Outbreak of Nosocomial Urinary Tract Infections Caused by *Serratia marcescens*

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A prolonged outbreak (December 1980 to July 1982) of nosocomial urinary tract infections appeared to be due to strains of *Serratia marcescens* that were resistant to currently available antibiotics. The serotyping and antibiotic susceptibility patterns suggested a few endemic strains of serotypes O13, O2/3, O12/14, and nontypable strains. These strains were isolated from the urine samples of inpatients with urinary tract infections in the urology ward and in other wards. The strains of O12/14 (gentamicin susceptible) were replaced with those of O2/3 (gentamicin resistant) between June and September 1981, whereas the other serotypes were isolated continuously. They were resistant to sulbenicillin, cefmetazole, gentamicin, and amikacin, and susceptible to micronomicin and ofloxacin, a new quinolone antibiotic. Most of them were also resistant to the disinfectant chlorhexidine, which had been used widely for hand washing in the hospital.

During the past decade, *Serratia marcescens* has been encountered as a formidable nosocomial pathogen, particularly of the urinary tract and respiratory tract (1, 2, 8-11, 16, 17, 20). In some hospitals, a definite strain or serotype with an unusual antimicrobial susceptibility pattern has caused an epidemic of limited duration (11, 17). Most of the strains reported have been highly resistant to the currently available antibiotics, except to a few aminoglycosides, including gentamicin and amikacin (4, 18, 19). Recently, however, increasing numbers of reports have pointed out the rise in epidemics due to strains resistant even to gentamicin or to amikacin (3, 5, 6, 12, 15, 18), thus posing difficulties in the control of *S. marcescens* infections.

We recently experienced a nosocomial outbreak of urinary tract infection (UTI) with a few serologically different strains of *S. marcescens*. During the events, a bacterial substitution possibly due to an antibiotic employed was noticed. Through the susceptibility testings, ofloxacin, an antibiotic of greater potency than the currently available antibiotics, was found to be useful.

MATERIALS AND METHODS

Bacteria. A total of 192 strains of *S. marcescens* isolated from the urine samples of patients with UTI hospitalized in Kyorin University Hospital, Tokyo, Japan, from 1980 to 1982 were used. In all cases, the colony counts exceeded 10^5 /ml, and no colony was pigmented. After being serotyped, they were preserved at room temperature in a semi-solid agar medium made by adding 0.4% Oxoid agar no. 3 (Oxoid Ltd., London) to brain heart infusion (BHI) broth (Nissui Seiyaku Co., Ltd., Tokyo).

Serotyping. The serotype of each strain was determined by an agglutination test, using the specific antisera to O antigens of *S. marcescens* (Denka Seiken Co., Ltd., Tokyo). Overnight cultures of each strain on heart infusion agar medium (Nissui Seiyaku Co.) at 37°C were suspended in physiological saline and killed by heating at 120°C for 20 min. After cooling, the bacterial suspensions were centrifuged at 3,000

rpm for 30 min, and the sediments were resuspended in physiological saline at a concentration of 1 mg/ml for use as antigens. Samples (0.5 ml) of the antigens were poured into small test tubes containing 0.1 ml of specific antisera, and the test tubes were placed overnight in a refrigerator after incubation at 50°C for 2 h with shaking. The antigen that aggregated macroscopically in one test tube was designated as the O antigen corresponding to the specific O antiserum in the test tube.

MICs. For each strain, the MICs of the antibiotics and of a disinfectant, chlorhexidine, were measured by the agar dilution method. The overnight cultures in BHI broth at 37°C were adjusted to 0.5 McFarland standard by the addition of fresh BHI broth to the cultures, equivalent to 10^8 CFU/ml, and then were diluted 1:100 (10^6 CFU/ml) with fresh BHI broth. By using a semiautomatic inoculator, Micro Planter (Maeda Factory Ltd., Tokyo), we spotted the bacterial suspensions on BHI agar plates containing antibiotics or chlorhexidine. After incubation of the plates, the minimal drug concentrations that prevented the formation of more than five colonies on the plates were defined as the MICs.

Antibiotics and disinfectant. The antibiotics tested were as follows: sodium sulbenicillin (Takeda Chemical Industries, Ltd., Osaka), sodium cefmetazole (Sankyo Co., Ltd., Tokyo), gentamicin sulfate (Shionogi & Co., Ltd., Osaka), amikacin sulfate (Banyu Pharmaceutical Co., Ltd., Tokyo), micronomicin sulfate (Kyowa Hakko Co., Ltd., Tokyo), and ofloxacin (Daiichi Seiyaku Co., Ltd., Tokyo). For a disinfectant, we used chlorhexidine gluconate (ICI Pharmaceutical Inc., Tokyo). Chlorhexidine and the antibiotics other than ofloxacin were dissolved in sterile distilled water at concentrations of 4,000 and 1,000 µg/ml and diluted serially twofold to 31.2 and 2 µg/ml, respectively. Ofloxacin was dissolved in 0.1 N sodium hydroxide and diluted with sterile distilled water in the same manner. The agar plates were prepared by mixing 2-ml quantities of each dilution of the drugs with 18 ml of BHI agar medium in a plastic petri dish (90 mm in diameter), so that the concentrations of chlorhexidine and the antibiotics ranged from 3.12 to 400 and from 0.2 to 100 µg/ml, respectively.

