

Nosocomial Infection with *Serratia marcescens*:  
Comparison of Bacteriocin Types and Antibiograms  
between Two University Hospitals

Masaru NASU, Jun GOTO, Yoichiro GOTO,  
Takayoshi TASHIRO and Takashi ITOGA

Second Department of Internal Medicine, Medical College of Oita,  
Oita, Japan

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Nosocomial distribution of *Serratia marcescens* at two university hospitals was studied by bacteriocin typing and antibiogram from January 1981 to July 1983, and the results were compared. The 126 strains isolated from Medical College Hospital of Oita (MCHO) consisted mainly of bacteriocin types 4 (29%), 9 (23%), 52 (19%) and 42 (6%), and the 86 from Nagasaki University Hospital (NUH) were mainly of types 14 (20%), 4 (17%), 9 (12%) and 26 (12%); these four types amounted to 77% and 61% respectively. Types 4 and 9 which formed a high proportion of isolates at both hospitals were apparently the most common bacteriocin types. Using 154 strains of the bacteriocin types 4, 9, 14, 26, 42 and 52 of *S. marcescens*, antibiogram with 13 antimicrobial agents was tested. The strains isolated from MCHO were found to be more resistant than those from NUH. The isolates of *S. marcescens* at each hospital showed characteristic, different distribution in bacteriocin type and antibiogram. The close relation was not always observed between antibiograms and bacteriocin types.

Key words: *Serratia marcescens*, Bacteriocin typing, Antibiogram, Nosocomial infection

## INTRODUCTION

*Serratia marcescens*, a member of the tribe *Klebsiellae*, has been reported with increasing frequency as a cause of nosocomial infection. Urinary tract infection, bacteremia, respiratory tract infection and wound infection involving *S. marcescens* have

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那須 勝, 後藤 純, 後藤陽一郎, 田代 隆良, 糸賀 敬

Requests for reprints should be addressed to Dr. Masaru NASU, 2nd Department of Internal Medicine, Medical College of Oita, 1-1506, Hazama-cho, Oita 879-56, Japan.

been often encountered in hospitalized patients with severe underlying diseases<sup>5)18)19)</sup>. Most of *S. marcescens* strains are resistant to  $\beta$ -lactam compounds, aminoglycosides, nalidixic acid and colistin. The mechanism of antimicrobial resistance is mostly due to drug inactivating enzymes such as  $\beta$ -lactamase mediated by R-plasmids<sup>2)8)</sup>.

The authors recently obtained the indicator strains for bacteriocin typing of *S. marcescens* from Professor TRAUB, and the typing was carried out according to the method of TRAUB, RAYMOND and STARTSMAN<sup>17)</sup> for the isolates from the patients admitted to two university hospitals, Medical College Hospital of Oita (MCHO, 600 beds) and Nagasaki University Hospital (NUH, 780 beds). This paper presents the results of a comparison of bacteriocin types and antibiograms of *S. marcescens* isolates at two university hospitals.

## MATERIALS AND METHODS

**Bacteria:** A total of 212 strains of *S. marcescens* used in this study consisted of 126 isolates from MCHO and 86 from NUH. These bacteria were all collected from hospitalized patients during the period from January 1981 to July 1983, excluding reisolates from the same patient. These organisms were identified and stored at room temperature in the dark as previously described<sup>10)12)</sup>. The sources of *S. marcescens* from patients are shown in Table 1. The indicator strains used for bacteriocin typing were those reported by TRAUB, W. H., *et al.*<sup>17)</sup>.

**Bacteriocin typing of *S. marcescens*:** Bacteriocin solutions were prepared by a method similar to that of TRAUB, W. H., *et al.*<sup>17)</sup> and the isolates were typed by bacteriocin sensitivity as previously reported<sup>12)</sup>. The bacteriocin types were determined by the bacteriocin sensitivity pattern of TRAUB, W. H., *et al.*<sup>14)15)16)</sup>.

**Antimicrobial agents:** The following 14 compounds used in the current study were kindly supplied as follows; ceftazidime (Nihon Glaxo Co.), ceftriaxone (Nihon Roche Co.), latamoxef (Shionogi Co.), azthreonom (Taito-Pfeizer Co.), imipenem (Merk-Banyu Co.), norfloxacin (Kyorin Yakuhin Co.), ciprofloxacin (Bayer Yakuhin Co.), ofloxacin (Daiichi Seiyaku Co.), enoxacin (Dainihon Seiyaku Co.), gentamicin (Shionogi Co.), amikacin (Merk-Banyu Co.), tobramycin (Shionogi Co.), sisomicin (Bayer Yakuhin Co.) and astromicin (Kyowa Hakko Co.).

