DISTRIBUTION OF *SERRATIA MARCESCENS* SEROTYPES IN CANCER PATIENTS

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THERE have been frequent reports about *Serratia marcescens* as an opportunistic pathogen (Davis, Foltz and Blakemore, 1970; Traub *et al.*, 1976) or as a micro-organism capable of causing outbreaks of nosocomial infection (Allen and Conger, 1969; Sanders *et al.*, 1970; Wilfert *et al.*, 1970; Wilkowske *et al.*, 1970; Traub, 1972; Maki *et al.*, 1973; Schaberg *et al.*, 1976; Traub *et al.*, 1976). It is also a bacterium that readily develops multiple antibiotic resistance (Schaberg *et al.*, 1976; Traub *et al.*, 1976; Ostenson, Fields and Nolan, 1977). That such a multiply resistant micro-organism might emerge in the setting of a highly susceptible cancer-patient population where antibiotics are necessarily used for prophylactic and for therapeutic purposes led to an interest in the development of methods for monitoring *Serratia* strains isolated from the cancer patients and their environment in the Baltimore Cancer Research Program (BCRP). A study was made of *S. marcescens* strains isolated from surveillance, environmental, and diagnostic cultures during 51 months to determine their prevalence in cancer patients and their environment. Representative strains were serotyped to establish the serotypic distributions among these isolates and the usefulness of the technique as an epidemiological tool in a cancer centre.

MATERIALS AND METHODS

Patients. A total of 1314 patients with malignancies were admitted to the BCRP during the period of study, Sept. 1972–Jan 1977; of these, 296 had leukaemias, 444 had lymphomas and 574 had solid tumours.

Collection of specimens. Two sets of surveillance cultures were obtained from all patients entering the hospital and twice weekly thereafter from patients with leukaemia; the surveillance cultures were taken from the nose, gums, axilla, and rectum. Diagnostic samples were cultured at the request of the physician. Environmental samples for culture were taken from 10–30 sites in protected-environment (isolation) rooms twice weekly and from 20 sites in the intensive-care unit once weekly. An average of 30 other environmental sites also were sampled each month.

Rectal cultures were made from two swabs that had been pre-moistened with Todd-Hewitt broth; the swabs were immediately replaced into 2–3 ml of the same medium for transport to the laboratory. The methods for environmental cultures depended on the site: flat surfaces were sampled with Rodac plates containing horse- or sheep-blood agar; crevices, sink drains, inner rims of toilets etc. were swabbed; air was sampled by means of fallout (sedimentation) plates of blood agar. Results from the environmental sites were reviewed for the presence of *Serratia* from 6 Jan. 1974 until 1 Jan 1977, as were results from 175 food samples.

Bacteriological methods. Identification of *S. marcescens* was performed according to the methods described by Ewing and Martin (1974).

Of the strains isolated during a two-year period, 220 were serotyped. The 15 somatic (O) and 13 flagellar (H) antisera were obtained from Lee Laboratories, Grayson, Georgia 30221, USA. The product circular directive was used as a guideline for the serological procedures, but some modifications were necessary to improve results. For the preparation of O antigen, colonies were selected from growth on 5% horse-blood agar plates, inoculated into 5 ml of

Received 21 June 1979; accepted 2 Aug. 1979

trypticase soy broth (TSB), and incubated for 4–6 h at 37°C. The broths were autoclaved for 90 min. and centrifuged at 700 *g* for 20 min. The supernatant fluids were decanted and the pellets resuspended in 5 ml of 0.3% phenolised 0.85% sodium chloride. These preparations could be used immediately or kept refrigerated at 4°C where they remained stable for at least 2 months; they were not stable if left at room temperature longer than 6 h. It was usually necessary to enhance the flagellar (H) antigen by transferring the strain through three semisolid agar plates which contained agar of increasing concentrations: 0.4%, 0.6%, and 0.8%, respectively. From the outer edge of the growth from the third passage, 4 ml of TSB were inoculated and this was incubated at 37°C for 4–6 h; 1 ml was then removed from the broth culture, to be used later in confirmation studies. To the remaining 3 ml, an equal volume of 0.5% phenolised 0.85% sodium chloride was added. The phenolised bacterial suspension was allowed to stabilise at 4°C for at least 1 h before use, but not longer than 1 week because prolonged refrigeration leads to deterioration.

