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図の説明

図1　追跡調査対象者（児）20名の口腔ケア変更前後における歯垢からの菌種別検出状況および概算菌数

□：－（菌検出なし），　▨：＋（10^3 cfu/mL），

▩：＋＋（10^3～10^5 cfu/mL），　■：＋＋＋（10^5 cfu/mL）

Evaluation of Oral Care Practice Based on Opportunistic Pathogens Identified in Dental Plaque of Adults/children with Severe Motor and Intellectual Disabilities

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Abstract

We examined the prevalence of major opportunistic pathogens identified in the dental plaque of adults/children with severe motor and intellectual disabilities who were residents of a rehabilitation and nursery center. Subsequently, oral care practice was changed to reduce the incidence of opportunistic pathogens in dental plaque expecting higher effects of disinfection, and the effectiveness was evaluated by bacteriological examination; identification of targeted organisms and approximate number of colonies evaluated as (+), (++), (+++). Of 56 residents, 11 and 45 were cared in each of the intensive-care and day-care rooms, respectively. One to three types of targeted organisms were identified in the dental plaque of 24 residents, and the major opportunistic pathogens MRSA, *Pseudomonas aeruginosa* and *Serratia marcescens* were detected in 14 (25.0%), 14 (25.0%) and 5 (8.9%) residents, respectively. Oral care practice was changed to two new methods for the residents in the intensive-care and day-care rooms, and bacteriological examination was performed for 20 residents in whom any of these 3 types of organisms was detected. Before the change of oral care practice, MRSA was found in 1 (+++), 2 (++) and 11 (+) residents, *P. aeruginosa* in 8 (+++), 5 (++) and 1 (+) residents, and *S. marcescens* (+++) in 5 residents. After the change of oral care practice, these three organisms tended to persist in the same residents, but 5 months later, MRSA was found in 0 (+++), 0 (++) and 7 (+) residents, *P. aeruginosa* in 0 (+++), 10 (++) and 2 (+) residents, and *S. marcescens* 3 (+++) and 1 (+) residents. Although the changed oral care practice was effective in removing or reducing opportunistic pathogens in dental plaque, *P. aeruginosa* was hard

to remove. The present study indicates that further development is needed for oral care practice based on the individual condition of the oral cavity, bacterial species detected, and viable counts in adults/children with severe motor and intellectual disabilities.

Key wards: adults/children with severe motor and intellectual disabilities, dental plaque, oral care, *P. aeruginosa*, MRSA

表1　療育センター障害者(児)の口腔ケア変更前における歯垢内日和見病原菌の検出状況

	全対象者(56名)		追跡対象者(20名)		追跡外対象者(36名)	
			観察室(11名)	デイルーム(9名)	観察室(0名)	デイルーム(36名)
	名	%	名	名	名	名
検査対象菌						
陽性 (1～3菌種)	24	42.9	11	9	0	4
1菌種	12	21.5	1	7	0	4
2菌種	7	12.5	5	2	0	0
3菌種	5	8.9	5	0	0	0
陰性	32	57.1	0	0	0	32
検出菌内訳						
S. aureus (MRSA)	14	25.0	9	5	0	0
P. aeruginosa	14	25.0	10	4	0	0
S. marcescens	5	8.9	4	1	0	0
S. aureus (MSSA)	3	5.4	0	0	0	3
K. pneumoniae	3	5.4	1	1	0	1
β溶血性*Streptococcus*属	2	3.6	2	0	0	0

表2　追跡調査対象者(児)20名の背景と口腔内の状態

対象 No.	性別	年齢	管理状況	基礎疾患	JCS *1	大島分類*2	気管切開	人工呼吸器	肺炎既往	内服薬	食事方法	嚥下状態	口腔内の状態
1	M	11	観察室	脳性麻痺	0	1	+	−	頻回 1ヶ月以内	抗菌薬微量 抗痙攣剤	経管 (経鼻)	不可	歯肉腫脹
2	M	10	観察室	脳性麻痺	0	1	喉頭気管 分離術	夜間	頻回 1ヶ月以内	抗菌薬微量 抗痙攣剤	経管 (胃瘻)	不可	歯石
3	F	15	観察室	脳性麻痺	0	1	+	−	頻回 1年以内	抗菌薬微量 抗痙攣剤	経管 (経鼻)	(不可) 誤嚥(+)	歯列不正
4	M	5	観察室	溺水後脳障害	200	1	+	+	頻回 半年以内	抗痙攣剤	経管 (胃瘻)	不可	特記事項なし
5	M	8	観察室	先天性ミオパチー	0	4	+	+	2年以上	抗菌薬微量 抗痙攣剤	経管 (経鼻)	不可	歯の石灰化不全
6	F	31	観察室	アルギナーゼ欠損症	0	1	−	−	1年以内	抗菌薬微量 抗痙攣剤	経管 (経鼻)	不可	特記事項なし
7	M	10	観察室	溺水後遺症 脳性麻痺	0	1	+	−	半年以内	抗痙攣剤	経管 (経鼻)	不可	特記事項なし
8	M	4	観察室	先天性緊張性ジストロフィー	0	2	−	−	2年以上	抗痙攣剤	経管 (経鼻)	不可	歯肉腫脹
9	M	11	観察室	頭部外傷後	0	1	+	−	頻回 1ヶ月以内	抗痙攣剤	経管 (経鼻)	不可	歯肉腫脹
10	F	8	観察室	頭蓋内出血後遺症	0	1	+	−	1年以内	抗痙攣剤	経管 (経鼻)	不可	特記事項なし
11	M	17	観察室	脳性麻痺	0	1	−	−	半年以内	抗痙攣剤	経管 (経鼻)	不可	歯石
12	M	19	デイルーム	脳性麻痺	0	1	−	−	半年以内	抗痙攣剤	経管 (経鼻)	不可	歯石
13	M	52	デイルーム	脳性麻痺	0	4	−	夜間	半年以内	抗痙攣剤	経口 (全介助)	可	義歯着脱困難 歯槽膿漏
14	M	58	デイルーム	脳性麻痺	0	2	−	−	1年以内	抗菌薬微量 抗痙攣剤	経管 (経鼻)	嚥下困難 誤嚥(+)	歯肉膨脹
15	F	28	デイルーム	脳性麻痺	0	1	−	−	無	抗痙攣剤	経口 (全介助)	可	歯肉肥厚(全周)
16	M	22	デイルーム	レンノックス ガスト−症候群	0	2	−	−	1年以内	抗痙攣剤	経口 (全介助)	可	歯肉膨脹
17	M	24	デイルーム	蘇生後脳症後遺症	0	1	−	−	無	抗痙攣剤	経管+経口 (全介助)	嚥下困難	歯列不正 歯石
18	M	29	デイルーム	頭蓋内出血後遺症	0	5	−	−	無	抗痙攣剤	経口 (部分介助)	可	歯肉腫 歯周ポケット形成
19	F	57	デイルーム	脳性麻痺	0	5	−	−	無	抗痙攣剤	経口 (部分介助)	可	義歯着脱困難
20	F	38	デイルーム	脳性麻痺	0	1	−	−	無	抗痙攣剤	経口 (全介助)	嚥下困難	特記事項なし

