Distribution of oral mucosal bacteria with *mecA* in patients undergoing hematopoietic cell transplantation

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Abstract

Purpose:

We recently reported frequent detection of antibiotic-resistant bacteria on the oral mucosa during the period of hematopoietic cell transplantation (HCT), and suggested an association between oral mucositis and antibiotic-resistant bacterial infection. Methicillin-resistant *Staphylococcus* spp. were frequently detected, and the oral cavity may be a reservoir of the gene mediating methicillin resistance, *mecA*. Here, we examined the frequency of *mecA* carriers in patients undergoing HCT.

Methods:

Fifty-nine patients (M: 37, F: 22, 47.3 ± 11.0 y) receiving HCT were enrolled in this study. Buccal swab samples were obtained 4 times from day –7 to day +20 (once/week), and *mecA* was detected by PCR. Fifty-two subjects without systematic disease, who completed dental treatment, especially periodontal treatment (M:21, F:31, 55.4 ± 14.2 y) were also enrolled as controls, and checked for *mecA* on the oral mucosa.

Results:

Seventy-six percent (45/59) of HCT patients carried *mecA* at least once in the study period (day –7 to +20), while no control subjects had *mecA*. The frequency of *mecA* carriers was 19.2% from day –7 to –1, while it was significantly increased on

days +7 to +13 and +14 to +20, with frequencies of 60.9% and 63.2%, respectively ($P < 0.01$, ANOVA).

Conclusions:

mecA was detected in oral mucosa of patients undergoing HCT. The high detection frequency of staphylococci resistant to penicillin and beta-lactams in our recent report was supported.

Keywords:

hematopoietic cell transplantation, oral mucosa, bacteria, *mecA*, antibiotic-resistant

Introduction

Oral mucositis is one of the most common symptomatic complications associated with chemotherapy, especially hematopoietic stem cell transplantation (HCT) [12, 13]. Severe mucositis is associated with not only intolerable pain but also the possible risk of systemic bacteremia. Oral mucositis is a significant cause of suffering and morbidity in patients receiving myeloablative chemotherapy [1].

Severe mucositis is associated with a risk of systemic infection related to bacteremia. Our recent studies showed that not only normal oral flora but also opportunistic bacteria appear on the oral mucosa. Bacterial substitution of mainly coagulase-negative staphylococci (CoNS) for streptococci occurred frequently on the oral buccal mucosa after HCT, and other bacterial species not usually found in the normal flora were also identified [9]. We reported that multidrug-resistant opportunistic bacteria appearing in the gingiva may be involved in fatal sepsis [11]. Furthermore, many antibiotic-resistant bacteria were detected in the oral cavity after HCT, especially during the period in which the severity of oral mucositis reaches its peak [10]. CoNS and *Staphylococcus aureus* with penicillin and beta-lactam resistance were detected [10].

Penicillin and beta-lactam resistance among staphylococci are mediated by point mutations in penicillin binding proteins (PBPs) [2]. Methicillin resistance in staphylococci is mediated by the *mecA* gene complex, which is located on a unique molecular vector called the staphylococcal chromosome cassette (SCC*mec*) [3]. SCC*mecs* carry mobility genes and integrate in a site-specific manner into a highly conserved locus in the *Staphylococcus* chromosome.

The present study was performed to determine the distribution of oral mucosal bacteria with *mecA* in patients undergoing hematopoietic cell transplantation.

Materials and Methods

Subjects

Fifty-nine patients (M: 37, F: 22, 47.3 ± 11.0 y) receiving HCT at Okayama University Hospital from 2011 to 2012 were enrolled in this study. The diseases of these patients are shown in Table 1. Autologous HCT, conventional allogeneic HCT, and reduced-intensity stem cell transplantation (RIST) were administered to 12 (M: 8, F: 4, 56.8 ± 9.5 y), 13 (M: 9, F: 4, 42.4 ± 10.6 y), and 34 (M: 20, F: 14, 54.6 ± 11.4 y) patients, respectively. Fifty-two patients without systematic diseases, who visited the Department of Periodontics, Okayama University Hospital (M: 21, F: 31, 55.4 ± 14.2 y), were also enrolled as controls. A total of 111 subjects were enrolled in the study. Informed consent for examination of oral bacteria was obtained from each subject, and the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences approved this study (No. 457).

Oral management

Intensive oral care was performed for all HCT subjects as our previous report [10]. All HCT subjects received referred to dentists, and necessary dental treatment was completed before HCT. All subjects received instruction regarding 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 these oral managements in cases in which the patient's condition was poor. No antibiotics rinses were used.