Tube-agglutination tests were performed with 0.5 ml of antigen and 0.5 ml of antiserum diluted so that final dilutions in the tests were 1 in 400 for the O antiserum and 1 in 1000 for the H antiserum, as recommended. Because many weakly reactive or non-reactive results were encountered, all the strains were retested at a final dilution of 1 in 200 of the O antiserum and 1 in 500 of the H antiserum; the avidity of the antibody for the antigen was more clearly demonstrable at these dilutions. Cross reactions between serotypes O12 and O14 were observed occasionally, as described by Edwards and Ewing (1972). The strains that gave these reactions were retested with twofold dilutions of sera and the serum in which the highest agglutination titre was obtained was considered to indicate the serotype of the strain. All tests were performed in duplicate on separate occasions and were found to give reproducible results.

RESULTS

Isolation of Serratia from patient samples

Strains of *Serratia* were isolated from 114 (8.7%) of 1314 patients in the BCRP during the 51-month study period (table I). The 220 strains that were serotyped were isolated from 85 of these patients and 9 environmental sources.

S. marcescens was infrequently isolated from patients who had lymphoma or solid tumours, 5% and 6% respectively. Patients with haematological neoplasms had a higher isolation rate, 19%. Of the patients who carried *S. marcescens*, as revealed by surveillance cultures, rectal cultures yielded the highest isolation rate both from patients with lymphoma (32%) and those with leukaemias (43%). However, in the latter patients there was no significant difference between isolation from gingival and from rectal (39%) cultures, and the gingival cultures had a higher rate (30%) than rectal (17%) in patients with solid tumour. The source that gave the highest isolation rate from diagnostic cultures in these solid-tumour patients was the urine (39%); only a few of these urine cultures were taken for surveillance purposes. While bacteraemias caused by *S. marcescens* were infrequent (0.7%), there was an appreciable occurrence of bacteraemias among the cancer patients in whom this organism was isolated from another site (lymphomas, 5%, haematological neoplasms, 12.5%, and solid tumours, 3%). The isolation rates of *S. marcescens* strains varied but little throughout the study, and neither the rates nor the serotyping results indicated an outbreak.

Isolations from environmental and food samples

Only 10 strains of *S. marcescens* were isolated from the 7852 environmental samples; they were found in cultures of the sink or tub drains, or other areas near water outlets or where water splash could occur.

From the 40 samples of fruits and vegetables, *S. marcescens* was grown in small numbers from one culture each of orange juice and fresh tomatoes, but one culture of green peppers yielded more than 10⁶ *S. marcescens* colonies/ml of homogenate, as did samples of one water-

TABLE I
Isolations of Serratia marcescens from patients with lymphoma, haematological neoplasms and solid tumours

Diagnosis	Number of patients investigated	Number (%) of patients with <i>Serratia</i>	Number of patients (and percentage of those yielding <i>Serratia</i>) who yielded <i>Serratia</i> from:											
			surveillance cultures from						diagnostic cultures from					
			nose	gums	axilla	rectum	throat	sputum	urine	blood	lesions	blood	lung	spleen
Lymphoma	444	22 (5)	5 (22)	5 (22)	3 (14)	7 (32)	1 (5)	6 (27)	5 (22)	1 (5)	0	0	0	0
Haematological neoplasms	296	56 (19)	10 (18)	22 (39)	11 (20)	24 (43)	13 (23)	11 (20)	9 (16)	7 (12.5)	7 (12.5)	2 (3.6)	2 (3.6)	2 (3.6)
Solid tumours	574	36 (6)	5 (14)	11 (31)	8 (22)	6 (17)	1 (2.8)	3 (8.3)	14 (39)	1 (2.8)	1 (2.8)	4 (11)	4 (11)	3 (8.3)

