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REVIEW ARTICLE

Serratia marcescens

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Over the last 30 years, *Serratia marcescens* has become an important cause of nosocomial infection. There have been many reports concerning the identification, antibiotic susceptibility, pathogenicity, epidemiological investigations and typing of this organism. Accurate identification is important in defining outbreaks. The API 20E system has been used widely, but is not individually satisfactory. The growth of *S. marcescens* in the environment has been investigated in relation to water, disinfectants and plastics such as blood bags. Certain extracellular products are unique to *S. marcescens*. Pigment (prodigiosin) biosynthesis by *S. marcescens* has been investigated fully since the emergence of the organism as a cause of infection. Many other aspects of the pathogenicity and virulence of *S. marcescens* have been studied, including adherence and hydrophobicity, lipopolysaccharide (LPS) and extracellular products. Two modes of adhesion to host epithelial surfaces have been suggested. These are mannose-resistant (MR) pili and mannose-sensitive (MS) pili. LPS, which is responsible for the biological activity of endotoxin, has been investigated fully and 24 somatic antigens have been described. The production of different enzymes by *S. marcescens* as virulence factors has also been reported, including chitinase, lipase, chloroperoxidase and an extracellular protein, HasA. Antibiotics used to treat serratia infection include β-lactam agents, aminoglycosides and fluoroquinolones and a variety of different resistance mechanisms have been demonstrated. Typing methods used to study the epidemiology of *S. marcescens* include biotyping, bacteriocin typing, phage typing, plasmid analysis, polymerase chain reaction amplification of enterobacterial repetitive intergenic consensus sequences (ERIC-PCR) and ribotyping. Serological typing has also been used and this method seems to be a suitable first-line typing method for *S. marcescens*, although some strains remain untypable. RAPD-PCR has also been applied to a small number of isolates and seems to be a promising method, especially for rapid monitoring of an outbreak and tracing the source of initial infection.

Introduction

Serratia marcescens, a gram-negative bacillus classified as a member of the Enterobacteriaceae, has been recognised as a cause of hospital-acquired infection for the last two decades. It is a widely distributed saprophytic bacterium, and has been found in food, particularly in starchy variants which provide an excellent growth environment. While this organism was known formerly by a variety of names, including *Chromobacterium prodigiosum* [1], Gaughran *et al.* [2] used the name *S. marcescens* that had been assigned by Bizio in 1823.

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S. marcescens was considered originally to be an innocuous, non-pathogenic saprophytic water organism and was often used as a biological marker because of its easily recognised red colonies. After a review in 1896 of a small number of incidents, Professor Scheurlen of the University of Strasbourg concluded that this organism contributed to more deaths than many pathogenic bacteria. The first description of nosocomial infection caused by *S. marcescens* was Wheat's report of 11 cases over a 6-month period in 1951 at Stanford University Hospital [3]. Infections caused by this organism have been reported with increasing frequency since 1960 [4]. In 1966, McCormack and Kunin [5] reported a nursery epidemic involving 27 babies, although only 15 cases of *Serratia* bacteraemia had been recorded by 1968 [6]. Its ability to cause infection was once thought to be limited to patients with chronic debilitating disorders,

but *S. marcescens* has now been implicated as an aetiological agent in every conceivable kind of infection, including respiratory tract infection, urinary tract infection (UTI), septicaemia, meningitis and wound infections [7–9]. *S. marcescens* has been reported to cause infective endocarditis acquired in the community [10] and in hospitals. In contrast to other gram-negative bacteria, it usually affects the left side of the heart [11]. *S. marcescens* endocarditis acquired in the hospital is usually an exogenous infection associated with cardiac surgery [11]. Today, *S. marcescens* has attained the status of a fully fledged pathogen that causes infections particularly in two disparate groups: heroin addicts and hospitalised patients.

Environmental isolates of *S. marcescens* characteristically produce a red pigment, prodigiosin, and in early times such growth was often mistaken for fresh blood [2]. The pigmented bacterium is found in various ecological niches, including soil, water, air, plants and animals [12]. The ability to form prodigiosin is characteristic of *S. marcescens* [12], but the function of this red pigment remains unclear because clinical isolates are rarely pigmented.

Culture and identification

S. marcescens was defined by Grimont and Grimont [12] as an oxidase-negative gram-negative bacillus producing DNAase. Over the last two decades, the API 20E system has been used widely for the identification of gram-negative bacilli, but this system was found to correctly identify only 85% of *S. marcescens* isolates to the species level (personal unpublished results). *S. marcescens* is unable to ferment arabinose in peptone water, whereas all *S. liquefaciens* strains are arabinose-positive. However, *S. marcescens* strains can oxidise arabinose in the API system, giving a false-positive reaction leading to misidentification as *S. liquefaciens* [13]. Therefore, to confirm the identity of *S. marcescens*, a short series of sugars, including arabinose and raffinose, should be tested in tubes, regardless of the API 20E result.

Growth in the environment

The potential of *S. marcescens* to utilise a wide range of nutrients is expressed clearly by its ability to survive and grow under extreme conditions, including in disinfectant [14, 15], antiseptics [16] and double-distilled water [17]. Szewzyk *et al.* [17] studied the survival and growth of *S. marcescens* strain K202, isolated from blood bags, at different oxygen concentrations in de-ionised water containing materials derived from the blood bags. The rate of survival and growth was highest under anaerobic conditions, in which growth occurred with all materials and even in de-ionised water alone. In contrast, *S. marcescens* did not survive in control cultures under semi-anaerobic and aerobic conditions, an observation which is in agreement with our own unpublished findings. Growth was observed under both aerobic and semi-anaerobic conditions in the presence of each of the tested blood bag materials. An initial high number of bacteria may lead to better survival following cryptic growth [18] and utilisation of oxygen by respiration, thereby eliminating the toxic effect of oxygen radicals. Other studies have demonstrated that extremely low concentrations of organic material can be sufficient for survival and active growth of other bacteria [19].

The capacity to multiply in blood components varies for different bacterial species. The bactericidal effect of humoral factors in plasma [20] and the growth kinetics of the bacteria at different storage temperatures play a significant role. Gong *et al.* [21] demonstrated that white cells in freshly collected blood act to clear the blood of contaminating bacteria, although the effect varies among bacterial species [22–24]. These results, and those mentioned above, clearly show a toxic effect of oxygen on *S. marcescens* in low-nutrient conditions and reflect the importance of the oxygen concentration and the type of nutrients leaching out of the materials. When whole-blood units become contaminated, there is a high risk of rapid growth in red blood cells stored at 4°C, as well as in platelet concentrates stored at 22°C, which indicates that protective mechanisms involving phagocytosis and complement killing are not effective for *S. marcescens*.

Extracellular products

Aside from the production of marcescins, *S. marcescens* is unique among enteric bacteria in many respects. It secretes extracellular chitinase, several proteases, a nuclease and a lipase [25], and produces a wetting agent or surfactant called 'serrawettin' which helps in the colonisation of surfaces [26–28]. In keeping with its varied habitat, *S. marcescens* produces alternate forms of differentially flagellate cells; these display different types of motility depending on whether the growth medium is liquid or solid [29]. Non-flagellate cells of *S. marcescens* can also translocate efficiently over the surface of low-agar media [30]. Matsuyama *et al.* [31] showed that flagellate but non-swarming *dps* mutants of *S. marcescens* with defects in serrawettin production do not swarm on media solidified with Difco agar. The spreading deficiency can be overcome by serrawettin supplied extracellularly. Introduction of *fla* defects into chemotaxis mutants does not affect this mode of surface translocation.

