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Prevalence of Methicillin-resistant *Staphylococcus epidermidis* in New Hospital Residents : A Cohort Study of New Resident Physicians in a University-affiliated Hospital

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SUMMARY

Methicillin-resistant *Staphylococcus epidermidis* (MRSE) is known to cause various nosocomial infections. However, there is limited data available regarding its prevalence, especially among the cohorts of new resident physicians. Our aim in this study was to describe the carriage rate of MRSE in the anterior nares of new resident physicians in the hospital over a 12-month period. We used a prospective cohort design that examined the relationship between duration of work and the MRSE carriage rate. The volunteer participants consisted of 64 new resident physicians working full time in the hospital with no previous work experience and without anterior nares infections. Of the 64 participants, 45 completed the data collection at 12 months. Nasal swabs were obtained from the residents at 0, 6, and 12 months, yielding a total of 517 strains. All *S. epidermidis* strains were examined for the minimum inhibitory concentration (MIC). For MRSE strains, staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed. Strains that were only resistant to oxacillin were analyzed using multilocus variable-number tandem repeat analysis (MLVA) for genetic testing. MRSE prevalence was 56.2%, 70.6%, and 82.2% at 0, 6, and 12 months, respectively. Data related to the drug-resistance profile and SCC*mec* type revealed a possible dominant strain among the isolated MRSE strains. Moreover, the prevalence of MRSE increased over time, and MLVA analysis verified that there was a dominant strain among the isolated MRSE strains. In addition, the duration of work directly correlated with the MRSE carriage rate, suggesting that the duration of the work is related to MRSE acquisition.

Keywords : methicillin-resistant *Staphylococcus epidermidis* (MRSE), prevalence, resident physicians, cohort, university affiliated hospital

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INTRODUCTION

Although *Staphylococcus epidermidis* (*S. epidermidis*) is a component of normal skin and mucosal microflora, it has emerged as a major cause of healthcare-associated infections. In addition, according to the National Nosocomial Infections Surveillance System in the United States, the rate of methicillin resistance has increased over the past 2 decades[1]. With the recent increase in use of intravenous antibiotic treatments, coagulase-negative staphylococci (CNS) have emerged as healthcare-associated pathogens[2,3]. Among CNS, methicillin-resistant *S. epidermidis* (MRSE) is known to cause various healthcare-associated infections. However, limited data are available on the prevalence of MRSE in physicians[4,5] despite reports that healthcare workers may be reservoirs of such pathogens[6]. Several studies have reported that nurses' hands quickly become the carriers of methicillin-resistant CNS[6,7].

Here, we have investigated the prevalence of MRSE in a cohort of new resident physicians over 12 months to examine the relationship between the length of time worked in a hospital and the MRSE carriage rate.

PATIENTS AND METHODS

Recently graduated resident physicians who were newly hired (n=64) at a 1,020-bed university-affiliated hospital in Tokyo, Japan volunteered to participate in this prospective study, which was conducted between April 2010 and March 2011. Individuals who had an infection of the anterior nares or who could not participate in the data collection were excluded from the study. Nasal swabs were collected from the participants prior to their commencing work at the hospital (baseline) and again at 6 and 12 months. Each swab was cultured using mannitol salt agar and egg yolk, with or without 10 mg/L of ceftizoxime, for 48 h at 37℃[8]. If colonies were present on both plates (with and without ceftizoxime), 2 colonies from each plate were collected, and all 4 strains were tested for minimum inhibitory concentration (MIC). Each collected strain was then stored at −80℃ until further analysis.

Approval was obtained from the ethical review board of the Juntendo University Faculty of Medicine (#196). Each participant provided a written consent before each round of data collection.

Bacterial identification

Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper ver. 3.0, Bruker Daltonik GmbH, Leipzig, Germany) using either the direct smear or the modified on-plate method[9]. A log score >2.0 was accepted as identification, as recommended by the manufacturer.

