-labelled proteins of *S. marcescens* from a hospital outbreak [13]. This system was unable to differentiate strains from the outbreak despite them being of two different serotypes; it was however able to separate an unrelated strain (from a different hospital) with the same serotype as some of the outbreak strains.

High-resolution polyacrylamide gel electrophoresis (PAGE) of bacterial proteins has been used for identification at the species, sub-species and infra sub-specific levels [14, 15]. The technique using either conventionally stained or radiolabelled proteins, has been applied increasingly to the typing of a variety of clinically important species that have included *Acinetobacter calcoaceticus* [16], *Campylobacter pylori* [17], *Clostridium difficile* [18, 19], *Enterobacter cloacae* [20], *Providencia alcalifaciens* [21], and methicillin-resistant *Staphylococcus aureus* [22, 23]. In the present study a computerized analysis, of high resolution, was used to gain an objective evaluation of protein patterns as a typing method and to compare its efficacy with O-serotyping and phage typing.

MATERIAL AND METHODS

Bacterial cultures

The 25 cultures used in this study are listed in Table 1 together with their respective O-serotype, phage sensitivity pattern (where these are available) and

Table 1. *Strains analysed by whole cell SDS–PAGE protein patterns*

Ref. no. in dendrogram	Strain no.	Patient: source	O-Serotype	Phage sensitivity pattern	Protein type
Serratia marcescens					
1	NCTC 10211*	Pond water, Czechoslovakia	NT	—	1
Hospital I, Special care baby unit: South Glamorgan, Wales					
2	3024	–: Cooker room, used for disposal of milk	NT	NT	6
3	3025	–: Blood gas analyser	NT	NT	6
4	3026	A: Urine	14	3/5^{w}/8/9/11	7
5	3027	B: Expressed breast milk	14	3/5^{w}/8/9/11	7
6	3029	C: Urine	21	1/4^{w}	4
7	3030	D: Sputum	NT	1/2^{w}/3/4/5/6/7/8/9/10/11	3
Hospital II: London, England					
8	3061	E: Faeces, intensive therapy unit	14	2^{w}/4/5/9	7
9	3064	F: Faeces	14	4/5	7
10	3066	G: Faeces	14	4/5/9	7
11	3067	H: Faeces, special care baby unit	14	2^{w}/7	2
12	3068	I: Faeces	14	1^{w}/2^{w}/3/4/5/6^{w}/7/8/9/10	7
13	3069	J: Faeces	14	2^{w}/3/4/5/7/8/9	7
14	3071	K: Faeces	14	10	7
15	3072	L: Faeces	14	4^{w}/9	7
16	3073	M: Faeces	14	2^{w}/4/8^{w}	7
17	3074	N: Faeces	21	1/4	5
18	3075	O: Faeces	NT	1^{w}/2/3/4/7^{w}/8/11	8
19	3076	P: Faeces	14	1/2^{w}/4/7/9/10/11	7
Serratia liquefaciens					
20	NCTC 10861	Leg ulcer, Eire	—	—	—
Serratia marinorubra					
21	NCTC 10912	Sea water, U.S.A.	—	—	—
22	NCTC 10845	Pig faeces, England	—	—	—
Serratia odorifera					
23	NCTC 11214*	Sputum, France	—	—	—
Serratia plymuthica					
24	NCTC 8015	Canal water, England	—	—	—
Serratia sp.					
25	NCTC 4619	Water, France	—	—	—

* Type strain; w, weak (+ or ±); NT, non-typable

PAGE types. Nineteen of the cultures were *S. marcescens* and included 18 hospital isolates: 6 from a special care baby unit in South Glamorgan, Wales (Hospital I; Ref. nos. 2–7) and 12 from a hospital in London, England (Hospital II; Ref. nos. 8–19), together with the type strain, NCTC 10211. In addition 6 other strains, 1 of which was a type strain, of different species of *Serratia* were also included as study references.

Strains were grown in Nutrient Broth No. 2 (Oxoid: CM67, 25 g/l), with shaking for 3 h at 37 °C.