Pigmentation

Prodigiosin biosynthesis in *S. marcescens* depends on growth conditions [32]. Rjazantseva *et al.* [32] found that pigment synthesis started later in the presence of

low concentrations of NaCl, but that prodigiosin accumulation/biomass unit was increased at NaCl concentrations of up to 5% w/v. However, there was no growth in a culture medium containing NaCl 8% w/v. Visible light (2000 lux) influenced pigmentation without changing the growth characteristics of the culture. The maximum prodigiosin content in dark and light cultures was observed on days 3–4 and 2–3, respectively. The influence of illumination on pigmentation of *S. marcescens* was also demonstrated. Light affects the pigment synthesised by the culture directly. Growing cells contain mono and dimer forms of prodigiosin in glycerol, with light influencing these pigments. The highest increase in biomass and maximal pigmentation was found in cultures grown on glycerol medium. A culture medium containing glucose as the carbon and energy source did not allow prodigiosin synthesis. The investigation supported the conclusion that strains of *S. marcescens* differ in their sensitivity to the effect of NaCl on prodigiosin biosynthesis. Although proline was present in the medium, *S. marcescens* grown on mineral media did not produce pigment when the carbon source was glucose or the nitrogen source was ammonium chloride.

The pigment has a role in respiration [33] and has some antibiotic properties [34]. It is presumed that pigment biosynthesis acts as a protective mechanism in unfavourable conditions when the growth of cells is delayed [35]. While the utilisation of light energy by pigmented *S. marcescens* has been suggested [32], attempts to exploit the pigment as a commercial dye have failed because of its sensitivity to light [36]. Pigment biosynthesis, as with other physiological processes, is affected significantly by factors such as temperature [37], ions [38], anionic detergents [39] and amino acids [40]. However, most strains that cause infection fail to produce pigment and form colourless colonies which are difficult to distinguish from other coliform organisms [41]; thus pigment would not appear to be a virulence factor of advantage to clinical isolates.

Antibiotic susceptibility

Infections caused by *S. marcescens* may be difficult to treat because of resistance to a variety of antibiotics, including ampicillin and first and second generation cephalosporins [42]. Aminoglycosides have good activity against *S. marcescens*, but resistant strains have also been reported recently [43]. As the killing effect of β-lactam antibiotics is time dependent, the length of time the bacteria are exposed to antibiotic concentrations above the MIC is an important parameter when assessing likely clinical outcome. Data obtained from a rabbit model suggest that if a β-lactam and aminoglycoside are combined, the aminoglycoside induces rapid killing and reduction of the inoculum, while the β-lactam antibiotic prevents regrowth between the doses of aminoglycosides [44].

β-Lactam resistance

While β-lactamase-mediated carbapenem resistance is rare among the Enterobacteriaceae, it has been described recently for clinical isolates of *S. marcescens* and *Enterobacter cloacae* [45, 46]. This resistance may arise from two mechanisms: first, high-level production of chromosomal AmpC cephalosporinases combined with substantially decreased outer-membrane permeability [47, 48]; and second, the synthesis of β-lactamases able to hydrolyse carbapenems [49].

Palomar *et al.* [50] studied the effect of the O-side chain on the permeability of the *S. marcescens* outer membrane to β-lactam antibiotics, and found that O-side chain-defective spontaneous mutants of *S. marcescens* had lower MICs for various β-lactam agents than their parent strains. Recovery of the ability to produce O-antigen restored the original MIC values, as well as phage susceptibility. The permeability coefficients of wild-type, O^- mutants and revertants indicated that the permeability to antibiotics of the *S. marcescens* outer membrane depends on the O-somatic antigen.

Ito *et al.* [51] studied the distribution of strains producing metallo-β-lactamase among 105 strains of *S. marcescens* and concluded that 19% of *S. marcescens* clinical isolates were resistant to imipenem (MICs > 2 mg/L), while strains that showed high-level imipenem resistance following acquisition of a plasmid-mediated bla_{IMP}-like metallo-β-lactamase gene proliferated as a cause of nosocomial infection in a general hospital. This observation suggests that imipenem-resistant strains producing the metallo-β-lactamase IMP-1 [52] may become prevalent in the near future if the present style of chemotherapy is continued without careful consideration.

Naas *et al.* [44] showed by cloning and subcloning of the carbapenem-hydrolysing β-lactamase gene ($bla_{Sme\text{-}1}$) from a strain of *S. marcescens* that the β-lactamase conferred resistance to carbapenems, aztreonam, cefamandole and cephalothin, but conferred no significant resistance (more than one dilution increase in MIC) to cefotaxime, ceftazidime, moxalactam or cefoxitin. Hybridisation results further indicated that only one copy of $bla_{Sme\text{-}1}$ was present in *S. marcescens* strains S6 and S8, and that the carbapenem-hydrolysing properties of these *Serratia* strains do not result from gene amplification. In the course of determining the DNA sequence of the cloned gene for $bla_{Sme\text{-}1}$, Naas *et al.* [53] found an open reading frame (ORF) divergent in transcription. This ORF was identified as encoding a novel 31.1-kDa LysR family protein analogous to other LysR proteins, especially β-lactamase regulators.

Cefepime, an extended-spectrum cephalosporin, is active against organisms such as *E. cloacae* and *S. marcescens* which are frequently resistant to broad-spectrum cephalosporins; it has therefore been described as a fourth-generation cephalosporin [54]. The extended activity of cefepime results from its low affinity for type I β-lactamase and the ease with which the molecule passes through porin channels [55]. Chong *et al.* [56] found that the MIC90s of cefepime and amikacin (both 8 mg/L) were lower for *S. marcescens* than for the other gram-negative bacilli studied.

Aminoglycoside resistance

Bacteria acquire resistance to aminoglycosides by preventing the drug from reaching the target site in the ribosome in one of two ways: firstly, alterations in the cell envelope can prevent uptake of the drug; and secondly, the drug itself can be modified by 'inactivating enzymes' that adenylate, acetylate, or phosphorylate the aminoglycoside hydroxyl or amino groups [1]. The bacterial activity of aminoglycosides is concentration-dependent; hence high doses given infrequently may be the best method of administration [9]. The netilmicin-sensitive, gentamicin-resistant pattern of resistance in *S. marcescens* is probably caused by acetyltransferase AAC(3)-I, an enzyme that inactivates gentamicin, but not netilmicin or amikacin. Aminoglycoside resistance in *S. marcescens* caused by inactivating enzymes is commonly mediated by plasmids and is often transferable [57].

Garcia *et al.* [58] found that 90% of their *S. marcescens* isolates were gentamicin-resistant. The *aac(6')-Ia* gene was not found in 10 of these isolates, whereas the *aac(6')-Ic* gene was detected by dot-blot hybridisation in all *S. marcescens* isolates examined and was the only amikacin resistance marker in 72% of these isolates. The *aac(6')-Ib* gene was found in five isolates. Most (75%) *Serratia* isolates had the triple combination of AAC(3)-V + AAC(6')-I + APH(3')-I. The combination of these enzymes causes resistance to gentamicin, tobramycin, netilmicin and amikacin. Amikacin resistance was associated with the presence of plasmids between 10 and 20 kb in size. When plasmid DNA from 27 transformants carrying the *aac(6')-Ib* gene was digested with *Bam*HI, *Bgl*I and *Eco*RV + *Sst*I and hybridised with a 3-kb *Bam*HI DNA fragment specific for Tn*1331*, 26 of the plasmids exhibited a hybridisation pattern characteristic of Tn*1331*.