Susceptibility to the antimicrobial agents was performed for all collected strains using a microdilution technique employing a DP32 plate (Eiken Chemical Co. LTD, Tokyo, Japan), and phenotypic resistance was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100-S19. MRSE was confirmed if the MIC of oxacillin was >0.5 μg/mL, and *mecA* was confirmed by multiplex polymerase chain reaction, as described by Kondo et al. (2007)[10].

If at least 1 strain of MRSE was identified, the person was categorized as a MRSE carrier. Identification of the same antibiogram at the same time point in the same person was counted as 1 strain. If different antibiograms were found in one person, the result was categorized as different strains and subsequently analyzed.

Antibiograms of hospital and resident patterns were compared. The antibiogram at each time point was created with the strain demonstrating the highest resistance to oxacillin from each person. The antibiogram for the hospital *S. epidermidis* strain was the result of the collection of positive inpatient blood culture samples from April 2010 to March 2011, for a total of 113 strains obtained from inpatient positive blood cultures. Susceptible (S), intermediate (I), and resistant (R) strains were classified using the 2011 guidelines defined by CLSI M100-S19, with a standard laboratory workflow by employing the MicroScan WalkAway panel (Siemens, Sacramento, CA, USA).

Staphylococcal cassette chromosome *mec* (SCC*mec*) type was determined as described by Kondo et al. (2007)[10] and designated as either type I, II, III, IV, V, VI, or VIII, according to the current nomenclature

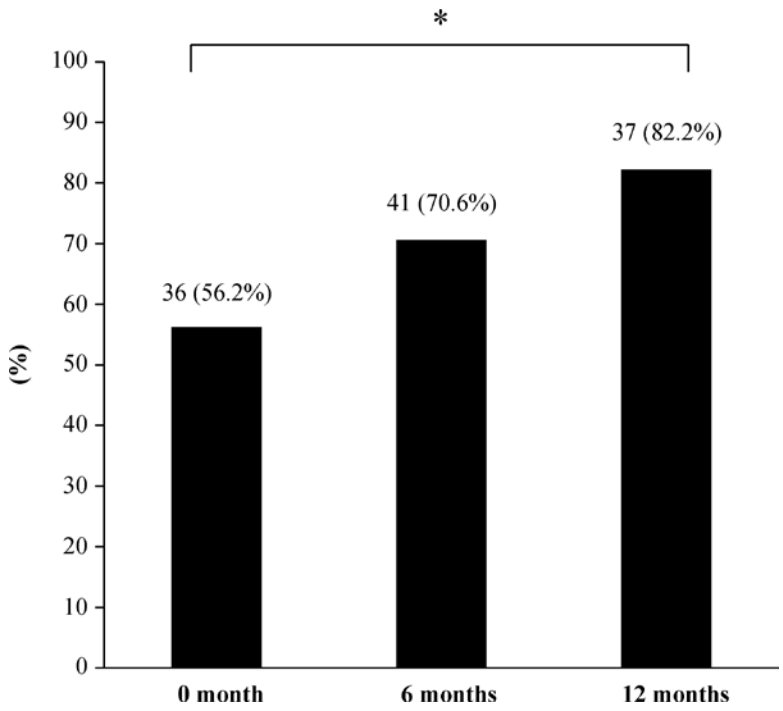


Figure 1

Comparison of MRSE prevalence at each time point (0, 6, and 12 months) in recently graduated medical residents. Number of participants were 64, 58 and 45 for 0, 6, 12 months respectively. *P = 0.0044 between 0 and 12 months.

used for methicillin-resistant *S. aureus*. Since the method was designed for MRSA, if the results could not be categorized as previously described, they were categorized as non-typable (NT).

Multilocus variable-number tandem repeat analysis (MLVA) of MRSE isolates was performed on groups only resistant to oxacillin. Twenty strains with this specific resistance were typed by MLVA, a method that assesses the length of the polymorphisms of four chromosomal variable-number tandem repeats designated Se1 to Se4[11]. The phylogenetic relationship between MLVA patterns were evaluated using the unweighted pair group method with arithmetic mean (UPGMA) by employing BioNumerics software Version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium)[11].

