A survey of potential virulence factors in clinical and environmental isolates of *Serratia marcescens*

S. P. FRANCKEK, R. P. WILLIAMS, and S. I. HULL

Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030, USA

Summary. One hundred and forty seven isolates of *Serratia marcescens* were collected from diverse clinical and environmental sources in south-east Texas. Natural isolates were compared with hospital strains for the occurrence of 12 potential virulence determinants. Their overall frequency was as follows: haemolytic activity 48%; lecithinase 95%; lipase 95%; motility 99%; pigmentation 24%; plasmid carriage 46%; proteolytic activity 98%; siderophore activity 99%; urease activity 5%; mannose-sensitive haemagglutination 96%; mannose-resistant haemagglutination 61%; and mannose-resistant type-K haemagglutination (MR/K-HA) 68%. Clinical strains demonstrated a significantly higher occurrence of MR/K-HA ($p < 0.001$) and non-pigmentation ($p < 0.01$) than environmental isolates.

Introduction

Serratia marcescens, long regarded as an innocuous saprophyte, is an opportunist pathogen of rising nosocomial significance. Its pathogenicity for man was first noted in 1913 in a pulmonary infection (Woodward and Clarke, 1913), but its prevalence in human disease has been underestimated for years (Ball *et al.*, 1977; Tabaqchali *et al.*, 1977). Failure to recognise non-pigmented strains that constitute a majority of clinical isolates has contributed to this inaccurate assessment (Fulton *et al.*, 1959). In a study by the Diagnostic Laboratory of the Massachusetts Department of Public Health, a typical non-pigmented strain was incorrectly identified as *Enterobacter*, *Klebsiella* or several other genera by 60% of the 121 laboratories surveyed (Wilfert *et al.*, 1970). Misconceptions of the non-pathogenic nature of *S. marcescens* also may be attributed to its variable potential as an aetiological agent of disease. Used as a harmless indicator strain in early studies of bacteraemia following dental extractions, *S. marcescens* was applied to marginal gingiva before operative trauma and subsequently cultured from the blood (McEntegart and Porterfield, 1949). This indiscriminate use of *S. marcescens* is of questionable safety because more recent evidence indicates that the mortality rate from *serratia* septicaemia is comparable to that from other gram-negative bac-

teria (Davis *et al.*, 1970; Wilkowske *et al.*, 1970). *Serratia* infections are typically nosocomial, affecting compromised patients receiving broad-spectrum antibiotic therapy and often with indwelling urinary catheters, endotracheal tubes or other foreign bodies (Wilfert *et al.*, 1970; Ball *et al.*, 1977). New evidence from ophthalmic infections, however, indicates that even healthy contact-lens wearers may be at risk of *serratia* keratitis (Cooper and Constable, 1977; Lass *et al.*, 1981). Improved techniques for the selective isolation, identification, and epidemiological characterisation of *Serratia* have aided health-care laboratories in more accurately recognising the occurrence and capacity for disease of this opportunist pathogen.

S. marcescens possesses several traits that are known to be virulence determinants in other genera. Amongst uropathogenic factors, haemolytic activity and piliation are important in *Escherichia coli* (Welch *et al.*, 1981; Hagberg *et al.*, 1983), as are urease activity and motility in *Proteus mirabilis* (Pazin and Braude, 1974; Parsons *et al.*, 1984). Lipase and lecithinase activity, commonly found in *S. marcescens*, are known to contribute to the virulence of *Staphylococcus aureus* and *Clostridium perfringens* respectively (Duncan, 1975; Willett, 1980). In *Pseudomonas aeruginosa* the pyoverdinin pigment exhibits siderophore activity that may be related to pathogenicity as are the iron-binding catechols of *E. coli* (Rogers, 1973; Cox and Adams, 1985). Some plasmids encode virulence determi-

nants; Smith and Huggins (1976) correlated the presence of the ColV plasmid with increased pathogenicity in invasive *E. coli* strains. To date, however, only antibiotic resistance and proteolytic activity have been shown to be virulence factors in *Serratia* infections (Hedges *et al.*, 1975; Lyerly and Kreger, 1983).

