

Antibiotic-Resistant *Serratia marcescens* Infection in a Hospital

R. E. AMBROSIO, A. J. VAN WYK, H. C. DE KLERK

SUMMARY

Over a 12-month period, 74 isolates of *Serratia marcescens* were obtained from various sources at Tygerberg Hospital. The majority of these isolates were from catheterized patients with urinary tract infections, and were non-pigmented and resistant to all antibiotics tested, excepting amikacin and neomycin. All isolates transferred resistance to tobramycin, gentamicin and tetracycline by conjugation to *Escherichia coli* recipients as separate markers at low frequency. A non-self-transmissible plasmid conferring resistance to kanamycin, ampicillin and gentamicin was mobilized from *Serratia* species to *E. coli*, and became fully self-transmissible in subsequent matings.

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During the past decade there has been an increase in the reported isolation of *Serratia marcescens* from hospital-acquired infections.^{1,2} This organism has been associated with various types of infection.^{1,2}

Strains of *S. marcescens* isolated from infective lesions may differ from those from other sources in their pigment production and antibiotic resistance,¹ and the infrequent recognition of this organism as a pathogen has been ascribed to inadequate identification,¹ owing to variations in pigment production.

Multiple antibiotic resistance in *Serratia* species^{3,4} has been found to be plasmid-mediated,^{5,6} and in certain cases these plasmids may confer an additional degree of resistance to already resistant strains.⁶

In recent months *S. marcescens* has been increasingly isolated in this laboratory as a result of a localized outbreak of urinary tract infection. In this article some of the characteristics of these isolates are reported.

MATERIALS AND METHODS

Bacteria and Plasmids

S. marcescens strains were isolated from a number of sources (Table I). Initial isolation was on MacConkey agar and identification was according to standard techniques.⁷ The environment was investigated for the presence of the organism by taking agar impressions⁸ of general ward utensils as well as hand washings, and throat and

rectal swabs from patients, medical and nursing personnel.⁹ Samples of disinfectants were incubated in brain-heart infusion broth containing Tween 80 (0,05%). Antibiotic sensitivity patterns were determined according to the method of Bauer *et al.*¹⁰ Plate minimal inhibitory concentrations (MICs) for gentamicin, tobramycin and amikacin were determined on 31 isolates representing different sources and disc sensitivity patterns.

Recipients for plasmid transfer experiments were *Proteus mirabilis* strains PM5006 *str*^r and PM5006 *nal*^r,¹¹ *Escherichia coli* strains J62 *str*^r and J62 *nal*^r,¹² J53 *rif*^r,⁵ as well as streptomycin-resistant *Klebsiella pneumoniae* (strains KP104, KP107, KP133, KP118, KP123) isolated from clinical material in this laboratory. R477-1 is a self-transmissible, S-group plasmid, isolated from *S. marcescens* which encodes resistance to tetracycline, sulphonamide and streptomycin.⁵

Media

Materials and growth media for the isolation and identification of isolates, antibiotic sensitivity testing and plasmid transfer experiments have been described.^{10,11} Antibiotics for transfer experiments were used at the following concentrations: streptomycin 1000 mg/l; tetracycline 50 mg/l; tobramycin 50 mg/l; cephalothin 150 mg/l; kanamycin 50 mg/l; gentamicin 20 mg/l; nitrofurantoin 100 mg/l; carbenicillin 50 mg/l; and chloramphenicol 40 mg/l.

Plasmid Transfer and Mobilization

The methods for mating on solid media have been described.¹² Experiments were performed at 30°C. Plasmid mobilization experiments were performed by the triple mating technique, which consisted of mixing equal volumes of the strain carrying the presumptive plasmid, a strain carrying the mobilizing plasmid and a suitable plasmid-negative recipient. These matings were on solid medium and selection was for the antibiotic markers of the presumptive plasmid, the mobilizing plasmid and the chromosomal resistance of the final recipient. The transfer frequency was calculated relative to the number of donors.

Segregation Studies

These were performed according to the method of Coetzee.¹¹

RESULTS

During the year June 1977 to May 1978, 74 isolates of *S. marcescens* were obtained from 46 patients (see Table I). Attention was focused on this organism because of the increased frequency of its isolation from urine speci-

Department of Medical Microbiology, Tygerberg Hospital and University of Stellenbosch, Parowvallei, CP

R. E. AMBROSIO, M.Sc., D.Sc.

A. J. VAN WYK, M.B. CH.B., M.MED., (MICROBIOL. PATH.)

H. C. DE KLERK, M.B. CH.B., M.D.

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TABLE I. ORIGIN OF *S. MARCESCENS* ISOLATES