Fluoroquinolone resistance

Korner *et al.* [9] reported a case of endocarditis caused by a ciprofloxacin-resistant strain of *S. marcescens* (MICs: azlocillin, 16 mg/L; ciprofloxacin, 4 mg/L) which was isolated from blood cultures taken from a peripheral vein and from the Hickman line. The MIC of ciprofloxacin for Enterobacteriaceae is usually 0.01 mg/L, while for *S. marcescens* it is 0.5 mg/L. Consequently, a ciprofloxacin-resistant mutant of *S. marcescens* is more likely to be selected than other Enterobacteriaceae.

Juvin *et al.* [59] studied the MICs (and MBCs) of pefloxacin and ciprofloxacin for a *S. marcescens* strain and concluded that killing curves for the *S. marcescens* strain studied (but possibly not for others) were essentially time-dependent. Thus, the excellent intrinsic activity *in vivo* was probably caused mainly by the pharmacokinetics of the two drugs (long half-lives combined with rapid and homogeneous diffusion). However, killing did not increase at antibiotic concentrations between 8- and 32-fold greater than the MIC. When the same dose of ciprofloxacin (4 mg/kg) was administered by continuous infusion over 24 h, a steady state was reached after about 3 h, which means that the in-vivo data obtained by continuous infusion correlated well with the in-vitro data.

Pathogenicity and virulence

Adherence and hydrophobicity

Piliation has been shown to be a determinant of microbial adherence to host epithelial surfaces [60]. *S. marcescens* is a cause of nosocomial UTI [8], possesses pili and adheres to uroepithelial cells [61]. Two classes of adhesins have been suggested. One class, designated mannose-resistant (MR) pili, agglutinates chicken erythrocytes in the presence of D-mannose; the other class, mannose-sensitive (MS) pili, exhibits mannose-sensitive haemagglutination of guinea-pig and chicken erythrocytes. It has been reported that renal damage following acute pyelonephritis is not related directly to bacterial growth in the kidney, but is related closely to the inflammatory process, including infiltration by polymorphonuclear leucocytes (PMNLs) [62–64]. Mizunoe *et al.* [65] assessed the effect of bacterial piliation on interaction with human PMNLs and found that *S. marcescens* strain US46, a human urinary tract isolate, seemed to possess both MR and MS pili. This finding suggested that MS-piliate bacteria stimulate PMNLs to produce active oxygen radicals, leading to tissue damage of the infected organ.

S. marcescens was reported to possess hydrophobic surface properties by Mudd and Mudd [66] more than 70 years ago. Since then, cell surface hydrophobicity has been linked to partitioning of *S. marcescens* at air:water and oil:water interfaces, as well as adhesion to solid surfaces including catheters and other plastics [67]. Ness-Greenstein *et al.* [68] tested the feasibility of increasing the cell surface hydrophobicity of a commonly used strain of *Escherichia coli* by transformation with DNA from *S. marcescens*, a microorganism with pronounced adhesion to hydrophobic

substrates [69]. Their results supported the concept of rendering *E. coli* cells more hydrophobic by transformation with DNA from other, more hydrophobic species. The results extended previous data showing that *E. coli* cells can be rendered hydrophobic by chemical modification [70] as well as by addition of organic cations [71].

Palomar *et al.* [72] compared the efficiency of adhesion of wild-type and O-deficient spontaneous mutants of *S. marcescens* to plastic, glass and Foley catheters, and also to human uroepithelial cells. The BATH test was used, in which migration of bacteria from the aqueous phase depends upon their ability to adhere to the organic phase. The results demonstrated that the O-antigen has a strong influence on the adhesion of *S. marcescens* to both inert and biological surfaces.

Lipopolysaccharide

Lipopolysaccharide (LPS) is responsible for the biological activity of endotoxin and is located in the outer membrane of gram-negative bacteria. LPS O-polysaccharides may contribute to the virulence of a bacterium by enabling it to resist serum killing [73]. *S. marcescens* serotype O16 produces two neutral O-polysaccharides: D-galactan I and a polymer containing 2-substituted β-D-ribo-furanosyl (Rib*f*) residues [74]. The question of the evolutionary origin of *rfb* clusters in different bacterial species that produce identical O-antigen structures was addressed by Szabo *et al.* [75] who cloned the *rfb* cluster from *S. marcescens* serotype O16 (rfb_{SmO16}) and compared its genetic organisation with that of the *rfb* cluster from *Klebsiella pneumoniae* serotype O1 (rfb_{KpO1}). Cloning and sequencing of rfb_{KpO1} revealed that six genes (*rfbA–F*) are responsible for the synthesis and export of D-galactan I, which results in resistance to phage Ffm. Southern hybridisation experiments did not detect any signals at high stringency, but three probes detected an 8-kb *Pst*I fragment from *S. marcescens* serotype O16 and a 7-kb *Pst*I fragment from *S. marcescens* serotype O20 at low stringency, suggesting that the *rfb* genes in *S. marcescens* are clustered on the chromosome. It was anticipated that recombinant cosmids obtained following cloning of the rfb_{SmO16} genes would express O-polysaccharide D-galactan I and therefore would be resistant to phage Ffm.

The bacterial surface components of pathogenic bacteria are the primary factors determining the outcome of contact with the host. Gram-negative bacteria are surrounded by an outer-membrane layer, which protects the cell from toxic agents by slowing their penetration and hindering their access to target site. The penetration of nutrients and antibiotics takes place through the outer-membrane proteins (e.g., unspecific porins, specific porins). The external face of the outer membrane is formed by LPS, which has a central role in both pathogenicity and virulence of many gram-negative bacteria [76]. LPS comprises three regions, i.e., lipid A, the O-antigen and the core. The O-antigen is a repetitive saccharide chain which is the most prominent immunogenic agent determining the O-serotype of bacteria. The presence of the O-side chain confers resistance to non-immune serum of this bacterial species [77], but O-antigen may affect the antibiotic susceptibility and the efficiency of transformation [78]. The structure of LPS in *S. marcescens* is variable as >24 somatic antigens have been described for this species [79].

Palomar *et al.* [77] presented evidence showing that bactericidal action on *S. marcescens* depends upon the O-side chain length. Both classical pathway (CPC) and alternate pathway (APC) are activated by *S. marcescens* strains, and both pathways have active roles in the serum killing of strains of *Serratia* with defective O-side chains. When APC or CPC were inhibited selectively and separately, there was a decrease in serum killing activity. These results suggested that the major role corresponding to APC was active. When CPC was active and APC was inhibited, the decrease was more apparent.

Extracellular products

S. marcescens produces several extracellular enzymes [80] and is one of the most efficient organisms for the biological degradation of chitin [81]. Chitinolytic enzymes are of biotechnological interest, as their substrate, chitin, is a major structural component of fungal cell walls. Thus, chitinolytic enzymes could, in principle, be employed as natural anti-fungal agents by, for example, expressing their genes in crop plants or in bacteria used in fermentation processes prone to fungal attack [82]. Sundheim *et al.* [83] cloned two chromosomal fragments encoding chitinolytic (and antifungal) activity from *S. marcescens* strain BJL200. Brurberg *et al.* [84] analysed one of these fragments and determined the nucleotide sequence of the chitinase gene. Protein engineering could be used to improve the stability and activity of the naturally occurring *Serratia* chitinases in order to improve their applicability. SDS–PAGE showed that the chitinase peak contained a pure protein of *c.* 62 kDa.