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TABLE 2. Incidence of isolation of strains of serotype O13 and NT from urine samples of patients in each ward

Serotype	Source ^a	No. of strains isolated during:																			
		1980 Dec.	1981 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	1982 Jan.	Feb.	Mar.	Apr.	May	June	July
O13	Building A																				
	INT-1												1		1	1		2	3	3	3
	INT-2														1		2	1	3	3	1
	GYN																				
	Building B																				
	ICU	2																1	4	3	3
	ORT																			1	1
	URO			1	1			1	3	2			1							1	1
	NER	3	2					2	1					1				2	1		1
	SUR-1																		1		
	INT-3		1	1															1	1	
	CAR																		1		
NT	Building A																				
	INT-1																		1		
	OTO																		1		
	INT-2																	1	1	1	
	Building B																				
	ICU	1		1		1	1	1													
	ORT							2		1											
	URO					1		1		1	1	1		1				1			
	NER		1								1								1		
	INT-3	1					1	1	1	1									1		

^a Abbreviations: First Department of Internal Medicine (INT-1), Second Department of Internal Medicine (INT-2), Third Department of Internal Medicine (INT-3), Gynecology (GYN), Intensive Care Unit (ICU), Orthopedic Surgery (ORT), Urology (URO), Neurosurgery (NER), First Department of Surgery (SUR-1), Cardiac and Thoracic Surgery (CAR), and Otorhinolaryngology (OTO).

DISCUSSION

S. marcescens has been established clearly as a cause of serious UTIs in hospitalized patients (1, 4, 5, 9, 11, 16, 17, 20–22). The predominant serotypes of clinical isolates varied greatly (11, 16, 20), except in a few cases in which a single serotype predominated (17). The present prevalence survey revealed an epidemic of four or more serotypes, including a nontypable one, with an interesting possible mode of transmission within a hospital.

It is conceivable that the indigenous *Serratia* sp. organisms (drug susceptible), living in water or in soil, are brought into a hospital and cause opportunistic infections in high-risk

patients, thus defining the serotypes predominating in the hospital (9). Then the organisms in patients acquire resistance to drugs as a result of indiscriminate extensive dosing (9, 16). Many strains of clinical isolates of *S. marcescens* are highly resistant to the current antibiotics (3, 5, 6, 15, 17–19, 22), and the drug resistance is known to be transferred to other susceptible bacteria via R plasmids (4, 14, 15, 18), thus introducing a problematic situation in which aminoglycoside antibiotics, including gentamicin and amikacin, which have been employed for the treatment of infections with *S. marcescens*, may no longer be effective. The decrease in the susceptibility of O13 over time (group A versus group B) to

TABLE 3. Incidence of isolation of strains of serotype O12/14 and O2/3 from urine samples of patients in each ward

Serotype	Source ^a	No. of strains isolated during:																			
		1980 Dec.	1981 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	1982 Jan.	Feb.	Mar.	Apr.	May	June	July
O12/14	Building A																				
	INT-1	1				1															
	INT-2						1	1													
	GYN		1																		
	Building B																				
	ICU					1		3		1											
	ORT						2		1												
	URO					2		1	1	1	1										
	SUR-1			1				1													
INT-3	1	1	2			1															
O2/3	Building A																				
	INT-1													2				1			
	INT-2									1		2	1	1	1						
	GYN									1	1										
	Building B																				
	ICU									4	1	1	1	2	1	2					
	ORT													4							
	URO													1		2	2	5	1	2	4
	NER										1			1	1	1			1	2	1
	SUR-1								1												
SUR-2							1														
INT-3													1	1	1	1					

^a Abbreviations are the same as in Table 2.

TABLE 4. Susceptibilities to antibiotics of the major strains of *S. marcescens* isolated from patients in each ward

Drug	Serotype	No. of strains tested	MIC ($\mu\text{g/ml}$)		
			Range	MIC ₅₀	MIC ₉₀
Sulbenicillin	O12/14	15	25->100	>100	>100
	O2/3	52	12.5->100	>100	>100
	O13	47	25->100	>100	>100
	NT	25	6.25->100	>100	>100
Cefmetazole	O12/14	15	25->100	>100	>100
	O2/3	52	50->100	100	>100
	O13	47	12.5->100	>100	>100
	NT	25	1.56->100	100	>100
Gentamicin	O12/14	15	1.56-100	6.25	25
	O2/3	52	3.12->100	50	>100
	O13	47	1.56->100	100	>100
	NT	25	1.56->100	25	>100
Amikacin	O12/14	15	1.56-100	25	50
	O2/3	52	6.25-100	25	50
	O13	47	0.78-50	25	50
	NT	25	3.12-25	25	25
Micronomicin	O12/14	15	0.78-12.5	3.12	6.25
	O2/3	52	1.56-25	6.25	25
	O13	47	≤ 0.20 -50	6.25	25
	NT	25	≤ 0.20 -25	1.56	12.5
Ofloxacin	O12/14	15	1.56-12.5	3.12	3.12
	O2/3	52	≤ 0.20 -25	0.78	3.12
	O13	47	≤ 0.20 -25	6.25	12.5
	NT	25	≤ 0.20 -12.5	1.56	12.5

