MICROBIAL PATHOGENICITY

Different O and K serotype distributions among clinical and environmental strains of *Serratia marcescens*

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Recent revision of the O serotyping scheme for *Serratia marcescens* has allowed the definitive serological identification of a collection of 511 epidemiologically distinct strains in terms of both lipopolysaccharide (O) antigens and capsular (K) antigens. High levels of typability were achieved, 88% and 91% respectively, with only 2% failing to type with either method. In most cases, non-typability was due to a lack of antigen, i.e., the strains produced only rough LPS or were acapsular, suggesting that typability would be little improved by the discovery of additional serotypes. The distribution of the 58 O:K serotypes was very uneven, with O14:K14 accounting for 30% of the 423 clinical strains in the collection, but only 5% of the 88 non-clinical, environmental strains. Thus, the prevalence of O14:K14 strains in hospitals is not reflected in the environment. Similar conclusions were valid for O27:K14, O21:K3 and O21:K14 strains, as well as those with rough lipopolysaccharide. Conversely, the proportions of O6:K3, O6:K14, O8:K14 or O28:K28 strains were significantly lower among the clinical collection than among their environmental counterparts (12% in total rather than 65%). This suggests that O14:K14 may have a selective advantage in colonising or infecting hospitalised patients and, therefore, that the O14 and K14 polysaccharides themselves may contribute towards the apparent pathogenicity of these serotypes.

Introduction

Serratia marcescens is a small, gram-negative bacillus belonging to the family Enterobacteriaceae. For many years it was considered a harmless saprophyte and has frequently been isolated from plant materials [1] as well as water and soil [2]. It is a primary pathogen of insects [3, 4], but rarely, if at all, of man. However, since the late 1950s this organism has been increasingly identified as the cause of various hospital-acquired infections, and it is now recognised as a significant opportunist human pathogen.

In common with other nosocomial pathogens, *S. marcescens* has been the subject of many epidemiological investigations to identify possible outbreaks and common sources of infection. In the process, several typing systems have been described. One such is O serotyping, based on the detection of lipopoly-

saccharide (LPS) O antigens. In other gram-negative bacteria, O typing is a highly definitive technique, but the *S. marcescens* scheme has been plagued with cross-reactions and poor reproducibility [5, 6]. Recent chemical and immunological studies of the LPSs of the O serotype strains [7, 8] have allowed the development of a new O typing scheme so that, for the first time, the LPS O types of this species can be identified with reasonable accuracy. These studies also revealed the presence of acidic polysaccharides in the form of capsules and derived from them a complementary K-typing scheme.

Highly discriminatory systems exist for typing *S. marcescens*, generating DNA fingerprints ideal for strain comparisons, but unsuited for definitive strain typing of large populations. Serotypes, in addition to providing information on strain relationships, reveal biologically valid groups of strains that may be of value in population studies. This present study used the new O and K serotyping assays to characterise a collection of clinical and environmental strains; firstly to determine the typability and discrimination of these methods, and secondly to compare serotype distributions between the two populations.

Materials and methods

Bacterial strains

The 423 clinical strains used in this study were also utilised in the development of the O and K typing schemes [8], having been chosen from > 3000 isolates sent to the Central Public Health Laboratory (CPHL) between 1 Jan. 1980 and 30 June 1990 for epidemiological typing. They were selected to represent distinct strains based on their origin and the original serotyping and phage typing data [5, 6].

Twenty-nine strains from insects, plants and rodents were a gift from Drs P. and F. Grimont, Institut Pasteur, Paris, France. A further 42 environmental strains from insects and soil samples were received from Dr M. O'Callaghan, AgResearch (Pastoral Agricultural Research Institute), Lincoln, New Zealand. Seventeen other strains, from plants, animals and water, were obtained either from the National Collection of Type Cultures or were selected from those sent to CPHL for identification or typing.

All strains were maintained on nutrient agar or glycerol agar [9] and stored at -20°C on 2-mm glass beads in glycerol 20% w/v broth.

Species confirmation

Tests for the differentiation of the genus *Serratia*, as described by Farmer *et al.* [10], were used to confirm the identity of the *S. marcescens* strains. The various media were inoculated from an overnight tryptone soya broth (TSB) culture and incubated at 30°C for up to 5 days, as recommended by Grimont *et al.* [2]. Isolates that produced atypical results were tested further by the CPHL Computer Identification Laboratory [11].