The extracellular lipase of *S. marcescens* strain Sr41, lacking a typical N-terminal signal sequence, is secreted *via* a signal peptide-independent pathway [85]. A subcloned 6.5-kb *Eco*RV fragment contained three ORFs of 588, 443 and 437 amino-acid residues that together constituted an operon (*lipBCD*). The three proteins functioned to allow secretion of the extracellular proteins of *S. marcescens* in a style analogous to that of the $prtDEF_{EC}$ system [85]. Deletion mutation analysis indicated that all three *lipBCD* genes were essential for extracellular secretion of the *S. marcescens* lipase in *E. coli*, and that these

genes constituted an operon like the *prtDEF*$_{EC}$ system [85].

Hiroyuki *et al.* [85] isolated a lipase-nonsecreting mutant, 414, from a wild-type strain of *S. marcescens*. The mutant did not secrete either lipase or metalloprotease when grown on either lipase or LB medium, but secretion was recovered by the introduction of the *lipBCD* genes.

A non-haem chloroperoxidase was purified by Burd *et al.* [86] from *S. marcescens* even though this bacterium is not known to produce halogenated metabolites. The native chloroperoxidase has a molecular mass of 58 kDa and consists of two identical subunits of 29 kDa. The absorption spectrum of purified chloroperoxidase showed no absorption bands in the visible region of the spectrum and halogenating activity was not inhibited by acid, showing that the enzyme does not contain haem as a prosthetic group. The enzyme was very resistant to alcohols such as methanol, ethanol and 2-methoxyethanol. The enzyme did not lose activity when incubated at 65°C for 2 h, but was inactivated slowly at 70°C. Chloroperoxidase was only active in acetate or propionate buffer and showed no activity when phosphate buffer was used. The isolation of halogenating enzymes from *S. marcescens* is surprising, as no halogenated metabolites have ever been isolated from this organism. Although it is possible that *S. marcescens* produces an as yet unknown halogenated metabolite, it is very unlikely that this strain should also synthesise a nitro compound, especially as nitro compounds are very rare natural products [87].

Letoffe *et al.* [88] identified a novel type of extracellular protein, HasA [89], in *S. marcescens*. HasA does not have a signal peptide and does not show sequence similarities to other proteins. When HasA secretion was reconstituted in *E. coli*, it was shown that, like many proteins lacking a signal peptide, HasA has a C-terminal targeting sequence and is secreted by a specific ATP-binding cassette (ABC) transporter consisting of three proteins: an inner-membrane protein with a conserved ATP binding domain, the ABC; a second inner-membrane protein; and a third, outer-membrane component.

Epidemiological typing

Several methods have been described for typing *S. marcescens*, including serotyping, phage typing, biotyping, bacteriocin typing and plasmid analysis [90]. Ribotyping has also been used [91, 92], and has been reported to be more discriminatory than biotyping, serotyping and bacteriocin typing, with equivalent discriminative power to total DNA analysis [93]. However, because of technical difficulties and the prolonged time needed for Southern blot analysis, its clinical use has been limited. Esterase electrophoretic typing of *S. marcescens* is reliable, but its use in clinical laboratories has been limited because of technical difficulties [94]. Serotyping, phage typing, and bacteriocin typing require the availability of specific reagents. Recently, Liu *et al.* [95] used PCR amplification of enterobacterial repetitive intergenic consensus (ERIC) sequences to investigate nosocomial *S. marcescens* isolates; however, neither ERIC-PCR nor analysis of PCR-amplified rDNA spacer polymorphisms allowed sufficient discrimination of the *S. marcescens* isolates examined.

Biotyping [96] has the advantage of using bacteriological techniques that can be employed on a routine basis, although the method is time-consuming and laborious. Biotyping cannot always distinguish between different epidemiological types. Many strains within the genus may give very similar reactions, while others can give irregular results for certain tests such as arabinose fermentation. We have found that only 85% of profile numbers generated by the API 20E system give good identification at the species level (personal unpublished results). rRNA gene restriction analysis has proved discriminatory, but is expensive and technically demanding [97].

Serotyping is the basic method for typing *S. marcescens*, but has limitations associated with the tedious determination of both O and K antigens, and the presence of a few untypable mutants [90]. Bacteriocin typing is a powerful method, but may not distinguish between different epidemiological types [90], while phage typing may only be of value in subdividing strains of the same O group from the same incident of infection [98].

Serological typing

The importance of *S. marcescens* as an opportunist pathogen and an agent of nosocomial infections has stimulated the development and evaluation of O-serology for typing clinical isolates, leading to a composite scheme of 20 O-serotypes by 1978 [13]. The number of serotypes had risen to 24 by 1985 [99], additional serotypes represented by strains S1254 and S3255 were described in 1989 [100], and other serotypes (O25 and O26) were proposed in 1991 [101]. The surface polysaccharides of the two most recently proposed O-serotype strains of *S. marcescens*, O25 and O26, were characterised in terms of their chemical structure and immunological reactions by Aucken *et al.* [102]. No polymer was isolated from O25, which was shown to lack both capsular K-antigen and smooth, O-antigenic LPS. A neutral polysaccharide was isolated from O26 and shown to be a polymer of rhamnose and *N*-acetylgalactosamine of the same type found previously in the O9 and O15 reference strains. Serological cross-reactions among all three strains were demonstrated by whole-cell enzyme-linked immuno-

sorbent assay and immunoblotting of LPS resolved by SDS-PAGE. No acidic polysaccharide was found in O26, and this was consistent with the absence of an immunogenic capsule. It was concluded that neither strain qualified for inclusion as a new serotype in either an O-typing or a K-typing scheme.

RAPD-PCR typing

Bosi *et al.* [103] reported an outbreak of *S. marcescens* infection in a neurosurgery intensive care unit. To investigate these *S. marcescens* isolates, random amplification of polymorphic DNA (RAPD) [104] was used in conjunction with primer AP12h (5′-CGGCCCCTGT-3′). The RAPD patterns for each isolate were identified on the basis of identical numbers and sizes of the bands. As proposed by Woods *et al.* [105] in their study of *Citrobacter diversus* isolates, a difference of more than two bands was taken to indicate different strains. A review of patient charts revealed that the strains were isolated from microbiological samples collected on admission and were, therefore, probably community-acquired. RAPD typing provided results in agreement with antibiotic susceptibility profiles. In contrast, Liu *et al.* [95] recently tried RAPD typing, but the primers selected were not suitable for the study of *S. marcescens* isolates.

