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PROPERTIES OF A HIGH RATE OF MRSA COLONIZATION IN THE NASAL CAVITY OF INTENSIVE CARE UNIT DOCTORS

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ABSTRACT

Background: Methicillin-resistant Staphylococcus aureus (MRSA) is a major causative agent of healthcare-associated infections. **Aims:** To survey S. aureus/MRSA colonization in the nasopharyngeal cavities of intensive care unit (ICU) doctors at a university hospital. **Methods**: Surveys on nasopharyngeal S. aureus/MRSA colonization in 29 ICU doctors at a university hospital were conducted during July 2011 and January 2012. Polymerase chain reaction (PCR) analysis revealed mecA-positive strains as MRSA. The antimicrobial susceptibilities and toxin gene profiles of the isolates were additionally examined. Results: A total of 52% of the doctors examined during the first survey and 64% during the second survey showed S. aureus colonization, and 81% of the isolates were confirmed to be MRSA. Most of the MRSA strains had partially mutated mecA, as determined by PCR. The MRSA isolates, except for three, were susceptible to oxacillin, suggesting that these isolates could be misidentified as methicillin-sensitive S. aureus (MSSA) in hospital laboratories, whereas several resistant colonies appeared after an additional 3 days of incubation in the presence of oxacillin. Among the MRSA isolates, only four were tst-positive, and none were eta/etb-positive. **Conclusion**: A high rate of MRSA colonization in the nasal cavity of ICU doctors at a university hospital was observed. Most MRSA isolates, as determined through mecA detection, were susceptible to oxacillin, but produced resistant mutants in the presence of oxacillin. Therefore, we strongly suggest monitoring and/or eradication of colonized MRSA in the nasal cavity of ICU doctors.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) has been a major causative agent of healthcare-associated infections [1,2]. The prevalence of MRSA in hospitals, although variable, comprises a proportion as high as 60%–70% of S. aureus isolates in Japan. Patients with severe underlying conditions are at a significantly greater risk of invasive staphylococcal infection than normal people [3]. MRSA infections such as septicemia and surgical site infection are occasionally found in intensive care unit (ICU) patients who have undergone major surgery. Moreover, an ICU is a cross-contamination site for MRSA because of the typical high turnover of patients from various wards in a hospital to the ICU.

Therefore, particular care of ICU patients with the MRSA virus is necessary for the prevention of cross infections among patients. Because the transmission of MRSA among ICU patients is largely caused through the unwashed hands of ICU doctors and nurses, improved hand hygiene practices are particularly required, in the ICU. Moreover, MRSA colonized in the nasal cavity of ICU doctors and nurses is a potential source of MRSA infection in patients. ICU doctors and nurses are at higher risk of MRSA exposure than normal people. Therefore, we examined the MRSA colonization in the nasal cavity of ICU doctors and found a high rate of MRSA colonization in ICU doctors at a university hospital in central Japan.

Objectives: The aim of this study is to survey S. aureus/MRSA colonization in the nasopharyngeal cavities of ICU doctors at a university hospital.

MATERIALS AND METHODS

Study design and setting: This study is a descriptive survey based research the MRSA colonization of ICU doctors.

Ethics approval: The study was approved by IEC of our university, and the subjects' informed consent was obtained.

Sample size: Surveys on S. aureus/MRSA colonization in the nasal cavity of 29 ICU doctors at a university hospital in central Japan were conducted during July 2011 and January 2012.

Inclusion criteria: Sample target of one time experiment was decided to doctors who work six months in this ICU, The second sample was decided to doctors to continue working from the first time.

Methodology:

Bacteriological examination [4.5.6] : Nasal swab

specimens were plated onto mannitol salt agar plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 7.1% NaCl. After 48 h of incubation at 37°C, suspected colonies were applied to a VITEK® 2 automated system with a GP card (bioMerieux Inc., Durham, NC, USA) for identification. Colonies identified as S. aureus were confirmed with the coagulase test for rabbit plasma (EIKENCHMICAL Co., Tokyo, Japan). Further, the identified S. aureus were amplified in tryptic soy broth (BD Japan, Tokyo, Japan) and stored in 20% glycerol solution at −80°C until use. Antibiotic susceptibility was measured with the Sensi-Disc (BD Japan) against oxacillin and cefoxitin according to the instructions of the manufacturer and the CLSI recommendation (CLSI M100-S20, 2010).

Polymerase chain reaction procedure.

