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Long-term periodical isolation of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from Japanese children's oral cavities

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Abstract INTRODUCTION: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen causing nosocomial infections. To control nosocomial infection in a dental hospital, periodical surveillance of MRSA from the oral cavity is very important. Furthermore, this surveillance might reveal any alteration of the incidence of MRSA among healthy children in a particular area, because many children having no systemic disease except for dental problems come to dental offices. METHODS: Totally 741 children were participated in the present study; 307 in 1987–88, 103 in 1992–93, 129 in 1997–98 and 202 in 2002–2003. *S. aureus* was isolated from the tongues of children without systemic diseases. All isolates were investigated for coagulase types and susceptibilities to five antibiotics. RESULTS: *S. aureus* was isolated from 33–44% of children during the examination period. The incidence of MRSA was maximal in 1992–93, and then constant at around 3%. MRSA that have type II or III coagulase were relatively dominant. CONCLUSION: Although outbreaks of MRSA in Japan was decreased in the early 90's, more than 3% of children without particular risks are considered positive for MRSA.

Key words
Child,
Methicillin-resistant *Staphylococcus aureus*,
Oral cavity,
Periodical isolation

Introduction

Staphylococcus aureus is an indigenous bacterium that colonizes at the skin, naris or oral cavity. It is also the most common pathogen in various infectious diseases such as impetigo or pneumonia. Further, it was reported that *S. aureus* causes osteomyelitis and alveolar abscesses[1,2].

Since the first case of methicillin-resistant *S. aureus* (MRSA) was reported in the UK in early 60's[3–5], it has spread around the world. In 1980's, many reports had been published that described MRSA isolated frequently in Japan. MRSA is considered to be a critical pathogen that causes nosocomial infection not only in the medical but also the dental field. To control these nosocomial infections, numerous surveys were carried out to try to isolate MRSA from hospitalized patients, the medical environment or medical equipment. We periodically isolated *S. aureus* and MRSA from children who visited our clinic having no systemic disease but dental problems, because we thought that it might be more important for this purpose to survey the children who harbor MRSA without any infectious symptoms than simply dealing carefully with patients who already have infectious disease caused by MRSA. This protocol is also very interesting because it would reveal the sequential alterations in the incidence of community-acquired MRSA among healthy children in Japan.

In the present study, we isolated *S. aureus* and MRSA from the oral cavities of children who were in good health except for dental disease in four experimental periods. Furthermore, we examined the coagulase types and the susceptibilities against several antibiotics of all isolated *S. aureus*.

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Table 1 Number of children harboring *S. aureus* and MRSA

Period of isolation	Number of subjects		
	Total (male, female)	*S. aureus**	MRSA
1987–88	307 (156, 151) (4y1m)§	100 (32.6)¶	6 (2.0)
1992–93	103 (54, 49) (6y1m)	44 (42.7)	13 (12.6)
1997–98	129 (65, 64) (6y8m)	50 (38.8)	4 (3.1)
2002–03	202 (106, 96) (6y3m)	82 (40.6)	8 (4.0)

*: *S. aureus* containing MRSA, §: Average age of participated children, ¶: Percent of total subjects

Table 2 Coagulase types of isolated *S. aureus* and MRSA

		Coagulase types									
		I	II	III	IV	V	VI	VII	VIII	NT*	Total
1987–88	*S. aureus*§	4	23	17	4	16	4	27		5	100
	MRSA		3	1	2						6
1992–93	*S. aureus*	7	9	8	2	4		11	1	2	44
	MRSA	1	3	4	2			2		1	13
1997–98	*S. aureus*	6	11	9	1	6	1	12		4	50
	MRSA		2	2							4
2002–03	*S. aureus*	10	22	14	4	12	2	16		2	82
	MRSA		4	3				1			8

*: Strains that showed more than two coagulase types were classified into "non-typable" (NT).
§: *S. aureus* contains MRSA.

Materials and Methods

Subjects

We isolated *S. aureus* and MRSA from children who visited the clinic of Pediatric Dentistry in Hiroshima University Dental Hospital. Children were considered to be in good health at the examination a side from having dental disease. Children and their guardians participated after receiving sufficient explanation and giving their informed consent. The number of children who joined each examination is shown in Table 1.

Isolation and identification of *S. aureus*

Bacteria were isolated from the tongue of each child by swabbing with a sterilized cotton bud described previously[6], and inoculated onto Staphylococcus selective medium agar plate (Nissui #110, Tokyo, Japan). After a 2-day-incubation, well-separated colonies were re-inoculated to the same medium. *S. aureus* was identified with Gram staining, testing of coagulase productivity and hemolysin productivity on sheep blood agar plates.

Antibiotics susceptibility and determination of MRSA

All isolated *S. aureus* were measured for susceptibilities to five antibiotics that are often used clinically. Oxacillin, cefalexin, tetracycline, ofloxacin and vancomycin were used in a micro-liquid dilution assay to determine the minimal inhibitory concentration (MIC). The strains that showed resistance against oxacillin (MIC$\geq 4\mu g/ml$) were identified as MRSA following the recommendation of National Committee for Clinical Laboratory Standards[7].

Coagulase typing

Coagulase typing is a conservative classification method that has been frequently applied to current epidemiological studies[8], and was determined immunologically using an inhibition assay kit (Denka Seiken, Tokyo, Japan) as described previously[9].