Not all species of *Serratia* are isolated from man as commonly as others; most clinical isolates are *S. marcescens* with some biotypes predominating (Johnson and Ellner, 1974; Farmer *et al.*, 1976; Grimont and Grimont, 1978). These studies suggest that pathogenic strains represent a select subpopulation of *Serratia* with special virulence properties. To test this hypothesis, 12 virulence-associated traits were surveyed in a collection of clinical and environmental isolates from widely diverse sources but all located within a limited geographical area. All of the determinants were assessed for statistical correlation with plasmid carriage and site of isolation. Demonstration of increased occurrence or plasmid carriage of these factors would provide impetus and valuable insight for future work detailing the pathogenic mechanisms important in *Serratia* infections.

Materials and methods

Isolation and identification of *Serratia* isolates

One hundred and forty seven isolates of *S. marcescens* were collected over an 18-month period from south-east Texas. Clinical isolates were from patients from four hospitals and a private doctor's office in the Texas Medical Center, Houston, Texas. Species identification was confirmed by the Enterotube II system (Roche Diagnostic Systems, Nutley, NJ, USA), deoxyribonuclease (DNAase) activity, and growth on minimal media with caprylate, arabinose or xylose as the sole source of carbon (Grimont *et al.*, 1977). The site of isolation was noted as respiratory, wound, urinary, blood or other (table I). In cases of duplicated cultures from the same patient, only the earliest isolated strain was kept.

Environmental isolates were obtained by screening widely diverse biotic specimens as well as water and soil samples from several urban and rural locations in the greater Houston vicinity; the hospital environment, however, was specifically excluded. Environmental specimens were disrupted and mixed vigorously in Nutrient Broth (Difco, Detroit, MI, USA), incubated at 15 °C for 3–5 days, washed with phosphate-buffered saline (PBS) (Curtiss, 1965) on 0.45 µm membrane filters (Millipore) and inoculated on to Caprylate-Thallos (CT) Agar (Starr *et al.*, 1976). Plates were incubated at 30 °C for 1 week, and colonies were streaked for isolation onto DNAase test agar with methyl green (Difco) and incubated for 24 or 48 h at 30 °C. Genus and species determinations of DNAase-positive colonies were made as mentioned above with the Enterotube II system and carbon-source utilisation.

Media

Motility medium consisted of Nutrient Broth (Difco) 0.8 w/v, agar 0.5% w/v, and triphenyl tetrazolium chloride 0.005% w/v (Lankford, 1966). Carbon-source utilisation medium of Grimont and Grimont (1978) was modified for chelated iron minimal (CIMM) agar. This medium consisted of two solutions which were autoclaved separately and then combined to yield 1 L of total volume. Solution A contained: KH₂PO₄ 0.68 g; K₂HPO₄ 2.61 g; ethylenediamine di-0-hydroxyphenylacetic acid (EDDA) 29.95 g; 5.0 ml of 1 N NaOH and 480 ml of distilled H₂O. Solution B contained: NaCl 7.0 g; (NH₄)₂SO₄ 1.0 g; agarose 15.0 g and 500 ml of distilled H₂O. Autoclaved solutions were cooled to 55 °C and to solution B were added: 1.0 M CaCl₂ 0.132 ml; 1.0 M MgSO₄ 1.0 ml; M70 inorganic mixture of Véron (Véron, 1975, cited by Grimont and Grimont, 1978) 10 ml; and glucose 40% w/v, 5.0 ml. Solutions A and B were then mixed, dispensed, and stored for 3 days at 4 °C before use. L-broth (Lennox, 1955) without glucose consisted of (/L) tryptone 10 g, yeast extract 5 g, NaCl 5 g and adjusted to pH 7 with 1 N NaOH. L-agar consisted of the same medium solidified with Difco agar 1.5% w/v.

