MICROBIAL PATHOGENICITY

Antibiotic resistance and putative virulence factors of *Serratia marcescens* with respect to O and K serotypes

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*Serratia marcescens* serotypes O6:K14, O8:K14 and O28:K28 are common in the natural environment, but rare in hospitals. Serotypes O14:K14 and O27:K14 predominate among clinical strains, but not in the environment, suggesting that the latter serotypes may be more suited for survival in the clinical setting. Consequently, 469 epidemiologically distinct strains of *S. marcescens* were tested for various putative virulence factors and analysed for associations with serotype. The factors positively associated with serotype O14:K14 were agglutination of five different species of red blood cells and expression of type 1 fimbriae. These were found in 63% and 53% of O14:K14 strains, respectively, compared with 7% and 12% of the three ‘environmental serotypes’. Almost a quarter of the collection expressed the mannose-resistant haemagglutinin indicative of type 3 fimbriae, but this was not associated with any serotype. The production of DNAase, haemolysin, lipase, lecithinase, proteases and siderophores was almost universal and showed no serotype correlations. Almost half of the strains (46%) were resistant to serum and serotypes O27:K14 and O6:K14 were strongly associated with this characteristic. Serotype O27:K14 was also associated with higher proportions of antibiotic-resistant strains than other serotypes, but the same was not true of serotype O14:K14. All three ‘environmental serotypes’ were associated with low frequencies of antibiotic resistance; <12% were resistant to gentamicin, carbenicillin or piperacillin, or any combination of these three, compared with 20–25% of O14:K14 strains and >42–51% of O27:K14 strains. Pigment production was strongly associated with serotype. None of the O14:K14 or O27:K14 strains produced prodigiosin, but frequencies for the three ‘environmental serotypes’ ranged from 31% of O28:K28 strains to 85% of O6:K14 strains. The results of this study suggest that the adherence capability of *S. marcescens* strains may play a role in the colonisation of hospital patients, while the production of prodigiosin is a marker of environmental origin.

Introduction

The gram-negative bacillus *Serratia marcescens* is not normally considered highly pathogenic for man because it rarely causes primary infections. However, it is a cause for concern in hospitals, because as an opportunistic pathogen, it can be responsible for serious infections and occasional fatalities in compromised patients. Such infections often occur in clusters and their epidemiology can be investigated by typing techniques [1]. Serotyping is a well-established method in which differences in the chemistry of bacterial cell surface structures are detected with panels of antisera. Most schemes are based on either the O antigens of lipopolysaccharide (LPS) or the K antigens of capsular polysaccharide. Recently, new O and K typing schemes have been described for this species [2], and applied to a collection of both clinical and environmental *S. marcescens* isolates selected to represent distinct strains [3]. The latter study showed a marked difference in serotype distribution between the two populations. Serotypes common in the natural environment were rare in hospitals and *vice versa*. In particular, the O14:K14 serotype accounted for almost a third of clinical strains, but only 5% of the environmental collection. This would suggest that some kind of selection process occurs in the transfer of strains from the non-clinical environment to hospitals. The aim of the current investigation was to determine whether virulence factors such as haemagglutination as a...
measure of adhesion, extracellular enzyme production, serum resistance and antibiotic resistance, could account for the apparent prevalence of O14:K14 strains over those of other serotypes.

Materials and methods

Bacterial strains

The collection of 423 distinct clinical strains was selected as described previously [3]. It included isolates from 77 hospitals representing 46 different towns and cities, 29 of which were in England. Four Welsh or Scottish hospitals were represented as well as eight hospitals in six other European countries; these 12 hospitals accounted for 93 isolates. Twenty isolates were from four non-European countries: Oman, India, Australia and New Zealand.

In addition, 46 environmental strains were available for this study: 29 strains from insects, plants and rodents were received as a gift from Drs P and F. Grimont, Institut Pasteur, Paris, France; 17 strains were obtained from the National Collection of Type Cultures or were submitted to this laboratory for identification or epidemiological typing.

All strains were maintained on nutrient agar or glycerol agar [4] and stored at −20°C on 2-mm glass beads in glycerol broth 20% w/v.