Table 1: Polymerase chain reaction primers and conditions

Target gene	Primer	Sequence	Expected sizeof product (bp)	Annealing temperature (°C)	Positive-control strain	Reference
mecA	A-F	5′-TGC TAT CCA CCC TCA AAC AGG-3′	280	56	N315	5
	A-R	5′-AAC GTT GTA ACC ACC CCA AGA-3				
mecA	B-F	5′-AAA ATC GAT GGT AAA GGT TGG C-3′	523	55	N315	6
	B-R	5′-AGT TCT GCA GTA CCG GAT TTG C-3′				
mecA	C-F	5′-GTT GTA GTT GTC GGG TTT GG-3′	1818	55	N315	7
	C-R	5′-CCA CCC AAT TTG TCT GCC AGT TTC TCC-3′				
tst	TST-F	5′-TGT AGA TCT ACA AAC GAT AAT ATA AAG GAT-3′	700	52	N315	4
	TST-R	5′-ATT AAG CTT AAT TAA TTT CTG CTT CTA TAG TT-3′				
seg	SEG-F	5′-AAT TAT GTG AAT GCT CAA CCC GAT C-3′	642	59	N315	4
	SEG-R	5′-AAA CTT ATA TGG AAC AAA AGG TAC TAG TTC-3′				
eta	ETA-F	5′-CTA TTT ACT GTA GGA GCT AG-3′	741	47	MH	4
	ETA-R	5′-ATT TAT TTG ATG CTC TCT AT-3′				
etb	ETB-F	5′-ATA CAC ACA TTA CGG ATA AT-3′	629	50.5	NA1	4
	ETB-R	5′-CAA AGT GTC TCC AAA AGT AT-3′				

Polymerase chain reaction (PCR) was performed for the identification of the mecA and staphylococcal toxin genes tst, seg, eta, and etb. Primers and PCR conditions for identification of the genes are shown in Table 1 [4.5.6.7]. Template DNA of S. aureus was prepared as described previously [4], and PCR was performed in a 10 μL reaction mixture containing 0.4 μL template DNA, 1 μL of 10 × Taq buffer, 0.8 μL dNTP mixture, 0.5 μM of each primer, and 0.25 U Taq polymerase (Takara Bio, Otsu, Japan). The following amplification cycles were repeated 30 times using the Veriti Thermal Cycler (Applied Biosystems, LIFE TECHNOLOGIES Japan, Tokyo, Japan): denaturation for 30 s at 94°C, annealing of primers for 30 s at a suitable temperature for each amplification, and extension for 60 s at 72°C. Amplified products were analyzed by 1.4% agarose gel electrophoresis in TAE buffer at 135 V for 20 min. The gel was stained with ethidium bromide and then exposed to ultraviolet light to visualize the amplified products. When the bands were unclear, we repeated the experiment a few times to confirm the reproducibility. Therefore, mecA-positive strains were identified to be MRSA when one of the three sets of primers showed a positive reaction. Positive control strains N315, MH, and NA1 for PCR were obtained from university school of Medicine.

RESULTS

S. aureus colonization in the nasal cavity of ICU doctors, 12 out of 23 ICU doctors (52%) during the first survey and 14 out of 22 (64%) during the second survey showed S. aureus colonization in their nasal cavity. (Table 2)

Table 2: Carriage of *Staphylococcus aureus* in the nasal cavity of Intensive Care Unit doctors

Examinee	First survey	Second survey
1	ND	ND
2	*S. aureus* (2-1)	
3	*S. aureus* (3-1)	
4	ND	*S. aureus* (4-2)
5	*S. aureus* (5-1)	*S. aureus* (5-2)
6	ND	ND
7	*S. aureus* (7-1)	*S. aureus* (7-2)
10	*S. aureus* (10-1)	*S. aureus* (10-2)
11	ND	*S. aureus* (11-2)
13	*S. aureus* (13-1)	*S. aureus* (13-2)
14	*S. aureus* (14-1)	ND
15	*S. aureus* (15-1)	*S. aureus* (15-2)
16	*S. aureus* (16-1)	*S. aureus* (16-2)
17	ND	*S. aureus* (17-2)
18	ND	ND
19		ND
20	ND	*S. aureus* (20-2)
21	ND	ND
23	ND	*S. aureus* (23-2)
24	*S. aureus* (24-1)	
25		ND
28	*S. aureus* (28-1)	
29	ND	
30	*S. aureus* (30-1)	
31	ND	
33		S. aureus (33-2)
34		S. aureus (34-2)
35		ND

36	S. aureus (36-2)

ND; not detected

Table 3: Antibiotic susceptibility and toxin gene profile of *Staphylococcus aureus* isolates

