Re-evaluation of the serotypes of *Serratia marcescens* and separation into two schemes based on lipopolysaccharide (O) and capsular polysaccharide (K) antigens

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Chemical and serological analysis has revealed that many of the 29 O serotype reference strains of *Serratia marcescens* contain both neutral and acidic polysaccharides which correspond to LPS O antigens and capsular K antigens, respectively. New O and K antigen typing schemes have therefore been devised, based on the known chemical structures of the surface polysaccharides of the organism. These schemes were designed to allow the specific detection of these antigens on unknown strains using ELISAs. O antigens were detected using whole cells cultured in broth then autoclaved to remove capsular material, while K antigens were detected using formalized whole cells which had been cultured on glycerol agar to enhance capsule production. After testing with the 29 reference strains as well as 423 distinct clinical strains, it was apparent that different aspects of chemical structure were associated with different degrees of serological reactivity and the typing schemes were modified further to accommodate this. In general, the O antigen repeating unit structures were chemically simple with di- or trisaccharide backbones. Serological specificity was often provided solely by the presence or absence of an O-acetyl substituent, or a change in the linkage between two sugar residues. Five of the O serotypes in the new scheme were represented by 12 of the 29 reference strains, while three reference strains lacked O antigens altogether, resulting in the elimination of 10 of the original O types. In contrast, the K antigen repeating unit structures were more complex and chemically diverse, having at least four sugar residues. Three K types were each seen in two reference strains while 12 of the 29 reference strains were acapsular. Thus, the resulting schemes contain 19 O types and 14 K types and allow the definitive serotype identification of *S. marcescens*.

Keywords: *Serratia marcescens*, LPS, capsules, serotyping, O and K antigens

INTRODUCTION

Serratia marcescens is an opportunist Gram-negative bacterium that causes outbreaks of infection, and occasionally death, in hospitalized patients. The nature and extent of an outbreak can be determined by investigating the strain-relatedness of isolates using epidemiological typing methods. Such investigations

will not only identify episodes of cross-infection, but also will reveal possible common sources and unrelated sporadic infections (Farmer *et al.*, 1976). Serotyping schemes based on the O antigens of LPS or capsular K antigens have proved extremely useful for typing other Gram-negative species such as *Escherichia coli* and *Klebsiella* spp. (Ørskov & Ørskov, 1984, 1992). Generally, these antigens are highly stable and therefore are often used as 'gold standard' characteristics since isolates with different serotypes can confidently be assumed to be different strains in the vast majority of cases. Furthermore, by providing definitive type labels,

Abbreviations: KA, King's agar; NA, nutrient agar; NRS, normal rabbit serum; TSB, tryptone soya broth.

Table 1. New serotype designations of the 29 *S. marcescens* O serotype reference strains

Original serotype	Reference strain	Strain reference	Polysaccharide reference	New serotype*
O1	866-57	Davis & Woodward (1957)	Furn & Wilkinson (1985); Johansson <i>et al.</i> (1995)	O19:K1
O2	868-57	Davis & Woodward (1957)	Oxley & Wilkinson (1988f, 1992b)	O2:K2
O3	863-57	Davis & Woodward (1957)	Oxley & Wilkinson (1989b, 1992b)	O2:K3
O4	864-57	Davis & Woodward (1957)	Oxley & Wilkinson (1988c, e)	O4:K4
O5	867-57	Davis & Woodward (1957)	Oxley & Wilkinson (1988b)	O5:K28
O6	862-57	Davis & Woodward (1957)	Brigden & Wilkinson (1985a); Brigden <i>et al.</i> (1985)	O6:K14
O7	843-57	Ewing <i>et al.</i> (1959)	Oxley & Wilkinson (1988c)	O6:K7
O8	1604-57	Ewing <i>et al.</i> (1959)	Oxley & Wilkinson (1986)	O8:K—
O9	4534-60	Ewing <i>et al.</i> (1959)	Oxley & Wilkinson (1987)	O26:K9
O10	1289-59	Sedlák <i>et al.</i> (1965)	Oxley & Wilkinson (1989c)	O10:K—
O11	1914-63	Ewing <i>et al.</i> (1962)	—	O—:K—
O12	6320-58	Ewing <i>et al.</i> (1962)	Brigden <i>et al.</i> (1985)	O14:K12
O13	3607-60	Ewing <i>et al.</i> (1962)	Oxley & Wilkinson (1988a)	O—:K13
O14	4444-60	Ewing <i>et al.</i> (1962)	Brigden & Wilkinson (1983, 1985a)	O14:K14
O15	4523-60	Ewing <i>et al.</i> (1962)	Oxley & Wilkinson (1988d, 1989a)	O26:K15
O16	IP687	Le Minor & Pigache (1978)	Oxley & Wilkinson (1989d)	O16:K—
O17	IP374	Le Minor & Pigache (1978)	Oxley & Wilkinson (1990b)	O19:K—
O18	IP333	Le Minor & Pigache (1978)	Oxley & Wilkinson (1989e, 1991d)	O18:K18
O19	IP451	Le Minor & Pigache (1978)	Oxley & Wilkinson (1990b)	O19:K—
O20	IP785	Le Minor & Pigache (1978)	Oxley & Wilkinson (1989d)	O20:K—
O21	IP1521	Le Minor & Sauvageot-Pigache (1981)	Oxley & Wilkinson (1991b)	O21:K—
O22	HOM SH59	Traub (1981)	Oxley & Wilkinson (1990c, 1992a)	O22:K22
O23	IP1160	Le Minor <i>et al.</i> (1983)	Oxley & Wilkinson (1990a, d)	O23:K23
O24	HOM US1	Traub (1985)	Oxley & Wilkinson (1989f)	O24:K—
O25	P12	Traub (1991)	Aucken <i>et al.</i> (1995)	O—:K—
O26	SB15	Traub (1991)	Aucken <i>et al.</i> (1995)	O26:K—
O27	S1254	Gaston & Pitt (1989b)	Oxley & Wilkinson (1991a)	O27:K—
O28	S3255	Gaston & Pitt (1989b)	Oxley & Wilkinson (1991c)	O28:K28
O29	S111	Holst <i>et al.</i> (1997)	Holst <i>et al.</i> (1997)	O29:K4

*O—, rough LPS; K—, acapsular.

these methods may allow the identification of virulent types, as well as investigations into the global epidemiology of a species.