Serotyping and phage typing

O-serotyping and phage typing were performed using methods described previously [10, 24]. Isolates were divided on the basis of O-serotype and were then further sub-divided by phage typing. Minor variations in phage susceptibility pattern (i.e. up to two reaction differences) were not considered significant [10].

Preparation of protein samples and electrophoresis

For each protein sample, approximately 0·02–0·04 g wet weight of the bacteria were harvested after centrifugation (2500 ***g***) from the nutrient broth and suspended in about 60 μl of double strength lysis buffer (20% v/v glycerol, 1·5% v/v 2-mercaptoethanol, 4% w/v sodium dodecyl sulphate (SDS) and 70% v/v stacking gel buffer). The protein samples were extracted as described previously [25].

Samples were run on discontinuous SDS–polyacrylamide gels which were cast to allow for a 10 mm stacking gel. The final polyacrylamide concentrations were 10% w/v for the separation gel and 5% w/v for the stacking gel. Full details of the methods used in gel preparation and electrophoresis were described previously [26].

Scanning of gels and computations

The stained protein patterns in the dried gels were scanned using a LKB Ultroscan XL laser densitometer (Pharmacia-LKB Biotechnology, Sweden). Absorbance was recorded at 160 μm intervals along the gel yielding 625 values per 10 cm gel. The absorbance range was set from 0·15–0·6 absorbance units (full scale). A rectangular line beam (800 μm × 50 μm) was used to scan each track three times (with no overlap in scan positions) resulting in a multiple track scan of 2·4 mm width. Multiple scanning was carried out in order to reduce the effect of inconsistencies which may be encountered across a track. The mean absorbance of the area scanned was recorded, via an RS232C interface, as raw data on the magnetic disc of a computer.

The initial (stacking gel/separation gel interface) and final (bromophenol blue marker) bands were deleted and protein patterns corrected for gel-to-gel variation using a reference bacterial standard (*S. marcescens* 3030: Ref. no. 7). A replicate of the reference bacterial standard on the subsequent (second) gel was used to calibrate patterns against the reference on the first gel. Segmented linear correction was performed using a total of 18 discernible marker positions (usually peaks) on the reference pattern and by marking the same positions on the calibration pattern replicate. Linear correction (expansion or compression) to the reference distances was carried out within each of the 17 defined segments for each

track by three point quadratic interpolation [27]. The length-corrected traces on the reference gel were composed of 591 absorbance values after removal of the initial and final bands. A general background trend (0·3; fraction of absorbance) in each trace was removed to increase discrimination between patterns. Similarity between all possible pairs of traces was expressed as the Pearson product-moment correlation coefficient, which was converted for convenience to a percent value. The best fit between each pair of traces was obtained by laterally shifting one corrected trace with respect to the other in single point steps of 160 μm up to two points on either side of the initial alignment. Strains were then clustered by the method of unweighted pair group average linkage (UPGMA). Computations were carried out on a Compaq 386 microcomputer using a program package written in Turbo Pascal [22, 27].

RESULTS

O-serotyping and phage typing

The results for serotyping and phage typing of the outbreak strains are shown in Table 1.

Serotyping did not prove to be an entirely effective method for characterizing these sets of isolates. Twelve isolates belonged to serotype O14, the most frequent type in this study. Only one other serotype, O21, was represented by two strains and five further strains were non-typable using the antisera available. All except two isolates reacted with one or more of the typing phages. The isolates which were non-typable using phages were also non-typable using O-antisera.

Hospital I. Attempts were made to type 6 isolates, 4 of which were from different patients. Two of the patient isolates were serotype O14 but 3 strains (2 of which were isolated from the patient associated environment) were non-typable using O-antisera. Two of the patients (A and B) had isolates with identical O-serotypes and phage sensitivity patterns as did a third isolate (from a neonate) which was not included in the study. The 2 isolates from patients C and D each gave distinctive phage sensitivity patterns which served to distinguish them from the other strains.