Growth conditions

Static L-broth cultures (without glucose) and L-agar cultures were incubated for 48 h at 30 °C and passed once for haemagglutination assays. Unless otherwise indicated all other tests were conducted at 30 °C for 48 h. Strains were stored and maintained on L-agar.

Enzyme assays

Both lipase and lecithinase activity were evaluated visually from colonies grown on egg-yolk agar (Smibert and Krieg, 1981). Urease activity was determined after incubation for 24 h of strains inoculated on to Christensen urea agar (Smibert and Krieg, 1981). Although four patterns of haemolysis have been observed for various biotypes of *S. marcescens* grown on horse-blood agar (Grimont and Grimont, 1978), data presented below have been combined to describe general haemolysis. Dialysed brain-heart infusion milk agar (Sokol *et al.*, 1979) was used for the detection of protease activity. DNAase activity was assayed on DNAase test agar with methyl green.

Haemagglutination tests

Human blood was collected in a Vacutainer with EDTA (Becton-Dickinson, Rutherford, NJ, USA) and red blood cells were washed and treated for 10 min at 37 °C with tannic acid 0.003% w/v (Boyden, 1951). Tanned and untreated human RBC were washed three times and resuspended to 3% v/v in PBS with D-mannose

3% w/v. RBC from defibrinated horse and guinea pig blood (Colorado Serum Co., Denver, Colorado, USA) were washed 3 times and resuspended to 3% v/v in PBS with or without D-mannose 3% w/v.

Mannose-sensitive haemagglutination (MS-HA) was demonstrated by mixing guinea pig RBC and plate-grown bacteria with toothpicks on glass plates chilled over ice. Preliminary tests revealed that the greatest contrast in mannose sensitivity was achieved from plate-grown colonies (data not shown). Mannose-resistant haemagglutination (MR-HA) was minimised in such conditions, whereas no difference in MS-HA was noted between agar- and broth-grown cultures. Mannose-resistant type-K haemagglutination (MR/K-HA) was assayed with tanned and fresh human RBC in the presence of mannose; ox cells were not required because untreated human cells were not agglutinated by *Serratia* in the presence of mannose (Duguid, 1959). Bacterial suspensions and RBC were mixed for 10 min at 200 rpm on a reciprocating shaker at room temperature. MR-HA of horse RBC in D-mannose 3% w/v was demonstrated with broth cultures as described above.

Supplementary tests

Isolates were cultured individually on peptone-glycerol agar at 30°C (Dauenhauer *et al.*, 1984) and checked visually for prodigiosin production after 96 h. Dark red, red, pink, as well as various striated and sectorial patterns of pigmentation were all scored as positive. After growth for 48 h at 30°C, tubes of motility medium were evaluated for diffuse growth away from the inoculation stab. Isolates incubated for 48 h at 30°C on CIMM agar were used to assay iron acquisition from an EDDA-chelated source.

Plasmid screening

Plasmid DNA was purified by the procedure of Portnoy *et al.* (1981). Half of the plasmid preparation was electrophoresed on an 11-cm-long gel of agarose 0.7% w/v in Tris-borate buffer and the remaining sample was run on a 17-cm-long gel of agarose 1% w/v (Meyers *et al.*, 1976). Gels were stained with ethidium bromide and photographed.

Statistical analysis

Virulence factors were evaluated for correlation with plasmid carriage, site of isolation, and other virulence factors by determining the Yates corrected value of χ^2 .

Results

S. marcescens isolates were obtained from widely diverse sources. Table I reflects only a small portion of the specimens screened; *S. marcescens* was cultured from less than one-fifth of the environmental items analysed. *Providencia rettgeri* was com-

Table I. Sources of *S. marcescens* isolates

Source	Number of isolates
Clinical	122
Wound	50
Respiratory	46
Urinary	11
Blood	9
Other*	6
Environmental	25
Insects and spiders	10
Mushrooms	8
Plants	4
Fresh water	2
Minnow	1
Total	147

* Other clinical isolates were from cases of peritonitis, conjunctivitis, and neonatal meningitis.

monly isolated on DNAase-test media from colonies growing on CT agar; other bacteria isolated were *P. stuarti*, *Enterobacter cloacae*, and *Proteus vulgaris*.