＊1 JCS (Japan coma scale)

I. 覚醒している
- 0: 意識清明
- 1: 見当識は保たれているが意識清明ではない
- 2: 見当識障害がある
- 3: 自分の名前・生年月日が言えない

II. 刺激に応じて一時的に覚醒する
- 10: 普通の呼びかけで開眼する
- 20: 大声で呼びかけたり、強く揺するなどで開眼する
- 30: 痛み刺激を加えつつ、呼びかけを続けると辛うじて開眼する

III. 刺激しても覚醒しない
- 100: 痛みに対して払いのけるなどの動作をする
- 200: 痛み刺激で手足を動かしたり、顔をしかめたりする
- 300: 痛み刺激に対し全く反応しない

＊2 大島分類: 重症心身障害児区分(知的障害および生活活動能力) 1: IQ20以下 寝たきり, 2: IQ20以下 座位可, 3: IQ20〜35 座位可, 4: IQ20〜35 寝たきり, 5: IQ20以下 座位可・歩行不可

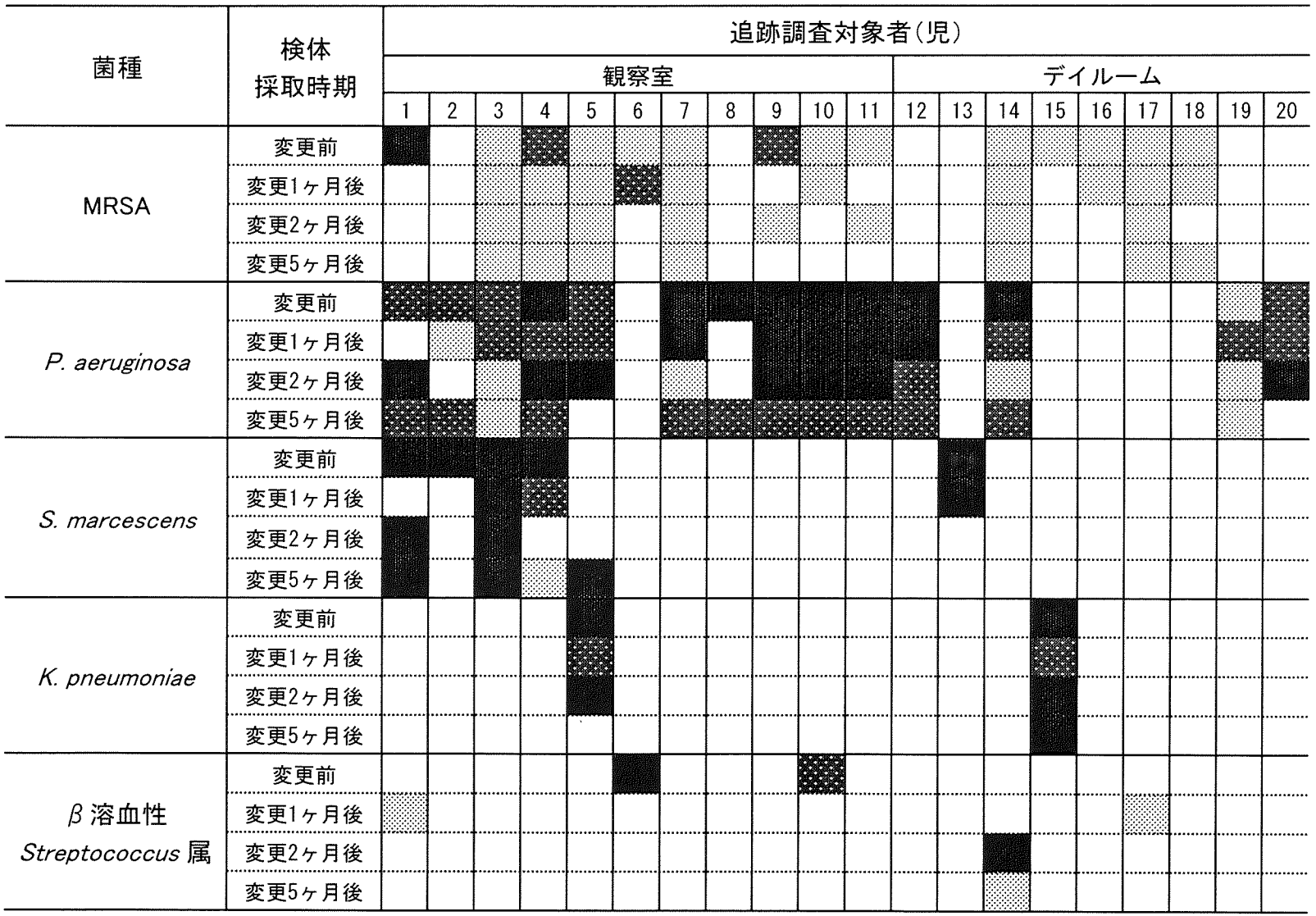
菌種
検体採取時期
追跡調査対象者(児)
観察室
デイルーム
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
MRSA
P. aeruginosa
S. marcescens
K. pneumoniae
β溶血性 Streptococcus 属
変更前
変更1ヶ月後
変更2ヶ月後
変更5ヶ月後

図 1

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SHORT COMMUNICATION

Total bacterial counts on oral mucosa after using a commercial saliva substitute in patients undergoing hematopoietic cell transplantation

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Abstract
Purpose The commercial saliva substitute Oralbalance® has been reported to alleviate symptoms of postradiotherapy xerostomia in head and neck cancer patients. Oralbalance® may also be effective for xerostomia in patients undergoing hematopoietic cell transplantation (HCT) with high-dose chemotherapy and total-body irradiation. However, HCT patients are in a severely compromised condition, and saliva substitute must not promote infection. We reported previously that Oralbalance® has antimicrobial effects against microbial species detected during HCT in vitro. This study was performed to determine the in vivo effects of Oralbalance® on oral mucosal total bacterial counts in patients undergoing HCT.
Methods A total of 18 neutropenic patients undergoing HCT were enrolled in this study. Before and after 1 week of Oralbalance® use, bacterial samples were obtained from patients by wiping an area of ϕ1 cm on the buccal mucosa with sterilized cotton swabs. Total bacterial counts of the obtained samples were examined by quantitative polymerase chain reaction amplification of the bacterial 16S ribosomal RNA gene. As controls, bacterial samples were also obtained from ten healthy subjects, and total bacterial counts were examined.
Results No significant increase in bacterial count was observed with use of Oralbalance®. None of the patients showed bacterial counts above the range found in healthy controls after using Oralbalance®.
Conclusions In neutropenic patients undergoing HCT, Oralbalance® did not increase the total counts of oral mucosal bacteria beyond the range found in healthy controls. Oral-care using Oralbalance® may alleviate the symptoms induced by hyposalivation without promoting infection.