Source	Outbreak		Sporadic		Total
	Number of isolates	Pigmented	Number of isolates	Pigmented	
Urine	29 (14)*	0	9 (8)	3	38 (22)
Pus swabs	10 (3)	0	6 (6)	1	16 (9)
Sputum	0	—	12 (10)	3	12 (10)
Throat swabs					
Blood	3 (2)	0	0	0	3 (2)
Other†	0	—	5 (3)	3	5 (3)
Total	42 (19)	0	32 (27)	10	74 (46)

* Figures in parenthesis indicate number of patients.
 † Isolated from postoperative drainage, contaminated cerebrospinal fluid and cervical swabs.

mens during July 1977. All these strains were isolated from a single urological ward, and were non-pigmented and resistant to all antibiotics tested, except amikacin, neomycin and chloramphenicol (Fig. 1). This outbreak abated after the institution of barrier nursing procedures, but sporadic isolates of resistant 'outbreak' strains subsequently occurred. Despite intensive bacteriological investigation of the ward environment, only a single, pigmented, predominantly sensitive strain of *S. marcescens* was isolated from the hands of a nurse. Resistant strains remained confined to this urological ward.

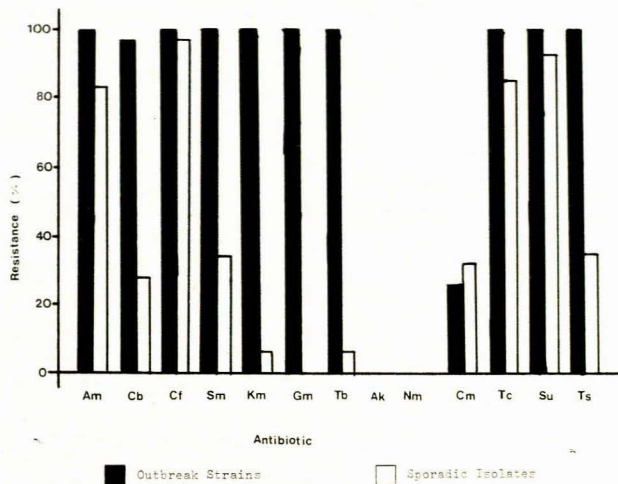


Fig. 1. Antibiotic resistance of *S. marcescens* isolates (Am — ampicillin; Cb — carbenicillin; Cf — cephalothin; Sm — streptomycin; Km — kanamycin; Gm — gentamicin; Tb — tobramycin; Ak — amikacin; Nm — neomycin; Cm — chloramphenicol; Tc — tetracycline; Su — sulphonomide; Ts — co-trimoxazole).

The majority caused urinary tract infections in catheterized patients, most of whom (22/32) had urinary bacterial counts of 10⁶ organisms per ml or more. Two patients had wounds infected with the 'outbreak' strains; one had postoperative prostatectomy wound infection, and the other an infected arteriovenous shunt. The latter patient became infected while undergoing surgery to the shunt in the urology unit. Subsequent blood culture showed *S. marcescens* bacteraemia.

Other isolates not associated with the outbreak described above were obtained from throat, pus and cervical swabs, as well as from sputum specimens from various sources throughout the hospital. The antibiotic sensitivity pattern of all the isolates is shown in Fig. 1. *S. marcescens* strains from other sources (i.e. the 'non-outbreak' strains) were predominantly sensitive to the antibiotics tested, regardless of their pigmentation. MICs of 3 aminoglycoside antibiotics for 20 'outbreak' strains were within the following ranges: gentamicin 32-256 mg/l; tobramycin 64-256 mg/l; and amikacin 2-16 mg/l, whereas the ranges for 11 strains from other sources ('non-outbreak' strains) were gentamicin <1-2 mg/l; tobramycin <1-8 mg/l; and amikacin <1-4 mg/l.

R-Plasmid Studies

A number of multiply-resistant isolates were tested for their ability to transfer their resistances to *E. coli*, *P. mirabilis* and *K. pneumoniae* recipients, and the results of these experiments are presented in Table II. All isolates transferred tobramycin, gentamicin or tetracycline resistance to *Klebsiella* recipients at low frequency. Transfer of these resistances to *Klebsiella* did not occur as a single unit, but as individual markers, and no transfer was detected to *Proteus* or *E. coli* recipients. A lower transfer frequency was obtained when matings were at 37°C, and no transfer was detected with mating in liquid medium.