Statistical analysis

Microsoft Excel was used to perform chi-square tests or Fisher's exact tests to analyze the MRSE carriage rate. A p-value <0.05 was considered significant.

RESULTS

The mean age of the participants was 24.9 ± 0.82 years at 0 months (n = 64 ; men, 33 ; women, 31), 25.3 ± 1.2 years at 6 months (n = 58 ; men, 29 ; women, 29), and 25.7 ± 0.88 years at 12 months (n = 45 ; men, 24 ; women, 21). There appeared to be no difference in the number of the participants who had taken antimicrobials prior to each collection : 15 (23.4%), 11 (18.9%), and 9 (20.0%) at 0, 6, and 12 months, respectively. The dropout rates at 6 and 12 months were 9.4% and 29.7%, respectively.

Of the 517 strains obtained, *S. epidermidis* accounted for 493 (95.3%), and *S. capitis* accounted for 12 (2.32%). Both *S. lugdunensis* and *S. schleiferi* accounted for 2 (0.38%) strains each, while *S. intermedius*, *S. cohnii*, *S. haemolyticus*, *S. pettenkoferi*, and *Corynebacterium propinquum* all accounted for 1 (0.19 %) strain each. After excluding methicillin-susceptible *S. epidermidis* and overlap for strains of the same antibiogram in the same person, 207 strains were classified as MRSE strains.

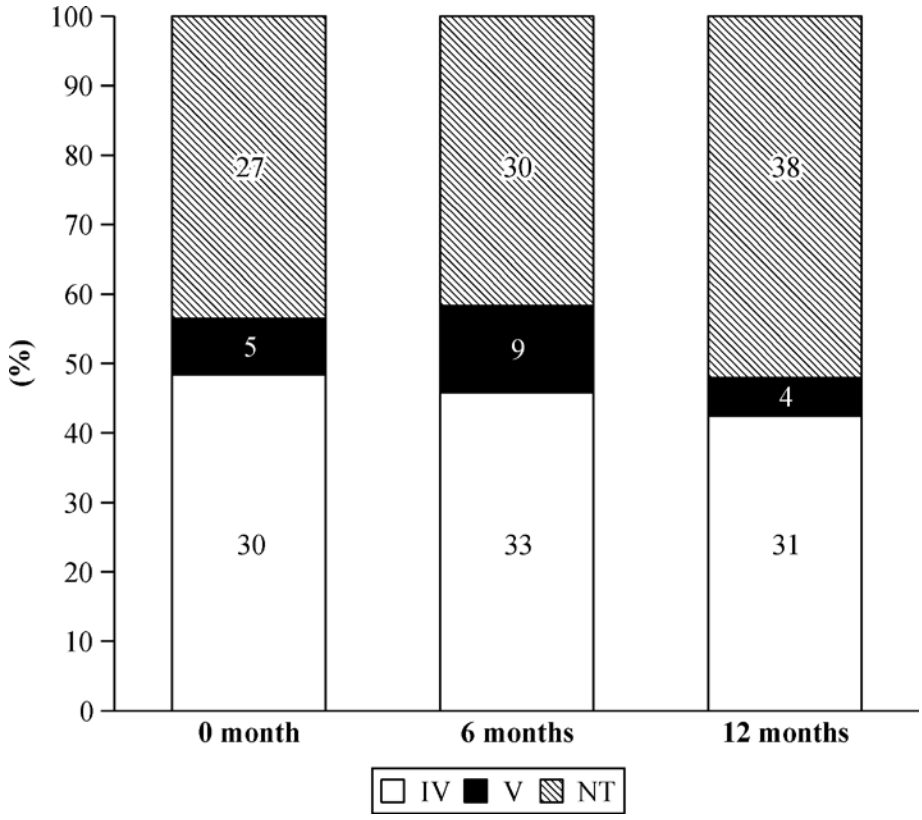


Figure 2

Changes in SCC*mec* patterns of MRSE strains at each time point. A 100% ratio of SCC*mec* type is shown. The number in the figure indicates the actual number of strains analyzed in this study. NT, non-typeable.