Serotype identification

O and K serotypes were identified by ELISA with adsorbed antisera [2] and the frequency distributions were reported in a separate study [3].

Haemagglutination

Chicken (CPHL), guinea-pig (CPHL), horse (TCS Biologicals, Botolph Claydon), cynomolgus monkey (Shamrock Great Britain, Brighton), human A positive and O positive (CPHL), rabbit (CPHL), rat (CPHL) and goat (TCS) blood were supplied either in Alsever’s solution or with sodium citrate 0.4 %/3 ml blood as an anti-coagulant. The red blood cells (RBC) were washed three times in phosphate-buffered saline (PBS) at 400 g for 10 min and resuspended in PBS at a concentration of 50%. Tanned human group A cells were prepared by adding 10 ml of sterile-filtered tannic acid 0.005% in saline to 10 ml of 2.5% washed cells in PBS [5]. After 10 min at 37°C, the cells were centrifuged, washed once and resuspended in PBS as above.

A 3% suspension of RBC was prepared by adding 0.5 ml RBC 50% to either PBS or PBS + mannose 0.5%. Bacterial suspensions were prepared from overnight growth at 37°C from colonisation factor agar [6] containing bile salts (Oxoid, Unipath, Basingstoke) 0.15%. The growth was covered with PBS and left at room temperature for c. 1 h to detach from the agar. After gentle resuspension, 50-μl drops of cell suspensions were placed on a dimpled perspex tray by row, and equal drops of the RBC 3% suspensions were added by column. After rocking on ice for 1 min, the trays were left on ice for a further 10 min and rocked again before the haemagglutination was recorded as negative, weakly positive or strongly positive.

Tests with and without mannose were performed simultaneously. Agglutination of equal degree of strength in the absence or presence of mannose was reported as mannose-resistant haemagglutination (MR-HA), while mannose-sensitive haemagglutination (MS-HA) was completely abolished by mannose. If the presence of mannose resulted in only partial inhibition of haemagglutination, the strain was considered to exhibit both mannose-sensitive and mannose-resistant activity. The presence of MS-HA of guinea-pig RBC and of MR-HA tanned human A-positive cells (MRK-HA) were considered indicative of type 1 and type 3 fimbriae, respectively [7].

Extracellular products

DNA-toluidine blue agar plates, Tween 80 agar plates, nutrient gelatin stabs, Columbia agar plates (Oxoid) with horse blood 5%, and skimmed milk powder casein plates were used for the detection of DNAase, lipase, gelatinase, haemolysin and caseinase respectively [8]. Lecithinase activity was assessed with lecithovitellin agar plates, prepared by adding 50 ml of egg yolk emulsion (Oxoid) to 500 ml of molten nutrient agar at 55°C. Siderophore production was detected with Schwyn and Neilands’ universal siderophore agar [9].

For each test strain, 20 μl of an overnight, 37°C tryptone soya broth culture were diluted in 2 ml of sterile distilled water to c. 10⁷ cells/ml and 25 μl of this were inoculated on to the agar media and incubated at 30°C. Results were recorded as the radius of the reaction halo on days 1, 2 and 5. DNAase changed the colour of the agar from blue to pink; lipase and lecithinase activity resulted in an opaque or opalescent halo; gelatinase liquefied agar stabs; haemolysin and caseinase produced zones of clearing; and siderophores produced a yellowing of the blue-green agar. Reproducibility studies with 18 strains showed that the variation of zone radii was within 3 mm of the original.

Serum resistance

Normal human serum (NHS) was prepared from clotted blood samples from four laboratory volunteers and stored at −70°C in 0.5-ml aliquots for up to 1 month. An overnight, 37°C tryptone soya broth culture was used to prepare a suspension of c. 10⁷ cells/ml by dilution of 10 μl in 1 ml of Hanks’s Balanced Salts.
Solution (HBSS). For the assay, 50 μl of this suspension were added to an equal volume of either HBSS (control wells) or NHS (test wells) in rigid, polystyrene microtiter trays (Bibby Sterilin, Stone, Staffs). After incubation at 37°C for 3 h, the plates were shaken for c. 1 min, and three 20-μl drops from each well were spotted on nutrient agar plates. These were incubated at 30°C overnight and the growth was scored as confluent, semi-confluent, >50 discrete colonies, or as the number of discrete colonies if <50. The test and control wells were prepared in duplicate and repeated if discrepant results were observed. Three control strains (resistant, intermediate and sensitive) were included in each batch of tests. The serum-resistant control strain produced confluent growth and the highly sensitive strain <120 cells/ml (usually none at all). Viable counts of the intermediate sensitive strain varied from 120 to 5000 cells/ml and these values were used to define strains as either highly sensitive, intermediate sensitive or resistant.