Keywords Hematopoietic cell transplantation · Xerostomia · Saliva substitute

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Introduction

High-dose chemotherapy and total-body irradiation, which are performed as the conditioning regimen for hematopoi-

etic cell transplantation (HCT), are associated with xerostomia. Oralbalance® (Laclede, Inc., Rancho Dominguez, CA, USA), which is a commercially available saliva substitute, has been reported to alleviate the symptoms of postradiotherapy xerostomia in head and neck cancer patients [1, 2]. Therefore, this product may be effective in HCT patients. However, as these patients are in a markedly compromised condition throughout the period of HCT, saliva substitute must not promote infection.

Previously, we reported the in vitro antimicrobial effects of Oralbalance® against microbial species detected during HCT [3]. Oralbalance® does not facilitate increases in microorganisms detected in the HCT period in vitro [3]. In the present study, we determine the in vivo effects of Oralbalance® on total bacterial counts of oral mucosa in neutropenic patients undergoing HCT.

Subjects and methods

Subjects

A total of 18 neutropenic patients (neutrophil counts< 1,000/μL) undergoing allogeneic conventional (not reduced intensity regimen) HCT with a conditioning regimen composed of total-body irradiation (TBI) and high-dose chemotherapy at Okayama University Hospital (M, 12; F, 6; age, 42.9±16.2 years), who elected to use Oralbalance® to alleviate their symptoms of xerostomia, were enrolled in this study. The diseases in these patients were as follows: malignant lymphoma, nine; acute myeloid leukemia, four; acute lymphocytic leukemia, three; myelodysplastic syndromes, one; and solid tumor, one. Ten of the 18 subjects did not require antibiotics, while the remaining eight subjects needed antibiotics on at least 1 day during the examination period. Informed consent was obtained from all subjects, and the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences approved this study.

Oral managements and use of Oralbalance®

All subjects were referred to dentists, and necessary dental treatment had been completed before HCT. All subjects were taught about the self management of oral hygiene, tooth brushing after every meal and before going to bed, and oral rinsing with normal saline solution every 3 h during the day was also indicated. Nurses, dental hygienists, and dentists performed this oral management in patients with poor general condition. They used Oralbalance® from the day on which the patients felt xerostomia (all patients began use of Oralbalance® from 5 to 1 days prior to HCT, which corresponded to the period of the conditioning regimen for HCT composed of TBI and high-dose chemotherapy) at least four times per day, i.e., after every meal and before going to bed.

Total counts of bacteria on the buccal mucosa

Bacterial samples were obtained about 2 h after breakfast by wiping the buccal mucosa with sterilized cotton swabs over an area of ϕ1 cm before and after 1 week of using Oralbalance®. Samples were obtained from areas without ulcer. As controls, samples were also obtained from ten healthy members of the hospital staff (M, 5; F, 5; age, 30.5± 4.2 years). Controls did not use Oralbalance® and were only sampled once. Total bacterial counts were examined by quantitative polymerase chain reaction (PCR) amplification of the bacterial 16S ribosomal RNA gene (16S rDNA) as described previously [4] with minor modifications as follows. Cotton swab samples were suspended in 1 mL of PBS(-) (Gibco BRL, Grand Island, NY, USA). Aliquots of 500 μL from each suspension were transferred into new tubes and pelleted. Pelleted samples were resuspended in 200 μL of InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA) to extract total bacterial DNA. Aliquots of 5 μL of extracted DNA were quantified by real-time PCR amplification of the 16S rDNA with SYBR Green. Real-time PCR and data analysis were performed using a GeneAmp® 5700 Sequence Detection System and GeneAmp 5700 SDS software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Total bacterial counts before and after the use of Oralbalance® were compared by Wilcoxon's signed-rank test. *P* values were calculated using the statistical software StatFlex (Artech, Osaka, Japan).

Results

Changes in total bacterial counts on the buccal mucosa after using Oralbalance®

As shown in Fig. 1, there were no significant changes in total counts of bacteria on the buccal mucosa after use of Oralbalance®. The group without antibiotic use (Fig. 1b) tended to have higher total bacterial counts than the group treated with antibiotics (Fig. 1c), although the difference was not significant. After using Oralbalance®, none of the patients showed bacterial counts above the range found in healthy controls ($10^{4.2}$–$10^{5.6}$, n=10).

