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Original Article

Comparative Characterization of *Staphylococcus aureus* Isolates from Throats and Noses of Healthy Volunteers

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SUMMARY: *Staphylococcus aureus* isolates in 2001 from the nose and the throat of an adult population were characterized for their incidence and type. The incidence was 51%, present in 80 out of 157 individuals examined, consisting of 34 nasal carriers, 24 throat carriers, and 22 who carried the isolates in both the nose and throat. Among these isolates, 2 and 5 from the nose and the throat, respectively, were identified as methicillin-resistant *S. aureus*. *S. aureus* from the nose and throat of the same individuals were characterized for identification. Examination of their phenotypes revealed that in 11 individuals the clone of *S. aureus* in the throat was different from the nasal clone. These results suggested that staphylococcal flora in the nose and the throat were independently formed, and that attention should also be directed to the carriers of *S. aureus* in the throat for the control of nosocomial infection.

INTRODUCTION

Staphylococcus aureus as a causative organism of nosocomial infection is now often multidrug resistant, as represented by methicillin-resistant *S. aureus* (MRSA). Since the therapy for its infection has become more problematic than ever, prevention is now essential. *S. aureus* is one of the regular flora of human body surface, and is transmitted from person to person by touch, which is an important infection route of nosocomial infection. Therefore, an effort to find MRSA carriers among hospital staff members has been made in order to eliminate MRSA (1,2). In the studies on *S. aureus* carriers, samples from the nasal vestibulum have usually been examined, although *S. aureus* is widely distributed in the human body, including the nose, throat, intestine, skin, etc. Therefore, only a few detailed studies on *S. aureus* in the throat and/or intestine, etc. have been reported. Our previous studies on the incidence of *S. aureus* from the nasal vestibulum and the throat in children indicated that *S. aureus* was isolated more frequently from the throat than from the nasal vestibulum (3). Therefore, it was suggested that surveying *S. aureus* in only the nasal vestibulum is not sufficient to control nosocomial infection. In addition, it is of interest to compare data obtained from children with those from adults. The aims of this study were to survey the incidence of *S. aureus* in the throat of adults, and to investigate the difference between *S. aureus* in the nose and throat of the same individual.

MATERIALS AND METHODS

Subjects: A total of 157 adult volunteers who were not aware of any illness at the time of sampling were examined in 2001. They included 104 nursing students who had experienced a short-term rotation in the hospital of University of the Ryukyus, 18 medical students without hospital practice, and 35 teaching and administrative staff members in the Faculty of Medicine, University of the Ryukyus.

Sampling and isolation of *S. aureus*: Samples were taken from the surface of the tonsils and the nasal vestibulum using cotton swab. The swabbed samples were directly inoculated onto a sheep blood agar (Nissui Phamaceutical Co., Tokyo) and mannitol-salt agar (Eiken Chemical Co., Tokyo), and incubated at 37°C for 24 h.

Identification of *S. aureus*: Two yellow colonies on the mannitol-salt agar and two colonies with β-hemolysis on the blood agar were subcultured on a heart infusion agar plate. The proliferated organisms were characterized by the following examinations: Gram staining, catalase production, coagulase production, glucose and lactose fermentations in kligler iron agar (BBL, Becton Dickinson and Co., Cockeysville, Md., USA), and acetoin production in VP semisolid agar (Eiken Chemical). The organisms identified as *S. aureus* were examined for urease production using urea medium (Eiken Chemical), and if necessary, further characterization was carried out.

Toxin production: The productivity of staphylococcal enterotoxin was examined using an enterotoxin detection kit, SET-RPLA (Denka Seiken Co., Tokyo), and its antigen types A, B, C, and D were determined. The productivity of toxic shock syndrome toxin (TSST-1) was examined by using a toxic shock toxin detection kit, TST-RPLA (Denka Seiken). The detection procedures were performed according to the manuals accompanying the kits.

Coagulase type: The antigenic-type of coagulase was examined by neutralization tests using the coagulase-type specific antiserum. Inhibition of rabbit plasma coagulation was examined as described by Tajima et al. (4), using a coagulase typing immune sera kit (Denka Seiken).

Detection of PBP2': The expression of *mecA* was confirmed by detecting the gene product, PBP2'. A kit for the detection of PBP2', MRSA Screen (Denka Seiken), was used according to the manufacturer's instructions.