Serotype identification

Both O and K serotypes were determined by ELISA with adsorbed rabbit antisera as described previously. Autoclaved organisms grown in tryptone soya broth at 37°C overnight were used for the detection of O antigens, and formalinised cells grown on glycerol agar overnight at 30°C for the detection of K antigens [8]. Briefly, the ELISA plates were coated with whole-cell suspensions at an absorbance of 0.06 at 600 nm in carbonate buffer, before incubation with the panel of antisera. Positive reactions were detected with a goat anti-rabbit IgG-alkaline phosphatase conjugate and Sigma 104 phosphatase substrate.

Serotypes O10 and O22, O16 and O20, and O18 and O29 were merged for convenience as O10/22, O16/20 and O18/29, as within each pair the O antigens are similar both chemically and serologically and all had frequencies of < 2%.

SDS-PAGE and silver staining

Crude LPS extracts were prepared, electrophoretically separated and silver-stained as described previously [12]. The resulting LPS profiles were categorised as 'rough' if only low mol. wt material was visible and 'smooth' if the typical LPS ladder pattern was present.

Capsule detection

The presence or absence of capsules was determined by the microscopic examination of cell suspensions mixed with Indian ink as a negative stain, as well as indirectly by the Cetavlon technique [7]. This method involves horizontal electrophoresis of crude capsular extracts followed by precipitation with Cetavlon, a cationic detergent that binds acidic but not neutral polysaccharides.

Statistical methods

Associations between the serotype and source were tested for statistical significance by the χ^2 test or Fisher's exact test. The Yates' correction for non-continuous data was applied to the χ^2 test with one degree of freedom, while Fisher's exact test was used when one or more of the expected values was ≤ 0.5 .

Results

Species identification

In all, 485 (95%) of the 511 strains met the criteria for identification as *S. marcescens*. The remaining 26 strains were confirmed as *S. marcescens* by extended biochemical tests. The most frequently discrepant test was non-fermentation of sorbitol, which occurred in nine strains. Negative gelatinase, lysine decarboxylase or ornithine decarboxylase test results occurred in c. 1% of the collection, whereas only two strains failed to produce DNAase or lipase (Table 1).

Two further tests (pigment production and adonitol fermentation), which aid the differentiation of the species within the genus *Serratia*, are variable characteristics in the species *S. marcescens*. Of this collection, 93 were pigmented while 51 fermented adonitol. It was notable that a much higher proportion of the environmental collection (61%) was pigmented than the clinical collection (9%).

Source

The 88 environmental strains originated from insects (63%), the natural environment (plants and water, 23%) and animals (14%). The 423 clinical strains, i.e., those which originated from hospitals, included 114 for which a source was not known. Among the others, only the urine (81/423, 19%), blood (69/423, 16%) and respiratory tract (61/423, 14%) strains accounted for > 7% of the clinical collection and were therefore large enough to allow inter-source comparisons of their

Table 1. Biochemical reactions of 511 strains of *S. marcescens*

Biochemical reaction	Patterns of results given by n (%) isolates						
	485 (94.9)	9 (1.7)	6 (1.2)	5 (1.0)	4 (0.8)	1 (0.2)	1 (0.2)
DNAase	+	+	+	+	+	+	-
Lipase	+	+	+	+	+	-	+
Gelatinase	+	+	+	+	-	+	+
Lysine decarboxylase	+	+	+	-	+	+	+
Ornithine decarboxylase	+	+	-	+	+	+	+
Potato-like odour	-	-	-	-	-	-	-
Prodigiosin	v	v	v	v	v	v	v
Fermentation of							
arabinose	-	-	-	-	-	-	-
arabitol	-	-	-	-	-	-	-
sorbitol	+	-	+	+	+	+	+
adonitol	v	v	v	v	v	v	v
dulcitol	-	-	-	-	-	-	-

v, variable.

serotype distributions. The remaining 98 clinical strains (from wound, hospital environment, peritoneal fluid, faeces, skin, eye, breast milk and cerebrospinal fluid specimens) were grouped together as ‘other’. As the collection was not random, the distribution of sources could only be taken as a rough guide to the true clinical distribution. The relatively high numbers of strains from urine, blood and respiratory tract were consistent with the fact that *S. marcescens* is typically a non-enteric nosocomial pathogen. This feature of *S. marcescens* also explains the absence of statistically significant associations between clinical source and either O serotype or K serotype ($p > 0.05$). Thus Fig. 1 compares the O and K serotype distributions between clinical strains as a single group and environmental strains. No significant difference was observed for the K-type distributions ($p > 0.05$), but the O serotype frequencies varied markedly ($p < 0.001$) between clinical and environmental strains.