TABLE II
Distribution of *Serratia marcescens* serotypes among 220 isolates

O antigen*	H antigen†													Non-reactive	Total
	1	3	4	5	6	7	8	10	11	12	13				
1	1	...	14	3	...	12	...	...	...	...	...	...	...	30	
2	6	...	1	...	...	...	...	...	1	...	...	...	...	8	
3	...	...	...	...	...	...	1	...	...	...	...	...	...	1	
4	6	1	23	...	...	8	...	...	...	6	...	1	...	45	
5	2	...	1	...	...	...	...	...	...	...	1	...	...	4	
7	...	...	...	...	...	...	...	...	1	...	...	...	...	1	
9	...	...	1	...	...	...	...	...	...	...	...	...	...	1	
10	...	...	...	...	2	...	...	...	...	...	...	...	...	2	
11	1	...	...	...	...	...	...	...	...	...	...	...	...	1	
12	...	...	...	...	...	...	...	...	...	...	...	1	...	1	
13	...	...	1	...	...	...	...	...	...	...	...	2	...	3	
14	5	7	2	...	...	...	...	9	...	83	...	6	...	112	
15	2	...	...	...	...	...	1	...	1	...	...	2	...	6	
Non-reactive	2	...	1	...	...	...	...	...	...	...	...	2	...	5	
Total	25	8	44	3	2	20	2	9	3	89	1	14	...	220	

* There were no serotypes of O antigens 6, 8.

† There were no serotypes of H antigens 2, 9.

melon; other food samples (135 foods from lunch and dinner trays) yielded abundant *S. liquefaciens* in two sandwiches (turkey and tuna fish).

Serological studies

There were 38 distinct antigenic types among the 220 strains studied. The distribution of these O serotypes is shown in table II: the commonest types were O14* (112 strains), O4 (45

TABLE III
Sources of predominant *Serratia* serotypes

Serotype	Total number of patients yielding serotype/total number of isolates	Number of patients yielding serotype/number of isolates from					
		upper resp. tract	rectum	blood	urine	skin	lesions
O14:H12*	32/83	16/35	11/15	7/12	9/12	4/8	1/1
O14:H10	4/9	2/4	1/2	1/1	1/1	1/1	0
O4:H4	8/23	4/8	3/7	0	1/1	2/3	2/4
O4:H1*	4/6	4/5	1/1	0	0	0	0
O1:H4	4/14	1/1	—	3/11	1/1	1/1	0
O1:H7	2/12	1/7	1/2	0	0	1/3	0
All others	50/73	17/30	10/10	7/7	7/12	9/10	2/4
Total	104†/220	45/90	27/37	18/31	19/27	18/26	5/9

* One strain of O14:H12 and 3 strains of O4:H1 were recovered from the hospital environment. Six strains from that source had other serotypes.

† The number of patients who yielded *Serratia* of any type at any time was 85.

strains) and O1 (30 strains). These three O groups accounted for 84% of the isolates that were serotyped; strains of serotypes of O6 and O8 were not found.

The most prevalent H types among the isolates were 12 (40%), 4 (20%), 1 (11%), and 7 (9%); H types 2 and 9 were not found among the test isolates. Somatic antigen O1 (14%) was associated with four different H types, and antigens O4 (20%) and O14 (51%) with five H types each. The most frequently encountered antigenic types were O14:H12 (38%), O4:H4 (10%), O1:H4 (6%), O1:H7 (5%), O14:H10 (4%) and O4:H1 (3%).

Five (2%) of the *Serratia* strains were nonreactive in the O antisera, but three of these were typed with H antisera, and 14 strains (6%) were untypable in H antisera but 12 of these were typed in O antisera. Two strains (1%) did not type in either of the antisera.

The strains of *S. liquefaciens* recovered from the watermelon and from two patients were readily serotyped with the *S. marcescens* antisera.