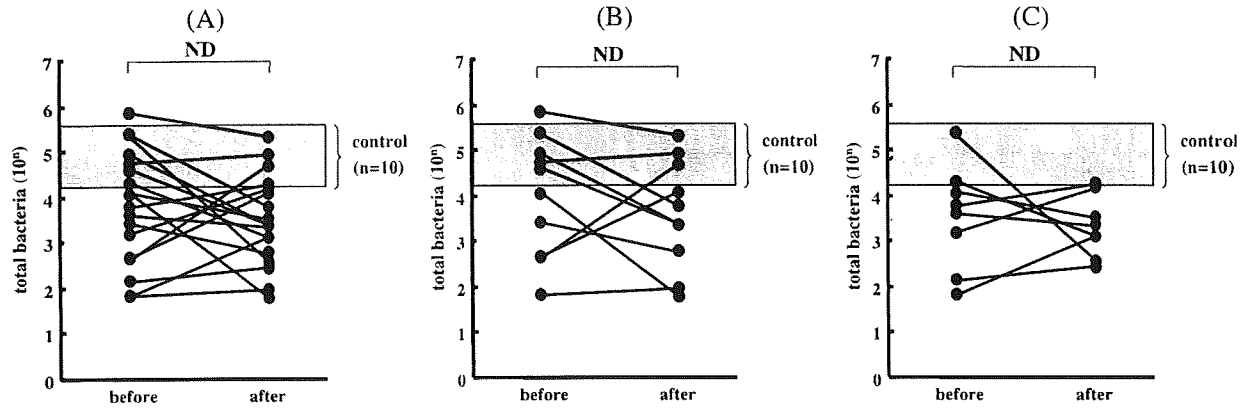

Fig. 1 Changes in total bacterial counts on the buccal mucosa with use of the mouth moisturizing gel, Oralbalance® (n=18). All patients began use of Oralbalance® from 5 to 1 days prior to HCT. "Before" using Oralbalance® samples were obtained at these points. "After" samples were obtained 1 week after starting use of Oralbalance®. **a** All subjects (n=18). **b** No antibiotics (n=10). **c** Subjects used antibiotics at least once (n=8). No significant increase in bacterial counts was observed associated with use of Oralbalance® (Wilcoxon's signed-rank test; *ND* no significant difference, $P>0.05$). Bacterial counts of healthy controls are shown in the *gray area* ($10^{4.2}$–$10^{5.6}$, n= 10). None of the patients' counts were *above* the range of those in healthy controls

Discussion

Our previous study demonstrated antimicrobial activity of Oralbalance® against the bacterial species detected during HCT in vitro and suggested that this product would not promote infection. In the present study, we performed further examination of the in vivo effects of Oralbalance® on total bacterial counts of the oral mucosa in patients undergoing HCT. No significant increases in bacterial counts were observed associated with use of Oralbalance®. It would be better to examine the differences with and without use of Oralbalance® as a case-control study. However, when this study was performed, the rumor that oral management with Oralbalance® alleviated oral pain spread among the patients in the ward. As a result, almost all patients requested oral management with Oralbalance® and a case-control study could not be performed. Therefore, we compared total bacterial counts on the oral mucosa of HCT patients with those of healthy subjects. None of the patients showed bacterial counts above the range found in healthy controls after using Oralbalance®.

In this study, quantitative PCR amplification of 16S rDNA was performed to evaluate the total counts of bacteria on the oral mucosa. Samples were obtained from areas without ulcer because of pain, and oral ulceration may influence colonization by microorganisms. Oral care was performed intensively, and no visible colonization was observed in any of the subjects during the examination period. The gene encoding the small subunit of bacterial 16S rDNA has been used frequently as a target of PCR examination because of its structural characteristics [5, 6]. The nucleotide sequences of some portions of the 16S rDNA are highly conserved through evolution [4]. The conserved sequences can provide PCR primers for amplification of 16S rDNA from all bacterial species [4]. Quantitative real-time PCR has been demonstrated to be a powerful tool for quantitative microbiological examination [4]. Therefore, we evaluated the changes in total bacterial counts on the buccal mucosa after using Oralbalance® by real-time PCR quantification of 16S rDNA. Culture and colony counting are common methods of determining bacterial counts. However, it can be difficult to culture some species of bacteria. Our results based on the bacterial DNA counts were reliable compared with culture-based methods.

The results of this study confirmed that Oralbalance® does not promote infection not only in vitro but also in vivo. Hyposalivation results in uncomfortable oral dryness, and may also increase the severity of the oral mucositis induced by chemotherapy and/or irradiation, because patients with xerostomia lose one of the most important factors involved in protecting the oral mucosa, saliva, which contains many components of the innate and acquired defense systems and not only eliminates microorganisms from the oral cavity [7, 8] but also moderates mechanical contact between the teeth and the oral mucosa. Indeed, we often see the development of ulcerative mucositis on the mucosa in contact with dry teeth clinically. Oral care using saliva substitute may alleviate the symptoms induced by hyposalivation without promoting infection. Furthermore, as Oralbalance® does not contain antibiotics, it does not contribute to the appearance of antibiotic-resistant bacteria.

In conclusion, in neutropenic patients undergoing HCT, the commercially available saliva substitute Oralbalance® did not increase the total counts of oral mucosal bacteria beyond the range found in healthy controls.

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ORIGINAL ARTICLE

Rapid detection of *mecA* and *spa* by the loop-mediated isothermal amplification (LAMP) method

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Keywords
loop-mediated isothermal amplification, *mecA*, Methicillin-resistant *Staphylococcus aureus*, naked-eye inspection, *spa*.

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Abstract

Aim: To develop a detection assay for staphylococcal *mecA* and *spa* by using loop-mediated isothermal amplification (LAMP) method.

Methods and Results: *Staphylococcus aureus* and other related species were subjected to the detection of *mecA* and *spa* by both PCR and LAMP methods. The LAMP successfully amplified the genes under isothermal conditions at 64°C within 60 min, and demonstrated identical results with the conventional PCR methods. The detection limits of the LAMP for *mecA* and *spa*, by gel electrophoresis, were 10^2 and 10 cells per tube, respectively. The naked-eye inspections were possible with 10^3 and 10 cells for detection of *mecA* and *spa*, respectively. The LAMP method was then applied to sputum and dental plaque samples. The LAMP and PCR demonstrated identical results for the plaque samples, although frequency in detection of *mecA* and *spa* by the LAMP was relatively lower for the sputum samples when compared to the PCR methods.

Conclusion: Application of the LAMP enabled a rapid detection assay for *mecA* and *spa*. The assay may be applicable to clinical plaque samples.

Significance and Impact of the Study: The LAMP offers an alternative detection assay for *mecA* and *spa* with a great advantage of the rapidity.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) including *Staphylococcus epidermidis* (MRSE) are an important cause of healthcare and community-acquired infections (Secchi *et al.* 2008; Makgotlho *et al.* 2009). Rapid identification of MRSA and MR-CoNS in hospitalized patients is essential for administration of appropriate antibiotic therapy and infection control regimens. In addition, the increasing numbers of community-acquired infections necessitate the screening of carriers, rather than simply detecting the infected patients (Cookson 1997).

It is generally accepted that oral infectious disease and oral hygiene status are associated with general health. Colonization of pathogens such as *Staphylococcus* spp. in the oral cavity acts as a reservoir for infection of systemic organs (Sumi *et al.* 2007). Oral hygiene of the functionally impaired elderly is generally poor, and the population of individuals aged 65 and older is growing rapidly in Japan and is expected to increase 135% by 2050. For these reasons, the requirement of professional oral care by dentists or dental hygienists for hospitalized patients or dependent elderly patients is expanding dramatically, and the care is known to be effective to reduce the potential pathogens in oral cavity (Yoneyama *et al.* 1999; Ishikawa *et al.* 2008). As the opportunistic infection of MRSA or MR-CoNS in these elderly or compromised hosts can be a significant cause of morbidity and mortality, dentists must pay attention to the distribution of methicillin resistance in these strains. The rapid and accurate diagnosis of these strains is sometimes difficult because of the lack of facilities, especially in visiting home-nursing patients.