Distribution of O and K serotypes

Among the clinical strains, both distributions were very uneven, with O14 and K14 by far the most common types (Fig. 1). Frequencies $> 5\%$ were only found for three other O serotypes (O21, O27 and O28) and two other K serotypes (K28 and K3). Similar percentage frequencies were found among the K serotypes of the environmental collection, but the O serotype distribution was clearly different, although still uneven. Among this collection, O6 and O8 were the most common; together they accounted for almost half of these strains, while a further 17% were O28. In contrast, these three serotypes accounted for only 14% of the clinical collection. Conversely, O14, the predominant clinical type, was only represented by 5% of the environmental strains. This difference in distribution of O14 strains between clinical and environmental strains was highly statistically significant, $p < 0.0001$. One serotype, O24, was not represented in either collection.

Typability was 88% for the O-ELISA and 91% for the

K-ELISA, but 98% overall. Of the 59 strains that failed to O serotype, 45 (76%) were shown by SDS-PAGE and silver staining to have rough LPS profiles, indicating that these strains lacked O antigen. Similarly, 29 (69%) of the 42 K-non-serotypable strains were found to be acapsular by Indian ink staining and the Cetavlon technique. A further difference between the O serotype distributions of clinical and environmental collections was that non-typable strains among the former were generally rough (44/53, 83%), whereas non-typable strains from the natural environment tended to have smooth LPS and may have represented undiscovered serotypes.

The original O serotype of 356 of the clinical strains had been obtained by agglutination with unadsorbed antisera [5]. These results were compared with the ELISA results to determine whether there was any improvement in typability and discrimination. By agglutination, 101 strains had been either non-typable or polyagglutinable giving only 72% typability, compared with 87% and 91% of these 356 strains by the O typing and K typing ELISAs respectively, and 97% when combined. Over half (187, 53%) of this smaller collection were O14 by agglutination, and a third of these were re-allocated to other O types, notably O27, O21, O8 and O6, leaving only 34% as O14. There were very few obvious correlations between O type and original agglutination type, which seemed to correlate better with K type. All but six of the 187 O14-agglutinating strains were shown to be K14, while ‘O3’, ‘O4’ and ‘O5’ were highly correlated with K3 (18/20), K4 (12/14) and K28 (12/12), respectively.

Associations between O and K serotype

Table 2 shows the relationships between O type and K type which were clearly non-random, although there were only four 100% correlations: all 37 O8 strains had a K14 capsule, the three K2 strains were O2, the 10 K9 strains were O26, and the three K12 strains were all

Fig. 1. Distributions of 511 clinical (■) and environmental (□) strains of *S. marcescens*: (a) O serotype; (b) K serotype.

O14. In total there were 58 combinations of O and K type among the 511 strains (if rough and acapsular strains are included as non-typable), with 19 combinations represented by only a single strain. Most of the O14 strains were also K14 while other strong correlations were O4 and O27 with K14, O5 and O28 with K28, and O6 and O21 with both K3 and K14.

Most O types were found with only three or four different K types, although O26 occurred in six combinations. This number of combinations was also found among the K3, K13 and K28 strains. K14 was the most widely distributed type, as would be expected from its high frequency and its history of involvement in cross-reactions.

The distribution of O:K serotype combinations was also significantly associated with source in that 30% of clinical strains were O14:K14 compared with only 5% of environmental strains ($p < 0.0001$). A similarly skewed result was seen with O27:K14 with frequencies of 12% and 1% respectively ($p < 0.01$). Four serotypes were found with frequencies of 10% or more among the environmental collection, namely O6:K3, O6:K14, O8:K14, O28:K28 which together accounted for 65% of these strains, but only 15% of the clinical strains (for each serotype, $p < 0.001$).