The number of new resident MRSE carriers was 36/64 (56.2%) people at 0 months, 41/58 (70.6%) people at 6 months, and 37/45 (82.2%) people at 12 months (Figure 1). The prevalence of MRSE strains significantly increased from 0 to 12 months ($p=0.0044$) ; however, there was no significant increase from 0 to 6 months ($p=0.09878$) or from 6 to 12 months ($p=0.1956$). With an increase in employment time at the hospital, there was a significant increase in the MRSE carriage rate.

The SCC*mec* analysis resulted in the identification of types IV and V, and non-typeable strains (Figure 2). In our study, type IV was the dominant type, and this trend did not change over the 12 months. The number of isolated non-typeable strains increased at 12 months ; however, there were no other marked changes.

Antibiograms of *S. epidermidis* from inpatient blood cultures for the year 2010-2011 were compared between each time period (Figure 3). Oxacillin resistance was found to have been acquired quickly, matching the levels found in blood cultures from 36/60 (60%) strains at 0 month, 44/58 (75.8%) strains at 6 month, 37/43 (86%) strains at 12 month and 96/113 (84.9%) strains at blood culture. In contrast, gentamicin resistance did not increase in parallel with oxacillin resistance from 17/60 (28.3%) strains at 0 month, 16/58 (27.5%) strains at 6 month, 15/43 (34.8%) strains at 12 month and 74/113 (65.4%) strains at blood culture. There was only a marginal change in erythromycin resistance between the 3 time points, in contrast to the blood culture results from 37/60 (61.6%) strains at 0 month, 33/58 (56.8%) strains at 6 month, 26/43 (54.1%) strains at 12 month and 73/113 (64.6%) strains at blood culture. Clindamycin (14/60 (23.3%) strains at 0 month, 17/58 (29.3%) strains at 6 month, 15/43 (34.8%) strains at 12 month and 55/113 (48.6%) strains at blood culture) and levofloxacin (19/60 (31.6%) strains at 0 month, 20/58 (34.4%) strains at 6 month, 19/43 (41.1