Most serological typing schemes, including the 29 currently recognized *S. marcescens* O serotypes (see list of strain references, Table 1), were derived empirically, by dividing strain collections into groups based on shared agglutination reactions with their corresponding antisera and selecting one serotype reference strain for each group. However, the *S. marcescens* scheme has always suffered from cross-reactions which have proved difficult to resolve. Gaston & Pitt (1989a) suggested that this scheme must include non-LPS antigens, since SDS-PAGE and immunoblotting revealed that two of the reference strains are rough (i.e. lack O antigen) and several pairs of reference strains have the same LPS antigen. Chemical analysis of polymers present in 'LPS' extracts from the reference strains has shown that the

polysaccharide repeating units can be divided into neutral and acidic structures and that many of the reference strains possess both (Wilkinson, 1990). It has been suggested that the LPS O antigens correlate with the neutral polysaccharides (Gaston *et al.*, 1988; Gaston & Pitt, 1989a) while the acidic polysaccharides have now been shown to correspond to capsular antigens (Aucken *et al.*, 1997). The current study was undertaken to rationalize the existing O typing scheme and to propose a complementary capsular K typing scheme. We present here the immunochemical basis of these O and K antigen schemes for the serological typing of *S. marcescens*.

METHODS

Bacterial strains. The 29 reference strains were as listed in Table 1. For clarity, new serotype designations are printed in bold typescript in both text and tables. In addition, 423 clinical

Table 2. O antigen serotyping scheme for *S. marcescens* and adsorptions necessary to obtain serospecificity

Reference strain to which serum is raised	Serotype antigen	Serotype antibody to be adsorbed		Adsorbing strain
868-57	O2	O21	K2	IP1521 (O21:K-) + S117 (O19:K2)
864-57	O4	O27	K4	S1254 (O27:K-) + S1725 (O-:K4)
867-57	O5	-	K28	S3255 (O28:K28)
862-57	O6	-	K14	4444-60 (O14:K14)
1604-57	O8	-	-	4444-60 (O14:K14)*
1289-59	O10	O22	-	HOM SH59 (O22:K22)
4444-60	O14	-	K14	862-57 (O6:K14)
IP687†	O16	O20	-	IP785 (O20:K-)
IP333	O18	O29	K18	S111 (O29:K4) (O18-:K18 unavailable)
IP451	O19	O26	-	4534-60 (O26:K9)
IP785	O20	-	-	4444-60 (O14:K14)*
IP1521	O21	O2+O3	-	868-57 (O2:K2) + 863-57 (O2:K3)
HOM SH59	O22	O10	K22	1289-59 (O10:K-) + S2164 (O-:K22)
IP1160	O23	-	K23	S4093 (O28:K23)
HOM US1	O24	-	-	4444-60 (O14:K14)*
SB15	O26	O19	-	IP451 (O19:K-)
S1254	O27	O4	-	864-57 (O4:K4)
S3255-1‡	O28	-	-	None
S111	O29	O18	K4	IP333 (O18:K18) + 864-57 (O4:K4)

* Used as default adsorbing strain - could be omitted.

† Strain IP687 contains two neutral polysaccharides (Oxley & Wilkinson, 1989c).

‡ Acapsular variant.

strains were selected from over 3000 isolates sent to the Laboratory of Hospital Infection between 1 January 1980 and 30 June 1990 for epidemiological typing. One representative of each distinct strain within each incident of hospital infection was chosen using the O agglutination and phage typing results obtained at the time of initial investigation (Pitt *et al.*, 1980). An incident was defined as one or more isolates sent for typing from the same centre and isolates were considered to represent distinct strains if they differed in serotype or if they were of the same serotype but differed by at least three major reactions in their patterns of phage sensitivities. These criteria were more stringent than those normally recommended for phage typing (Pitt *et al.*, 1980) to reduce the likelihood of selecting isolates representing the same strain. Among these clinical strains were some that were subsequently selected for further adsorption of the typing sera. These were S117 (O19:K2), S1072 (O29:K4), S1725 (O-:K4), S2164 (O-:K22) and S4093 (O28:K23).

Phage-resistant acapsular variants of 11 of the 29 reference strains had been produced for a previous study (Aucken *et al.*, 1997). These variants were used here for adsorption of O antibodies from sera to the encapsulated reference strains to produce K-specific typing reagents. Prior to each use, each variant was tested both for phage resistance and for absence of capsule in the Quellung reaction and reselected with the appropriate phage when necessary.

All cultures were maintained on nutrient agar and stored at -20 °C on 2 mm glass beads in nutrient broth containing 20% (v/v) glycerol.

Polysaccharides. LPS, neutral polysaccharides and acidic polysaccharides from the serotype reference strains were prepared from cells grown in nutrient broth at 37 °C for 16 h. After preparation of cell walls (Brigden & Wilkinson, 1985a) and the extraction of lipids with chloroform/methanol (2:1, v/v), surface polysaccharides were isolated by treatment with hot aqueous phenol (Westphal & Jann, 1965). Reference strains O2, O3 and O21 were unusual in that the LPS remained in the phenolic phase rather than the aqueous phase (Oxley & Wilkinson, 1991b, 1992b). Lipid A was released from the purified LPS by mild acid hydrolysis and the water-soluble polymeric products were isolated by Sephadex G-50 chromatography. Where appropriate, neutral and acidic polysaccharides were separated by chromatography on DEAE-Sepharose CL-6B (Brigden & Wilkinson, 1985a). In most cases, their structures were determined by component analysis, NMR spectroscopy, methylation analysis and appropriate chemical degradations. Detailed information is presented in the polysaccharide references cited in Table 1.

Antisera. Rabbit antisera were raised against the reference strains as previously described using cells which had been either heated at 100 °C for 1 h or autoclaved at 121 °C for 15 min (Aucken *et al.*, 1996). Normal rabbit control serum (NRS) was collected by exsanguination of a non-immunized rabbit.