Plasmid analysis

Although some traits were tested soon after each isolate was received, all traits were tested in identical conditions once the collection was complete. Some unstable plasmids were lost; in ten clinical isolates which were initially scored positively for plasmid carriage, no plasmids could be detected in subsequent assays. A small plasmid of mol. wt $<2.6 \times 10^6$ was recovered in variable amounts depending on the host strain; this size of plasmid was the most commonly isolated, being present in 19 of 122 clinical and 1 of 25 environmental strains. Individual strains harboured from 0 to 5 different plasmids ranging in size from $<2.6 \times 10^6$ to $>62 \times 10^6$ mol. wt. None of the traits listed in tables II or III were found to be correlated with plasmid carriage ($p > 0.05$).

Occurrence of virulence factors

Environmental and clinical strains were compared to determine any statistically significant differences in occurrence of potential virulence factors. Table II lists nine traits that were examined. In five clinical strains siderophore activity could not be assayed because of slow growth on control plates, probably due to uncharacterised auxotrophic mutations. There was no difference in the

Table II. Trait occurrence among *S. marcescens* isolates

Trait	Percentage (and number) of isolates exhibiting each trait from	
	clinical sources	environmental sources
Haemolysin	47 (57)	56 (14)
Lecithinase	96 (117)	92 (23)
Lipase	96 (117)	92 (23)
Motility	99 (121)	100 (25)
Pigmentation	19 (23)	52 (13)
Plasmid carriage	46 (56)*	48 (12)
Protease	99 (121)	92 (23)
Siderophore	99 (116)	100 (25)
Urease	4 (5)	8 (2)

* Plasmids could not be recovered in subsequent assays from ten of these isolates.

occurrence of lecithinase or lipase activity; the same seven strains lacked both these properties. Nearly all isolates tested were motile and capable of scavenging iron from EDDA, a chelator with an association constant for ferric iron of $10^{33.9}$ (L'Ep-lattenier *et al.*, 1967). Urease activity was noted in seven strains, five from clinical and two from environmental sources.

Grimont and Grimont (1978) described four patterns of haemolysis produced by *S. marcescens* on horse-blood agar. Although all four patterns were observed in our collection, the data reported in table II merely indicate whether haemolysis of any type was observed. This trait is quite unstable in some strains as was evidenced by clearly segmented patterns of haemolysis beneath four colonies. Although a few isolates exhibited segmented patterns of pigmentation, the four strains with segmented haemolysis patterns were all non-pigmented. There was no difference in the occurrence of haemolytic activity with regard to source of isolation. Proteolytic activity was assayed by casein hydrolysis; its activity could not be correlated with haemolytic activity nor was there a significant difference in occurrence between sources of isolation. The frequency of nonpigmented strains, however, was notably higher from clinical strains ($\chi^2 = 10.60$, $df = 1$, $p < 0.01$).

Haemagglutinin assays

Nearly all isolates demonstrated MS-HA that was visualised by either a partial reduction or complete inhibition of haemagglutinin by D-man-

Table III. Haemagglutination with respect to source of isolation of *S. marcescens*

Source	Percentage (and number) of isolates positive for haemagglutination		
	MS-HA	MR-HA	MR/K-HA
Environmental	100 (25)	48 (12)	36 (9)
Clinical	95 (112)*	64 (78)	75 (91)
Respiratory	93 (42)	67 (31)	72 (33)
Wound	98 (46)	70 (35)	76 (38)
All others	92 (24)	46 (12)	77 (20)

* Four clinical isolates have been excluded from the MS-HA data because MR-HA may have blocked the observation of mannose sensitivity in these cases.

nose (table III). In four strains, strong MR-HA activity prevented the determination of MS-HA. There was no difference in the frequency of MS-HA or MR-HA between clinical and environmental sources. In contrast to a 96% overall frequency of MS-HA, MR-HA occurred in only 61% of all isolates. Clinical strains exhibited MR/K-HA more frequently than did environmental strains ($\chi^2 = 12.49$, $df = 1$, $p < 0.001$). Although the intensity of reaction was highly variable, all 11 urinary isolates (from three separate hospitals) agglutinated tanned human RBC in the presence of mannose.