For the rapid examination of methicillin resistance in *Staphylococci*, polymerase chain reaction (PCR)-based molecular techniques have been developed by targeting the *mecA*, the gene for penicillin-binding protein 2a

(Hiramatsu *et al.* 1992), and real-time PCR is now widely used for the diagnosis (Makgotlho *et al.* 2009). In general, compared to the conventional culture method, PCR can be performed in relatively rapid and simple fashion, but special regents and apparatus such as thermalcycler are needed for the method.

The loop-mediated isothermal amplification (LAMP) method was originally developed by Notomi *et al.* (2000). The LAMP reaction can be conducted under isothermal condition ranging from 60 to 65°C, and specificity is attributable to four primers that recognize six distinct sequences. Continuous amplification under isothermal condition produces an extremely large amount of target DNA within 30 to 60 min, and the method enables simple visual (naked-eye) judgment of the DNA amplification through a colour change of the reaction mixture with SYBR green I (Iwamoto *et al.* 2003). As the method requires only one type of enzyme and special apparatus is not needed, LAMP may be suitable for onsite diagnosis of methicillin resistance in isolates of home-nursing patients or bed-side diagnosis of hospitalized patients. In the current study, the LAMP method was applied to detect the *mecA* gene in both cultivated cells and clinical samples. In addition, the method was used for detection of *spa*, the gene for protein A, unique to *S. aureus* (Hallin *et al.* 2009).

Materials and methods

Bacterial strains and clinical isolates

Three MRSA strains and five methicillin-resistant *S. epidermidis* (MRSE) were isolated from patients in Okayama University Hospital. Six MRSA strains (NCTC 10 442, N 315, 85/2082, JCSC 4744, JCSC 4788 and WIS) with each type of staphylococcal cassette chromosome *mec* (SCC*mec* type-I, II, III, IVa, IVc and V; Okuma *et al.* 2002) were kindly donated by Dr T. Ito of Juntendo University. In

Table 1 Detection of *mecA* and *spa* from clinical isolates and reference strains

Bacterial strains	No. of strains tested	LAMP		PCR	
		mecA	*spa*	*mecA*	*spa*
MRSA (SCCmec* type)					
Clinical isolates† (unknown)	3	+	+	+	+
NCTC 10442 (type I)	1	+	+	+	+
N315 (type II)	1	+	+	+	+
85/2082 (type III)	1	+	+	+	+
JCSC 4744 (type IVa)	1	+	+	+	+
JCSC 4788 (type IVc)	1	+	+	+	+
WIS (type V)	1	+	+	+	+
MSSA					
NBRC 14462	1	−	+	−	+
NBRC 15035	1	−	+	−	+
FDA 209	1	−	+	−	+
MRSE					
Clinical isolates†	5	+	−	+	−
MSSE					
ATCC 155	1	−	−	−	−
ATCC 12228	1	−	−	−	−
ATCC 14990	1	−	−	−	−
Streptococcus salivalius JCM 5707	1	−	−	−	−
Streptococcus sanguis ATCC 10556	1	−	−	−	−
Streptococcus pyogenes IID 866	1	−	−	−	−
Streptococcus mutans ATCC 700610	1	−	−	−	−
Enterococcus faecalis NBRC 100481	1	−	−	−	−
Escherichia coli ATCC 25922	1	−	−	−	−
Aggregatibacter actinomycetemcomitans ATCC 29523	1	−	−	−	−
Porphyromonas gingivalis FDC 381	1	−	−	−	−
Fusobacterium nucleatum ATCC 25586	1	−	−	−	−

LAMP, loop-mediated isothermal amplification; MRSA, Methicillin-resistant *Staphylococcus aureus;* MSSA, Methicillin-susceptible *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis.*; MSSE, Methicillin-susceptible *Staphylococcus epidermis*

*Staphylococcal cassette chromosome *mec*.

†Isolated in Okayama University Hospital.

addition to the clinical isolates, 21 reference strains listed in Table 1 were used in the current study.

Bacterial colonies on agar plates were suspended in phosphate-buffered saline (PBS) (Invitrogen), and the cell numbers were determined using Petroff-Hauser counting chamber and phase-contrast microscope. For determination of detection limits, ten-fold serial dilutions of cultivated cells (10–10^5 cells per tube) in PBS were prepared and subjected to DNA extraction.

Culture conditions

MRSA and MRSE were cultured on mannitol-salt oxacillin (6 mg ml^{-1}) agar plate (MSO agar: Nissui, Co., Ltd.) at 37°C for 48 h. Methicillin-susceptible *S. aureus* (MSSA) and methicillin-susceptible *S. epidermis* MSSE were grown on the mannitol-salt agar (Nissui, Co., Ltd.). *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were cultured in modified general anaerobic medium (GAM) broth (Nissui Seiyaku Inc.), and *A. actinomycetemcomitans* was cultivated in brain heart infusion broth (Becton, Dickinson and Company) supplemented with 0·5% yeast extract (Becton, Dickinson and Company) and 0·4% sodium bicarbonate. *Streptococcus salivalius*, *S. sanguinis*, *S. mutans* and *E. faecalis* were cultivated in brain heart infusion broth supplemented with 0·5% yeast extract. *E. coli* was grown in Luria–Bertani (LB) broth.

Clinical samples

Eighty-seven dependent elderly hospitalized patients [mean age (year): 70·84 ± 11·52] in Mannari Hospital (Okayama city, Japan) participated in this study. The patients did not have a medical history of MRSA-infection and were routinely received professional oral care. During the care, 28 dental plaque samples and 59 sputum samples were collected from the surface of tooth and oro-pharyngeal swabs, respectively. DNA was extracted promptly from the clinical samples and was subjected to *mecA* and *spa* detection. The sampling and clinical studies were approved by Okayama University Hospital Ethics Committee (approved NO. 439).

DNA extraction

InstaGene Matrix (Bio-Rad) was used for DNA extraction from cultivated strains, clinical dental plaque and sputum samples according to manufacturer's instructions. Briefly, bacterial samples were suspended in PBS, and were pelleted by centrifugation at 10 000 *g* for 15 min and resuspended in 100 μl of InstaGene Matrix. The suspension was incubated at 56°C for 30 min and then 100°C for 8 min. After the incubation, the suspension was centrifuged and 2 μl of the resulting supernatant was used as template for the LAMP and the conventional PCR.