Serum adsorptions. The bacterial strains used for adsorption of the reference antisera were as listed in Tables 2 and 3. K-specific reagents were produced by adsorption of O antibodies using NA-grown cells, while O-specific reagents were pro-

Table 3. Proposed K antigen serotyping scheme for *S. marcescens*

Reference strain to which serum is raised	Serotype antigen	Serotype antibody to be adsorbed	Adsorbing strain
866-57	K1	O19	866-57-2* (O19:K-)
868-57	K2	O2	868-57-4* (O2:K-)
863-57	K3	O2	863-57-1* (O2:K-)
864-57	K4	O4	864-57-1* (O4:K-)
843-57	K7	O6	843-57-1* (O6:K-)
4534-60	K9	O26	4534-60-1* (O26:K-)
6320-58	K12	O14	4444-60 (O14:K14)
3607-60	K13	Rough LPS	1914-63 (O-:K11)
4444-60	K14	O14	6320-57 (O14:K12)
4523-60	K15	O26	4523-60-2* (O26:K-)
IP333	K18	O18	S1072 (O29:K4)
HOM SH59	K22	O22	1289-59 (O10:K-) in lieu of O22:K22-
IP1160	K23	O23	IP1160-2* (O23:K-)
S3255	K28	O28	S3255-1* (O28:K-)

* Acapsular variant of reference strain.

duced using growth from King's A agar (KA, King *et al.*, 1954) which contains glycerol and enhances capsule production (Aucken *et al.*, 1997). Overnight bacterial growth at 30 °C was harvested from four 15 cm plates into approximately 25 ml 0.4% formol saline. The cells were pelleted by centrifugation at 2500 *g* for 1 h and thoroughly resuspended in 4.5 ml PBS containing 0.02% Merthiolate as a preservative. After the addition of 0.5 ml of the serum to be adsorbed, the suspension was incubated at 37 °C for 2–3 h on a rotary mixer and then overnight at 4 °C. The suspension was centrifuged at 27 000 *g* for 1 h, filtered through a 2 µm membrane filter and stored at –20 °C.

ELISA. The ELISA for the detection of O antigens employed autoclaved whole cell cultures grown in tryptone soya broth (TSB) as the coating antigen and an alkaline phosphatase detection system as described previously (Aucken *et al.*, 1995). The optical densities were read at 405 nm using a Titertek ELISA plate-reader. This assay was modified for the detection of K antigen by the use of KA-grown cells exposed to 0.4% formol saline for approximately 1 h at room temperature as coating antigen (Aucken *et al.*, 1997). NRS at a dilution of 1 in 500 and PBS alone were used as negative controls, producing OD₄₀₅ readings of 0.3 and 0.15, respectively. Serum titres were recorded as the highest dilution giving a reading of 0.5 and were performed on three separate occasions. Working concentrations for each of the adsorbed sera were based on the titre, then optimized according to the pattern of positive and negative results seen with all reference strains. Assays using clinical strains were performed once only using serum panels at the previously established working dilutions. An individual test was considered positive when the OD₄₀₅ was ≥0.6. Tests on strains with negative or anomalous results were repeated and investigated by SDS-PAGE.

Quellung reaction. The Quellung reaction was seen when corresponding antiserum and bacterial cells were mixed on a microscope slide then viewed with phase-contrast illumination under a ×100 oil-immersion objective (Aucken *et al.*, 1995). A distinct grey halo around the short, dark bacilli indicated

recognition of capsule by the antiserum, since antibody binding produces a change in the refractive index of the capsular material, thus identifying the capsular serotype of the organism.

Cetavlon technique. Ørskov's method (Ørskov, 1976) for the indirect, non-immunological detection of acidic polysaccharides was as previously described (Aucken *et al.*, 1997). Briefly, cell suspensions of approximately 10⁹ c.f.u. ml⁻¹ were heated at 60 °C and subjected to horizontal electrophoresis, then allowed to diffuse towards a trough of 0.2% Cetavlon (hexadecyltrimethylammonium bromide, Sigma) which precipitates acidic but not neutral polysaccharides.

SDS-PAGE. The purified LPS extracts described above, or alternatively cruder extracts prepared using proteinase K (Hitchcock & Brown, 1983), were separated by SDS-PAGE and the profiles revealed either by silver staining (Aucken & Pitt, 1993) or by electrophoretic transfer to nitrocellulose paper and subsequent detection by immunoblotting (Aucken *et al.*, 1995).

RESULTS AND DISCUSSION

Antigen composition and structure

The chemical structures (or compositional data in some cases) established for the repeating units of polysaccharides isolated from the reference strains originally designated O1–O29 are summarized in Tables 4 and 5. The principal finding was that 14 strains produced two types of polymer: one polysaccharide made up of neutral sugars, the second polysaccharide containing an acidic residue. The remaining strains had neutral polysaccharides alone, except for reference strains O11 and O13 (which possessed only an acidic polymer) and O25 (from which no polymer was isolated). Six different neutral repeating unit structures were found in pairs of reference strains, namely O2 and O3, O6 and O7, O12 and O14,

Table 4. Neutral polysaccharide repeating units of *S. marcescens*

Reference strain (new serotype)	O antigen repeating unit*			
O16† (O16)	→ 2)-β-D-Ribf-(1 →			
O12 & O14 (O14)	→ 2)-β-D-Ribf-(1 → 4)-α-D-GalpNAc-(1 →			
O4 (O4)	α	OAc		
O27 (O27)	α	—		
O6 & O7 (O6)	β	OAc		
O1 (O19)	4	3	Glc	
O19 (O19)	3(4)	3	Glc	
O9 (O26)	3(4)	3	Gal	
O15 & O26 (O26)	4(3)	3	Gal	
O23 (O23)	4	4	Gal	
O5 (O5)	OAc	4	Glc (no z)	
O16† & O20 (O20)	—	3	Gal (no z)	
O24 (O24)	—	3	Gal (z = α-D-Galp)	
O2 & O3 (O2)	→ 4)-α-D-GlcpX-(1 → 4)-β-D-ManpNAc-(1 →			
O21 (O21)	NAc			
O28 (O28)	→ 3)-β-D-Manp-(1 → 2)-α-D-Manp-(1 → 2)-α-D-Manp-(1 →			
O10 (O10)	3	α-D-Glcp	3	NAc
O22 (O22)	3	—	3	NAc
O18 (O18)	2	—	6	NAc
O29 (O29)	2	—	6	—
O8 (O8)	β-D-GlcpNAc			
	1			
	↓			
	3			
	→ 6)-α-D-Glcp-(1 → 4)-α-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 →			

* Abbreviations: Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Hex, hexose; HexNAc, N-acetylhexosamine; Man, mannose; ManNAc, N-acetylmannosamine; Rha, rhamnose; Rib, ribose.