**Susceptibility testing:** The strains used for this study were selected for the most common bacteriocin types at the both hospitals, i.e. showing types 4, 9, 14, 26, 42 and 52. There were 154 strains composed of 101 isolates from MCHO and 53 from NUH. Susceptibility tests were made by the agar plate dilution technique. The strains were grown at 37°C for 14 hours in Mueller-Hinton broth (BBL) and further diluted in broth to provide 10<sup>5</sup> colony forming unit when delivered to the surface of test media with a multipoint inoculator (Sakuma Co., Japan). Doubling dilutions of antibiotics were prepared in 10 mM phosphate buffer (pH 7.2) to final concentrations of 100~0.05  $\mu$ g/ml in

Mueller-Hinton agar medium (BBL). Following incubation at 37°C for 14 hours the minimum inhibitory concentration (MIC) was defined as the lowest concentration resulting in the suppression of the growth.

## RESULTS

**Comparison of the bacteriocin types between two hospitals:** Table 1 and Fig. 1 show the distribution of *S. marcescens* bacteriocin types at both MCHO and NUH. The 126 strains isolated from MCHO were divided into eight types, mainly 4, 9, 52 and 42; 15 (12%) were nontypable. These four types amounted to 77%. The 86 strains isolated from NUH were classified into 18 types, mainly 14, 4, 9 and 26; 14 (16%) were nontypable. These four types amounted to 61%. Bacteriocin type 42 which was one of the major types at MCHO was not isolated from NUH; on the other hand, type 26 which was one of the major types at NUH was not isolated from MCHO. *S. marcescens* types 4, 9 and 52 were more frequently isolated from MCHO than from NUH. Type 26 of *S. marcescens* which was not found at MCHO was isolated mainly from urine at NUH, and type 42 which was not found at NUH was isolated from sputa at MCHO.

**The distribution of *S. marcescens* bacteriocin types:** The distribution of bacteriocin types

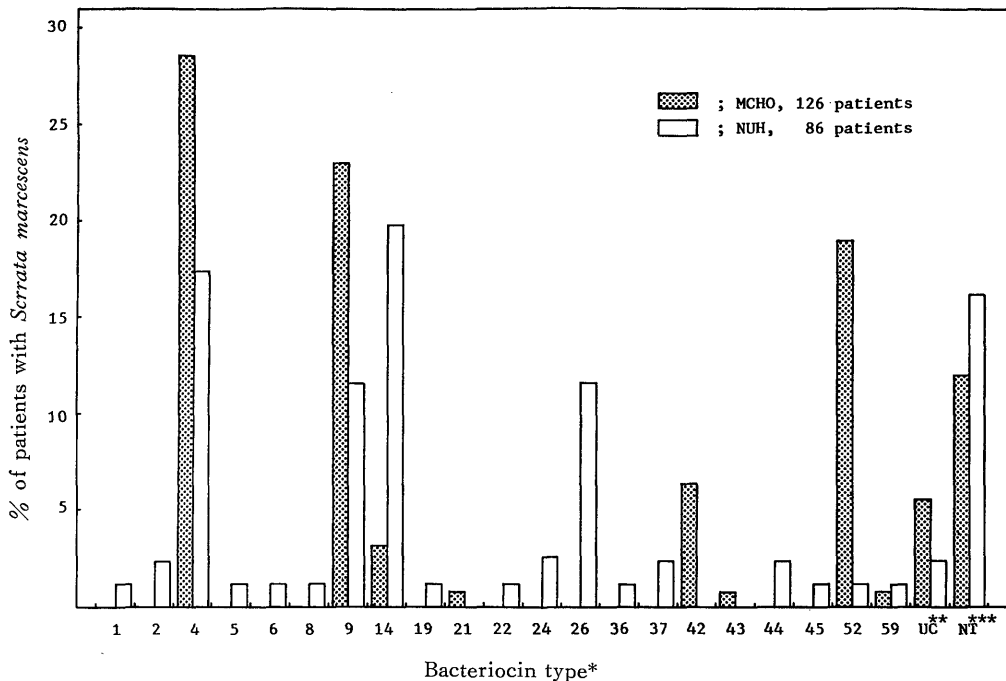


Fig. 1. Distribution of *Serratia marcescens* at Medical College Hospital of Oita (MCHO) and Nagasaki University Hospital (NUH) during 1981–1983.

\* See TRAUB & RAYMOND, 1971; TRAUB, 1972; TRAUB & KLEBER, 1974.

\*\* Unclassifiable.