Incident size

The clinical strains were also classified in terms of the size of the incident from which they originated, on the

Table 2. Associations between the O and K serotypes of *S. marcescens*

O type	Number of isolates with K type																NT	A
	K1	K2	K3	K4	K7	K9	K12	K13	K14	K15	K18	K22	K23	K28				
O2	1	3	4	6	0	0	0	0	0	0	0	0	0	0	0	0	0	
O4	1	0	0	0	0	0	0	1	9	0	0	0	0	0	0	0	0	
O5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0	
O6	0	0	12	0	0	0	0	1	24	0	0	0	0	0	0	1	2	
O8	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	
O10/22	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	
O14	0	0	0	0	0	0	3	3	133	0	0	0	0	0	0	0	5	
O16/20	0	0	0	0	0	0	0	2	0	0	0	0	0	0	5	3	1	
O19	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	
O18/29	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	1	
O21	0	0	22	0	0	0	0	0	20	2	0	0	0	0	1	0	3	
O23	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	
O26	0	0	2	2	0	10	0	0	1	0	0	0	0	2	2	1	2	
O27	0	0	1	0	0	0	0	0	53	0	0	0	0	0	0	0	4	
O28	0	0	0	0	0	0	0	0	0	0	0	0	1	36	1	3	3	
NT	1	0	5	0	0	0	0	1	4	0	0	0	1	1	0	1	1	
R	0	0	2	5	1	0	0	1	16	0	0	1	0	10	4	5	5	

NT, non-typable; A, acapsular; R, rough LPS.

assumption that strains producing significant cross-infection may be more pathogenic in man than strains affecting single individuals. This analysis revealed that almost half (198/423, 47%) were apparently unique, i.e., produced sporadic infection only. This number included 10 strains from the hospital environment that did not appear to have been isolated from any patients. About a third (134/423, 32%) were representatives of small outbreaks (two to four patients), while 22% (91/423) of strains were from outbreaks involving at least five patients; thus over 50% of the collection were associated with cross-infection.

The differences in distribution of sporadic and epidemic strains within each O type were of only borderline statistical significance ($p = 0.04$), but there was a tendency for the more common types, such as O14 and O27, to be associated with epidemic rather than sporadic strains (*c.* 60:40). The converse was true of the less frequent serotypes such as O6, O8 and O28, which were more prevalent in the sporadic category (*c.* 40:60). There were no statistically significant associations among the K types, although it was noticeable that the acapsular group was the one with the highest proportion of sporadic strains (68%). Rough strains were also more frequently associated with sporadic than epidemic strains (66%).

Discussion

When used in combination, the O and K serotyping ELISAs provided a high level of typability with only 2% of the 511 strains proving to be completely non-

typable. Given that, particularly among clinical strains, most non-typability was due to antigen absence (i.e., rough or acapsular strains) rather than the presence of new antigens, it is unlikely that the addition of further types to the schemes would prove of much benefit in terms of additional discrimination. This conclusion is supported, at least for the O serotype scheme, by the fact that there was no predominant LPS profile among the 14 smooth non-typable strains. The high level of typability contrasted favourably with the 72% typability rate of the smaller collection for which O agglutination results were available, and with previously published rates (Table 3) [6, 13–22]. Comparison between old and new serotypes confirmed that the original O typing scheme was a mixture of O and K antigens, and that the agglutination method tended to detect K antigens, where present. One result was an apparent reduction in frequency of the O14 antigen, from 53% by agglutination to 28% by ELISA, largely because the use of adsorbed antisera allowed the detection of O antigen independently of capsule. This revealed that the K14 capsule occurred in combination not only with O6, O8, O14 and O21 as had been described previously by Gaston *et al.* [23], but also with O4, O23, O26 and O27, as well as both capsular and acapsular K-non-typable strains.