Discussion

This study assessed the occurrence of potential virulence factors found in *S. marcescens* based upon the representative sampling of a defined bacterial population. Our survey is unique in its collection of environmental isolates from the same geographical setting as the patient population. Suspected epidemic strains were received on two occasions. To prevent an uncharacteristic skewing of the data, the collection included only a single representative isolate for each variant of an epidemic strain, and it excluded duplicate cultures from the same patient. Species other than *S. marcescens*, that might have different virulence determinants, were excluded as well.

Plasmid carriage is a factor associated with virulence that is especially subject to change under appropriate selective pressures. Both the acquisition and disappearance of resistance plasmids in hospital strains of *S. marcescens* have shown to be influenced by the selective pressure of antimicrobials used in therapy (O'Brien *et al.*, 1980; Rubens *et al.*, 1980). Antimicrobial resistance in *S. marcescens*

was not analysed but has been well characterised by others. Virulence determinants other than antimicrobial resistance, however, could be encoded on *Serratia* plasmids, in which case these plasmids might be enriched by the selective pressures of infection. In *E. coli*, other virulence determinants such as adherence pili and iron-uptake systems are known to be plasmid encoded in certain instances (Williams and Warner, 1980; Duguid and Old, 1980). In this survey the proportion of clinical strains harbouring plasmids was compared to that of environmental strains. The proportions were similar and the presence of the virulence factors could not be correlated with plasmid carriage. This evidence suggests that, in terms of plasmid occurrence, the *Serratia* strains cultured from patients in this study were no different from environmental isolates.

Of the 12 virulence factors assayed, only MR/K-HA and pigmentation were found to be statistically different in occurrence between clinical and environmental isolates. Our findings confirm the observations of others (Farmer *et al.*, 1976; Grimont and Grimont, 1978) that there is an increased prevalence of non-pigmented strains among clinical isolates. Whether prodigiosin production is selectively disadvantageous in *serratia* infections has yet to be demonstrated. The higher frequency of MR/K-HA would suggest that type-3 fimbriae offer a selective advantage to *S. marcescens* in nosocomial infections or that the gene or genes encoding type-3 fimbriae is genetically linked to such a factor. Old *et al.* (1983) found that all of 11 *S. marcescens* strains demonstrated all three types of haemagglutination. Their findings are in contrast to our results in which the overall occurrence of haemagglutinins were as follows: MS-HA 96%, MR-HA 61%, and MR/K-

HA 68%. This discrepancy may be accounted for by a larger sample size with different strain types, variable expression under modified culture conditions, or a less sensitive assay procedure. Even in *Serratia* cultures from a single strain, expression of these haemagglutinins is highly variable (Adegbola and Old, 1982). Nevertheless, under the given assay conditions, clinical strains of *S. marcescens* exhibited MR/K-HA more often than environmental isolates. Additional experiments are necessary to determine whether type-3 fimbriae have a role in pathogenesis and what that role might be.

For many strains, virulence-associated properties were found to disappear and, in some instances, reappear with passage; the most obvious of these were pigmentation, haemolysin, haemagglutinins, and carriage of a small ($<2.6 \times 10^6$ mol. wt) plasmid. Without in-vivo selective pressure some strains may have lost such determinants during laboratory handling, as was true for plasmid carriage in ten clinical isolates. Other strains may have temporarily ceased to exhibit such traits. Further investigation into the sectoring patterns of pigmentation and haemolysis may provide a better understanding of the regulatory mechanisms controlling gene expression in *Serratia*.

We are grateful to J. Clarridge, E. Mason, M. McBride, D. Moore, and C. Stager for providing clinical isolates and to T. Blasdel of the Houston Zoological Gardens for contributing variety to the environmental samples. We thank the numerous others who collected environmental specimens for screening and Michelle Grant for her technical assistance.