Detection of *mecA* and *spa* by PCR

PCR amplification of the *mecA* and *spa* gene was accomplished with the specific primers under the conditions as described previously (Hiramatsu *et al.* 1992; Shopsin *et al.* 1999). The sequences of those primers were as follows, *mecA* forward (mA1): 5′-TGCTATCCACCCTCAAACA GG-3′, *mecA* reverse (mA2): 5′-AACGTTGTAACCACCC CAAGA-3′, *spa* forward (1095F): 5′-GACGATCCTTCAG TGAGCAAAG-3′, *spa* reverse (1517R): 5′-GCAGCAATTT TGTCAGCAGTA-3′. The PCR products were electrophoretically detected on 2% agarose gels.

LAMP primers

The candidates of LAMP primer sets were nominated from the nucleotide sequence of *mecA* and *spa* by Primer Explorer version 2 (Fujitsu) at the Net Laboratory website (http://www.venus.netlaboratory.com/partner/lamp/index.html). Nucleotide sequences of *mecA* (AB033763, D86934, AB037671, AB063172, AB063173, AB096217) and *spaA* (X61307, U54636, M18264, J01786) were obtained from the GenBank at the National Center for Biotechnology Information (NCBI) website and were aligned by GENETYX ver. 8 (Genetyx, Tokyo, Japan). A set of four primers, a forward inner primer (FIP), a backward inner primer (BIP) and two outer primers (F3 and B3) were selected for each target gene from the candidate primer sets, possibly not to include the polymorphic regions (Table 2).

LAMP reaction

The LAMP reaction was carried out using a Loopamp DNA amplification kit (Eiken Chemical) in a 25-μl volume. The reaction mixture contained 40 pmol each of

Table 2 Primers of LAMP for *mecA* and *spa*

Target gene	Primer	Sequence
mecA	F3	5′-aagatggcaaagatattcaact-3′
	B3	5′-aggttctttttatcttcggtta-3′
	FIP	5′-acctgtttgagggtggatagcatgatgctaaagttcaaaagagt-3′
	BIP	5′-gcacttgtaagcacaccttcacttcgttactcatgccatac-3′
spa	F3	5′-ggtgatacagtaaatgacattgc-3′
	B3	5′-acgctaatgataatccaccaa-3′
	FIP	5′-cttgaccaggtttgatcatgttttttactgctgacaaaattgctg-3′
	BIP	5′-aaccatgcagatgctaacaaagctacagttgtaccgatgaatgg-3′

FIP, forward inner primer; BIP, backward inner primer; LAMP, loop-mediated isothermal amplification.

FIP and BIP, 5 pmol each of F3 and B3 primers, 2 μl of template, 1 μl of *Bst* DNA polymerase (8 units) and 12·5 μl of reaction mix prepared in the kit. The reaction was carried out under isothermal condition at 64°C for 60 min. After the incubation, the reaction was terminated by heating the reaction mixture at 80°C for 5 min.

Detection of LAMP products

The LAMP product was detected by naked-eye inspection or agarose gel electrophoresis. For naked-eye detection, 1·0 μl of 10^{-1}-diluted SYBR Green I (Takara Bio, Otsu, Japan) was added to the reaction mixture, and the colour change was observed under natural light. For the electrophoretic analysis, 2 μl of reaction mixture was loaded on 2% agarose gel. The gels were stained with ethidium bromide (50 μg ml^{-1}) and assessed photographically under UV light (302 nm).

Results

Specificity of LAMP

Specificity of the LAMP for *mecA* and *spa* was examined using 29 cultivated strains (Table 1). DNA samples were extracted from 10^5 cells of each strain, and the LAMP products were electrophoretically detected. The representative results of electrophoretic detections were shown in Fig. 1. The LAMP for *mecA* successfully amplified the DNA from the strains of MRSA and MRSE. No amplicons were seen in other tested strains. Successful LAMP reactions were also seen with the primer set for *spa* and template DNA from MRSA and MSSA strains. The *mecA* and *spa* were simultaneously detected by conventional PCR using the same DNA templates. The results of PCR were consistent with the results of LAMP (Fig. 1 and Table 1).

Detection limit of LAMP and conventional PCR

For the determination of detection limit, the LAMP reactions were performed using serial diluted DNA templates of MRSA strain (NCTC 10442), and the amplicons were detected by both agarose gel electrophoresis and naked-eye inspection. The results of electrophoretic detection were shown in Fig. 2. The detection limit of the LAMP for *mecA* was $<10^2$ cells (Fig. 2a), while the detection limit for *spa* was <10 cells (Fig. 2b).

The results of naked-eye inspection were shown in Fig. 3. The colour of reaction mixture of LAMP for *mecA*