\*\*\* Nontypable.

of *S. marcescens* at NUH has been previously reported<sup>12)</sup>. The distribution at MCHO is, therefore, reported in this paper (Table 2). Patients admitted to the wards of Urology, Medicine and Surgery were found to be the major hosts of *S. marcescens* infections, which were mostly of types 4, 9 and 52. The main infection sites of *S. marcescens* were urinary tract catheterized in Urology (types 4, 9, 52 and 14), respiratory tract with respiratory assistance and bacteremia in Medicine (types 4, 52, 9 and 42), and abscess and post-operative wounds in Surgery (types 4, 52 and 9). Each ward showed a specific bacteriocin type distribution of the isolates, and these data seemed to indicate that *S. marcescens* are spread by cross infection.

**Comparison of the proportion of resistant strains between two hospitals:** The strain with

Table 1. Distribution of the bacteriocin types of *Serratia marcescens* according to clinical sources

Clinical source	No. of patients	bacteriocin type							
		4	9	14	26	42	52	others	
Urine	MCHO**	76	25	20	3	—*	2	13	13
	NUH	39	6	1	7	8	—	—	17
Sputum	MCHO	17	2	3	1	—	3	2	6
	NUH	29	6	6	6	—	—	1	10
Pus & exudate	MCHO	11	5	3	—	—	1	1	1
	NUH	3	—	—	1	1	—	—	1
Others	MGHO	22	4	3	—	—	2	8	5
	NUH	15	3	3	3	1	—	—	5

\* — : Not isolated

\*\* MCHO : Medical College Hospital of Oita

NUH : Nagasaki University Hospital

Table 2. Distribution of the bacteriocin types of *Serratia marcescens* at each ward of Medical College Hospital of Oita during Jan., 1981 to July, 1983

Ward	Number of patients	bacteriocin type					
		4	9	14	42	52	others
Urology	48	14	14	3	2	7	8
Medicine	47	12	7	1	5	11	11
Surgery	13	5	2	—*	—	3	3
Pediatrics	6	1	1	—	1	1	2
Orthopedic Surgery	3	—	2	—	—	—	1
Otorhinology	3	—	2	—	—	1	—
Others	6	4	1	—	—	1	—

\* — : Not isolated

MIC of more than 25  $\mu\text{g/ml}$  was identified as the resistant one. The antimicrobial resistance acquired at each hospital is shown in Fig. 2. There were no resistant strains at both hospitals for three antimicrobials, imipenem, astromicin and ciprofloxacin. The isolates from NUH were found to be generally less resistant than those from MCHO. The *S. marcescens* of bacteriocin type 9 and the strains originated from sputa at NUH were all sensitive to the compounds tested. Of the strains 67~77% at MCHO and 10~33% at

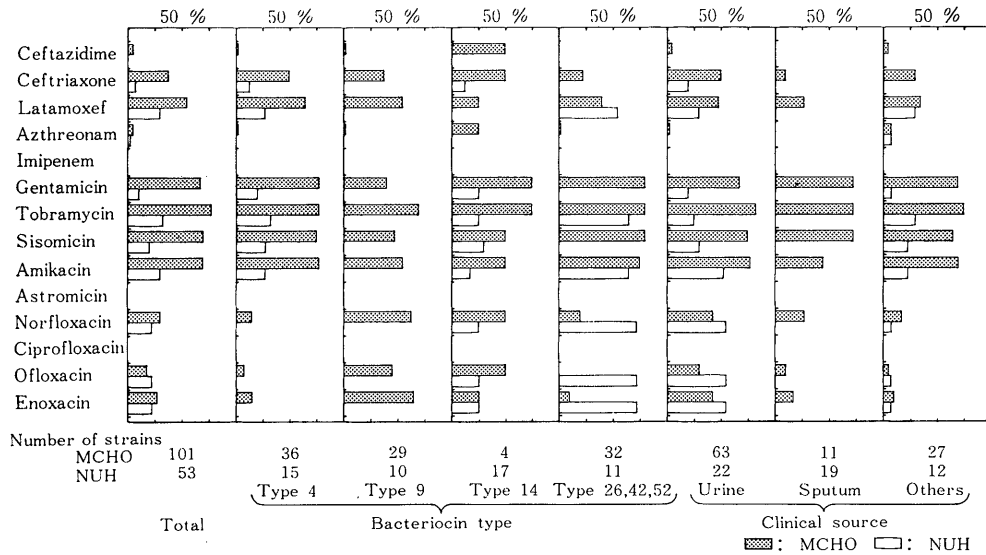


Fig. 2. Antimicrobial resistance (MIC;  $\geq 25 \mu\text{g/ml}$ ) of *Serratia marcescens* isolated from Medical College Hospital of Oita (MCHO) and Nagasaki University Hospital (NUH)

Table 3. Relationship between resistance patterns (MIC:  $\geq 25 \mu\text{g/ml}$ ) of *Serratia marcescens* to aminoglycosides and bacteriocin types