† Two neutral polysaccharides (O16 and O20) found in the O16 reference strain IP687.

Table 5. Acidic polysaccharide repeating units of *S. marcescens*

Reference strain (new serotype)	K antigen repeating unit*	
O1 (K1)	Pentasaccharide of Glc, Man, GlcNAc and GalA	+ OAc
O2 (K2)	$\rightarrow 3\text{-}\alpha\text{-D-Rhap}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-Galp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Rhap}\text{-(1}\rightarrow 4\text{)-}\alpha\text{-D-GalpA}\text{-(1}\rightarrow$	+ OAc
O3 (K3)	$\begin{array}{c} \beta\text{-D-Manp} \\ 1 \\ \downarrow \\ 4 \end{array}$ $\rightarrow 3\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 2\text{)-}\alpha\text{-D-GlcpA}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Manp}\text{-(1}\rightarrow$	+ OAc
O4 (K4)	$\begin{array}{c} \text{PyrA} \\ \wedge \\ 4\ 6 \\ \alpha\text{-D-Galp} \\ 1 \\ \downarrow \\ 3 \end{array}$ $\rightarrow 3\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-Galp}\text{-(1}\rightarrow 2\text{)-}\alpha\text{-D-Manp}\text{-(1}\rightarrow$	+ OAc
O5 (K28) O28 (K28) O23a (K23) O23b (K23)	<p>Hexasaccharide of Glc, Man, GlcA and an amino sugar</p> <p>Possibly O23 backbone plus the O5 amino sugar linked to GlcA</p> $\rightarrow 3\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 2\text{)-}\alpha\text{-D-GlcpA}\text{-(1}\rightarrow$ $\rightarrow 3\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 2\text{)-}\alpha\text{-D-GlcpA}\text{-(1}\rightarrow$ $\begin{array}{c} \uparrow \\ 6 \\ \text{L-Glu} \end{array}$	+ OAc
O6 & O14 (K14)	$\begin{array}{c} \alpha\text{-D-GlcpA} \\ 1 \\ \downarrow \\ 4 \end{array}$ $\rightarrow 4\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Glcp}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-Glcp}\text{-(1}\rightarrow$	+ OAc
O7 (K7)	Hexasaccharide of Glc, Man, Rha, Gal and GlcA	+ OAc
O11 (none)	Complex oligosaccharide of Man, Gal, Rha and GalA	
O12 (K12)	Tetrasaccharide of Glc, Man, Gal and GlcA	
O13 (K13)	$\begin{array}{c} \beta\text{-D-GlcpNAc} \\ 1 \\ \downarrow \\ 4 \end{array}$ $\rightarrow 3\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-Glcp}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-GlcpA}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 6\text{)-}\alpha\text{-D-Manp}\text{-(1}\rightarrow$	
O15 (K15)	$\begin{array}{c} \alpha\text{-D-Manp} \\ 1 \\ \downarrow \\ 4 \end{array}$ $\rightarrow 3\text{-}\beta\text{-D-Manp}\text{-(1}\rightarrow 3\text{)-}\beta\text{-D-Glcp}\text{-(1}\rightarrow 2\text{)-}\alpha\text{-D-GalpA}\text{-(1}\rightarrow 3\text{)-}\alpha\text{-D-Manp}\text{-(1}\rightarrow$	

Table 5. (cont.)

Reference strain (new serotype)	K antigen repeating unit*
O18 (K18)	$\begin{array}{c} \rightarrow 3)\text{-}\beta\text{-D-Galp}\text{-(1}\rightarrow 4)\text{-}\alpha\text{-D-Manp}\text{-(1}\rightarrow 3)\text{-}\alpha\text{-D-Galp}\text{-(1}\rightarrow \\ 3 \\ \uparrow \\ 1 \\ \alpha\text{-D-GlcpA} \\ 4 \\ \uparrow \\ 1 \\ \alpha\text{-L-Rhap} \\ 4 \\ \uparrow \\ 1 \\ 4\text{-O-lactyl-}\beta\text{-D-Glcp} \end{array}$
O22 (K22)	$\begin{array}{c} \beta\text{-D-Glcp} \\ 1 \\ \downarrow \\ 6 \\ \rightarrow 3)\text{-}\beta\text{-D-Galp}\text{-(1}\rightarrow 3)\text{-}\alpha\text{-D-Galp}\text{-(1}\rightarrow 6)\text{-}\beta\text{-D-Galf}\text{-(1}\rightarrow \\ 4 \\ \uparrow \\ 1 \\ \beta\text{-D-GlcpA} \\ 4 \\ \uparrow \\ 1 \\ \alpha\text{-L-Rhap} \\ 2 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glcp} \end{array}$

* Abbreviations: Gal, galactose; GalA, galacturonic acid; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Glu, glutamic acid; Man, mannose; PyrA, pyruvic acid; Rha, rhamnose.

O15 and O26, O16 and O20, and O17 and O19. The serotype heterogeneity of the first four pairs can be explained by their acidic polysaccharides, which are different for the first three pairs and absent from O26. Reference strain O16 possesses a second neutral polymer that differentiates it from strain O20. However, as no other polysaccharides were obtained from reference strains O17 and O19, some other kind of epitope must be responsible for differentiating these strains.

There were also many neutral polysaccharides sharing very similar structures. Thus, the repeating unit in reference strains O2 and O3 differs from that in strain O21, as do those in reference strains O18 and O29, by the presence of *N*-acetylglucosamine rather than glucose, while the four neutral polymers in reference strains O1, O17 and O19, O9, and O15 and O26 all consist of a (1 → 3)- or (1 → 4)-linked α -L-rhamnose residue and (1 → 3)-linked *N*-acetyl- β -D-glucosamine or *N*-acetyl- β -D-galactosamine. The polysaccharides in O27 and O4 are

even more similar in that the neutral polymer of the latter is an *O*-acetylated version of the former. In contrast, the acidic structures are all unique to individual reference strains, with the single exception of the glucomannan in O6 and O14. Although structural data are incomplete, the only similarities observed were the backbone structures of reference strains O23 and O28 and the sharing of an amino sugar side-chain by reference strains O5 and O28. In addition to their acidity and uniqueness, these polysaccharides differ from their neutral counterparts in size and complexity, as well as in the possession of some unusual sugars. For a more detailed review of the chemistry of these polysaccharides, see Wilkinson (1990).