gentamicin and amikacin may be due in part to the conjugational transfer of the resistance. Gentamicin was active against most of the strains of O12/14 and O13 isolated by September 1981 (group A), and thus the strains of the former serotype may have been eradicated by application of the antibiotic in each ward of the hospital. Furthermore, the strains of O2/3 resistant to gentamicin began to be isolated from patients in June 1981, and they spread rapidly throughout the hospital, appearing to replace the strains of O12/14. However, most strains of the isolates were susceptible to micronomicin and ofloxacin, and so these two antibiotics are expected to take the place of aminoglycoside antibiotics for the treatment of *S. marcescens* infections.

Futhermore, it has been known that outbreaks of nosocomial infections with the organisms are also caused by incorrect use of disinfectants (7, 17). Marrie and Costerton (13) described an outbreak of nosocomial infection due to contamination of disinfectant hand soap with *S. marcescens* resistant to 2% chlorhexidine. This may be the same strain as that observed in this study; most of the isolates, except for those of O12/14, were highly resistant to chlorhexidine.

In a hospital affiliated with a university, it is a major problem that, in addition to the bacterial contamination of medical instruments, the careless movement of medical students or undergraduate nurses from ward to ward for medical or educational purposes can facilitate the transmission of drug-resistant mutants of causative organisms (8, 11, 16, 17). In addition, the bed density of a ward has been correlated with the occurrence of nosocomial infections (1). To control nosocomial infections, more careful epidemiological surveys should be required in addition to correct drug selections for patients.

TABLE 5. Changes in MICs of drugs for clinical isolates of *S. marcescens* isolated at different times^a

Drug	Serotype	Group	No. of strains tested	MIC ($\mu\text{g/ml}$)		
				Range	MIC ₅₀	MIC ₉₀
Sulbenicillin	O12/14	A	15	25->100	>100	>100
		B	11	50->100	>100	>100
	O2/3	A	41	12.5->100	>100	>100
		B	12	50->100	50	>100
	O13	A	35	25->100	>100	>100
		B	17	6.25->100	>100	>100
		A	8	50->100	>100	>100
		B				
Cefmetazole	O12/14	A	15	25->100	>100	>100
		B	11	50->100	100	>100
	O2/3	A	41	50->100	100	>100
		B	12	100->100	>100	>100
	O13	A	35	12.5->100	>100	>100
		B	17	1.56->100	100	>100
		A	8	12.5->100	100	>100
		B				
Gentamicin	O12/14	A	15	1.56-100	6.25	12.5
		B	11	12.5->100	100	>100
	O2/3	A	41	3.12->100	25	>100
		B	12	1.56->100	1.56	100
	O13	A	35	3.12->100	100	>100
		B	17	1.56-100	12.5	100
		A	8	6.25->100	25	>100
		B				
Amikacin	O12/14	A	15	1.56-100	25	50
		B	11	12.5-50	25	50
	O2/3	A	41	6.25-100	25	50
		B	12	0.78-50	3.12	6.25
	O13	A	35	3.12-50	25	50
		B	17	3.12-25	25	25
		A	8	6.25-25	25	25
		B				

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TABLE 5—Continued

Drug	Serotype	Group	No. of strains tested	MIC (μ g/ml)		
				Range	MIC ₅₀	MIC ₉₀
Micronomicin	O12/14	A	15	0.78–12.5	3.12	6.25
		A	11	3.12–25	25	25
		B	41	1.56–25	6.25	12.5
	O13	A	12	≤0.20–50	0.78	12.5
		B	35	0.39–25	12.5	25
	NT	A	17	0.39–12.5	1.56	12.5
		B	8	≤0.20–25	6.25	25
Ofloxacin	O12/14	A	15	1.56–12.5	3.12	6.25
		A	11	0.39–12.5	0.78	0.78
		B	41	≤0.20–25	0.78	3.12
	O13	A	12	1.56–25	3.12	12.5
		B	35	≤0.20–12.5	6.25	12.5
	NT	A	17	≤0.20–6.25	1.56	6.25
		B	8	≤0.20–12.5	0.39	12.5
Chlorhexidine	O12/14	A	13	≤3.12–12.5	3.12	12.5
		A	9	≤3.12–200	100	200
		B	40	12.5–200	100	200
	O13	A	12	≤3.12–100	6.25	100
		B	34	≤3.12–400	12.5	200
	NT	A	15	≤3.12–200	6.25	200
		B	8	≤3.12–400	6.25	400

^a The strains of each serotype were divided into two groups: group A consisted of strains isolated by September 1981, when the bacterial substitution of O2/3 for O12/14 was observed, and group B consisted of those isolated subsequently, from October 1981 to July 1982.

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