Hospital II. A total of 12 isolates, each from separate patients, from 10 different wards or units were typed. Ten of the isolates were of serotype O14 and 1 of the remaining strains was serotype O21; the other was non-typable. The phage sensitivity pattern was different for each isolate but similar patterns were evident for some groups of strains, Ref. nos. 8, 9 and 10; Ref. nos. 12 and 13. Few serotypes were represented and most strains belonged to a single serotype. This, coupled with difficulties in interpretation of phage sensitivity patterns and those in comparing objectively between patterns, made the assessment of possible cross-infection difficult to ascertain.

General features of PAGE protein patterns

One-dimensional SDS–PAGE of whole cell protein extracts of the 25 cultures included in this study produced patterns containing approximately 40 discrete bands with molecular weights of 18–100 kDa. Proteins of < 18 kDa were not resolved under the electrophoretic conditions used in this study. PAGE protein

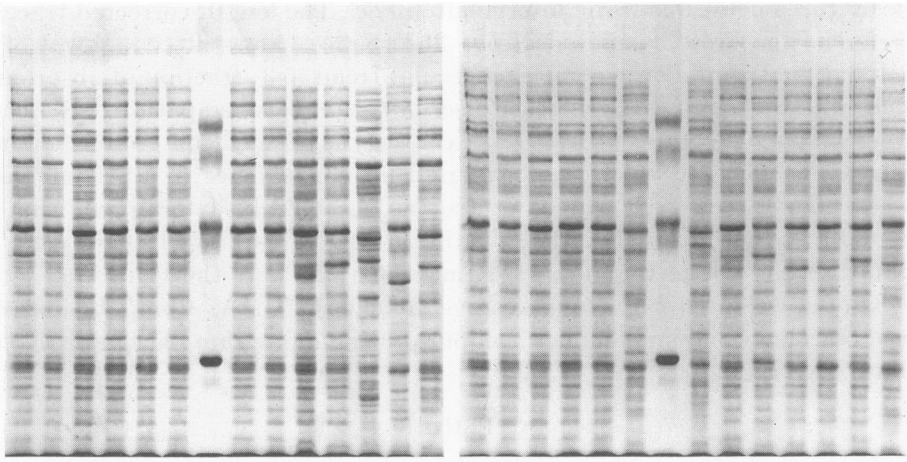

Fig. 1. Electrophoretic protein patterns of *Serratia marcescens* and reference strains of other species of *Serratia*. The numbers refer to those used in Table 1 and Fig. 2. Molecular-weight markers (track labelled X) are (from top to bottom): ovotransferrin, 76–78 kDa; albumin 66·25 kDa; ovalbumin, 42·7 kDa; carbonic anhydrase 30 kDa; myoglobin, 17·2 kDa.

patterns are illustrated in Fig. 1. The protein patterns of the *S. marcescens* isolates were in general very similar to each other. Qualitative differences between isolates were evident principally in the protein bands with molecular weights in the range 35–45 kDa. The protein patterns of the reference strains of other species of *Serratia* (Ref. nos. 20–25) did differ markedly from those of *S. marcescens*, reflecting the phenetic relationships between the species included in the genus.

Reproducibility

The protein patterns of the isolates examined were highly reproducible both within and between gels. Duplicate protein samples of *S. marcescens* 3030 (Ref. no. 7) run on different gels gave similarity values of 97%. Molecular-weight protein standards were also included on each gel and in this case estimates of their similarity were 99% although they provided a less objective measure of reproducibility as they were based on only four bands. The level of reproducibility achieved in this study was similar to that quoted as acceptable by Jackman [14] and was well above the minimum acceptable value [28]. Previous studies using similar methods have reported levels of at least 93% similarity between duplicate samples in separate electrophoretic runs [17, 21, 25, 26]. The dendrogram and protein types recognized in the analysis proved to be extremely robust when the computations were repeated using different levels of trace alignment and background subtraction.