Resistance Transfer to *E. coli*

The apparent inability to transfer resistances from *Serratia* species to *E. coli* was investigated further by means of the triple mating technique. Four *Serratia* species isolates which could transfer gentamicin or tobramycin resistance to *Klebsiella* species were selected, and these were used as intermediate recipients in matings with *E. coli* strains containing R477-1 and *E. coli* J53 as the final recipient. The results of these experiments are summarized in Table III. Resistance to kanamycin, ampicillin and gentamicin was mobilized by R477-1 and transferred to *E. coli* as a single unit at a frequency of 10⁻⁶/donor cell. In all cases, mobilization was accompanied by transfer of R477-1-encoded tetracycline resistance at a fre-

TABLE II. RESISTANCE TRANSFER TO *KLEBSIELLA*, *E. COLI* AND *PROTEUS* RECIPIENTS

Donors	Recipients	Selected markers*	Transfer frequency†
SM1 - SM17, SM19 - SM25	KP104, KP107, KP118 KP113, KP123	Gm	2×10^{-7}
		Tb	1×10^{-7}
		Tc	2×10^{-6}
		Tc Tb	0
		Tc Gm	0
		Tc Tb Gm	0
		Tb Gm	0
		Tc Tb	—‡
		Tc Gm	—
		Tc Tb Gm	—
SM1 - SM17, SM19 - SM25	J53 rif ^r , J62 str ^r PM5006 str ^r	Gm	—
		Tc	—
		Tb	—
		Tb Gm	—

* Symbols as in Fig. 1.

† Per donor cell. Average of 3 experiments at 30°C.

‡ No transfer detected.

TABLE III. TRIPLE-MATING MOBILIZATION OF *S. MARCESCENS* RESISTANCES

Donors	Recipients	Selected markers	Transferred markers	Transferred frequency§
SM1, SM17, SM23, SM21	*J53 rif ^r	Gm	Am Km Gm‡	2×10^{-6}
		Km	Am Km Gm	2×10^{-6}
		Gm Km	Am Km Gm	2×10^{-6}
		Tc†	Tc	2×10^{-3}
J65 rif ^r (STy-1)	J62 nal ^r	Gm Km	Am Km Gm	2×10^{-4}
		Gm	Am Km Gm	2×10^{-4}
		Km	Am Km Gm	2×10^{-4}

* Final recipient. Intermediate strain was J53-2 (R477-1).

† R477-1 — encoded tetracycline resistance.

‡ Symbols as in Fig. 1.

§ Per donor cell (average of 3 experiments).

|| Plasmid designation described in text.

quency of 10^{-3} /donor cell. Segregation studies showed that these resistances were stable in *E. coli*; however, the segregation rate of R477-1-encoded tetracycline resistance differed from that of the other markers, which had identical segregation rates.

These results suggested that resistance to kanamycin, ampicillin and gentamicin was located on a single plasmid which did not carry tetracycline resistance. This was confirmed by matings between the J53 transconjugants and *E. coli* J62 recipients. Resistance to kanamycin, ampicillin and gentamicin was transferred as a single unit at a frequency of 10^{-4} /donor cell (Table III). Co-transfer of R477-1 was not always detected in these matings. This confirmed that the mobilized *Serratia* species resistances were located on a self-transmissible plasmid not directly self-transmissible from *Serratia* species to *E. coli*, but fully self-transmissible once mobilized to *E. coli*. The designation STy-1 is proposed for this plasmid.

DISCUSSION

Previous investigations into the role of *S. marcescens* in nosocomial infections have shown an association between genito-urinary tract manipulation, especially catheterization, and subsequent infection.^{1,3} This organism has rarely been found in the gastro-intestinal tracts of hospi-

talized patients.^{1,4} Most of the isolates reported here were from catheterized male patients with significant bacteriuria. The relative apathogenicity of the 'outbreak' strains was evident from mild clinical signs and symptoms of infection observed in all these patients, including the one with bacteraemia.

In contrast to previously described outbreaks, there was extensive resistance to sulphonamides, and to all β -lactam and aminoglycoside antibiotics tested, except amikacin and neomycin. All strains were resistant to cotrimoxazole and gentamicin, which differs from previous findings.^{1,2,14} The MICs for gentamicin and tobramycin were well in excess of attainable blood levels, whereas for amikacin they were within the normal therapeutic range.

Routine identification of *Enterobacteriaceae* should include sufficient biochemical tests to differentiate between members of the *Klebsiella-Enterobacter-Serratia* tribe. Reliance on pigment production only could lead to considerable misdiagnosis. All our multiply-resistant isolates were non-pigmented, while pigmented isolates were predominantly antibiotic-sensitive. Similar findings were reported by Farmer *et al.*,¹⁵ and the isolation of 181 non-pigmented, multiply-resistant strains was also reported by Clayton and von Graevenitz.⁴ The results of the transfer experiments showed that resistance to 3 of the 11 anti-

biotics tested were self-transmissible to *Klebsiella* species recipients. The inability of *Serratia* species to transfer resistances to *E. coli* has been reported.⁵ Of 236 *S. marcescens* strains tested by Hedges *et al.*,⁵ only a small proportion could transfer their resistances directly to *E. coli*. It is possible that these resistances are not carried on self-transmissible plasmids, but on chromosomally integrated transposable elements which are known to be common in some *Enterobacteriaceae*.¹⁶ Alternatively, the *Serratia* species resistances could be on plasmids with a very limited host range. It has been reported that some *Serratia* species plasmids have a temperature-sensitive transfer system which influences the frequency of plasmid transfer.⁵ The STy-1 plasmid described here was not self-transmissible between *Serratia* species and *E. coli*; however, once mobilized to an *E. coli* host, it became fully self-transmissible in subsequent matings. Similar findings have been reported by Cooksey *et al.*,¹⁷ who found that some *Serratia* species isolates transferred their resistances to *Klebsiella* species, and then to *E. coli*.

