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Continued Surveillance of *Serratia marcescens* Infections by Bacteriocin Typing: Investigation of Two Outbreaks of Cross-Infection in an Intensive Care Unit

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During an 8.5-month period, 198 additional isolates of *Serratia marcescens* were typed by bacteriocin sensitivity; 154 isolates were typable and were categorized according to our current system of 54 provisional bacteriocin sensitivity patterns. Two outbreaks of nosocomial infection due to *S. marcescens* occurred in our intensive care unit, involving two and five patients, respectively. The latter outbreak was caused by a strain of *S. marcescens* which was not sensitive to any of the 10 bacteriocins normally used. Therefore we developed a supplementary procedure based on bacteriocin production rather than bacteriocin sensitivity. Bacteriocin production was induced with mitomycin C, and the crude lysates were applied to 15 provisional bacteriocin indicator strains. The reverse typing procedure was necessary to determine the spread and ultimate subsidence of this particular outbreak of cross-infection.

Recently we developed a simple procedure for typing clinical isolates of *Serratia marcescens* by bacteriocin sensitivity (7). The application to the study of hospital-acquired infections was presented in a subsequent communication (6). Here we wish to present further data. The previous scheme of 37 bacteriocin sensitivity patterns now has been expanded to 54, based on the results obtained from 198 additional clinical isolates. A strain of *S. marcescens* that had acquired nosocomial significance in our intensive care unit was found to be resistant to all 10 bacteriocins employed in our standard procedure. Therefore, a supplementary reverse typing procedure was developed.

MATERIALS AND METHODS

From 16 April through 31 December 1971, 198 isolates of *S. marcescens* were recovered from clinical specimens derived from 144 patients and the hospital environment. The isolates were identified and typed by bacteriocin sensitivity as previously described (5-7). Those isolates from patients in the intensive care unit which were resistant to all 10 of our standard bacteriocins were reverse typed by bacteriocin production as follows. Bacteriocins were prepared from each nontypable isolate after induction with mitomycin C in the same way as in our usual procedure (7). The crude lysates were then dropped onto *S. marcescens* indicator isolates no. 5, 10, 12, 16, 17, 18, 31, 33, 43, 46, 1, 9, 21, 274, and 326. Isolates that yielded bacteriocins of identical host range against these 15 selected indicator organisms *and* which were resistant to the killing action of homologous bacteriocins (1, 2) were interpreted to be the same strain.

Two outbreaks of *S. marcescens* cross-infection in the intensive care unit were investigated as follows. Patient specimens (sputum, tracheal aspirates; throat, wound, and rectal swabs; clean-voided or catheterized, or both, urine specimens; stools), throat swab and fingertip skin specimens from intensive care unit personnel (nurses, physicians, students, nursing aides, maids, etc.), and environmental specimens (swab technique) were inoculated directly into tubes that contained 2 ml of Tryptic Soy broth (Difco Laboratories, Detroit, Mich.) plus 50 μg of polymyxin B per ml (Aerosporin, injectable; Burroughs Wellcome and Co., Inc., Tuckahoe, N.Y.). The tubes were incubated at 35 C overnight; tubes with growth were subcultured to MacConkey agar (Fisher Scientific Co., Raleigh, N.C.) and further processed for isolation of *S. marcescens*. All isolates of *S. marcescens* were typed by bacteriocin sensitivity, and nontypable isolates were reverse typed as described above.

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RESULTS AND DISCUSSION

A total of 154 of the 198 isolates of *S. marcescens* were typable with our routine procedure. Nineteen of the typable isolates had previously undetected bacteriocin sensitivity patterns. Table 1 lists the new sensitivity patterns which we have designated 38 through 54; patterns 1 through 37 were reported previously (6). The remaining 135 typable isolates were of bacteriocin types 1, 3, 4, 5, 7, 9, 10, 13, 14, 16, 18, 19, 21, 24, 28, 33, and 37, as shown in Table 2. The majority of the isolates were of bacteriocin types 1, 4, 9, 14, 16, 18, and 21, as noted previously (6). A total of 44 isolates of the current series proved nontypable. Thirteen of these latter isolates (plus 6 of 12 isolates, designated S-ICC 1 through S-ICC 12) were recovered from patients in our intensive care unit during the period of October through December 1971. The bacteriocin production pattern (host range) of all isolates comprising this strain is listed in Table 3. Relevant epidemiological data concerning this strain were presented in Table 4.