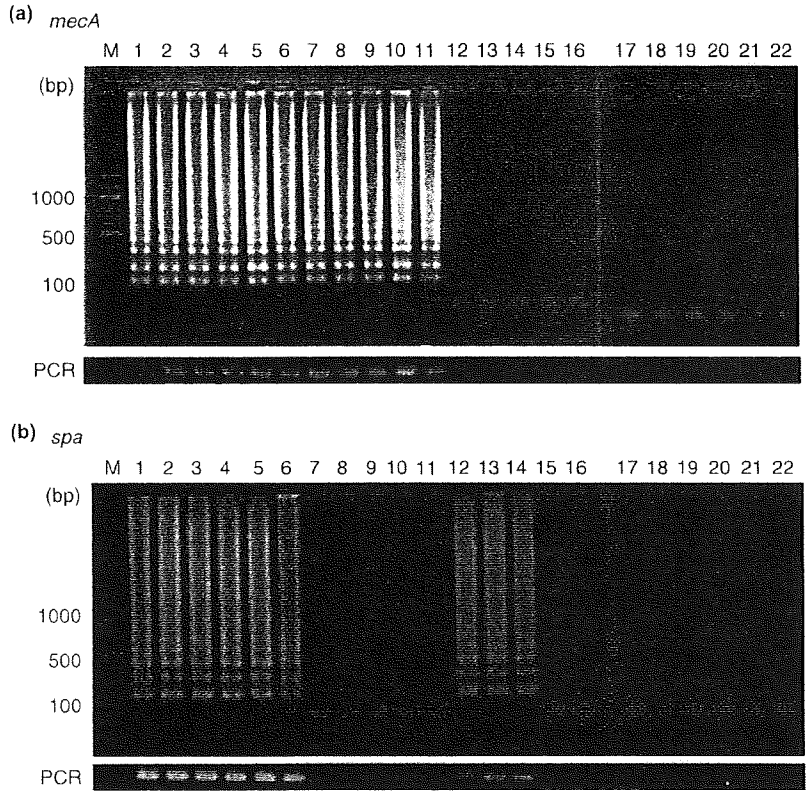

Figure 1 Specificity tests of the LAMP for *mecA* (a) and *spa* (b). All bacterial species prepared in the current study were tested, and the representative results were shown. The *mecA* and *spa* were simultaneously detected by PCR, and the amplicons were shown below the LAMP results. Lane M: DNA size marker, Lane 1–6: Methicillin-resistant *Staphylococcus aureus* (MRSA) (NCTC 10442, N 315, 85/2082, JCSC 4744, JCSC 4788 and WIS), Lane 7–11: methicillin-resistant *Staphylococcus epidermidis* (MRSE) (clinical isolates), Lane 12–14: MSSA (NBRC 14462, NBRC 15 035 and FDA 209), Lane 15–17: MSSE (ATCC 155, ATCC 12228 and ATCC 14990), Lane 18: *S. mutans* ATCC 700610, Lane 19: *P. gingivalis* FDC 381, Lane 20: *E. coli* ATCC 25922, Lane 21: *A. actinomycetemcomitans* ATCC 29523, Lane 22: water (negative control).

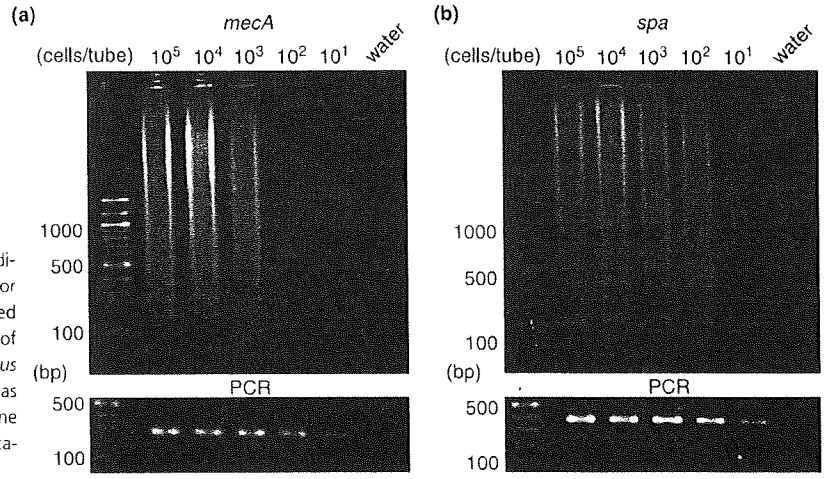

Figure 2 Detection limits of the loop-mediated isothermal amplification (LAMP) for *mecA* (a) and *spa* (b). DNAs were extracted from serial diluted cells (10–10^5 cells) of Methicillin-resistant *Staphylococcus aureus* (MRSA) (NCTC 10442) and were used as templates. The detection limits of the conventional PCR methods were simultaneously examined and were shown below.

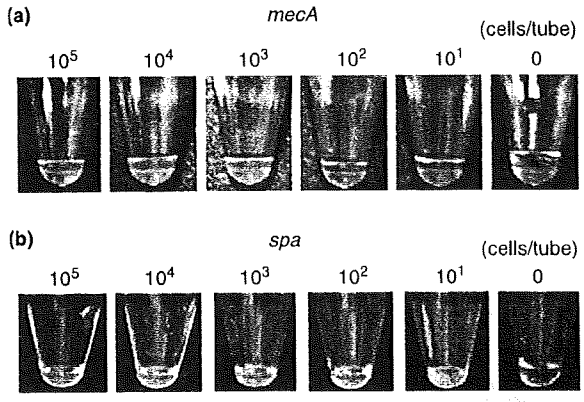

Figure 3 Naked-eye inspection of the LAMP for *mecA* (a) and *spa* (b). The original orange colour of SYBR green I turned to be green in the positive reaction mixture.

exhibited green by the addition of SYBR Green I when the mixture contained DNA templates from more than 10^3 cells, whereas original orange colour did not change when the cell number was less than 10^2 (Fig. 3a). Similarly, naked-eye inspection was feasible for detection of *spa* with the detection limit of ten cells (Fig. 3b). The detection limits of the conventional PCR method were less than ten cells for both *mecA* and *spa* (Fig. 2).

Application of LAMP to clinical samples

The LAMP for *mecA* and *spa* were applied to the clinical plaque and sputum samples. The conventional PCR methods were simultaneously performed, and the results of both methods were compared (Table 3 and 4). By the LAMP methods, *mecA* was detected in 6 plaque samples (21·4%), and *spa* was detected in 7 (25·0%) of the 28 plaque samples. The LAMP method demonstrated 100% agreement with the conventional PCR in analysing the plaque samples (Table 3).

In analysing the sputum samples, the LAMP detected *mecA* in nine samples (15·3%), whereas PCR detected the gene in 13 samples (22·0%). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the LAMP for *mecA* were 69·2, 100, 100, 92·0%, respectively. The LAMP for *mecA* showed 93·2%

Table 3 Detection of *mecA* and *spa* from clinical plaque samples

	PCR assay						
LAMP assay	Positive	Negative	Percent agreement (kappa value)	Sensitivity	Specificity	PPV*	NPV†
mecA							
Positive	6	0	100% (1·00)	100%	100%	100%	100%
Negative	0	22					
spa							
Positive	7	0	100% (1·00)	100%	100%	100%	100%
Negative	0	21					

LAMP, loop-mediated isothermal amplification.
*Positive predictive value.
†Negative predictive value

Table 4 Detection of *mecA* and *spa* from clinical sputum samples

LAMP assay	PCR assay		Percent agreement (kappa value)	Sensitivity	Specificity	PPV*	NPV†
	Positive	Negative					
mecA							
Positive	9	0	93·2% (0·78)	69·2%	100%	100%	92·0%
Negative	4	46					
spa							
Positive	9	0	86·4% (0·62)	52·9%	100%	100%	84·0%
Negative	8	42					

LAMP, loop-mediated isothermal amplificationl.
*Positive predictive value.
†Negative predictive value.