This research was supported by Public Health Service grant AI21009 from the National Institutes of Health. Computational assistance was provided by the CLINFO project, supported by the Division of Research Resources of the NIH under grant number RR-00350.

REFERENCES

- Adegbola R A, Old D C 1982 New fimbrial hemagglutinin in *Serratia* species. *Infection and Immunity* **38**:306-315.
- Ball A P, McGhie D, Geddes A M 1977 *Serratia marcescens* in a general hospital. *Quarterly Journal of Medicine, New Series* **46**:63-71.
- Boyden S V 1951 The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. *Journal of Experimental Medicine* **93**:107-120.
- Cooper R L, Constable I J 1977 Infective keratitis in soft contact lens wearers. *British Journal of Ophthalmology* **61**:250-254.
- Cox C D, Adams P 1985 Siderophore activity of pyoverdine for *Pseudomonas aeruginosa*. *Infection and Immunity* **48**:130-138.
- Curtiss R 1965 Chromosomal aberrations associated with mutations to bacteriophage resistance in *Escherichia coli*. *Journal of Bacteriology* **89**:28-40.
- Dauenhauer S A, Hull R A, Williams R P 1984 Cloning and expression in *Escherichia coli* of *Serratia marcescens* genes encoding prodigiosin biosynthesis. *Journal of Bacteriology* **158**:1128-1132.
- Davis J T, Foltz E, Blackmore W S 1970 *Serratia marcescens*: A pathogen of increasing clinical importance. *Journal of the American Medical Association* **214**:2190-2192.
- Duguid J P, 1959 Fimbriae and adhesive properties in *Klebsiella* strains. *Journal of General Microbiology* **21**:271-286.
- Duguid J P, Old D C 1980 Adhesive properties of Enterobacteriaceae. In: Beachey E H (ed) Bacterial adherence. (Receptors and recognition, series B, vol 6.) Chapman and Hall, London, pp 185-217.
- Duncan C L 1975 Role of clostridial toxins in pathogenesis. In: Schlesinger D (ed) Microbiology—1975. American Society for Microbiology, Washington D.C., pp. 283-291.
- Farmer J J *et al.* 1976 Detection of *Serratia* outbreaks in hospital. *Lancet* **2**:455-459.
- Fulton M, Forney C E, Leifson E 1959 Identification of *Serratia*