Numerical analysis

Numerical analysis of PAGE total protein profiles based on the determination of the Pearson product-moment correlation coefficient and UPGMA clustering revealed, at the 92% (S) similarity level, a total of 8 distinct protein types for the

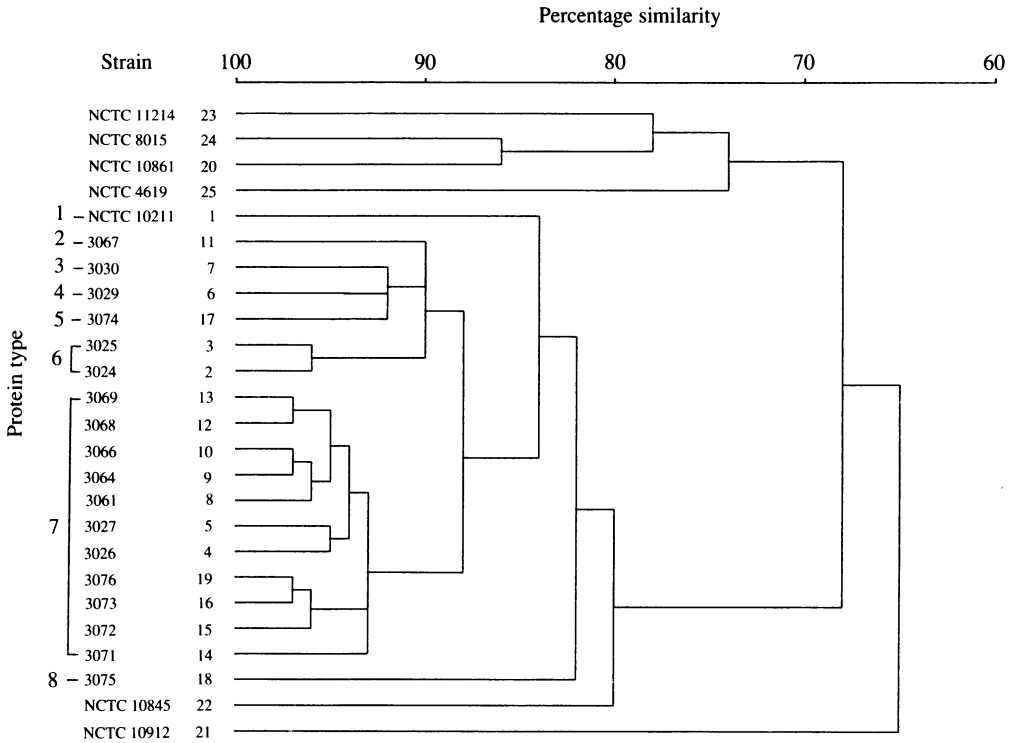

Fig. 2. Dendrogram of the cluster analysis based on total protein content of strains (vertical axis) listed in Table 1. The numbers on the horizontal axis indicate the percentage similarities as determined by the Pearson product-moment correlation coefficient and UPGMA clustering. Protein types were formed at the 92% similarity level.

19 *S. marcescens* strains (7 protein types for the 18 hospital isolates). The *S. marcescens* type strain had a unique pattern distinct from the patterns of the hospital isolates, but more similar to these than to the other species examined. In addition, all six reference strains representing different species gave distinct patterns as shown in the dendrogram in Fig. 2.

Only two of the protein types contained two or more isolates, the remainder being unique for each isolate. Protein type 6 was represented by two isolates, both from the patient associated environment. Neither strain was typable using O-antisera or phages. Protein type 7 contained 11 isolates and included representatives from both hospitals. All 11 strains in this type were of serotype O14. The sub-clustering within protein type 7 reflected the phage patterns of the isolates with groups of strains having similar or identical phage sensitivity patterns clustering more closely together. The serotype O21 strains, which also had similar phage patterns, were similar but not identical in protein pattern which was reflected in their close clustering (types 4 and 5).

DISCUSSION

The analysis of bacterial SDS–PAGE protein patterns is an effective means of differentiating medically important bacteria at the species and infra sub-specific

levels and can provide a novel method of typing, especially where other typing methods are not entirely satisfactory [17, 18, 21, 26]. In this study we have compared the effectiveness of this technique against other methods for typing a number of isolates of *Serratia marcescens*.