**Antibiotic resistance**

The minimum inhibitory concentrations (MIC) of the 14 antibiotics listed in Table 2 for the collection of strains were determined by an agar dilution method [10]. Briefly, bacteria were grown on nutrient agar and five colonies were resuspended in 5 ml of IsoSensitest broth to produce a suspension of c. 10⁶ cells/ml. After 4 h at 37°C, 0.03-μl volumes containing 10⁵–10⁶ bacteria were spotted on IsoSensitest agar supplemented with lysed horse blood 2% and the appropriate antibiotic, with a multipoint inoculator. The plates were incubated for 18–24 h at 37°C and the growth was recorded with the aid of a Mastascan image analyser (Mast Diagnostics, Merseyside). The MIC of an antibiotic for each strain was taken as that concentration for which there was ‘no visible growth, disregarding one or two colonies or hazes of uninhibited growth’ [10]. Each strain was then classified as either sensitive, intermediate or resistant according to the criteria suggested by the British Society of Antimicrobial Chemotherapy [10]. Control tests included antibiotic-free plates at the beginning and end of each batch, as well as 10 strains with known MIC values.

**Pigment**

Pigment production was assessed visually after incubation for 2 days at 30°C on glycerol agar.

**Statistical methods**

Data were analysed for the collection of 469 strains as a whole and also with respect to those serotypes which have significantly different frequencies among clinical and environmental strains [3]. Rough and acapsular strains were also analysed separately in order to assess any involvement of LPS and capsular polysaccharide. The χ² test and Fisher’s exact test were used to determine the statistical significance of associations between serotypes and virulence factors. Yates’ correction for non-continuous data was applied to the χ² test, while Fisher’s exact test was used when one or more of the expected values was ≤ 0.5. To allow for multiple testing, associations were considered significant for p values at or below 0.01 rather than 0.05.

**Results**

The results were analysed with respect to serotype and clinical or environmental origin. In addition, as 19% of the clinical strains were from urine, 16% from blood and 14% from the respiratory tract [3], the data from these strains were analysed with respect to clinical source. However, no significant associations were found and therefore these results are not presented.

**Haemagglutinins**

Haemagglutination of rabbit, rat, goat and human group O rhesus-positive RBC was not found with any of the 469 strains; only one strain agglutinated untreated human group A rhesus positive cells; 20% of strains agglutinated all five of the remaining species, while 42% failed to agglutinate any cells. The only other agglutination patterns seen in >5% of strains were agglutination of chicken, monkey, guinea-pig and horse RBC (12%) and agglutination of tanned A-positive cells only (8%). Only one serotype, O14:K14, was significantly associated with haemagglutination (p < 0.0001) (Table 1). When O and K serotypes were analysed independently (data not shown), both O14 and K14 retained the association. In contrast, rough strains showed fewer agglutination reactions than expected (p < 0.01).

Serotype O14:K14 (p < 0.0001) was also associated with MS-HA (indicative of type 1 fimbriae) (Table 2). MRK-HA (indicative of type 3 fimbriae) was not associated with any of the serotypes. Separate analysis of serotype showed associations between type 1 and O14, type 1 and K14, and type 3 and O14. Overall, MS-HA was found in 28% of the clinical collection and 17% of the environmental collection (p = 0.16). The corresponding frequencies for MRK-HA were 26% and 11% (p = 0.04).