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Susceptibility to antibiotics: The minimum inhibitory concentration (MIC) of the following five drugs was examined by means of plate dilution method: ampicillin (Meiji Seika Kaisha, Tokyo), oxacillin (Wako Pure Chemical Industries, Osaka) as a substitute for methicillin, tetracycline (Nacarai Tesque, Kyoto), erythromycin (Dainippon Pharmaceutical, Osaka), and cefdinir (Fujisawa Pharmaceutical, Osaka). A series of heart infusion agar plates containing twofold dilutions of the drugs at final concentrations from 100 μg/ml to 0.1 μg/ml were prepared. Ten-fold dilutions of overnight broth-cultured *S. aureus* strains were inoculated onto each plate using a Microplanter (model MITP #00257, Sakuma Co., Tokyo), and incubated at 37°C for 24 h. Susceptibility was expressed as the MIC of each drug. The MIC of oxacillin was determined using agar plates containing 2% NaCl and cultured at both 35°C and 32°C.

RESULTS

Incidence of *S. aureus*: *S. aureus* was detected from either the nasal vestibulum (nose) or throat in 80 out of 157 individuals examined (51%). *S. aureus*-positive nose was seen in 56 individuals (36%), and -positive throat was 46 (29%). There were 34 nose-positive and throat- negative cases (22%), 24 throat-positive and nose-negative cases (15%), and 22 cases were positive in both the nose and throat (14%) (Table 1).

Comparison of *S. aureus* isolates in the nose and throat: Fifty *S. aureus* isolates from the nose and 41 isolates from the throat were examined for enterotoxin, TSST-1, and urease (Table 2). These were positive in 42, 28, and 34% of the nasal isolates, respectively, whereas they were positive for 42, 17, and 44% of the throat isolates, respectively. PBP2' was detected in 4 and 12% of nasal and throat isolates, respectively.

Arbitrarily selected 16 pairs of *S. aureus* isolates from the nose and throat of the same individual showed different phenotypes in 11 cases (Nos. 1-8, 13, 15, 16) and the same

Table 1. Incidence of *S. aureus*

Isolation sites		Incidence (%) n = 157
Nose	Throat	
+	+	22 (14.0)
+	–	34 (21.7)
–	+	24 (15.3)
–	–	77 (49.0)

Table 2. Phenotype of *S. aureus*

	Detection rate		
		Nose	Throat
	No. examined	50 (%)	41 (%)
Enterotoxin positive		21 (42)	17 (42)
type A		7	2
B		11	10
C		8	1
D		3	3
TSST-1 positive		14 (28)	7 (17)
Urease positive		17 (34)	18 (44)
PBP2' positive		2 (4)	5 (12)
Negative for any of the above proteins		12 (24)	12 (29)

Table 3. Characterization of paired strain in 16 cases

No.[1)]	Strains[1)]	Phenotype			
		Coaglase[2)]	Enterotoxin[5)]	TSST-1[6)]	Urease[7)]
1	16N	I,VII	–	–	++
	16T	V	–	–	++
2	36N	III	B,C,D	–	(+)
	36T	III	B	–	++
3	41N	III	A	+	–
	41T	III	B	–	++
4	42N	II	B	–	++
	42T	III	B	–	–
5	72N	II	–	–	+
	72T	UT[3)]	–	+	–
6	95N	II	–	+	–
	95T	II	–	+	++
7	97N	IV	C	+	–
	97T	V	–	+	++
8	98N	VI	–	–	–
	98T	w[4)]	B	–	–
9	105N	III	–	–	+
	105T	III	–	–	+
10	106N	V	–	–	–
	106T	V	–	–	–
11	107N	II	D	–	+
	107T	II	D	–	+
12	114N	I,VII	B	–	–
	114T	I,VII	B	–	–
13	120N	VII	–	–	+
	120T	IV	–	+	–
14	127N	III	–	–	–
	127T	III	–	–	–
15	148N	I	–	–	–
	148T	V	–	–	–
16	152N	UT[3)]	–	–	–
	152T	V	–	–	+

[1)]: Each case provides two strains, one from the nose (N) and another from the throat (T).
[2)]: Serotypes were classified into I to VII.
[3)]: Untypable.
[4)]: Reaction was very weak, and the antigenic type was not determined. However, strain 98T had the *nuc* gene; therefore, it was identified as *S. aureus*.
[5)]: Enterotoxin serotypes were expressed by A to D.
[6)]: +, production; –, no production.
[7)]: +, production; –, no production; (+), weak production.