The future

S. marcescens has been recognised as a cause of wound infection, pneumonia, lung abscess, empyema, meningitis, UTI, endocarditis, septic arthritis, osteomyelitis, peritonitis, sinusitis and septicaemia [106, 107]. Patients most at risk are those with debilitating disorders, those treated with broad-spectrum antibiotics, and those in intensive care who are subjected to instrumentation such as tracheostomy tubes or indwelling catheters [108]. Infections have been associated with contaminated disinfectants and antiseptics. Parment *et al.* [15] isolated *S. marcescens* from contact lens washing solutions containing chlorhexidine. Nakashima *et al.* [109] isolated *S. marcescens* from benzalkonium chloride-soaked cotton balls and showed that the bacteria were responsible for septic arthritis in 10 patients during a 6-week period. Sautter *et al.* [110] reported a case of meningitis associated with contamination of a skin antiseptic solution containing benzalkonium chloride. Ehrenkranz *et al.* [111] demonstrated *S. marcescens* contamination in a dimethylbenzyl ammonium chloride solution that was sprayed pre-operatively in a heart surgery room. Barry *et al.* [112] demonstrated *S. marcescens* contamination of antiseptic soaps containing triclosan.

The 1981 survey of the Hospital Infection Society showed that 0.8% of UTIs are caused by *S. marcescens*. The risk of UTI in a catheterised patient varies with the proximity of that patient to other catheterised patients who are already colonised [113]. While some investigators used formalin as a disinfectant to control an outbreak of klebsiella infection [114], Thompson *et al.* [115] did not find this method to be of benefit. The respiratory tract is an important portal of entry for patients who undergo manipulative airway procedures. The gastrointestinal tract does not appear to be an important reservoir of infection in adults, but may be a primary reservoir in children [116]. The use of antimicrobial drugs has been documented as a risk factor for the emergence of drug-resistant strains [117]. It is not known whether these risk factors are truly independent or merely associated with prolonged hospitalisation, broad-spectrum antimicrobial use, and respiratory and urinary tract manipulation.

Contamination of donor blood or blood components with *S. marcescens* is fortunately a rare complication of blood transfusion, but has been reported regularly for decades [118, 119]. Transfusion-associated complications with *Serratia* spp. often show the picture of septic, endotoxic shock, as reported by Jeppson *et al.* [120] and Woodfield *et al.* [121]. Both of these reported cases were clinically severe, and the second was fatal. In a recent Danish–Swedish epidemic of transfusion-associated *S. marcescens* septicaemia [118, 119], four patients had severe hyperpyrexia but recovered, one patient died, and a sixth patient had relatively mild symptoms. Rapid growth occurred early in the storage of red blood cells at 4°C, and the bacteria seemed to multiply even faster in whole blood than in broth culture media. Recently, septicaemia caused by *Serratia* has been associated with contamination of the exterior surfaces of blood packs [118, 122].

Despite the identification of some sources of the organism – such as plastic nebulisers, disinfectant solutions, contaminated blood bags and mouth-wash solutions – the main reservoir has yet to be demonstrated. To monitor the isolation frequency of this organism, a good discriminatory typing method is required. The method should be easy, even for small laboratories, and should allow rapid tracing of the source of an outbreak strain. Despite the application of several methods, there is still a requirement for a convenient and speedy technique. While bacteriocin typing has been described as a powerful method for other Enterobacteriaceae, this approach was found to be inadequate for *S. marcescens* because of a lack of discrimination [90]. Serotyping is the best available first-line method for *S. marcescens*, although some strains remain untypable. Other methods like ERIC or Southern-blot analysis are unsuitable for use in small laboratories and are technically more difficult. The use of RAPD-PCR has been reported by Bosi *et al.* [103], but only a small number of isolates was investigated. More recent studies with a wider range of strains and different primers have confirmed that RAPD-PCR is a promising method [123]. By applying this technique to

all new isolates it should be possible to detect rapidly an outbreak of infection and allow the problem to be monitored and the source of the initial infection to be traced. Study of resistance mechanisms to new antibiotics is another field for study in *S. marcescens*. Although some authors have described induction of β-lactamase by combining a strong and a weak inducer, resulting in treatment failure, this resistance mechanism in *S. marcescens* requires further clinical and scientific evidence rather than anecdote.