**Extracellular products**

Tests for DNAase, lipase and gelatinase were performed as part of the initial identification for all strains [3], and, therefore, only qualitative and not quantitative results were obtained. Over 99% of the 469 strains were positive for all three enzymes, with four gelatinase-negative strains and two strains negative for either DNAase or lipase. The uniform nature of these results precluded any further analysis.
Table 1. Haemagglutination reactions of isolates of *S. marcensens* according to O and K serotype with respect to number of erythrocyte species

<table>
<thead>
<tr>
<th>Serotype</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Totals</th>
<th>p1</th>
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</thead>
<tbody>
<tr>
<td>O14:K14</td>
<td>37</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>16</td>
<td>58</td>
<td>133</td>
<td>&lt;10⁻⁴</td>
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<tr>
<td>O27:K14</td>
<td>28</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>7</td>
<td>53</td>
<td>0.19</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>0.02</td>
</tr>
<tr>
<td>O8:K14</td>
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<td>0</td>
<td>8</td>
<td>3</td>
<td>25</td>
<td>0.18</td>
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<tr>
<td>O28:K28</td>
<td>12</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>29</td>
<td>0.55</td>
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<tr>
<td>Rough</td>
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<td>6</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>39</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td>Acapsular</td>
<td>11</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>24</td>
<td>0.37</td>
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<tr>
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<td>35</td>
<td>7</td>
<td>9</td>
<td>18</td>
<td>16</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>198</td>
<td>59</td>
<td>27</td>
<td>21</td>
<td>68</td>
<td>96</td>
<td>469</td>
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</tr>
</tbody>
</table>

*When tested with chicken, monkey, guinea-pig, horse and tanned human A-positive RBC.

Table 2. Haemagglutination reactions of isolates of *S. marcensens* according to O and K serotypes with respect to fimbrial types

<table>
<thead>
<tr>
<th>Serotype</th>
<th>MS-HA</th>
<th>MR/K-HA</th>
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</thead>
<tbody>
<tr>
<td>O14:K14</td>
<td>70</td>
<td>41</td>
</tr>
<tr>
<td>O27:K14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>O6:K14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>O8:K14</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>O28:K28</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Rough</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Acapsular</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>42</td>
<td>54</td>
</tr>
<tr>
<td>Totals</td>
<td>141</td>
<td>128</td>
</tr>
</tbody>
</table>

MS-HA (mannose-sensitive haemagglutination of guinea-pig RBC) and MR/K-HA (mannose-resistant haemagglutination of tanned human A-positive RBC) indicative of type 1 and type 3 fimbriae respectively.

Serum resistance

Almost half of the strain collection (46%) was fully serum resistant with only 15% falling in the serum-sensitive category. Serum resistance was strongly associated with two serotypes (O27:K14 and O6:K14, p < 0.001, Fig. 2) in that >80% of strains of these types were fully serum resistant. The corresponding value for all other serotypes together was 40%. Only rough or acapsular strains were associated with significant levels (40%) of serum sensitivity (p < 0.0001). Strains that were both rough and acapsular (five in all) were invariably serum sensitive.

When the analysis was repeated with separate O and K serotypes, the distribution of strains into the three categories of serum resistance was significant for all except O8 (p < 0.001 compared with p = 0.18). No significant differences were seen between the clinical (15% sensitive, 50% resistant) and environmental collections (15% sensitive, 43% resistant).

Antibiotic resistance

Table 3 shows MIC50 and MIC90 values for clinical and environmental strains while Table 4a gives the
percentages of each collection that were antibiotic resistant. There was virtually no resistance to amikacin, cefazidime and ciprofloxacin and conversely little sensitivity to ampicillin, cefuroxime, chloramphenicol and tetracycline. However, the difference in tetracycline results was statistically significant \((p < 0.01)\): all 46 environmental strains were resistant to this antibiotic, compared with 84% of clinical strains. For six of the remaining antibiotics, i.e., gentamicin, neomycin, tobramycin, carbenicillin, piperacillin and cefotaxime, <10% of environmental strains were resistant compared with 24%-41% of the clinical strains. For all except neomycin \((p = 0.03)\), these differences were statistically significant \((p < 0.01)\). The same trend, i.e., a much higher proportion of resistant strains among clinical (56%) than environmental strains (22%) was demonstrable for trimethoprim \((p < 0.0001)\).