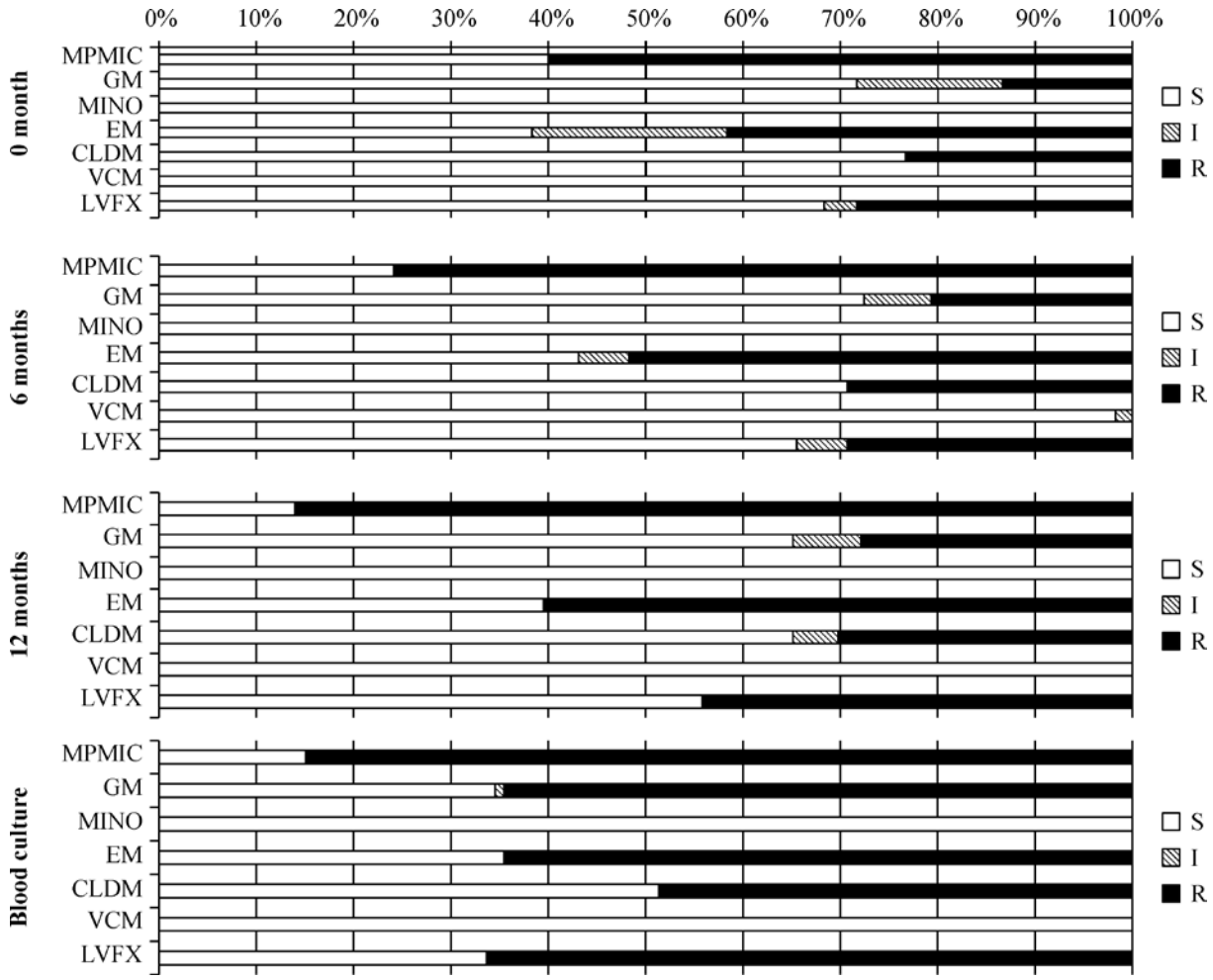


Figure 3

Antibiogram for each time point (0, 6, and 12 months) and hospital blood cultures. Susceptible (S), intermediate (I), and resistant (R) are used to identify the strains according to Clinical and Laboratory Standards Institute criteria. MPMIC, oxacillin ; GM, gentamicin ; MINO, minomycin ; EM, erythromycin ; CLDM, clindamycin ; VCM : vancomycin ; LVFX : levofloxacin.