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References

1. Sleigh JD. Antibiotic resistance in *Serratia marcescens*. *BMJ* 1983; **287**: 1651–1653.
2. Gaughran ERL. Division of microbiology from superstition to science: the history of a bacterium. *Trans NY Acad Sci*. The meeting of the division. January 26. 1968.
3. Wheat RP, Zuckerman A, Rantz LA. Infection due to Chromobacteria: report of eleven cases. *Arch Intern Med* 1951; **88**: 461–466.
4. Dodson WH. *Serratia marcescens* septicaemia. *Arch Intern Med* 1968; **121**: 145–150.
5. McCormack RC, Kunin CM. Control of a single source nursery endemic due to *Serratia marcescens*. *Pediatrics* 1966; **37**: 750–755.
6. Alexander RH, Reichenbach DD, Merendino KA. *Serratia marcescens* endocarditis: a review of the literature and report of a case involving a homograft replacement of the aortic valve. *Arch Surg* 1969; **98**: 287–291.
7. Gouin F, Papazian L, Martin C *et al.* A non-comparative study of the efficacy and tolerance of cefepime in combination with amikacin in the treatment of severe infections in patients in intensive care. *J Antimicrob Chemother* 1993; **32** Suppl B: 205–214.
8. Cox CE. Aztreonam therapy for complicated urinary tract infections caused by multidrug-resistant bacteria. *Rev Infect Dis* 1985; **7** Suppl 4: S767–S770.
9. Körner RJ, Nicol A, Reeves DS, MacGowan AP, Hows J. Ciprofloxacin resistant *Serratia marcescens* endocarditis as a complication of non-Hodgkin's lymphoma. *J Infect* 1994; **29**: 73–76.
10. Mills J, Drew D. *Serratia marcescens* endocarditis: a regional illness associated with intravenous drug abuse. *Ann Intern Med* 1976; **84**: 29–35.
11. Cohen PS, Maguire JH, Weinstein L. Infective endocarditis caused by gram-negative bacteria: a review of the literature, 1945–1977. *Prog Cardiovasc Dis* 1980; **22**: 205–242.
12. Grimont PAD, Grimont F. Genus VIII. *Serratia*. In: Krieg NR, Holt JG (eds) Bergey's Manual of systematic bacteriology, vol 1. Baltimore, Williams and Wilkins. 1984: 477–484.
13. Pitt TL. State of the art: typing of *Serratia marcescens*. *J Hosp Infect* 1982; **3**: 9–14.
14. Marrie TJ, Costerton JW. Prolonged survival of *Serratia marcescens* in chlorhexidine. *Appl Environ Microbiol* 1981; **42**: 1093–1102.
15. Parment PA, Ronnerstam R, Walder M. Persistence of *Serratia marcescens*, *Serratia liquefaciens* and *E. coli* in solutions for contact lenses. *Acta Opthalmol* 1986; **64**: 456–462.
16. Nakashima AK, Highsmith AK, Martone WJ. Survival of *Serratia marcescens* in benzalkonium chloride and in multiple-dose medication vials: relationship to epidemic septic arthritis. *J Clin Microbiol* 1987; **25**: 1019–1021.
17. Szewzyk U, Szewzyk R, Stenström T-A. Growth and survival of *Serratia marcescens* under aerobic and anaerobic conditions in the presence of materials from blood bags. *J Clin Microbiol* 1993; **31**: 1826–30.
18. Mason CA, Hamer G, Bryers JD. The death and lysis of microorganisms in environmental processes. *FEMS Microbiol Rev* 1986; **39**: 373–401.
19. Porter JF, Paton R, Wardlaw AC. Growth and survival of *Bordetella bronchiseptica* in natural waters and in buffered saline without added nutrients. *Appl Environ Microbiol* 1991; **57**: 1202–1206.
20. Kolmer JA. Preserved citrated blood "banks" in relation to transfusion in the treatment of disease with special reference to immunologic aspects. *Am J Med Sci* 1939; **197**: 442–452.
21. Gong J, Hogman CF, Hambraeus A, Johansson CS, Eriksson L. Transfusion-associated *Serratia marcescens* infection: studies of the mechanism of action. *Transfusion* 1993; **33**: 802–808.
22. Hogman CF, Gong J, Eriksson L, Hambraeus A, Johansson CS. White cells protect donor blood against bacterial contamination. *Transfusion* 1991; **31**: 620–626.
23. Hogman CF, Gong J, Hambraeus A, Johansson CS, Eriksson L. The role of white cells in the transmission of *Yersinia enterocolitica* in blood components. *Transfusion* 1992; **32**: 654–657.
24. Gong J, Hogman CF, Hambraeus A, Johansson CS, Eriksson L. Transfusion-transmitted *Yersinia enterocolitica* infection. Protection through buffy coat removal and failure of the bacteria to grow in platelet-rich or platelet-poor plasma. *Vox Sang* 1993; **65**: 42–46.
25. Hines DA, Saurugger PN, Ihler GM, Benedik MJ. Genetic analysis of extracellular proteins of *Serratia marcescens*. *J Bacteriol* 1988; **170**: 4141–4146.
26. Matsuyama T, Fujita M, Yano I. Wetting agent producing by *Serratia marcescens*. *FEMS Microbiol Lett* 1985; **28**: 125–129.
27. Matsuyama T, Kaneda K, Nakagawa Y, Isa K, Hara-Hotta H, Yano I. A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. *J Bacteriol* 1992; **174**: 1769–1776.
28. Matsuyama T, Murakami T, Fujita M, Fujita S, Yano I. Extracellular vesicle formation and bio-surfactant production by *Serratia marcescens*. *J Gen Microbiol* 1986; **132**: 865–875.
29. Alberti L, Harshey RM. Differentiation of *Serratia marcescens* 274 into swimmer and swarmer cells. *J Bacteriol* 1990; **172**: 4322–4328.
30. O'Rear J, Alberti L, Harshey RM. Mutations that impair swarming motility in *Serratia marcescens* 274 include but are not limited to those affecting chemotaxis or flagellar function. *J Bacteriol* 1992; **174**: 6125–6137.
31. Matsuyama T, Bhasin A, Harshey RM. Mutational analysis of flagellum-independent surface spreading of *Serratia marcescens* 274 on a low-agar medium. *J Bacteriol* 1995; **177**: 987–991.
32. Rjazantseva IN, Andreeva IN, Ogorodnikova TI. Effect of various growth conditions on pigmentation of *Serratia marcescens*. *Microbios* 1994; **79**: 155–161.
33. Allen EG. Conditions of the colour change of prodigiosin. *Nature* 1967; **216**: 929–931.
34. Cho LKN, Lowe IA, Maguire RB, Tsang JC. Relationship of prodigiosin condensing enzyme activity to the biosynthesis of prodigiosin and its precursors in *Serratia marcescens*. *Experientia* 1987; **43**: 397–399.
35. Akimenko VK, Trutko SM. Physiological role of the oversynthesis of intermediates and the biosynthesis of metabolites for microorganism-producers. *Prikl Biokhim Mikrobiol* 1991; **27**: 17–35.
36. Yu VL. *Serratia marcescens*: historical perspective and clinical review. *N Engl J Med* 1979; **300**: 887–893.
37. Williams RP, Gott CL, Qadri SMH, Scott RH. Influence of temperature of incubation and type of growth medium on pigmentation in *Serratia marcescens*. *J Bacteriol* 1971; **106**: 438–443.
38. Silverman MP, Munoz EF. Effect of iron and salt on prodigiosin synthesis in *Serratia marcescens*. *J Bacteriol* 1973; **114**: 999–1006.
39. Feng IS, Webb IW, Tsang IC. Enhancement of sodium dodecyl sulfate of pigment formation in *Serratia marcescens* O8. *Appl Environ Microbiol* 1982; **43**: 850–854.
40. Williams RP, Gott CL, Qadri SMH. Induction of pigmentation in nonproliferating cells of *Serratia marcescens* by addition of single amino acids. *J Bacteriol* 1971; **106**: 444–448.