Within the clinical collection, antibiotic resistances were compared between strains from the beginning and end of the 10-year collection period (Table 4b). Interestingly, more 1980 strains were resistant to gentamicin, tobramycin and carbenicillin than were the 1989/1990 representatives \((p < 0.01)\). The same
was true for neomycin and piperacillin, although in these cases the differences were not significant ($p > 0.1$). No significant differences were seen among the other antibiotics ($p > 0.2$), nor was there any difference in serotype distribution between the two time periods ($p > 0.5$).

Table 4c shows the antibiotic resistance frequencies with respect to serotype. Associations significant at the 1% level are indicated with *, and those significant at the 5% level with †. The main pattern to emerge was that rough strains and those of serotypes O27:K14 tended to have higher proportions of resistant strains than did other serotypes, while serotypes O6:K14, O8:K14 and O28:K14 were associated with lower than average proportions of resistant strains. This was most obviously the case for gentamicin resistance, but was also found with most of the antibiotics for which sensitivity or resistance was not universal. Within these serotypes, there was no significant difference in resistance with respect to clinical or environmental source.
Serotype O14:K14 did not fall into either group and was associated with higher than average percentages of ampicillin resistance and lower percentages of gentamicin and tetracycline resistance.

**Pigment**

Overall, 12% of the collection produced the red pigment prodigiosin and this was highly associated with serotype. All strains of serotypes O14:K14 and O27:K14 were non-pigmented. Conversely, 85% of O6:K14 strains, 72% of O8:K14 and 31% of O28:K28 strains were pigmented (p < 0.01). The frequencies of pigmented strains among other serotypes, acapsular and rough strains were 4%, 4% and 18% respectively; none of these serotype groups was significantly associated with pigment production. As would be expected from the differences in serotype distribution, the proportions of pigmented clinical and environmental strains were significantly different (9% and 6.1% respectively, p < 0.001). However, within each of the three serotypes associated with pigmentation there was no over-representation of either clinical or environmental strains, thus there was no serotype-independent association of pigmentation with source.

**Discussion**

*S. marcescens* is a typical opportunistic pathogen, i.e., one whose normal habitat is the natural environment [11] and, therefore, one with which mankind regularly comes into contact. Infections are only produced when either primary or secondary host defences, or both, are severely impaired, as in intensive care patients. Consequently, if the environment is the natural reservoir of hospital strains, the frequencies of epidemiological markers such as serotype should be similar among clinical and environmental strains. Differences in frequency distribution imply that the epidemiological markers are associated in some way with the ‘fitness’ of the bacteria to produce human infection. This is clearly the case for *S. marcescens*, among which O14:K14 and O27:K14 strains are markedly associated with human disease while O6:K14, O8:K14 and O28:K28 strains are not.

Most studies on the pathogenicity of *S. marcescens* describe work done on only one or two strains, and many of these papers have been reviewed recently by Hejazi and Falkiner [12]. Two other studies have looked at the virulence factors of a collection of *S. marcescens* strains. Gaston et al. [13] compared six epidemic neonatal strains with six epidemic adult strains and six sporadic clinical strains, and found associations for haemagglutination and antibiotic resistance, but not for serum sensitivity or survival on human skin. Franczek et al. [14] studied a collection of 122 clinical and 25 environmental strains and found associations for haemagglutination and
pigmentation but not for extracellular enzyme production, motility or plasmid carriage.

Table 5 summarises the associations that were found between the virulence factors expressed by the strains in this study and their serotypes. The serotypes chosen for analysis were those with frequencies >12% among either the clinical or environmental collections; rough and acapsular strains are also included separately.

It is striking that the only positive associations for the dominant clinical serotype O14:K14 were related to adherence. Strains of this serotype tended to agglutinate the red cells of a wider range of animal species and were more likely to possess MS-HA indicative of type 1 fimbriae. This finding underlines the importance of the initial colonisation of the host as a requirement for the establishment of infection, at least for S. marcescens. However, the studies by both Gaston et al. and Franczek et al. showed associations only with mannose-resistant haemagglutination (MR-HA), taken to be indicative of type 3 fimbriae [13, 14]. All six neonatal strains in the study by Gaston et al. [13] plus one each of the adult epidemic and sporadic strains showed MR-HA with at least one species of red cell, while only a third of the environmental strains of Franczek et al. [14] showed MR-HA compared with three-quarters of the environmental strains. These frequencies are higher than those found in the present study, but the differences may be the result of differences in method, particularly bacterial culture conditions, in addition to genuine differences between the strain collections.