All control subjects were in maintenance phase after completion of dental treatment, especially for periodontal treatment. Therefore, their oral hygiene was well maintained. Subjects who had systematic diseases were excluded. All subjects confirmed that they had not received antibiotic treatment for at least 3 months prior to enrolling in the study.

General infection control for HCT subjects

All HCT patients were isolated in a room equipped with a laminar airflow system and received trimethoprim-sulfamethoxazole as prophylaxis against *Pneumocystis carinii*. Fluoroquinolone for prophylaxis against bacterial infection and fluconazole for prophylaxis against fungal infection were administered orally. Prophylaxis against herpes virus infection with acyclovir was also given. Neutropenic fever was managed according to the guidelines of Hughes *et al*. [5]. Briefly, empirical antibiotic therapy was administered promptly in all neutropenic patients at the onset of fever and in afebrile patients who were neutropenic but who had signs or symptoms compatible with infection. A fourth-generation cephalosporin (*e.g.*, cefepime) or

carbapenem (*e.g.*, meropenem) was administered intravenously as empirical antibiotic therapy. G-CSF (lenograstim 5 μg/kg/day or filgrastim 300 μg/m^2) was given intravenously for 60 min starting on day 1 or 5, and was continued until the absolute neutrophil count exceeded 500/μL.

Collection of bacterial samples

Microbial samples were obtained from HCT patients about 2 h after breakfast by swabbing from the whole surface of the buccal mucosa regardless of whether mucositis was observed. Collection of bacterial samples was performed four times (day –7 to –1; day 0 to +6; day +7 to +13; day +14 to +20) for each patient (a total of 236 times in 59 patients). However, samples could not be collected 27 times because of the patents' conditions. A total of 209 samples were subjected to *mecA* detection procedures.

Microbial samples were also obtained from control subjects once just after a check-up and before any dental intervention at our hospital. Thus, the dental treatment on the check-up day could not affect the results of this study. A total of 52 samples from 52 control subjects were subjected to *mecA* detection procedures.

Detection of mecA

1) Bacterial DNA extraction

Cotton swab samples were suspended in 1 mL of PBS(–) (Gibco BRL, Grand Island, NY). 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) to extract total bacterial DNA. Aliquots of extracted DNA were subjected to polymerase chain reaction (PCR).

2) Confirmation of bacterial DNA

First, to confirm bacterial DNA was obtained appropriately, PCR amplification of the 16S ribosomal RNA gene (16S rDNA) was performed. The PCR mixture (25 μL) contained 12.5 μL of 2×AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Carlsbad, CA), 10 pmol of forward and reverse universal primers (forward: 5'-GTG STG CAY GGY TGT CGT CA-3', reverse: 5'-ACG TCR TCC MCA CCT TCC TC- 3') [7], and a 2.5-μL aliquot of extracted DNA. PCR cycles were as follows: initial cycle of 95°C for 10 min; 35 cycles of 95°C for 1 min, 56°C for 1 min and 72°C for 2 min; and a final extension at 72°C for 5 min. Amplified products were subjected to 2% agarose electrophoresis, and 120-bp DNA fragments were confirmed by ultraviolet light after ethidium bromide staining.

3) *mecA* detection by PCR

mecA detection by PCR was performed as described previously [4]. The PCR

mixture (25 μL) contained 12.5 μL of 2×AmpliTaq Gold® 360 Master Mix (Applied Biosystems), 10 pmol of primers (forward: 5'- TGC TAT CCA CCC TCA AAC AGG -3', reverse: 5'- AAC GTT GTA ACC ACC CCA AGA - 3'), and a 2.5-μL aliquot of extracted DNA. PCR cycles were as follows: initial cycle of 95°C for 10 min; 35 cycles of 95°C for 30 s, 52°C for 30 s and 72°C for 1 min; and a final extension at 72°C for 7 min. Amplified products were subjected to 2% agarose electrophoresis, and 284-bp DNA fragments were confirmed by ultraviolet light after ethidium bromide staining.

Statistical analysis

Differences in *mecA* carrier frequencies were compared by Fisher's exact test or ANOVA using the statistical software IBM® SPSS® Statistics Version 21 (IBM Corporation, NY). In all analyses, $P < 0.05$ was taken to indicate significance.

Results

Confirmation of bacterial DNA by PCR detection of 16S rDNA

The 16S rDNA PCR-amplified fragment was detected from 191 samples out of 209 prepared DNA samples from 59 HCT patients. The 16S rDNA was not detected in 18 samples, and *mecA* was also not detected from all these 18 samples in following analysis. These 18 samples were considered that bacterial gene sample could not be collected appropriately because something technical error could be occurred in the sample correction because of patients' condition, therefore excluded from further analysis. Collect rate of bacterial DNA from oral mucosal swab was 91.4%. The 16S rDNA fragment was successfully amplified by PCR from all samples from the control group ($n = 52$).