(κ = 0·78) agreement with the conventional PCR method. The *spa* was detected by the LAMP in 9 (15·3%) of the 59 sputum samples, whereas 17 (28·8%) samples were positive in PCR detection (Table 4). Sensitivity, specificity, PPV and NPV of the *spa* LAMP were 52·9, 100, 100, 84·0%, respectively, and the per cent observed agreement with the conventional PCR was 86·4% (κ = 0·62).

Discussion

As opportunities of oral care for elderly or hospitalized patients are increasing, dentists must give care to the distribution of antibiotic resistance such as MRSA. PCR is a rapid molecular technique for the microbiological diagnosis. However, the method is sometimes difficult to perform because of the lack of facilities, especially in case of home-nursing patients. Therefore, it is important for dentists to have a rapid and simple detection method for antibiotic resistance. In the current study, LAMP method was applied to the detection of *mecA* and *spa*.

The LAMP reactions were successfully accomplished within 60 min. Culture methods require 24–48 h until a result is known, whereas 2–4 h of reaction time is required for the PCR assay. When compared to these methods, the LAMP for *mecA* and *spa* demonstrated a great advantage in the rapidity. However, among PCR-based methods, real-time PCR with LightCycler System had also an advantage in the rapidity (Shrestha *et al.* 2002), as the capillary is designed to have an optimal surface-to-volume ratio to ensure rapid temperature control. By using SYBR Green chemistry, the reaction can be performed at similar cost with LAMP method ($4–5 per reaction), although an expensive equipment is required. Clinicians need to select appropriate methods according to the purpose of the examination and circumstance of the clinics.

The LAMP is a highly specific method attributable to a set of two specially designed inner and outer primers that recognize six distinct sequences (Notomi *et al.* 2000). As expected, specificity test using the cultured cells demonstrated the high specificity of the LAMP for both of *mecA* and *spa*. In addition, in analysing plaque samples, the LAMP demonstrated complete concordance with the conventional PCR method. As hundreds of bacterial species exist in oral cavity and dental plaque (Paster *et al.* 2001), these results may also support the specificity of the LAMP established in the current study.

The detection limits of the LAMP for *mecA* and *spa* by agarose gel electrophoresis were $<10^2$ cells and 10 cells, respectively, while the detection limits of the PCR methods were less than ten cells for both targets. The LAMP methods generally demonstrate equivalent or higher sensitivity with the PCR methods (Maeda *et al.* 2005; Miyagawa *et al.* 2008). However, the detection limit for *mecA* was inferior to that of the conventional PCR method because of unknown reason. The LAMP reaction can be accelerated, and higher sensitivity would be expected by using additional primer, termed loop primer (Nagamine *et al.* 2002). Redesign of the LAMP primer set including the loop primer may improve the detection limit for *mecA*.

The naked-eye inspection of LAMP for *spa* demonstrated the equal sensitivity to the electrophoretical detection (10 cells per tube), while the sensitivity of the LAMP for *mecA* declined to 10^3 cells. By increasing the reaction time to 90 min, the naked-eye inspection for *mecA* improved the sensitivity to 10^2 cells (data not shown). The quite simple and rapid eye inspection may be useful for application of the LAMP methods.

In analysis of the plaque samples, the LAMP and PCR demonstrated identical results, suggesting the clinical applicability of the method for plaque samples. However, in analysis of the sputum samples, the sensitivity of the LAMP was considerably declined in both *mecA* and *spa* detection. DNA extraction performed in the current study was based on a simple boiling method. Comparing to the plaque samples, the sputum samples may contain larger amount of inhibitors for the LAMP reaction. The DNA extraction procedure should be reconsidered. The use of lysostaphin (Schindler and Schuhardt 1964) will be a

good strategy for the DNA extraction. The analyses of clinical samples revealed the carrier of antibiotic resistance and verify the significance of examinations in clinical fields of dentistry.

Each LAMP methods for *mecA* and *spa* were accomplished in an hour with high specificity and sensitivity. In addition, through the naked-eye inspection, the LAMP obtained great advantages in simplicity and rapidity. Although further experiments will be required for the examination of sputum samples, the LAMP methods established in the current study have a potential to be beneficial tools for the detection of *mecA* and *spa*.

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原　著

歯科臨床における感染予防対策意識と行動についての現状と課題

―某県歯科衛生士会会員に対する意識調査から―

Survey on Understanding the Consciousness and Action of Infection Control in Treatments by Dental Hygienists

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和文抄録

歯科医療の多くは外科的処置を伴う行為であり，患者の唾液や血液に曝露されるリスクが高い。

現在，ヒト免疫不全ウイルス感染者の増加，肺結核の再燃などやＢ型あるいはＣ型肝炎ウイルスの持続感染をはじめ，様々な感染症の患者の歯科診療機会が多くなっている。さらに歯科医療の場は，要介護高齢者や易感染性長期入院患者の歯科治療や口腔ケアなどの領域にも広がってきている。そのため，コメディカルスタッフとしての歯科衛生士の感染予防対策に関わる役割はますます大きくなってきており，感染予防対策に対するより的確な知識と技能が求められている。

今回，我々は歯科臨床の現場で働く歯科衛生士143名（平均年齢38.9 ± 9.7歳）に対し，感染予防対策の意識に加えて行動を把握するべく意識調査を行った。その結果，感染予防対策に関する教育を受けたことがある，またはスタンダード・プリコーションなどの感染予防対策を知っている歯科衛生士は半数以下であった。すべての歯科衛生士は感染予防対策としてグローブ，マスクを着用しており，グローブの交換率は81.8%と高かったが，マスクの交換率は22.4%と低かった。一方，防護メガネの未着用は90.9%であり，感染予防対策の不十分さが示された。そして，不潔グローブで清潔域に触れた経験がある歯科衛生士は90%以上と多く，感染予防に対する意識の低さは，診療中の行動にも見られた。また，このような感染予防に対する行動の少なさは，診療環境や職場内の人間関係も大きく関わっていることが認められた。

歯科医療における感染予防対策の向上のために専門学校や大学卒業後も歯科衛生士に対する継続した感染予防対策教育が重要であると考える。そして今後，職場のより良いコミュニケーションの構築などの総合的な対応も感染予防対策には重要である。

キーワード　感染予防対策，歯科衛生士，歯科衛生士教育，意識調査

【緒　言】

歯科医療における感染予防対策は，患者と歯科医療従事者双方の安全を確保する上で重要で欠かせないものである。歯科医療の多くは，外科的処置を伴う行為であり，患者の唾液や血液に曝露されるリスクが高い。また現在，市中では病院内感染の原因菌の１つであるメチシリン耐性黄色ブドウ球菌（methicillin-resistant *Staphylococcus au-*

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