Our results support previous findings that *S. marcescens* is a bacterium of low virulence which may flourish in the compromised host. Furthermore, the low frequency of resistance transfer to other *Enterobacteriaceae* sug-

gests that the role of this organism in the spread of multiple antibiotic resistance may not be clinically important.

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REFERENCES

1. Ball, A. P., McGhie, D. and Geddes, A. M. (1977): *Quart. J. Med.*, **181**, 63.
2. Maskell, R., Crump, J. and Lee, R. (1977): *Lancet*, **1**, 1013.
3. Schaberg, D. R., Alford, R. H., Andersen, R. *et al.* (1976): *J. infect. Dis.*, **134**, 181.
4. Clayton, E. and von Graevenitz, A. (1966): *J. Amer. med. Ass.*, **197**, 1059.
5. Hedges, R. W., Rodriguez-Lemoine, V. and Datta, N. (1975): *J. gen. Microbiol.*, **86**, 88.
6. Medeiros, A. A. and O'Brien, T. F. (1968): *Antimicrobial Agents and Chemotherapy*, p. 30. Detroit: American Society for Microbiology.
7. Edwards, P. R. and Ewing, W. H. (1972): *Identification of the Enterobacteriaceae*, 3rd ed., p. 308. Minneapolis: Burgess Publishing.
8. Horwitz, B. M. (1974): *S. Afr. med. J.*, **48**, 271.
9. Casewell, M. and Phillips, I. (1977): *Brit. med. J.*, **2**, 1315.
10. Bauer, A. W., Kirby, W. M. M., Sherris, J. C. *et al.* (1966): *Amer. J. clin. Path.*, **45**, 493.
11. Coetzee, J. N. (1975): *J. gen. Microbiol.*, **86**, 133.
12. Clowes, R. C. and Hayes, W. (1968): *Experiments in Microbial Genetics*. Oxford: Blackwell Scientific Publications.
13. Dennison, S. and Baumberg, S. (1975): *Molec. gen. Genet.*, **138**, 323.
14. Editorial (1977): *Brit. med. J.*, **1**, 1177.
15. Farmer, J. J., Davis, B. R., Presley, D. B. *et al.* (1976): *Lancet*, **2**, 455.
16. Starlinger, S. and Saedler, H. (1977): *Curr. Top. Microbiol. Immunol.*, **75**, 111.
17. Cooksey, R. C., Thorne, G. M. and Farrar, E. W. jun. (1976): *Antimicrobial Agents and Chemotherapy*, p. 123. Detroit: American Society for Microbiology.

Clinicopathological Conference

Pulmonary Fibrosis and Pulmonary Hypertension

CLINICAL NOTES

A 34-year-old Black woman was admitted to hospital on 8 July 1977 with a 2-month history of dyspnoea. She was 37 weeks pregnant and although short of breath, was able to sweep, do washing and could walk uphill provided she rested along the way. The dyspnoea was more severe than during her previous pregnancy in 1975. She did not complain of paroxysmal nocturnal dyspnoea, cough or chest pain. She experienced mild pain behind her knees when walking, felt excessively tired and had minimal swelling of the ankles. Pulmonary tuberculosis was diagnosed in 1973 but it was not ascertained whether the

sputum was positive for acid-fast bacilli. In April 1974 an abdominal operation was done, ostensibly for 'TB of the womb', and she was treated as an outpatient with streptomycin for 3 months, with INH and PAS treatment continuing for 2 years. Her present pregnancy was her 7th. She did not smoke, was a teetotaler and lived on a chicken farm but did not handle the birds. She was able to continue working as a domestic servant until 2 months before admission when shortness of breath and the advanced stage of her pregnancy caused her to stop. Her mother had pulmonary tuberculosis.

When examined, she was neither breathless nor tachypnoeic at rest, and there was no clubbing, cyanosis or lymphadenopathy. The pulse rate was 100/min, regular and normal in volume. The blood pressure was 90/60 mmHg. The jugulovenous pressure was not elevated but prominent A and CV waves were present. The cardiac apex was normal and not displaced. A left parasternal heave was present and there was a palpable 2nd heart

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Clinicopathological Conference held at Groote Schuur Hospital, Cape Town, on 2 March 1978.