Table 4. Drug susceptibility of *S. aureus* from the nose and the throat

nose = 50 throat = 41

MIC	MPIPC[1)]		ABPC		CFDN		EM		TC	
μg/ml	N	T	N	T	N	T	N	T	N	T
≦0.1	1	1	1	0	5	3	1	0	1	0
0.2	13	15	5	1	21	22	0	0	14	13
0.39	12	12	6	10	22	10	32	15	33	23
0.78	14	5	5	4	0	1	15	20	0	0
1.56	8	3	8	3	0	0	0	0	0	0
3.13	0	0	7	5	2	1	0	1	0	0
6.25	0	0	7	2	0	1	0	1	1	0
12.5	1	0	4	8	0	0	0	0	0	2
25	0	0	3	2	0	1	0	0	1	1
50	1	1	2	3	0	1	0	0	0	2
100	0	1	2	1	0	1	0	0	0	0
>100	0	3	0	2	0	0	2	4	0	0

[1)]: Results at 32°C.
N, nose; T, throat.
MPIPC, oxacillin; ABPC, ampicillin; CFDN, cefdinir; EM, erythromycin; TC, tetracycline.

Table 5. Characterization of MRSA in the nose and the throat

Sites	Strains	PBP2'	*mec*A	Coagulase	Enterotoxin	TSST-1	Urease
Nose	52N	+	+	V	UD	–	–
	95N	+	+	II	UD	+	–
Throat	61T	+	+	II	C	+	+
	95T	+	+	II	UD	+	+
	98T[1)]	+	+	w[2)]	B	+	+
	103T	+	+	II	UD	+	–
	129T	+	+	III	D	–	–

[1)]: See footnote [4)] of Table 3.
[2)]: Reaction was too weak to type.

phenotype in 5 cases (Nos. 9-12, 14) as shown in Table 3. Different phenotypes were seen in coagulase (8 cases: Nos. 1, 4, 5, 7, 8, 13, 15, 16), enterotoxin (4 cases: Nos. 2, 3, 7, 8), TSST-1 (3 cases: Nos. 3, 5, 13), and urease (8 cases: Nos. 2-7, 13, 16).

Drug susceptibility: The drug susceptibility patterns of the nasal and throat isolates were compared and no marked difference was found between them (Table 4).

MRSA: MRSA was detected in six cases (3.8%) out of the 157 examined. In one case, MRSA was isolated from both the nose and throat. The biological properties of these strains are summarized in Table 5. The coagulase reaction of strain 98T was very weak, thus we failed to identify its type, although it was regarded as *S. aureus* since *nuc* gene was positive (11). Except this strain, four of the six MRSA revealed coagulase type II (4/6 = 66.7%) phenotype. In comparison, 84 methicillin-susceptible *S. aureus* (MSSA) were also examined, and showed coagulase type II in only four strains (4.8%).

DISCUSSION

Many reports have described the rates of nasal carriage in various populations (5). The present study revealed that the throat as well as the nasal vestibulum were the habitat of *S. aureus*, and that *S. aureus* isolates from both the nose and throat in the same individual turned out to contain different clones. The real incidence rate of *S. aureus* from the throat might be higher than the present data, because sampling from the throat is more difficult than from the nose. Actually, we have found that the incidence of *S. aureus* was higher in samples taken by a medical doctor than those taken by non-medical doctors who played the main role in samplings (swabbing the tonsil surface) in this study.

In the past few decades we have not been aware of any particular report describing a survey *S. aureus* in the throat of healthy adults, although the incidence of *S. aureus* was incidentally mentioned in investigations with other purposes. According to the throat culture reports, *S. aureus* was isolated from 12% of non-infectious adults (6). This percentage is much lower than the detection rate in the present study (29%) and that in the previous study on children (58%) in which all sampling was performed by a pediatrician (3). Moreover, attention should be paid to the ratio of MRSA in the throat (6 of 41 = 15% in this study), which was substantially higher than that in the nose (2 of 50 = 4%). Based on these results, it seems that MRSA favors the throat more than the nose, although a follow-up study of *S. aureus* in the throat is required. In addition, these results suggested that throat culture should be taken into account in the investigation of MRSA carriers, and that the eradication of MRSA from the nasal vestibulum using mupirocin is not always satisfactory.

The incidence of different *S. aureus* clones depending on the isolation sites implies that the staphylococcal flora in the nose and the throat were independently formed. In order to distinguish the *S. aureus* clone in the throat and the nose, we compared four phenotypes with AP-PCR using four primers we designed. Consequently, it was indicated that AP-PCR pattern is not superior to phenotype as an epidemiological marker (data of AP-PCR not shown). An examination of phenotypes revealed a difference in throat and nasal *S. aureus* isolates in 11 individuals, whereas the AP-PCR we employed (7-12) revealed a difference in only seven individuals.

This study showed that the throat is an important habitat of *S. aureus* including MRSA. Although a project to prevent nosocomial MRSA infection has been intensively pursued in the past decade, the frequency of MRSA infection in hospitals has not effectively declined. Directing attention to only the nasal vestibulum may be a reason why nosocomial MRSA infections have not been reduced. Ecological study should be considered in epidemiology.

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