Isolate	Susceptibility			mecA			Toxin gene			
	oxacillin	Resistant colony in inhibition zone	cefoxitin	Primer set A	Primer set B	Primer set C	tst	seg	eta	etb
S. aureus (2-1)	S	+	S	+	-	-	-	-	-	-
S. aureus (3-1)	S	+	S	+	-	-	+	+	-	-
S. aureus (5-1)	S	-	S	+	-	-	-	-	-	-
S. aureus (7-1)	S	+	S	+	-	-	-	-	-	-
S. aureus (10-1)	R	-	R	+	+	-	+	+	-	-
S. aureus (13-1)	S	+	S	+	-	-	-	+	-	-
S. aureus (14-1)	R	-	S	+	-	-	-	-	-	-
S. aureus (15-1)	S	+	S	+	-	-	-	+	-	-
S. aureus (16-1)	S	-	S	+	-	-	-	+	-	-
S. aureus (24-1)	S	-	S	-	-	-	-	-	-	-
S. aureus (28-1)	S	-	S	+	-	+	+	+	-	-
S. aureus (30-1)	S	-	S	+	-	-	+	+	-	-
S. aureus (4-2)	S	-	S	-	-	-	-	-	-	-
S. aureus (5-2)	S	-	S	-	-	-	-	-	-	-
S. aureus (7-2)	S	-	S	-	-	-	-	-	-	-
S. aureus (10-2)	R	-	S	+	-	-	+	-	-	-
S. aureus (11-2)	S	-	S	+	-	-	-	+	-	-
S. aureus (13-2)	S	+	S	+	-	-	-	+	-	-
S. aureus (15-2)	S	+	S	+	-	-	-	+	-	-
S. aureus (16-2)	S	-	S	+	-	-	-	+	-	-
S. aureus (17-2)	S	-	S	+	-	-	-	-	-	-
S. aureus (20-2)	S	+	S	+	-	-	-	+	-	-
S. aureus (23-2)	S	+	S	+	-	-	-	-	-	-
S. aureus (33-2)	S	-	S	+	-	-	-	-	-	-
S. aureus (34-2)	S	-	S	+	-	-	-	+	-	-
S. aureus (36-2)	S	-	S	-	-	-	-	-	-	-

Oxacillin-sensitive mecA-positive MRSA colonization in the nasal cavity of ICU doctors(Table 3)

Of the 12 S. aureus isolates identified during the first survey, two were oxacillin-resistant. However, mecA was detected in 11 isolates, including two oxacillin-resistant isolates. Similarly, 10 out of 14 isolates during the second survey were mecA-positive.

Among the oxacillin-sensitive mecA-positive isolates, four isolates had the tst gene. The seg gene, which is the representative toxin gene in the egc locus, was additionally detected in seven isolates during the first survey and six isolates during the second survey. All these isolates, except for one, were phenotypically oxacillin-sensitive but mecA-positive.

DISCUSSION

Survey results for S. aureus colonization in the nasal cavity of ICU doctors at a university hospital suggest that although the numbers of examinees were relatively small at both times, the colonization rates were high and seasonal change of colonization was small.

In total, 81% of S. aureus isolates were confirmed to be oxacillin-sensitive mecA-positive MRSA colonization in the nasal cavity of ICU doctors. Furthermore, mecA was detected with the primer set A but not with the primer set B or C in most of these isolates. Some of the oxacillin-sensitive mecA-positive isolates raised resistant colonies in the inhibition zone around the oxacillin disk after further 3 days culture, indicating that these isolates have a potential to become oxacillin-resistant. The primer set A has been most commonly used for the detection of the mecA gene, and the positive strains were identified as MRSA (definition in CLSI M100-S20, 2010). Among the oxacillin-sensitive mecA-positive isolates, four isolates had the tst gene. The seg gene, which is the representative toxin gene in the egc locus, was additionally detected in seven isolates during the first survey, and six isolates during the second survey. All these isolates, except for one, were phenotypically

oxacillin-sensitive but mecA-positive. We suggest that these isolates are pathogenic for compromised ICU patients; therefore, special caution is necessary for these potential MRSA. Because ICU doctors at the university hospital share the beds and the kitchen in the nap room of the ICU, the nap room may be a site for potential MRSA cross infection among ICU doctors. The eradication of the potential MRSA colonized in the nasal cavity of the ICU doctors was performed after the second survey, and the third survey confirmed that none subsequently showed S. aureus colonization (data not shown).

It was recently reported that certain proportion of community-acquired MRSA (CA MRSA) isolated in Japan expressed only a low revel resistance against oxacillin. These MRSA were termed borderline MRSA and were confirmed to be the resistance phenotype against cefoxitin [8, 9]. The potential MRSA identified in the current study is likely different because the potential MRSA isolates were sensitive to cefoxitin. The nucleotide sequences of the PCR primer sets used in the current study were derived from the nucleotide sequence of the classical mecA gene found in hospital-acquired MRSA. In our previous study, we found that only the primer set A could amplify the mecA gene of most CA MRSA isolates, including borderline MRSA; however, the primer sets B and C could not amplify it, suggesting the mecA nucleotide sequences of CA MRSA varied at the regions of these primers [8]. The primer set C detected only one isolate, and the primer set B detected none of the potential MRSA isolates. Therefore, the mecA gene of the potential MRSA has similar nucleotide changes to that of CA MRSA. None of the potential MRSA isolates had eta or etb genes, which are frequently observed in Japanese CA MRSA [10]. Finally, the potential MRSA isolated in the current study are barely identified with the routine susceptibility tests in clinical laboratories. Therefore, we recommend the introduction of the genetic identification methods, such as PCR for the identification of various types of MRSA.

CONCLUSION

A high rate of MRSA colonization in the nasal cavity of ICU doctors at a university hospital was observed. Most MRSA isolates, as determined through mecA detection, were susceptible to oxacillin, but produced resistant mutants in the presence of oxacillin. Therefore, we strongly suggest monitoring and/or eradication of colonized MRSA in the nasal cavity of ICU doctors

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Conflict of Interest: The authors have no conflict of interest to report.

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