41. Phillips I, King A. *Serratia marcescens* in hospital practice. *Lancet* 1977; 1: 538.
42. Ball AP, McGhie D, Geddes AM. *Serratia marcescens* in a general hospital. *Q J Med* 1977; **46**: 63–71.
43. Coria-Jiménez R, Ortiz-Torres C. Aminoglycoside resistance patterns of *Serratia marcescens* strains of clinical origin. *J Epidemiol Infect* 1994; **112**: 125–131.
44. Carbon C. Impact of the antibiotic dosage schedule on efficacy in experimental endocarditis. *Scand J Infect Dis* 1991; Suppl. **74**: 163–172.
45. Naas T, Vandel L, Sougakoff W, Livermore DM, Nordmann P. Cloning and sequence analysis of the gene for a carbapenem-hydrolyzing class A β-lactamase, Sme-1, from *Serratia marcescens* S6. *Antimicrob Agents Chemother* 1994; **38**: 1262–1270.
46. Nordmann P, Mariotte S, Naas T, Labia R, Nicolas MH. Biochemical properties of a carbapenem-hydrolyzing β-lactamase from *Enterobacter cloacae* and cloning of the gene into *Escherichia coli*. *Antimicrob Agents Chemother* 1993; **37**: 939–946.
47. de Champs C, Henquell C, Guelon D, Sirot D, Gazuy N, Sirot J. Clinical and bacteriological study of nosocomial infections due to *Enterobacter aerogenes* resistant to imipenem. *J Clin Microbiol* 1993; **31**: 123–127.
48. Ehrhardt AF, Sanders CC, Thomsom K, Watanakunakorn SC, Trujillano-Martin I. Emergence of resistance to imipenem in *Enterobacter* isolates masquerading as *Klebsiella pneumoniae* during therapy with imipenem/cilastatin. *Clin Infect Dis* 1993; **17**: 120–122.
49. Livermore DM. Carbapenemases. *J Antimicrob Chemother* 1992; **29**: 609–613.
50. Palomar J, Puig M, Montilla R, Loren JG, Vinas M. Lipopolysaccharide recovery restores susceptibility levels towards β-lactamase in *Serratia marcescens*. *Microbios* 1995; **82**: 21–26.
51. Ito H, Arakawa Y, Ohsuka S, Wacharotayankun R, Kato N, Ohta M. Plasmid-mediated dissemination of the metallo-β-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. *Antimicrob Agents Chemother* 1995; **39**: 824–829.
52. Osano E, Arakawa Y, Wacharotayankun R *et al.* Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrob Agents Chemother* 1994; **38**: 71–78.
53. Naas T, Livermore DM, Nordmann P. Characterization of an LysR family protein SmeR from *Serratia marcescens* S6, its effect on expression of the carbapenem-hydrolyzing β-lactamase Sme-1, and comparison of this regulator with other β-lactamase regulators. *Antimicrob Agents Chemother* 1995; **39**: 629–637.
54. Bellido F, Pechère J-C, Hancock REW. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells. *Antimicrob Agents Chemother* 1991; **35**: 68–72.
55. Nikaido H, Liu W, Rosenberg EY. Outer membrane permeability and β-lactamase stability of dipolar ionic cephalosporins containing methoxyimino substituents. *Antimicrob Agents Chemother* 1990; **34**: 337–342.
56. Chong Y, Lee K, Kwon OH. In-vitro activities of cefepime against *Enterobacter cloacae*. *Serratia marcescens*, *Pseudomonas aeruginosa* and other aerobic Gram-negative bacilli. *J Antimicrob Chemother* 1993; **32** Suppl. B: 21–29.
57. Platt DJ, Sommerville JS. *Serratia* species isolated from patients in a general hospital. *J Hosp Infect* 1981; **2**: 341–348.
58. Garcia DC, Woloj GM, Piñeiro S, Sordelli DO, Kaufman S. An 8-year study of resistance to amikacin in gram-negative bacilli isolates from patients with nosocomial infections at one hospital in Argentina. *J Med Microbiol* 1995; **42**: 283–290.
59. Juvin ME, Potel G, Caillon J *et al.* In vivo bactericidal activities of ciprofloxacin and pefloxacin in an experimental model of *Serratia marcescens* endocarditis. *Antimicrob Agents Chemother* 1994; **38**: 883–885.
60. Reid G, Sobel JD. Bacterial adherence in the pathogenesis of urinary tract infection: a review. *Rev Infect Dis* 1987; **9**: 470–487.
61. Yamamoto T, Ariyoshi A, Amako K. Fimbria-mediated adherence of *Serratia marcescens* US5 strain to human urinary bladder surface. *Microbiol Immunol* 1985; **29**: 677–681.
62. Glauser MP, Lyons JM, Braude AI. Prevention of chronic experimental pyelonephritis by suppression of acute suppuration. *J Clin Invest* 1978; **61**: 403–407.
63. Slotki IN, Asscher AW. Prevention of scarring in experimental pyelonephritis in the rat by early antibiotic therapy. *Nephron* 1982; **30**: 262–268.
64. Bille J, Glauser MP. Protection against chronic pyelonephritis in rats by suppression of acute suppuration: effect of colchicine and neutropenia. *J Infect Dis* 1982; **146**: 220–226.
65. Mizunoe Y, Matsumoto T, Haraoka M, Sakumoto M, Kubo S, Kumazawa J. Effect of pili of *Serratia marcescens* on superoxide production and phagocytosis of human polymorphonuclear leukocytes. *J Urol* 1995; **154**: 1227–1230.
66. Mudd S, Mudd EBH. The penetration of bacteria through capillary spaces. IV. A kinetic mechanism in interfaces. *J Exp Med* 1924; **40**: 633–645.
67. Ashkenazi S, Weiss E, Drucker MM. Bacterial adherence to intravenous catheters and needles and its influence by cannula type and bacterial surface hydrophobicity. *J Lab Clin Med* 1986; **107**: 136–140.
68. Ness-Greenstein RB, Rosenberg M, Doyle RJ, Kaplan N. DNA from *Serratia marcescens* confers a hydrophobic character in *Escherichia coli*. *FEMS Microbiol Lett* 1995; **125**: 71–75.
69. Rosenberg M, Blumberger Y, Judes H, Bar-Ness R, Rubinstein E, Mazor Y. Cell surface hydrophobicity of pigmented and nonpigmented clinical *Serratia marcescens* strains. *Infect Immun* 1986; **51**: 932–935.
70. Goldberg S, Doyle RJ, Rosenberg M. Mechanism of enhancement of microbiol cell hydrophobicity by cationic polymers. *J Bacteriol* 1990; **172**: 5650–5654.
71. Goldberg S, Konis Y, Rosenberg M. Effect of cetylpyridinium chloride on microbial adhesion to hexadecane. *Appl Environ Microbiol* 1990; **56**: 1678–1682.
72. Palomar J, Leranoz AM, Vinas M. *Serratia marcescens* adherence: the effect of O-antigen presence. *Microbios* 1995; **81**: 107–113.
73. Joiner KA. Studies on the mechanism of bacterial resistance to complement-mediated killing and the mechanism of action of bactericidal antibody. *Curr Top Microbiol Immunol* 1985; **121**: 99–133.
74. Oxley D, Wilkinson SG. Structures of neutral glycans isolated from the lipopolysaccharides of reference strains for *Serratia marcescens* serogroups O16 and O20. *Carbohydr Res* 1989; **193**: 241–248.
75. Szabo M, Bronner D, Whitfield C. Relationship between *rfb* gene clusters required for biosynthesis of identical D-galactose-containing O antigens in *Klebsiella pneumoniae* serotype O1 and *Serratia marcescens* serotype O16. *J Bacteriol* 1995; **177**: 1544–1553.
76. Straus DC, Atkisson DL, Garner CW. Importance of a lipopolysaccharide-containing extracellular toxic complex in infections produced by *Klebsiella pneumoniae*. *Infect Immun* 1985; **50**: 787–795.
77. Palomar J, Montilla R, Fuste MC, Vinas M. The role of O-antigen in susceptibility of *Serratia marcescens* to non-immune serum. *Microbios* 1993; **76**: 189–196.
78. Palomar J. El Lipopolysacharido de *Serratia marcescens* como factor de virulencia. PhD thesis, University of Barcelona. 1994.
79. Gaston MA, Pitt TL. O-antigen specificities of the serotype strains of *Serratia marcescens*. *J Clin Microbiol* 1989; **27**: 2697–2701.
80. Fuchs RL, McPherson SA, Drahos DJ. Cloning of a *Serratia marcescens* gene encoding chitinase. *Appl Environ Microbiol* 1986; **51**: 504–509.
81. Monreal J, Reese ET. The chitinase of *Serratia marcescens*. *Can J Microbiol* 1969; **15**: 689–696.
82. Brurberg MB, Haandrikman AJ, Leenhouts KJ, Venema G, Nes IF. Expression of chitinase gene from *Serratia marcescens* in *Lactococcus lactis* and *Lactobacillus plantarum*. *Appl Microbiol Biotechnol* 1994; **42**: 108–115.
83. Sundheim L, Poplawsky AR, Ellingboe AH. Molecular cloning of two chitinase genes from *Serratia marcescens* and their expression in *Pseudomonas* species. *Phys Mol Plant Pathol* 1988; **33**: 483–491.