Extracellular products do not appear to be important virulence factors for S. marcescens, because there were no significant associations with those serotypes most associated with human disease. However, this may be because the production of the seven enzymes tested for in this study is virtually universal for the species.

Higher proportions of serum-resistant strains were seen for one clinical serotype (O27:K14) and also one environmental serotype (O6:K14), suggesting that this factor is not of major importance in human infection with S. marcescens. This is confirmed by the fact that both rough and acapsular strains showed a negative association with serum resistance, i.e., tended to be serum sensitive, yet neither group occurred at a lower frequency among clinical than environmental strains. A negative association with the absence of LPS and capsular polysaccharide suggests that these polysaccharides play a similar role in S. marcescens as in Escherichia coli. By providing binding sites for C3b, they ensure that any resulting membrane attack complexes are physically distant from the cell wall [15, 16]. Twelve of the 18 strains in the survey by Gaston et al. were serum resistant compared with 92%
of 60 clinical strains studied by Carbonell et al. [17]. These percentages may well be due to differences in method, e.g., Gaston et al. used viable counts after incubation for 1, 4 and 24 h with human sera rather than the single count of c. 3 h used in the present study, while Carbonell et al. used A560 readings at 30-min intervals up to 3 h.

*S. marcescens* is intrinsically resistant to ampicillin, cefuroxime and tetracycline [18] and the data for the environmental collection reflect this, although chloramphenicol resistance was also common. However, resistance to aminoglycosides and β-lactams is clearly more common among clinical strains, as was also found in a Canadian study of 40 isolates from septicaemic patients [19]. The pattern of acquired resistance is likely to be a reflection of patterns of use, e.g., cefazidime was introduced in 1983 but has not been widely used except in serious sepsis. Similarly, the introduction of third-generation cephalosporins and quinolones has led to a reduction in the use of aminoglycosides and penicillins, and this may be the explanation for the lower frequencies of resistant strains seen in 1989/1990 compared with 1980. The most striking result from the analysis of the antibiotic resistance data was that all three ‘environmental serotypes’ were associated with antibiotic sensitivity. Therefore, it is possible that strains of these serotypes are either inherently less antibiotic resistant or alternatively less able to acquire resistance. Such strains would be at a selective disadvantage within the hospital environment and consequently under-represented among the clinical population. However, given that no association was found for O14:K14 strains, antibiotic resistance is unlikely to be the dominant factor determining a strain’s ‘fitness’ for infection.

It has been known for some time that non-pigmented, antibiotic-resistant strains of *S. marcescens* are much more common in the clinical setting than in the natural environment and one explanation for this is the fact that acquisition of resistance plasmids by pigmented strains results in higher frequencies of non-pigmented variants [20]. The data presented here show that this is not the whole story; pigmented and non-pigmented clinical strains come from different lineages as indicated by their different serotypes. Pigmentation therefore seems to be a marker of low virulence.

Taken together, the results of this study emphasise the opportunistic nature of *S. marcescens*. This is not an organism which causes primary invasive disease, nor even a specific condition, but rather produces infection when and wherever it gains access to a suitably compromised host. In this context, the increased ability of O14:K14 strains to colonise and infect hospitalised patients compared with other serotypes could be readily explained by a greater ability to adhere to host cells. Further studies are required to investigate adhesion to human cells and tissues, and to ascertain whether the polysaccharide structures that determine serotype are directly involved in adhesion or whether they are merely associated with other adhesins.

We thank Mrs Lyn Ball for performing the MIC testing; Drs R. George and D. Livermore for helpful discussions concerning the presentation and interpretation of antibiotic resistance data; and Dr P. Farrington of CDSC for advice on statistical analysis of the results.

References


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