Patient C.V.G., the probable index case, was admitted to North Carolina Baptist Hospital on 10 October 1971. Surgery was performed 10 days later. Before his transfer to the intensive care unit on 20 October 1971 this patient had been hospitalized in room no. 357. Subsequently, patient W.B.H. was admitted to this room on 28 October 1971. A sputum culture from patient W.B.H. on 2 November yielded isolate no. 320, but this strain yielded a bacteriocin that differed in host range from those elaborated by isolates that comprised the nosocomially significant strain (Table 3). During November 1971, three more patients of the intensive care unit became infected by the outbreak strain; patients B.P.C. and D.D. were colonized, but patient J.W.T. appeared to have a significant infection (Table 4). Extensive monitoring of the patients, personnel, and the environment of the intensive care unit disclosed the following. The outbreak strain was recovered from an overhead table of a patient without *S. marcescens* infection, and the fingertips of a maid (Maid S.). In December 1971, a fifth patient (J.B.B.) was colonized by the outbreak strain; it is noteworthy that this patient had been on reverse isolation since his admission to the intensive care unit on 14 September 1971. Finally, the outbreak strain was

TABLE 1. *Bacteriocin sensitivity/tolerance patterns of Serratia marcescens isolates comprising bacteriocin types no. 38 through 54*

Bacteriocin (marcescin) type	Bacteriocin sensitivity pattern: susceptibility to and tolerance for bacteriocins of *S. marcescens* isolate no.									
	5	10	12	16	17	18	31	33	43	46
38	−[a]	−	−	−	−	+	+	+	−	−
39	−	+	−	−	−	−	−	+	+	−
40	−	−	+	−	+	−	−	−	−	−
41	+	−	+	−	+	−	+	−	−	−
42	−	−	−	−	−	−	−	−	+	−
43	−	−	−	−	−	−	+	+	−	−
44	−	+	−	+	−	+	−	−	+	+
45	−	+	−	+	−	−	−	−	+	+
46	−	+	+	+	−	−	+	+	+	−
47	−	−	−	−	−	−	+	−	−	+
48	−	+	+	+	−	+	+	−	+	−
49	+	+	+	+	+	+	+	−	+	+
50	−	−	−	−	−	+	+	−	−	+
51	+	−	+	−	+	−	−	−	−	+
52	−	+	−	−	−	−	−	−	+	−
53	−	−	−	−	−	+	+	−	−	−
54	+	+	+	−	+	−	+	−	+	−

[a] Symbols: +, sensitive; −, resistant (tolerant) to respective bacteriocin.

TABLE 2. *Distribution according to bacteriocin sensitivity pattern of 198 isolates of Serratia marcescens*

Bacteriocin sensitivity pattern	No. of isolates	Bacteriocin sensitivity pattern	No. of isolates
1	15	31	
2		32	
3	4	33	1
4	18	34	
5	1	35	
6		36	
7	1	37	2
8		38	1
9	11	39	1
10	3	40	1
11		41	3
12		42	1
13	9	43	
14	14	44	
15		45	1
16	13	46	1
17		47	1
18	29	48	1
19	1	49	2
20		50	1
21	9	51	2
22		52	1
23		53	1
24	2	54	1
25			
26		Nontypable	44
27			
28	2	Total	198
29			
30			

TABLE 3. *Bacteriocin production patterns (host ranges) of two nontypable strains*

Bacteriocin-producing strain	Host ranges of bacteriocins against indicator isolates no.														
	5	10	12	16	17	18	31	33	43	46	1	9	21	274	326
Outbreak strain	−[a]	+	−	+	−	+	+	+	+	+	+	+	−	+	−
Isolate no. 320 (patient W.B.H.)	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−

[a] Symbols: +, inhibition of growth of indicator isolate; −, no inhibition.

TABLE 4. *Epidemiology of two outbreaks of cross-infection in the intensive care unit*