Sources of the predominant *Serratia* antigenic types are shown in table III. Of the 32 patients who had the predominant serotype, O14:H12, 16 (50%) carried this organism in their upper respiratory tract; 4–11 of them also yielded this serotype from their rectal, urine, skin, and blood cultures. The upper respiratory tract was the chief source, or the source of equal numbers, of all the other *Serratia* serotypes, with the exception of type O1:H4, which was isolated from 11 blood cultures from three patients. Type O4:H4 was isolated in similar numbers from the upper respiratory and rectal cultures of similar numbers of patients. The isolation rates of the less common serotypes isolated from these same sources varied little from those of the predominant serotypes, with the possible exception of isolations from blood; 16% of the predominant serotypes were isolated from blood, whereas 10% of the less common serotypes were grown from this source.

DISCUSSION

The majority of strains that were isolated from cancer patients at the BCRP were of type O14:H12 as were those reported from patients at the Mayo Clinic (Negut, Davis and Washington, 1975). Rubin *et al.* (1976), reported type O14:H12 to be prevalent among 93 (16%) of isolates serotyped, with O14:H5 (10%) and O14:H1 (8%) next in predominance. Roemisch and Kocka, 1976; Anderhub *et al.*, 1977, and Griffiths *et al.*, 1977, also reported the isolation of large numbers of serotype O14, but these investigators did not type the flagellar antigens.

On the other hand, serotype O14 was not predominant in the studies from Boston City Hospital (Wilfert *et al.*, 1970) and none of 73 strains serotyped at Peter Bent Brigham Hospital in Boston was of serotype O14 (personal communication). However, Wilfert and his colleagues isolated some strains with somatic antigen O4, the serotype second in predominance at BCRP. Other investigators have reported the isolation of this serotype (Rubin *et al.*, 1976; Anderhub *et al.*, 1977; Roemisch and Kocka, 1976), although their isolation rates were lower and the H antigenic types were either unknown or different from the predominant H antigens in the BCRP patient group, namely H:1, 4, and 12. The only other antigenic types isolated with some frequency at the BCRP were O1:H4 and O1:H7, types that have not appeared to be predominant in other studies. Most of the seven strains of *Serratia* that did not react with the O antisera and the 23 strains that did not react with H antisera could probably have been serotyped if antisera for six new H antigens (Le Minor and Pigache, 1977; Traub and Kleber, 1977) and two new O antigens (Le Minor and Pigache, 1978) had been available.

Other methods of subspeciating strains of *S. marcescens* have been described; among these are bacteriocine typing (Traub and Raymond, 1971; Farmer, 1972) and phage typing (Pillich, Hradečna and Kocur, 1964; Prinsloo, 1966). Either of these techniques or the biotyping suggested by Rubin *et al.*, 1976, can be used to subgroup further the predominant serotypes when outbreaks of infection caused by *S. marcescens* are encountered. No evidence of such an outbreak was seen at BCRP within the period of study.

SUMMARY

A study of 1314 patients with malignancies was made to determine the prevalence of *Serratia*

marcescens in surveillance, diagnostic, and environmental cultures. Sera obtained from a commercial source were used to determine serotypic distributions of the *S. marcescens* strains isolated during a 51-month period. *S. marcescens* was isolated from 19% of patients with haematological neoplasms, from 5% of patients with lymphoma, and from 6% of those with solid tumours. Among carriers, rectal cultures were the commonest source in patients with lymphoma (32%); rectal and gingival cultures in patients with leukaemia (43% and 39%, respectively), and gingival cultures in patients with solid tumours (30%). Bacteraemias (0.7%) were infrequent sources. Although seldom isolated from environmental or food samples, *S. marcescens* may occasionally be abundant in fresh fruit and vegetables. Serotyping of 220 strains of *S. marcescens* demonstrated 38 distinct antigenic types. The predominant serotype, O14:H12, was present in the upper respiratory tract of half of the persons who carried this serotype. Serotyping is a readily reproducible method of subspeciating *S. marcescens*; it can be satisfactorily used as an epidemiological tool.

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