Visualization of LPS

The SDS-PAGE ladder patterns produced by purified LPS from the reference strains are shown in Fig. 1 (except those of strains O25, O26 and O29) and were

Fig. 1. LPS profiles of the O serotype reference strains of *S. marcescens*.

Table 6. Effect of different *S. marcescens* antigens and serum adsorptions in ELISA

Tests were performed in duplicate and the mean OD₄₀₅ coded as >0.9 = 3, >0.6 = 2, >0.4 = 1; – no reaction; ads, adsorbed with.

Original serotype (new serotype)	TSB antigen (O-ELISA) Serum					KA antigen (K-ELISA) Serum				
	O6 adsO14	O14 adsO6	O7 ads*	O14 adsO12	O12 adsO14	O6 adsO14	O14 adsO6	O7 ads*	O14 adsO12	O12 adsO14
O6 (O6:K14)	3	1	3	–	–	2	1	–	2	–
O7 (O6:K7)	3	–	3	–	–	2	–	3	–	–
O12 (O14:K12)	–	3	–	1	3	–	–	–	–	3
O14 (O14:K14)	–	3	–	1	1	2	2	–	3	–
Antigens detected	O6	O14	O6	O14	O14	O6 + K14	K14	K7	K14	K12

* 843-57-1, acapsular variant of reference strain 843-57 (O7:K7).

indistinguishable from those of proteinase K extracts (not shown) in all cases. This included the reference strains O2, O3 and O21, for which LPS had been recovered from the phenolic phase of the Westphal extraction. All strains from which a neutral polysaccharide was isolated showed characteristic O antigen ladders of smooth LPS. The three reference strains deficient in neutral polysaccharide, O11, O13 and O25, contained only rough LPS. Reference strains possessing the same neutral polysaccharide showed identical or very similar profiles, namely O2 and O3, O6 and O7, O12 and O14, O15 and O26, O16 and O20, and O17 and O19. These results were confirmed by immunoblotting. Unadsorbed antisera to reference strains O11, O13 and

O25 revealed only rough LPS profiles while identical profiles were obtained for each of the six pairs above when antiserum to either one of the pair was used for detection. Serum raised against strains sharing identical or related acidic polysaccharides either failed to produce ladder profiles (e.g. serum O14 with reference strain O6, serum O28 with strains O5 and O23) or produced a profile different from that of the homologous strain (e.g. serum O6 on reference strain O14, serum O5 on O28).

O and K antigens in ELISA

Assays were required which could be used for the identification of O and K antigens in a routine typing

laboratory, thus it was necessary to use crude antigen extracts as coating antigens to obviate the need for costly and time-consuming polysaccharide purification of clinical isolates. Reference strains O6, O7, O12 and O14 have only two O antigens and three K antigens between them and were therefore used to develop O- and K-specific ELISAs. The coating antigens were, respectively, whole cells grown in tryptone soya broth then autoclaved to reduce the amount of capsular material present (Gaston *et al.*, 1988) and formolized whole cells grown on KA to maximize capsule production. Cross-adsorptions of the four sera to eliminate either anti-O or anti-K antibodies were carried out using the reference strains with matching O or K antigens, respectively, except for the anti-K7 reagent which was prepared by adsorption with an acapsular variant (843-57-1) of the O7 reference strain. The resulting reagents were tested in both ELISAs and the results are shown in Table 6. They indicate that both appropriate adsorption and an appropriate coating antigen are required for specific antigen detection. For example, the system which best detected the O antigen of reference strains O12 (O14:K12) and O14 (O14:K14) was serum O14 adsorbed with reference strain O6 (O6:K14) in the O-ELISA. Detection of the K14 antigen with this serum required adsorption with reference strain O12 (O14:K12) and use of the K-ELISA.

O antigen typing scheme

Use of unadsorbed sera in the O-ELISA produced numerous cross-reactions (Table 7), many of which could be ascribed to shared polysaccharides. To produce specific reagents for O serotyping based on the neutral polysaccharide structures alone, all O antisera were adsorbed with antigens chosen to minimize the cross-reactions predicted from their chemical structures. These sera were tested at their working concentrations against all reference strains in the O-ELISA. None of them gave non-specific background readings and, other than those due to shared O antigens, there was only one significant cross-reaction: serum O14 with reference strain O23 (O23:K23) which was not investigated further. These reagents were likewise shown to be O-antigen-specific by immunoblotting.

Preliminary experiments suggested that purified neutral and acidic polysaccharides did not bind well to ELISA plates so, unfortunately, could not be used in these adsorbed antisera assays to demonstrate directly the correlation of chemical structure with serological specificity. The separated polysaccharides were used in a number of immunoassays, namely inhibition of purified LPS-mediated indirect haemagglutination, double diffusion and indirect capture ELISA, in an attempt to obtain such data but with only partial success. For each assay, those positive results which were obtained could be easily explained by reference to the chemical structures. For example, the 12N (neutral polysaccharide from reference strain O12), 14N and 14A polysaccharides reacted with immunoglobulin purified from (unadsorbed) O14 antiserum in the capture ELISA,

whereas 7N, 7A and 12A did not (6N and 6A were not available). However, many reactions predicted by chemistry did not occur, e.g. the immunoglobulin purified from (unadsorbed) O12 antiserum failed to bind any of the separated polysaccharides, although strong positive reactions were seen with the purified LPS from reference strains O12 and O14, but not with O6 or O7. Further work is needed in this area but it is possible that removal of lipid A from the polysaccharides alters their conformation so that they are less immunoreactive.