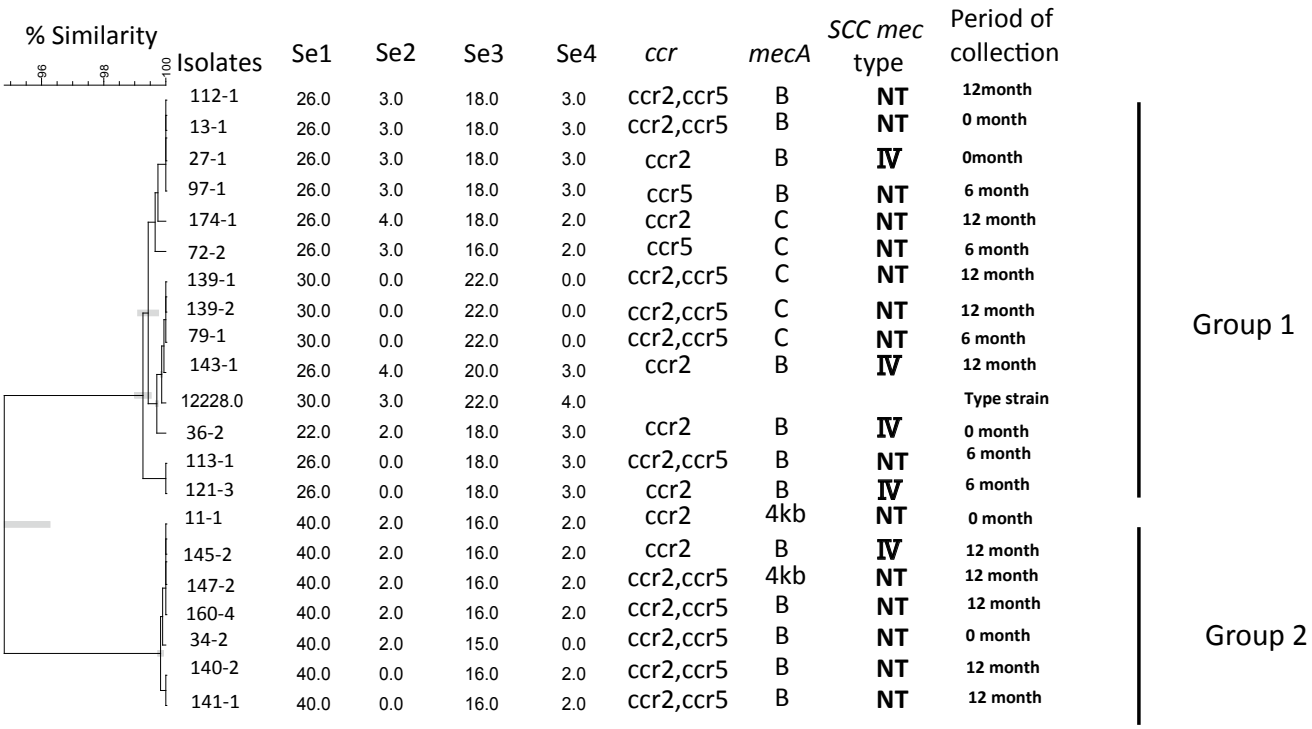
%) strains at 12 month and 75/113 (66.3%) strains at blood culture) resistance did not achieve the same level as that seen in blood cultures at 12 months. In all groups, almost no strains demonstrated minocycline or vancomycin resistance. The prevalence of oxacillin resistance increased more rapidly compared to all other antibiotics.

The strains resistant to oxacillin only, which were the most dominant strains, were analyzed using MLVA, and the results are shown in Figure 4. Only two dominant strains were found. Group 1 consisted of collections at 0, 6, and 12 months. On the other hand, group 2 consisted of collections only at 0 and 12 months. For the SCC*mec* type, most of the results from both groups were consistent with non-typable strains (NT). This indicated that there was a possibility that the same strain may have been circulating within the hospital environment.

DISCUSSION

In this study, the initial MRSE carriage rate in resident physicians with no previous hospital working experience was 56.2%. With an increase in employment duration, that prevalence increased to 70.6% at 6 months and 82.2% at 12 months, indicating a greater risk for acquiring MRSE strains.

The initial MRSE carriage rate in our study was higher than rates reported in previous studies con-



Isolates	Se1	Se2	Se3	Se4	*ccr*	*mecA*	*SCC mec* type	Period of collection	
112-1	26.0	3.0	18.0	3.0	ccr2,ccr5	B	NT	12month	
13-1	26.0	3.0	18.0	3.0	ccr2,ccr5	B	NT	0 month	
27-1	26.0	3.0	18.0	3.0	ccr2	B	Ⅳ	0month	
97-1	26.0	3.0	18.0	3.0	ccr5	B	NT	6 month	
174-1	26.0	4.0	18.0	2.0	ccr2	C	NT	12 month	
72-2	26.0	3.0	16.0	2.0	ccr5	C	NT	6 month	
139-1	30.0	0.0	22.0	0.0	ccr2,ccr5	C	NT	12 month	
139-2	30.0	0.0	22.0	0.0	ccr2,ccr5	C	NT	12 month	Group 1
79-1	30.0	0.0	22.0	0.0	ccr2,ccr5	C	NT	6 month	
143-1	26.0	4.0	20.0	3.0	ccr2	B	Ⅳ	12 month	
12228.0	30.0	3.0	22.0	4.0				Type strain	
36-2	22.0	2.0	18.0	3.0	ccr2	B	Ⅳ	0 month	
113-1	26.0	0.0	18.0	3.0	ccr2,ccr5	B	NT	6 month	
121-3	26.0	0.0	18.0	3.0	ccr2	B	Ⅳ	6 month	
11-1	40.0	2.0	16.0	2.0	ccr2	4kb	NT	0 month	
145-2	40.0	2.0	16.0	2.0	ccr2	B	Ⅳ	12 month	
147-2	40.0	2.0	16.0	2.0	ccr2,ccr5	4kb	NT	12 month	
160-4	40.0	2.0	16.0	2.0	ccr2,ccr5	B	NT	12 month	
34-2	40.0	2.0	15.0	0.0	ccr2,ccr5	B	NT	0 month	Group 2
140-2	40.0	0.0	16.0	2.0	ccr2,ccr5	B	NT	12 month	
141-1	40.0	0.0	16.0	2.0	ccr2,ccr5	B	NT	12 month	