The findings presented here indicate that, in the majority of cases, a combination of O and K serotyping should provide satisfactory discrimination of strains within hospital outbreaks. An additional typing method will be necessary to confirm strain identity among O14:K14 isolates, as this was by far the commonest type. The only other type to account

Table 3. Percentage frequency distribution of O antigens among clinical isolates of *S. marcescens*

Criteria	Percentage frequency of O antigens										
Country	USA	USA	USA	CZ	USA	USA	UK	FR	USA	UK	UK
Year	1957	1959	1962	1965	1970	1976	1977	1978	1980	1980	1989
Reference	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[6]	[22]
Number of isolates	50	115	69	90	95	86	178	583	220	273	104
O antigens											
O1	2	3	1	1	1	0	0	0	14	NR	0
O2	24	12	3	1	26	11	1	<1	4	1	1
O3	10	5	0	8	0	0	14	9	<1	13	–
O4	12	9	9	9	20	5	1	3	20	8	22
O5	34	28	55	21	10	6	3	2	2	1	0
O6	12	20	3	12	3	1	26	<1	0	NR	6
O7	–	2	0	6	1	0	0	<1	<1	NR	–
O8	–	9	0	2	0	0	13	<1	0	NR	9
O9	–	3	0	3	2	2	1	5	<1	NR	3
O10	–	–	0	12	0	8	0	0	1	NR	0
O11	–	–	0	–	19	3	0	0	<1	NR	–
O12	–	–	0	–	1	0	1	2	<1	4	–
O13	–	–	12	–	1	1	0	25	1	NR	–
O14	–	–	10	–	6	56	35	43	51	68	31
O15	–	–	6	–	0	0	1	<1	3	NR	–
O16–O20	–	–	–	–	–	–	–	2	–	–	4
O21	–	–	–	–	–	–	–	–	–	–	12
O22–O26	–	–	–	–	–	–	–	–	–	–	0
O27	–	–	–	–	–	–	–	–	–	–	11
O28	–	–	–	–	–	–	–	–	–	–	2
PA	–	–	–	25	–	–	–	4	–	1	7
NT	6	9	1	–	9	7	4	2	2	2	12

CZ, Czechoslovakia; FR, France; NR, not reported; –, not tested; PA, polyagglutinable, i.e., reacted with two or more antisera; NT, non-typable.

for > 10% of the clinical strains was O27:K14, while the corresponding environmental serotypes were O6:K3, O6:K14, O8:K14 and O28:K28. The characterisation of non-serotypable strains will also require a second typing method. In these cases it is likely that a genotypic method may prove the most satisfactory supplement. For example, ribotyping has been shown to produce results consistent with known epidemiology and biotyping [24], and to provide reliable strain discrimination when combined with biotyping or serotyping [25]. Pulsed-field gel electrophoresis of bacterial DNA digested with rare cutting enzymes also discriminates *S. marcescens* to strain level [26], as does DNA fingerprinting by the polymerase chain reaction [27]. For small investigations, the drawbacks of these methods – namely high cost, labour-intensiveness and lack of definitive strain labels – may well be outweighed by the advantage of good discrimination.

The uneven distribution of serotype, and in particular the predominance of O14:K14 clinical strains, suggested that these two types might be more prone to producing infections than other types. The alternative explanation, that this distribution of types simply reflects the frequencies with which these types are encountered in the environment, was clearly not supported by the serotype distribution seen among the environmental collection. Thus, the serotypes prevalent in the environment (O6:K3, O6:K14, O8:K14 and O28:K28) are uncommon in hospitals. Furthermore, the dominant clinical serotype, O14:K14, while accounting for a third of clinical strains, represented only 5% of the environmental material. This suggests that selective pressures have been brought to bear in the clinical environment, which have favoured the survival of O14 strains in preference to those of other O types. Consequently, it can be hypothesised that either O antigens themselves are virulence factors influencing the pathogenicity of *S. marcescens*, or that they are closely associated with such factors. In this context it is interesting to compare the serotype distributions reported for this species since 1957 (Table 3). Direct comparisons are difficult to make because the reports used agglutination techniques that fail to differentiate O and K antigens. However, it is notable that O14 was not added to the serotyping scheme until the third study of *S. marcescens* serotyping, when it accounted for only 14% of isolates tested. High frequencies of O14 were not seen until the 1970s; 20 years after the first report on serotyping of this species. Furthermore, the observation that acapsular and rough strains tended to be associated with sporadic rather than epidemic strains supports the idea that the surface polysaccharides typed as O and K antigens have a role in the pathogenicity of this species.

The results of this study demonstrated that the new O and K serotyping assays are discriminatory and no

longer suffer from high levels of non-typability. Furthermore, they also demonstrate a significant difference in the distribution of serotypes between clinical and environmental strains, suggesting that the surface polysaccharides represented by these antigens may be involved in the selection of strains within the clinical setting.

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