Episode no.	Cause	Isolate no.	Date isolated	Source	Patient, personnel (initials)	Underlying illnesses
1	Bacteriocin-resistant outbreak strain	305	10/26/71	Tracheal aspirate	C.V.G.	Chronic lymphocytic leukemia; adenocarcinoma of colon; intraperitoneal abscess. Splenectomy, subtotal colectomy, peritoneal drainage Deceased 11/4/71
		306	10/26/71	Sputum		
		314	10/28/71	Foley catheter		
		315	10/29/71	Tracheal aspirate		
		326	11/4/71	Wound (necropsy)		
		328	11/6/71	Tracheal aspirate	B.P.C.	Pulmonary emphysema
		329	11/6/71	Sputum	D.D.	Mitral valve replacement
		332	11/9/71	Overhead table		
		S-ICC 1	11/19/71	Fingertips	Maid S.	
		333	11/15/71	Wound	J.W.T.	Liver cirrhosis; portocaval shunt
		S-ICC 2	11/19/71	Rectal thermometer		
		339	11/22/71	Tracheal aspirate		
		341	11/23/71	Tracheal aspirate		
		S-ICC 3	11/30/71	Wound		
		S-ICC 4	11/30/71	Sputum		
		362	12/10/71	Sputum		
		363	12/10/71	Tracheal aspirate	J.B.B.	Guillain-Barré syndrome; tracheostomy
		S-ICC 5	12/11/71	Tracheal aspirate		
		S-ICC 6	12/14/71	Shaving brush		
2	Bacteriocin type 14 strain	S-ICC 7	11/19/71	Sputum	N.O.B.	Mitral valve replacement; tracheostomy
		338	11/22/71	Tracheal aspirate		
		S-ICC 8	11/30/71	Wound		
		367	12/10/71	Blood		
		S-ICC 9	12/14/71	Tracheal aspirate		
		S-ICC 10	12/14/71	Sputum		
		S-ICC 11	12/14/71	Stool		
		S-ICC 12	12/17/71	Tracheal aspirate		
		343	11/26/71	Tracheal aspirate	W.C.B.	Repair of left ventricular aneurysm

recovered from a shaving brush that had been in use for nonisolated male patients of the intensive care unit.

During November 1971, a second strain of *S. marcescens* (bacteriocin type 14) led to respiratory tract and postsurgical wound infection, including one episode of bacteremia, in patient N.O.B. (Table 4). In late November an isolate of *S. marcescens* of the same bacteriocin type was recovered once from the respiratory tract

of patient W.C.B. (Table 4).

On 30 November two more patients with infection due to *S. marcescens* were discovered. Patient J.L.H. (resection of aortic aneurysm) had acquired postsurgical wound infection due to *S. marcescens* of bacteriocin type 7. Patient C.B.R. (chronic alcoholism, hepatic coma) yielded *S. marcescens* of bacteriocin type 1 from sputum, tracheal aspirate, and rectal swab specimens prior to her death on 8 December. However, these latter two strains of *S. marcescens* did not acquire nosocomial significance. Thus, on 30 November 1971 there were four strains of *S. marcescens* prevalent in the intensive care unit, as judged by their different sensitivities to bacteriocins.

The data obtained with bacteriocin typing of 198 additional clinical isolates of *S. marcescens* allowed us to expand the system of categorization according to bacteriocin sensitivity from the previous 37 to the present 54 provisional bacteriocin types. The large number of nontypable isolates encountered during the current observation period was partly attributable to the emergence of a nontypable strain of *S. marcescens* that gained nosocomial significance. It may be added that *S. marcescens* isolate no. 326 (from patient C.V.G.) proved serologically nontypable (Betty Davis, Center for Disease Control, Atlanta, Ga., *personal communication*). If one were to examine all nontypable clinical isolates of *S. marcescens* with the supplementary reverse typing procedure, one might possibly be able to type roughly 75% of these isolates as based on the extent and specificity of host ranges of elaborated bacteriocins against selected indicator organisms. Previously we had found that roughly 75% of 50 isolates of *S. marcescens* proved bacteriocinogenic (7).

As noted previously, no major hospital-wide outbreaks of cross-infection due to *S. marcescens* were discernible during the present observation period. The value of the bacteriocin typing procedure was underscored by the finding that on 30 November 1971 there were four strains of *S. marcescens* prevalent among patients of our intensive care unit. Of these, two strains were shown to be nosocomially significant. One strain (bacteriocin type 14) spread from one patient with serious infection following open-heart surgery to the respiratory tract of another patient. The other outbreak of cross-infection involved five patients, two of whom had significant infection. The organism appeared to contribute toward mortality in one patient (C.V.G.), the probable index case. There were a number of disturbing findings in this outbreak. First, hand transmission of the outbreak was very likely since one of the maids of the intensive care unit carried this strain on her fingertips (3). Second, the strain was recovered from serveral inanimate sources. Thus, this strain had become established in a number of inanimate reservoirs, in contrast to findings on *Klebsiella pneumoniae* strains in an intensive care unit (4). Third, the finding that the outbreak strain had been transmitted to a patient who had been on protective isolation in the intensive care unit ever since his admission to the hospital indicated a breakdown of the technique of reverse isolation. These findings resulted in extensive and rigorously enforced combative prophylactic and educational measures, including strict isolation of every patient with known *S. marcescens* infection, which limited and ultimately brought under control this outbreak of cross-infection.

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