Resistance pattern	Number of strains	Bacteriocin type						
		4	9	14	26	42	52	
GM-TOB-SISO-AMK*	MCHO**	59	28	7	—***	—	7	17
	NUH	5	3	—	2	—	—	—
GM-TOB-SISO	MCHO	6	—	3	1	—	—	2
	NUH	0	—	—	—	—	—	—
TOB-SISO-AMK	MCHO	6	—	4	2	—	—	—
	NUH	3	—	—	3	—	—	—
TOB-AMK	MCHO	3	—	3	—	—	—	—
	NUH	8	1	—	—	7	—	—
Others	MCHO	4	—	4	—	—	—	—
	NUH	1	1	—	—	—	—	—

\* GM : Gentamicin, TOB: Tobramycin, SISO: Sisomicin, AMK: Amikacin

\*\* MCHO : Medical College Hospital of Oita, NUH: Nagasaki University Hospital

\*\*\* — : Not isolated

NUH were resistant against four aminoglycosides, gentamicin, tobramycin, sisomicin and amikacin.

**Relationship between aminoglycosides resistance patterns and bacteriocin types:** Relationship between aminoglycosides resistance patterns, excluding astromycin, and bacteriocin types of *S. marcescens* are shown in Table 3. Resistant strains for four antimicrobial drugs were most frequently isolated from MCHO, in 59 out of 101 strains (58%); they were classified into the bacteriocin types 4, followed by 52, 9 and 42 in decreasing order. Five strains (9%) of the isolates from NUH showed resistance for four compounds, three strains (which were all bacteriocin type 14) for three drugs, eight strains (of which seven were the bacteriocin type 26) for two agents, tobramycin and amikacin.

## DISCUSSION

*S. marcescens* is mostly resistant to cephalosporins and penicillins owing to chromosomal-mediated  $\beta$ -lactamase production with mainly cephalosporinase activity. Aminoglycoside-resistant strains are now increasingly found among clinical isolates<sup>2)8)</sup>. This bacterium, once used as a biological marker for pathogenicity studies, is now almost routinely encountered and play a role as an important opportunistic pathogen of compromised hosts<sup>18)19)</sup>. Following previous studies on *S. marcescens* infections with nosocomial distribution of bacteriocin types<sup>11)12)</sup>, comparative investigation was undertaken in two hospitals remotely situated from each other.

Epidemiologic study of infection with *S. marcescens* is usually carried out using serotyping, antibiogram, bacteriocin typing and biotyping as a marker<sup>1)4)</sup>. Bacteriocin typing is also frequently utilized in tracing the nosocomial infection<sup>3)6)7)9)11)13)14)16)</sup>. In this study, the bacteriocin typing method of TRAUB, RAYMOND and STARTSMAN<sup>17)</sup>, and the antimicrobial dilution method for antibiogram were used in order to compare *S. marcescens* distribution at both hospitals owing to their simple methodology. The results obtained from two university hospitals demonstrated characteristic distribution of bacteriocin types and antibiograms at each hospital, suggesting the different ecology in in-patients or hospital environments at the MCHO and NUH respectively. Bacteriocin types 4 and 9 constituted a high proportion of *S. marcescens* isolates at both hospitals, similar findings were reported by TRAUB and RAYMOND<sup>15)</sup>, indicating that these types are most common among clinical isolates. From 1976 to 1978 bacteriocin type 26 was most predominant at NUH, constituting 41% of isolates, followed by types 4 and 9<sup>12)</sup>. In the present study at the same hospital, however, types 14, 4 and 9 were the most common and the type 26 was only 12%. This apparently showed that as the hospital population changes a concomitant change in the nosocomial bacteria also occurs, suggesting the need for follow-up studies at intervals of some years. Of the strains isolated 12~16% were nontypable by bacteriocin typing method used in this study. However this method was considered to be useful in studies of nosocomial infection with *S. marcescens*, because of good reproducibility of results.

Antibiogram may be easily utilized as epidemiologic tool at the hospital laboratory level, but it changes in antimicrobial susceptibility related to environmental factors or R-plasmid. The strains isolated from NUH were found to be less resistant than those from MCHO. The antimicrobial resistance patterns of *S. marcescens* for aminoglycosides were different in distribution at each hospital. These facts may suggest that the resistant strains are selected by using antimicrobial agents and they are spread in each hospital. The close correlation between bacteriocin types and antimicrobial resistance patterns was not evident because of the phenotypic variation and transferable R-plasmid of the bacteria. It seems reasonable to conclude that bacteriocin typing and antibiogram are useful and effective for the surveillance and control of nosocomial infection with *S. marcescens* if they are used at the same time.

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