Table 3 *In vitro* susceptibilities of isolated *S. aureus* to five antibiotics

	1987–88		1992–93		1997–98		2002–03	
	MIC50*	MIC90§	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
Oxacillin	0.25	2	0.5	32	0.25	2	0.25	2
Cefalexin	4	16	4	32	4	16	4	16
Tetracycline	0.25	0.5	0.5	4	0.5	0.5	0.25	0.5
Ofloxacin	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.25
Vancomycin	1	1	1	2	1	2	1	2

*: The 50% strains of isolated *S. aureus* were inhibited with this concentration (μg/m*l*).
§: The 90% strains of isolated *S. aureus* were inhibited with this concentration (μg/m*l*).

Results

The numbers of children who harbored *S. aureus* and MRSA are shown in Table 1. During all examination periods, the incidence of *S. aureus* was steady at 33–44% and did not change significantly. On the other hand, the incidence of MRSA changed remarkably. It increased from 2.0% in 1987–88 to 12.6% in 1992–93, and then decreased to 3.1% in 1997 and 4.0% in 2002. There were no significant differences in the incidences of *S. aureus* and MRSA according to gender or age (data not shown).

Table 2 shows the coagulase types of isolated *S. aureus* and MRSA. Although *S. aureus* showing coagulase types I, II, III and VII were frequently isolated at all examinations, we could not find any particularly predominant coagulase type. Also, types II and III were comparatively prevalent in MRSA during all examinations.

The susceptibilities of isolated *S. aureus* to five antibiotics are shown in Table 3. MIC90 of oxacillin (32μg/m*l*) and tetracycline (4μg/m*l*) in 1992–93 showed a high value compared to other experimental periods, because MRSA was isolated frequently in this period. MIC of cefalexin was relatively stable during all periods, because many strains of methicillin-sensitive *S. aureus* exhibited tolerance to cefalexin. Although no isolated strain indicated resistance to vancomycin, one strain isolated in 1992 was resistant to ofloxacin (MIC = 16μg/m*l*).

Discussion

S. aureus is one of the most important pathogens causing suppurative disease. Even in the oral region, *S. aureus* is often isolated from infectious foci, such as osteomyelitis or alveolar abscesses[1,2]. Recently, MRSA was noted to be a critical pathogen causing horizontal infections in the medical field. To prevent nosocomial infection, we continuously performed surveillance of *S. aureus* and MRSA, because we believe it is very important to monitor the incidence of MRSA among the healthy people without systemic diseases. As bacteria and pathogens that induce nosocomial infections in dental clinics could come from the oral cavity, we attempted to isolate *S. aureus* from the tongue by swabbing with a cotton bud.

The incidence of *S. aureus* was maintained between 33 and 44% through out the examination period. The previous manuscripts reported this incidence to be 47.5% in the oral cavities of adults[10], 21% in saliva[11], 27% on dentures[12] among healthy people. In recent manuscripts, it was also reported to be 24% among healthy children in the US[13], 24% in Canada[14], and 23% in the UK[15]. From these data and the present study, the incidences of *S. aureus* in the oral region might be relatively stable all the time. It also seems to be similar in adults and children.

The ratio of MRSA-positive to total specimens altered dramatically during the examination. It increased from 2.0% in 1987–88 to 12.6% in 1992–93, fell down to 3.1% in next five years and then it remained constant until 2002. The prevalence of MRSA among healthy children varies very widely; 0.61%[13] or 11%[16] in the US, 8.7% in Nigeria[17]. Although we could find no reports that revealed the long-term alteration of prevalence of MRSA in the same place among healthy children, many papers have reported that the incidence of MRSA among hospitalized patients increases year by year. Hussain *et al.* reported that the incidence of community-acquired MRSA infection among hospitalized children without identified risk factors increased from

1988–90 to 1993–95 and then decreased slightly to 1998–99[13]. The reason why the incidence of MRSA peaked in 1992–93 in our survey is not clear, but it might have been due to doctors and dentists recognizing the risk of 3rd generation cephalosporin causing resistance of beta-lactams in *S. aureus* at the end of the 80's in Japan, and the consequent avoidance of needless administration of such antibiotics.

The susceptibilities of antibiotics did not change much during the experimental period, except for oxacillin and tetracycline in 1992–93 because MRSA were frequently isolated in this period. Although vancomycin resistant Enterococci was reported recently[18], and researchers identified the risk of transmission of tolerance from Enterococci to Staphylococci[18], all isolated *S. aureus* were susceptible to vancomycin. Only one strain that had tolerance for ofloxacin (MIC = 16μg/m*l*) was isolated in 1992; the other strains isolated in all experimental periods were susceptible to ofloxacin. It was reported that community acquired MRSA did not show cross-resistance to antibiotics other than beta-lactams. According to MIC50 for cefalexin showing 4μg/m*l*, resistance against cefalexin was spread not only to MRSA but also to methicillin-sensitive *Staphylococcus aureus*.

The coagulase types are often used as epidemiological markers to distinguish isolated *S. aureus*. In the present study, because there were no dominant coagulase types among the MRSA strains during all periods, we could confirm the endemic strain of MRSA, although several manuscripts have described endemic MRSA circulating in the community.

The incidence of MRSA among healthy children peaked at 12.6% in 1992–93 and then decreased to 3.1% in 1997. It was still steady at 4.0% in 2002. Although the risk of outbreak of MRSA was reduced, 3% of healthy children still harbored MRSA in their oral cavities. This means we should consider MRSA to be an indigenous bacterium. As it is very difficult to distinguish the patient who harbors MRSA in clinical field, it becomes more important to execute universal precautions to prevent nosocomial infection of MRSA.

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