- occurring in man and animals. *Canadian Journal of Microbiology* **5**:269-275.
- Grimont P A D, Grimont F 1978 Biotyping of *Serratia marcescens* and its use in epidemiological studies. *Journal of Clinical Microbiology* **8**:73-83.
- Grimont P A D, Grimont F, Dulong de Rosnay H L C, Sneath P H A 1977 Taxonomy of the genus *Serratia*. *Journal of General Microbiology* **98**:39-66.
- Hagberg L, Hull R, Hull S, Falkow S, Freter R, Svanborg Edén C 1983 Contribution of adhesion to bacterial persistence in the mouse urinary tract. *Infection and Immunity* **40**:265-272.
- Hedges R W, Rodriguez-Lemoine V, Datta N 1975 R Factors from *Serratia marcescens*. *Journal of General Microbiology* **86**:88-92.
- Johnson E, Ellner P D 1974 Distribution of *Serratia* species in clinical specimens. *Applied Microbiology* **28**:513-514.
- Lankford C E, Eklund C E 1966 Laboratory manual for public health bacteriology. University of Texas, Austin, pp 174-175.
- Lass J H, Haaf J, Foster C S, Belcher C 1981 Visual outcome in eight cases of *Serratia marcescens* keratitis. *American Journal of Ophthalmology* **92**:384-390.
- Lennox E S. 1955 Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190-206.
- L'Eplattenier F, Murase I, Martell A E 1967 New multidentate ligands. VI. Chelating tendencies of N,N'-di(2-hydroxybenzyl) ethylene-diamine-N,N'-diacetic acid. *Journal of the American Chemical Society* **89**:837-843.
- Lyerly D M, Kreger A S 1983 Importance of *Serratia* protease in the pathogenesis of experimental *Serratia marcescens* pneumonia. *Infection and Immunity* **40**:113-119.
- McEntegart M G, Porterfield J S 1949 Bacteraemia following dental extractions. *Lancet* **2**:596-598.
- Meyers J A, Sanchez D, Elwell L P, Falkow S 1976 Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *Journal of Bacteriology* **127**:1529-1537.
- O'Brien T F, Ross D G, Guzman M A, Medeiros A A, Hedges R W, Botstein D 1980 Dissemination of an antibiotic resistance plasmid in hospital patient flora. *Antimicrobial Agents and Chemotherapy* **17**:537-543.
- Old D C, Adegbola R, Scott S S 1983 Multiple fimbrial hemagglutinins in *Serratia* species. *Medical Microbiology and Immunology* **172**:107-115.
- Parsons C L, Stauffer C, Mulholland S G, Griffith D P 1984 Effect of ammonium on bacterial adherence to bladder transitional epithelium. *Journal of Urology* **132**:365-366.
- Pazin G J, Braude A I 1974 Immobilizing antibodies in urine. II. Prevention of ascending spread of *Proteus mirabilis*. *Investigative Urology* **12**:129-133.
- Portnoy D A, Moseley S L, Falkow S 1981 Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. *Infection and Immunity* **31**:775-782.
- Rogers H J 1973 Iron-binding catechols and virulence in *Escherichia coli*. *Infection and Immunity* **7**:445-456.
- Rubens C E, McGee Z A, Farrar W E 1980 Loss of an aminoglycoside resistance plasmid by *Serratia marcescens* during treatment of meningitis with amikacin. *Journal of Infectious Diseases* **141**:346-350.
- Smibert R M, Krieg N R 1981 General characterization. In: Gerhardt P (ed) Manual of methods for general bacteriology. American Society for Microbiology, Washington D.C., pp 409-443.
- Smith H W, Huggins M B 1976 Further observations on the associations of the Colicine V plasmid of *Escherichia coli* with pathogenicity and with survival in the alimentary tract. *Journal of General Microbiology* **92**:335-350.
- Sokol P A, Ohman D E, Iglewski B H 1979 A more sensitive plate assay for detection of protease production by *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology* **9**:538-540.
- Starr M P, Grimont P A D, Grimont F, Starr P B 1976 Caprylate-thallos agar medium for selectively isolating *Serratia* and its utility in the clinical laboratory. *Journal of Clinical Microbiology* **4**:270-276.
- Tabaqchali S, Chambers T J, Brooks H J L 1977 *Serratia marcescens* in hospital practice. *Lancet* **1**:306-307.
- Welch R A, Dellinger E P, Minshew B, Falkow S 1981 Haemolysin contributes to virulence of extra-intestinal *E. coli* infections. *Nature* **294**:665-667.
- Wilfert J N, Barrett F F, Ewing W H, Finland M, Kass E H 1970 *Serratia marcescens*: biochemical, serological & epidemiological characteristics and antibiotic susceptibility of strains isolated at Boston City Hospital. *Applied Microbiology* **19**:345-352.
- Wilkowske C J, Washington J A, Martin W J, Ritts R E 1970 *Serratia marcescens*: biochemical characteristics, antibiotic susceptibility patterns, and clinical significance. *Journal of the American Medical Association* **214**:2157-2162.
- Willett H P 1980 *Staphylococcus*. In: Joklik W K, Willett H P, Amos D B (eds) Zinser Microbiology 17th edn. Appleton-Century-Crofts, New York, pp 532-552.
- Williams P H, Warner P J 1980 ColV plasmid-mediated, colicin V-independent iron uptake system of invasive strains of *Escherichia coli*. *Infection and Immunity* **29**:411-416.
- Woodward H M M, Clarke K B 1913 A case of infection in man by the bacterium prodigiosum. *Lancet* **1**:314-315.