The 18 hospital isolates included in this study were divided on the basis of SDS–PAGE of protein patterns into 7 protein electropherotypes (although we refer to protein types throughout we do not here propose that they should be considered as definitive types). The SDS–PAGE technique enabled an electropherotype to be given to *S. marcescens* strains 3024 (Ref. no. 2) and 3025 (Ref. no. 3) both of which were non-typable by both O-serotyping and phage typing, as well as to strains 3030 (Ref. no. 7), 3075 (Ref. no. 18) and the type strain (Ref. no. 1) none of which were typable by O-serology. The level of discrimination was greater than that obtained by serotyping alone mainly because the five strains non-typable by serology were divided into four additional protein types. One of the major advantages of SDS-PAGE is that it offers potentially 100% typability. In general, there was an excellent correlation between strains defined by serotyping and electrophoretic typing (11/12 strains of serotype O14 belonged to a single protein type). However, phage typing could differentiate several strains of electropherotype 7 (or serotype O14). From a practical viewpoint phage typing, although apparently affording greatest discrimination, could not easily be used alone because of difficulties in interpreting the patterns. Clearly, at least two of these techniques must be used in combination for detailed epidemiological studies. In assessing which methods to use consideration must be given to typability, reproducibility, discrimination and the ease of interpretation of results of each method.

Serotype O14 accounts for more than 35% of isolates [2] and this distribution was reflected in the present study with 12/14 of the typable hospital isolates belonging to this type. Little significance can thus be attached to O-serotyping where isolates belong to type O14, but may be more useful when less common serotypes are represented. Protein typing did not offer great advantages over serotyping in this respect but was able to type all strains.

Within the special care baby unit of hospital I, strains from patients A and B (and another strain from a neonate not included in this study) were of identical O-serotype and phage sensitivity pattern and the first two were also of the same electropherotype. Although clearly related, and therefore possibly representing an episode of cross-infection, the three strains were different from the two environmental strains and the two from patients C and D. There was, therefore, some typing evidence to indicate that cross-infection had occurred from patient-to-patient but none to suggest that the original source was either of the environments sampled. This relationship was most clearly demonstrated by protein typing. In hospital II, although most strains were of O-serotype 14 and protein type 7, phage typing was able to discriminate between many of these strains. The strains from patients E, F and G (Ref. nos. 8, 9 and 10), although from different wards, nevertheless had similar phage sensitivity patterns so may have been related. It is of interest to note that the two strains of serotype O21, although from different hospitals, gave very similar phage sensitivity patterns and protein patterns.

In a previous study of a hospital outbreak of *S. marcescens* [13], 31 isolates from 11 patients and from associated equipment at a single hospital, plus one strain from a different hospital, were studied. SDS–PAGE was useful in showing that the latter strain, although of the same serotype as some from the outbreak, was clearly different. However, for the main outbreak the use of SDS–PAGE alone would not have given a clear epidemiological picture. Even strains isolated from the same site on a patient, but over a period of time, and those from different sites of the same patient at the same time, although giving high correlation coefficients (0·96 and 0·94, respectively) were found to belong to two different serotypes. This is not altogether surprising as the profiles shown were of very low resolution, the majority of minor bands being lost on scanning despite being visible on the published autoradiograms. Clearly, a high resolution scanning system is required to detect the minor features which may have significance in typing. In addition, the approach used has problems in using radioactive techniques which are overcome by the system described in the present study.

Where there is a requirement merely to determine whether strains are identical or not, which is often the case in outbreaks of infection, a simple visual interpretation of patterns on gels can be successfully used to differentiate isolates. However, it must first be established that there is protein pattern heterogeneity within a species and this must be done objectively. For definitive recognition of electrophoretic types using dendrogram analysis a high-resolution scanner and numerical analysis by computer are required.

At lower levels of similarity the cluster associated with the *S. marcescens* strains appears to be exclusive to this species and does not include other reference species of *Serratia* (Fig. 2). It is evident therefore that characterization of strains to species level may also be possible using this technique.

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