The O-ELISA was used to determine the O serotypes of 423 clinical strains to assess assay specificity: serotype frequencies will be published elsewhere. Representatives of all serotypes were tested by immunoblotting to confirm their O antigen and to check serotype homogeneity. Seventy-nine cross-reactions occurred but 36 of these were weak and did not prevent the correct identification of O serotype. Thirty-three strains reacted with either the O4, O23 or O28 reagents in addition to those identifying their true O serotypes. These reactions were shown to be due to capsular antigens and could be eliminated by the use of alternative reagents (see below). Eight strains reacted with both the O16 and O20 reagents but as immunoblotting confirmed that they possessed both antigens, as does the O16 reference strain, this cross-reaction must be considered justified. The remaining two strains reacted with both O4 and O14 reagents and were identified as O4:K14 strains. Since the other 253 K14 strains did not show this cross-reaction, which could in any case be readily investigated by Quellung, modification of the O14 reagent was considered unnecessary. Thus the final O antigen adsorption scheme, as shown in Table 2, produced only two (0.5%) non-specific results among the 423 strains.

Taken together, the chemical and serological results delineated eight structurally and serologically related groups of O serotypes, which are described in more detail below.

Group I

Each of the seven reference strains in this group has a disaccharide repeating unit consisting of a rhamnose residue with N-acetylglucosamine (O1, O17 and O19) or N-acetylgalactosamine (O9, O15, O23, O26). Strain O23 was the only member of the group to produce a clearly distinct LPS profile (Fig. 1) and no serological cross-reactions among the other type strains in the group by either ELISA or immunoblotting with unadsorbed sera. However, serum O23 required adsorption with an O28:K23 clinical strain, S4093, to eliminate cross-reacting K23 antibodies.

Reference strains O9, O15 and O26 possess two linkage variants of the same disaccharide in different proportions; in O9 the (1 → 3)-linked rhamnose-N-acetylgalactosamine predominates while in strains O15 and O26 the (1 → 4)-linked disaccharide is the major form. Consequently, it was not surprising that cross-reactions between these three strains occurred and that their LPS

Table 7. Serological reactions of the 29 O serotype reference strains of *S. marcescens* in the O-ELISA

Tests were performed on three separate occasions and the mean OD coded as $>0.9=3$, $>0.6=2$, $>0.4=1$. Results underlined are those obtained using the adsorbed sera listed in Table 3.

Strain	Serum													
	O1	O17	O19	O9	O15	O26	O23	O2	O3	O21	O4	O27	O5	O28
O1	3	2	2 <u>2</u>	1										
O17		3	3 <u>2</u>											
O19	3	3	3 <u>3</u>		1						<u>1</u>			
O9		1	1	3		3 <u>2</u>								
O15		1	1	2	3	3 <u>3</u>								
O26			1	2	2	3 <u>3</u>								
O23							3 <u>3</u>							
O2								3 <u>3</u>	3					
O3								3 <u>3</u>	3	1	2			
O21								1	1	3 <u>3</u>				
O4	2										3 <u>3</u>	3		
O21											2	3 <u>3</u>		
O5													3 <u>3</u>	3
O28													3	3 <u>3</u>
O16											2			
O20														
O24														
O6														
O7								<u>1</u>		<u>1</u>				
O12														
O14														
O8														
O10														
O22														
O18														
O29														
O11											3			
O13														
O25														

profiles were indistinguishable by silver staining and immunoblotting. Thus, no attempt was made to differentiate between them in the revised typing scheme. Cross-reactions occurred between all three of these strains when tested with unadsorbed O17 and O19 sera, although the reverse was much less common. Strains O17 and O19 have *N*-acetylglucosamine as their second sugar, not *N*-acetylgalactosamine, but this difference is presumably less significant immunologically than, for example, the second (1 → 4)-linkage of O23. The O26 reference strain was chosen to represent this serotype since it is acapsular and the O26 antiserum was adsorbed with O19 to remove cross-reactions. In both ELISA and immunoblotting, this reagent was shown to be specific for strains O9, O15 and O26.

The neutral polysaccharides of O17 and O19 are identical, as were their SDS-PAGE profiles, and there were strong reciprocal cross-reactions between them. The only difference between this neutral polysaccharide and that of O1 was the absence from the latter of any (1 → 3)-linked rhamnose. This difference proved to be insufficient to allow complete serological differentiation of these strains and as only five representatives were found among the clinical strains, they were considered as one, with the non-capsular O19 as the serotype strain and O9 as the adsorbing strain.

Group II

Reference strains O2 and O3 have the same neutral disaccharide repeating unit which differs from that of O21 only in its possession of an *N*-acetyl group. It was anticipated, therefore, that cross-reactions would occur among these three strains. However, although reference strains O2 and O3 showed strong reciprocal reactions, the reactions with unadsorbed serum O21 were generally weaker, suggesting that they are serologically distinguished by the *N*-acetyl group. This was borne out with adsorbed antisera; serum O2 adsorbed with reference strain O21 detected only the O antigen of reference strains O2 and O3 in ELISA and immunoblotting, while serum O21 adsorbed with reference strains O2 and O3 reacted only with strain O21, as shown in Fig. 2. Furthermore, the O21 LPS profile was significantly different from that of O2 and O3. Serum O21 did not require further adsorption since the reference strain is acapsular, but serum O2 was improved by adsorption with S117, an O19:K2 clinical strain, to remove anti-K2 antibodies.

Group III

As was shown in the study describing the O27 serotype strain (Aucken *et al.*, 1996), the neutral polysaccharides

Table 7. (cont.)

Serum															
O16	O20	O24	O6	O7	O12	O14	O8	O10	O22	O18	O29	O11	O13	O25	
2														3	
														3	
2				2		3						2			
												2			
												2			
3	3	3										2		3	
3		3										2			
1			3	3											
			3	3	3			3							
			3	3	3										
					3	3	3								
			2		3	3	3								
							3	3				2			
								3	3	1					
								3		3	3				
										3	3	2			
										2		3	3		
3												3	1	3	
												2	3	3	
														3	

of O4 and O27 differ only slightly in chemical structure: the O4 disaccharide is O-acetylated. In addition, they differ from that of reference strains O6 and O7 in having an α - rather than β -linked rhamnose. Immunologically, the difference between the α and β linkages seems to be highly significant, since no unadsorbed serum cross-reactions were found between reference strains O4 or O27 and either O6 or O7. The repeating unit structures of O4 and O27 differed only in an O-acetyl group, present on the former but not the latter, yet their cross-reactions could be eliminated completely with adsorbed sera in both O-ELISA and immunoblotting. The silver-stained LPS profiles of reference strains O4 and O27 were correspondingly similar but not identical (Fig. 1). Immunoblotting of clinical strains showed that the O4 serum also required adsorption with the O-:K4 clinical strain, S1725, before it was truly O-specific (Aucken *et al.*, 1996) but once this was done there were no difficulties in assigning the two types within the collection of *S. marcescens* strains.