Figure 4

MLVA analysis. The strains resistant to oxacillin only were the most dominant strains analyzed. Results indicate that there were two dominant strains among those resistant to oxacillin only and that these strains were similar.

ducted with medical professionals and students, which ranged from 17% to 48% using nonselective agar for the initial culture[12~15]. Our results were based on initial collection of MRSE strains that used ceftizoxime mannitol salt with egg yolk agar. When we made our analyses using only mannitol salt with egg yolk agar, the carriage rate was lower than that when ceftizoxime was included (data not shown). This may be due to the chance of picking MRSE is lower for the non-selective agar. In non-selective agar both MRSE and MSSE grow but selective agar grows ceftizoxime resistant strains. In general, all MRSE strains are resistant to ceftizoxime and higher rate of MRSE grows on selective agar. We pick randomly zero to two colonies from selective agar. Therefore, the selective agar resulted in increased sensitivity for the detection of multiple MRSE strains. However, our results regarding a trend in an increasing number of strains with longer working duration, were consistent with those of previous studies conducted with nurses and medical students[6,12,15]. Since the other study used only non-selective agar, our study results were not comparable.

Figure 3 shows antibiograms of the three time points and those of the blood cultures. It reveals a tendency for the results at the time points to approach those of the blood culture antibiograms. Moreover, Figure 4 indicates that there were two dominant strains found among the strains resistant only to oxacillin. These results indicate that MRSE strains were circulating both within the hospital environment and among the residents.

Based on the above-mentioned assumption, current safety precautions (i.e., hand-hygiene methods and the wearing of disposable gloves and gowns) did not hinder the rate of MRSE acquisition for resident physicians[5], especially when considering the fact that computer keyboards, stethoscopes, door handles, and towels may harbor MRSE strains[16-20]. We suggest, therefore, that the environmental factors within the hospital play an important role in the spread of MRSE in hospital workers.

In this study, the dropout rate was high, particularly at 12 months (n=19 ; 29.7%), which limited the accuracy of our outbreak analysis. Moreover, the number of data points was limited to 3 for a 1-year

period. Additional data points for the same time period could enable more accurate assessments of the prevalence of MRSE and its related factors.

Another limitation of our study was that we were unable to follow individual changes in the strain of MRSE owing to limitations set by the ethical review board. Therefore, this study followed participants as a group only. This limited the accuracy of the in-hospital prevalence of the MRSE strains. Future studies will allow us to obtain more detailed results.

In figure 2, SCCmec type did not change over time ; this may be because the method used was developed for MRSA, and may not be applicable for typing MRSE. In this study, comparison could not be performed between the blood culture strains and MRSE strains obtained for MLVA analysis since we could not obtain the MRSE strains of blood culture.

In conclusion, this is the first study analyzing a cohort of newly hired resident physicians for the prevalence of MRSE in their anterior nares. We report a stepwise increase in MRSE prevalence among these residents in relation to the length of their working hours ; i.e., longer employment duration was directly related to the acquisition of MRSE strains. Moreover, the MRSE strains found among the residents were similar to those found among inpatient blood cultures, indicating their general circulation. Therefore, we suggest that a cleaner hospital environment may decrease the MRSE carriage rate among new resident physicians and hospital workers in general.

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