84. Brurberg MB, Eijsink VGH, Nes IF. Characterization of a chitinase gene (*chi A*) from *Serratia marcescens* BJL 200 and one-step purification of the gene product. *FEMS Microbiol Lett* 1994; **124**: 399–404.
85. Akotsuka H, Kawai E, Omori K, Shibatani T. The three genes *lipB*, *lipC*, and *lipD* involved in the extraceullular secretion of the *Serratia marcescens* lipase which lacks an N-terminal signal peptide. *J Bacteriol* 1995; **177**: 6381–6389.
86. Burd W, Yourkevich O, Voskoboev AJ, van Pée KH. Purification and properties of a non-haem chloroperoxidase from *Serratia marcescens*. *FEMS Microbiol Lett* 1995; **129**: 255–260.
87. Turner WB. Nitro compounds. In: Laskin AI, Lechevalier HA (eds) CRC Handbook of microbiology, vol 3. Cleveland, CRC Press. 1973: 445–447.
88. Létoffé S, Ghigo J-M, Wandersman C. Identification of two components of the *Serratia marcescens* metalloprotease transporter: protease SM secretion in *Escherichia coli* is TolC dependent. *J Bacteriol* 1993; **175**: 7321–7328.
89. Létoffé S. Ghigo J-M, Wandersman C. Secretion of the *Serratia marcescens* HasA protein by an ABC transporter. *J Bacteriol* 1994; **176**: 5372–5377.
90. Larose P, Picard B, Thibault M, Grimont F, Goullet P. Nosocomial *Serratia marcescens* individualized by five typing methods in a regional hospital. *J Hosp Infect* 1990; **15**: 167–172.
91. Chetoui H, Delhalle E, Osterrieth P, Rousseaux D. Ribotyping for use in studying molecular epidemiology of *Serratia marcescens*: comparison with biotyping. *J Clin Microbiol* 1995; **33**: 2637–2642.
92. Bingen EH, Mariani-Kurkdjian P, Lambert-Zechovsky NY *et al.* Ribotyping provides efficient differentiation of nosocomial *Serratia marcescens* isolates in a paediatric hospital. *J Clin Microbiol* 1992; **30**: 2088–2091.
93. Alonso R, Aucken HM, Perez-Diaz JC, Cookson BD, Baquero F, Pitt TL. Comparison of serotype, biotype and bacteriocin type with rDNA RFLP patterns for the type identification of *Serratia marcescens*. *Epidemiol Infect* 1993; **111**: 99–107.
94. Picard B, Bruneau B, Goullet P. Demonstration of an outbreak of *Serratia marcescens* infections in medical intensive care unit by esterase electrophoretic typing. *J Hosp Infect* 1988; **11**: 194–195.
95. Liu PY-F, Lau Y-J, Hu B-S *et al.* Use of PCR to study epidemiology of *Serratia marcescens* isolates in nosocomial infection. *J Clin Microbiol* 1994; **32**: 1935–1938.
96. Grimont PAD, Grimont F. Biotyping of *Serratia marcescens* and its use for epidemiological studies. *J Clin Microbiol* 1978; **8**: 73–83.
97. Grimont F, Grimont PAD. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Ann Inst Pasteur/Microbiol* 1986; **137B**: 165–175.
98. Pitt TL, Erdman YJ. Serological typing of *Serratia marcescens*. *Methods Microbiol* 1984; **15**: 173–211.
99. Traub WH. Serotyping of *Serratia marcescens*: identification of a new O-antigen (O24). *Zentralbl Bakteriol Mikrobiol HygA* 1985; **259**: 485–488.
100. Gaston MA, Pitt TL. Improved O-serotyping method for *Serratia marcescens*. *J Clin Microbiol* 1989; **27**: 2702–2705.
101. Traub WH. Serotyping of *Serratia marcescens*: detection of two new O-antigens (O25 and O26). *Int J Med Microbiol* 1991; **275**: 495–499.
102. Aucken HM, Merkouroglou M, Miller AW, Galbraith L, Wilkinson SG. Structural and serological studies of lipopolysaccharides from proposed new serotypes (O25 and O26) of *Serratia marcescens*. *FEMS Microbiol Lett* 1995; **130**: 267–272.
103. Bosi C, Davin-Regli A, Charrel R, Rocca B, Monnet D, Bollet C. *Serratia marcescens* nosocomial outbreak due to contamination of hexetidine solution. *J Hosp Infect* 1996; **33**: 217–224.
104. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 1990; **18**: 6531–6535.
105. Woods CR, Versalovic J, Koeuth T, Lupski JR. Analysis of relationships among isolates of *Citrobacter diversus* by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 2921–2929.
106. Wilfert JN, Barrett FF, Kass EH. Bacteremia due to *Serratia marcescens*. *N Engl J Med* 1968; **279**: 286–289.
107. Davis JT, Foltz E, Blakemore WS. *Serratia marcescens*. A pathogen of increasing clinical importance. *JAMA* 1970; **214**: 2190–2192.
108. Wilkowske CJ, Washington JA, Martin WJ, Ritts RE. *Serratia marcescens*. Biomedical characteristics, antibiotic susceptibility patterns, and clinical significance. *JAMA* 1970; **214**: 2157–2162.
109. Nakashima AK, McCarthy MA, Martone WJ, Anderson RL. Epidemic septic arthritis caused by *Serratia marcescens* and associated with a benzalkonium chloride antiseptic. *J Clin Microbiol* 1987; **25**: 1014–1018.
110. Sautter RL, Mattman KH, Legaspi RC. *Serratia marcescens* meningitis associated with contaminated benzalkonium chloride solution. *Infect Control* 1984; **5**: 223–225.
111. Ehrenkranz NJ, Bolyard EA, Wiener M, Cleary TJ. Antibiotic-sensitive *Serratia marcescens* infections complicating cardiopulmonary operations: contaminated disinfectant as a reservoir. *Lancet* 1980; **2**: 1289–1292.
112. Barry MA, Craven DE, Goularte TA, Lichtenberg DA. *Serratia marcescens* contamination of antiseptic soap containing triclosan: implications for nosocomial infection. *Infect Control* 1984; **5**: 427–430.
113. Meers PD, Ayliffe GA, Emmerson AM *et al.* National survey of infection in hospitals 1980. Part 2: Urinary tract infection. *J Hosp Infect* 1981; **2**: 23–28.
114. Suryaprakash B, Rao MS, Panigrahi D, Vaidyanathan S, Goel AK. Ayyagari A. Formalin in the urinary bag: a cheap measure to control infection in urology wards. *Lancet* 1984; **2**: 104–105.
115. Thompson RL, Haley CE, Searcy MA *et al.* Catheter-associated bacteriuria: failure to reduce attack rates using periodic instillations of a disinfectant into urinary drainage systems. *JAMA* 1984; **251**: 747–751.
116. Christensen GD, Korones SB, Reed L, Bulley R, McLaughlin B, Bisno AL. Epidemic *Serratia marcescens* in a neonatal intensive care unit: importance of the gastrointestinal tract as a reservoir. *Infect Control* 1982; **3**: 127–133.
117. Lewis DA, Hawkey PM, Watts JA *et al.* Infection with netilmicin resistant *Serratia marcescens* in a special care baby unit. *BMJ* 1983; **287**: 1701–1705.
118. Heltberg O, Skov F, Gerner-Smidt P *et al.* Nosocomial epidemic of *Serratia marcescens* septicemia ascribed to contaminated blood transfusion bags. *Transfusion* 1993; **33**: 221–227.
119. Hogman CF, Fritz H, Sandberg L. Posttransfusion *Serratia marcescens* septicemia. *Transfusion* 1993; **33**: 189–191.
120. Jeppsson B, Lindahl S, Ingemansson S, Kornhall S, Sjoval S. Bacterial contamination of blood transfusion: an unusual cause of sepsis. *Acta Chir Scand* 1984; **150**: 489–491.
121. Woodfield DG. Transfusion acquired *Serratia liquefaciens* septicaemia. *N Z Med J* 1991; **104**: 141.
122. Hawkey PM, Constable HK. Selection of netilmicin resistance, associated with increased 6′ aminoglycoside acetyltransferase activity, in *Serratia marcescens*. *J Antimicrob Chemother* 1988; **21**: 535–544.
123. Hejazi A, Keane CT, Falkiner FR. The use of RAPD-PCR as a typing method for *Serratia marcescens*. *J Med Microbiol* 1997; **46**: 913–919.