Group IV

The O antigen of reference strain O24 contains the same disaccharide backbone as that found in the repeating unit shared by reference strains O16 and O20 but differs in having an extra galactose residue so that its repeating

unit is a branched trisaccharide. This results in a tertiary structure with sufficient differences to prevent marked serological cross-reactions. Weak reactions were noted only between serum O16 and strain O24 in the unadsorbed serum O-ELISA and the latter may well have been non-specific, given that serum O16 produced high background readings in this assay. Since the O24 reference strain is acapsular and serum O24 did not cross-react with any of the reference strains, it could be used without adsorption. However, for consistency, O14 was used as an adsorbing strain when no others were required. As with other groups of strains, minimal serological cross-reaction was correlated with differences in their LPS profiles. Thus, although the SDS-PAGE ladders of reference strains O16 and O20 were relatively similar, that of the non-cross-reacting O24 was clearly distinguishable. Strains O16 and O20 have the same disaccharide repeating unit, but O16 also produces a second neutral polysaccharide with a monomeric repeating unit (Table 4). As expected, therefore, these two strains showed strong cross-reactions in ELISA and when immunoblotted with unadsorbed antisera. Adsorption of serum O16 with reference strain O20 generated a specific reagent, while strain O14 was used with serum O20. Interestingly, both unadsorbed and adsorbed O20 sera revealed a ladder pattern when

Fig. 2. Immunoblotting results of the O2 (O2:K2), O3 (O2:K3) and O21 (O21:K-) serotype reference strains of *S. marcescens* with (a) serum O2, (b) serum O3, (c) serum O21, (d) serum O2 adsorbed with O21 and (e) serum O21 adsorbed with O2 and O3.

used to immunoblot strain O28. The explanation for this reaction remains unclear since the O-antigenic repeating unit of O28 is mannose trisaccharide, whereas that of O20 is a galactose disaccharide. However, since this cross-reaction did not occur in the O-ELISA, it did not give any problems for serotyping. Both types were rare in the clinical collection and no strains were found with the O16 antigen alone. Consequently, for the purposes of routine epidemiological typing, it would seem both economical and practical to use unadsorbed O16 serum for the detection of O16 and O20 antigens as a combined O16/O20 serotype.

Despite the similarities between the O16/O20 and O5 neutral polysaccharides, no cross-reactions occurred between them. The LPS profile of reference strain O5 was slightly different from others in this group (Fig. 1). Marked cross-reactions did occur, however, between reference strains O5 and O28. This cross-reaction was investigated in a previous study (Aucken *et al.*, 1996) and shown to be due to similarities in their acidic capsular polysaccharides. Both O5 and O28 sera could be made specific in immunoblotting by cross-adsorption, although serum O28 adsorbed with strain O5 continued to react with strain O5 in the ELISA. Adsorptions of the original serum with a variety of non-O28:K28 strains also failed to produce an O28-specific reagent, suggesting that cross-reacting K antibodies still remained after adsorption. Sera raised to the acapsular variants or clinical strains used in the K adsorption scheme would circumvent the problem of K antibody adsorption and this approach has now been successfully used to prepare a new rabbit antiserum which is a fully specific O28 serotyping reagent.

Group V

Only two neutral polysaccharides have been identified among reference strains O6, O7, O12 and O14: one shared by O6 and O7 and the other by O12 and O14. In addition, strains O6 and O14 share the same acidic polysaccharide, whereas O7 and O12 have different capsular polysaccharides. The SDS-PAGE results correlated with neutral polysaccharide structures, with O6 and O7 having the same LPS profile, which was clearly distinct from that shared by O12 and O14 (Fig. 1). Unadsorbed serum ELISA results accurately reflected the distribution of these structures; reciprocal cross-reactions were seen between reference strains O6 and O7 as well as between O12 and O14. Furthermore, serum O6 reacted with reference strain O14. However, some slightly anomalous results were obtained by immunoblotting. Although those strains with identical neutral polysaccharide structures clearly had the same LPS profile, a ladder pattern was also developed by serum O6 on strain O14 and even more surprisingly on strain O12. Adsorption of serum O6 with strain O14 and vice versa eliminated these reactions in both immunoblotting and ELISA, apart from the two O4:K14 clinical strains mentioned above which reacted with the O14 as well as the O4 reagent.

Group VI

The detection of the O8 antigen posed no problems, since the reference strain is acapsular and the neutral polysaccharide has a unique SDS-PAGE profile. This polymer has also been found together with the acidic glucorhamnan of reference strains O6 and O14 (see below) in a strain previously considered to be 'O14' (Brigden & Wilkinson, 1985b).

Group VII

The repeating unit structures of reference strains O10, O18, O22 and O29 are all based on a backbone of one glucose or *N*-acetylglucosamine and three rhamnose residues. In strains O10 and O22, these sugars are joined by two (1 → 2)- and two (1 → 3)-linkages, whereas O18 and O29 have three (1 → 2)- and one (1 → 6)-linkage instead. The neutral polysaccharide of reference strain O10 differs from that of O22 in having an additional, lateral glucose residue, while O29 has a glucose residue rather than the *N*-acetylglucosamine of O18. Results with the unadsorbed antisera showed that the differences in linkages seem to have more influence on tertiary structure than does the presence of the side-group. Not only were the profiles of reference strains O10 and O22 very similar in SDS-PAGE and that of O18 and O29 quite different, but cross-reactions in ELISA and immunoblotting only occurred within and not between each pair.

Adsorption of serum O22 with both reference strain O10 and the O-:K22 clinical strain, S2164, produced an O-specific reagent, but adsorption of serum O10 with strain O22 still left some residual cross-reactivity with

both reference strain O22 and the clinical O22 strains. The significant structural difference between the two antigens was considered sufficient to justify their inclusion in the scheme as distinct types, but, since both types were rare in the clinical collection, for routine epidemiological typing it seems reasonable to detect them together as O10/O22 using unadsorbed O10 serum.

Unfortunately, no O18-negative but K18-positive strain was identified among the clinical collection with which to render serum O18 O-specific by adsorption of K18 antibodies. At the time the clinical collection was tested, reference strain O29 had not been described and three 'O18' strains were found and confirmed by immunoblotting. Further testing by ELISA and immunoblotting with cross-adsorbed O18 and O29 sera showed that only one of these strains was O18, as were two of nine identified subsequently. The others were all O29. Thus, as has been suggested before with infrequent serotypes, the requirements of normal epidemiological typing would be satisfied by detection of a combined O18/O29 antigen using O29 serum adsorbed with reference strain O4 to remove K4 antibodies.

Group VIII

Chemical analysis failed to detect any neutral polysaccharide in reference strains O11, O13 and O25 and they were shown by SDS-PAGE to be rough strains lacking O antigen. Both whole-cell ELISA and immunoblotting with unadsorbed O11, O13 and O26 sera revealed cross-reactions between these three strains as well as with other reference strains. Immunoblotting confirmed that these were due to core or lipid A determinants on rough LPS. All three were non-typable in the O typing ELISA as they were negative with all the adsorbed antisera.

K typing scheme

An earlier study had identified 14 different K antigens among serotype strains O1–O28 of *S. marcescens* (Aucken *et al.*, 1997). Twelve of these were each found in only one of the serotype strains and cross-reacting O antibodies were adsorbed from the corresponding sera using either acapsular variants of the homologous strains or heterologous strains of the same O type (Table 3). Thus, adsorbed antisera to 8 of the 12 antigens were prepared using the phage-resistant acapsular variants described previously (Aucken *et al.*, 1997) which had been grown on NA to minimize any residual capsule production. Serum O12 was adsorbed by reference strain O14 since these two strains have identical O antigen structures, while serum O22 was adsorbed with strain O10 as an O22:non-K22 strain was not available and the O antigen repeating units of reference strains O10 and O22 are serologically very similar. Serum O18 was adsorbed with an O29:K4 clinical strain, S1072, as no O18:non-K18 was available, while serum O13 was adsorbed with strain O11 to remove rough LPS antibodies.

The O6 and O14 reference strains had chemically and serologically indistinguishable capsules and O14 was chosen as the K serotype strain since historically this has been the most frequent serotype among clinical *S. marcescens* isolates (Gaston & Pitt, 1989b; Le Minor & Pigache, 1978; Pitt *et al.*, 1980; Rubin *et al.*, 1976). A K-specific typing reagent was prepared by adsorption of serum O14 with strain O12. The fourteenth antigen is represented in the scheme by reference strain O28 (O28:K28). Although the acidic polysaccharide repeating units of the O5 and O28 reference strains are chemically distinct, it proved impossible to distinguish them serologically (Aucken *et al.*, 1997), suggesting their shared amino sugar side-group is an immunodominant determinant. A putative fifteenth K antigen was the acidic polysaccharide identified in reference strain O11. However, because it has not been possible to detect a capsule around this strain either directly with Indian ink or Quellung reaction, or indirectly by precipitation with Cetavlon, it has been excluded from the scheme (Aucken *et al.*, 1997).

In the K-ELISA, each of the 14 homologous reactions was distinct and gave acceptable OD levels, as did all the cross-reactions predicted from the chemical and immunological analyses, namely those between the two pairs of reference strains, O6 and O14 plus O5 and O28. There were 11 unforeseen cross-reactions but as all were weak and tended to be associated with individual strains producing higher background OD levels with all sera (0.3 rather than 0.15), they were not expected to interfere with K type determination.

K typing ELISA plates were prepared from the 423 clinical strains and tested with the adsorbed K sera. All weak results and cross-reactions were checked with the Quellung test and so were all non-K14 results. Five strains (1%) produced cross-reactions which fell into two groups. One group consisted of three strains which reacted with both K22 and K23 reagents with or without an additional K13 reaction. Quellung tests showed strong reactions with reagent K22 in two cases and K13 in the third, although faint, wide halos were seen with the other reagents. There is no obvious structural similarity among these three K antigens and the reason for the cross-reaction remains obscure. The second group of cross-reactions involved two strains which reacted with both the K3 and K15 reagents. Quellung tests identified them as K3 strains, although again, faint, wide halos could be seen with the second serum. Since K3 was one of the more frequent serotypes, this cross-reaction was investigated further. Fourteen other K3 strains were tested and all but three produced faint but clear halos in the Quellung reaction with reagent K15, including the O3 (O2:K3) reference strain. The O15 (O15:K15) strain gave a negative Quellung reaction with the K3 reagent, whereas faint halos were seen with the two K15 clinical strains. The K3 and K15 antigens have similar sugars in a tetrasaccharide repeating unit with a mannose side-chain. However, there are many differences in the linkages which could account for the serological distinction. Structural analysis of the poly-

saccharides of the clinical strains would be required to test this hypothesis.

Conclusions

Table 1 lists the 29 reference strains so far described as O serotype strains, together with their new serotype designations, each of which now represents a chemically defined repeating unit structure. Nineteen strains have been retained as serotype strains in the revised O typing scheme (Table 2) while 14 strains make up the K typing scheme (Table 3). Seven reference strains appear in both lists which leaves three strains not appearing in either of the new typing schemes; these are O11, O17 and O25. Reference strain O11 is an atypical strain of *S. marcescens*. It grows abnormally slowly and the individual cells are abnormally long, suggestive of some defect in the cell division process. It is possible that the capsule is present but in quantities too small to be detected by light microscopy. Alternatively, it could be a strain which bears the enterobacterial common antigen in an immunogenic state, although there is no evidence of its presence in any of the isolated polysaccharides. Reference strain O17 is acapsular and shares an O antigen with strain O19 which was chosen as the serotype representative, while strain O25 is both acapsular and O-antigen-deficient, suggesting that other determinants must be responsible for the original serotype specificities of these two strains.

Both O and K typing schemes have been in routine use at the Central Public Health Laboratory for over 4 years, allowing confident allocation of serotypes in the majority of cases. Cross-reactions are rare and when they do occur can usually be resolved using the Quellung reaction.

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