Frequencies of mecA carriers in groups of HCT patients and control subjects

The frequency of *mecA* carriers in whom *mecA* was detected at least once during the study period (day –7 to +20 from HCT) was compared with that of the control group. The results were shown in Table 2 and Figure 1. Seventy-six percent (45/59) of HCT patients carried *mecA*, while none of the control subjects had *mecA*. The difference in frequency of *mecA* carriers between HCT patients and control subjects was significant ($P < 0.01$ Fisher's exact test).

Transition of the frequency of mecA carriers in HCT patients

The transition of the frequency of *mecA* carriers on the oral mucosa before and after HCT is shown in Figure 1. The detection frequencies of *mecA* increased significantly with time after HCT. The frequency of *mecA* carriers was 19.2% on day –7 to –1 from HCT, while it was significantly increased from day +7 to +13 and day +14 to +20, with frequencies of 60.9% and 63.2%, respectively ($P < 0.01$, ANOVA).

Discussion

The results of the present study indicated the presence of *mecA* in the oral cavity after HCT. The detection frequencies of *mecA* increased significantly with time after HCT. These results support those of our recent study indicating the detection of many CoNS and *S. aureus* with penicillin and beta-lactam resistance in the oral cavity after HCT [6].

In our recent study on antibiotic sensitivity of bacteria on the oral mucosa after HCT, CoNS with high degrees of resistance to penicillins and beta-lactams and methicillin-resistant *S. aureus* (MRSA) were detected [6]. We expected *mecA* detection based on our recent study using the culture method, while the frequency of *mecA* carriers on the oral mucosa was very high, over 60% from day +7 to +20, which was beyond our expectation. This could be because a fourth-generation cephalosporin was mainly administered intravenously as empirical antibiotic therapy. A more in depth analysis of these patients compared to HCT patients who were negative before HCT as well as the patients who got positive during treatment might be interesting and might corroborate our assumption that the administration of a fourth-generation cephalosporin was responsible for this increase in *mecA* detection. We will try to perform multi-center study to increase subject number and would like to confirm our assumption.

The *mecA* gene complex is located on a unique molecular vector called the staphylococcal chromosome cassette (SCC*mec*) [3]. SCC*mecs* are considered to be transferred into *S. aureus* from a coagulase-negative species [6, 14]. The tendencies of *mecA* detection frequency may differ between institutes because of their policies of antibiotic use, while we speculate that the oral cavity just before and after HCT may be a reservoir and could be a transfer space of the genes regulating antibiotic resistance as *mecA*. Recent research strongly suggests that oral hygiene may also be a reasonable strategy to control methicillin-resistant CoNS to eventually lower the MRSA burden in medical facilities [8]. Maintenance of good oral hygiene after HCT may contribute to reducing the presence of genes regulating antibiotic resistance in the oral cavity and antibiotic-resistant bacterial infections.

In conclusion, *mecA*, which mediates penicillin and beta-lactam resistance, was detected from the oral mucosa immediate before and after HCT. The high detection frequency of staphylococci with resistance to penicillins and beta-lactams in our recent report was supported at the molecular level.

Sources of Funding and Conflict of Interest Statement

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Figure legends

Figure 1.

Frequency of HCT patients in whom *mecA* was detected at least once during the HCT period, and transition of the frequency of *mecA* carriers in HCT patients.

The difference in frequency of *mecA* carriers between HCT patients and control subjects was significant ($^{\#}P<0.01$, Fisher's exact test) (†Frequency of *mecA* carriers in whom *mecA* was detected at least once during day –7 to +20 from HCT). The detection frequencies of *mecA* increased significantly with time after HCT. The frequency of *mecA* carriers was 19.2% at day –7 to –1 from HCT, while it increased significantly at day +7 to +13 and day +14 to +20, with frequencies of 60.9% and 63.2%, respectively (*$P < 0.01$, ANOVA).

Tables

Table 1. Diseases of patients

Diseases	Type of HCT			Total
	Autologous	Allogeneic		
		Conventional	RIST	
Acute myelogenous leukemia		8	9	17
Acute lymphoblastic leukemia		3	1	4
Chronic myelogenous leukemia		1	1	2
Malignant lymphoma	12	1	12	25
Aplastic anemia			2	2
Myelodysplastic syndromes			8	8
Myelofibrosis			1	1
Total	12	13	34	59

Table 2. Frequency of *mecA* carriers in whom *mecA* was detected at least once during the study period (day –7 to +20 from HCT) and in control subjects

	mec A			Total
	+	–		
HCT group	45 (76.3%)	14 (23.7%)	*	59
control group	0 (0%)	52 (100%)		52
total	45	66		111

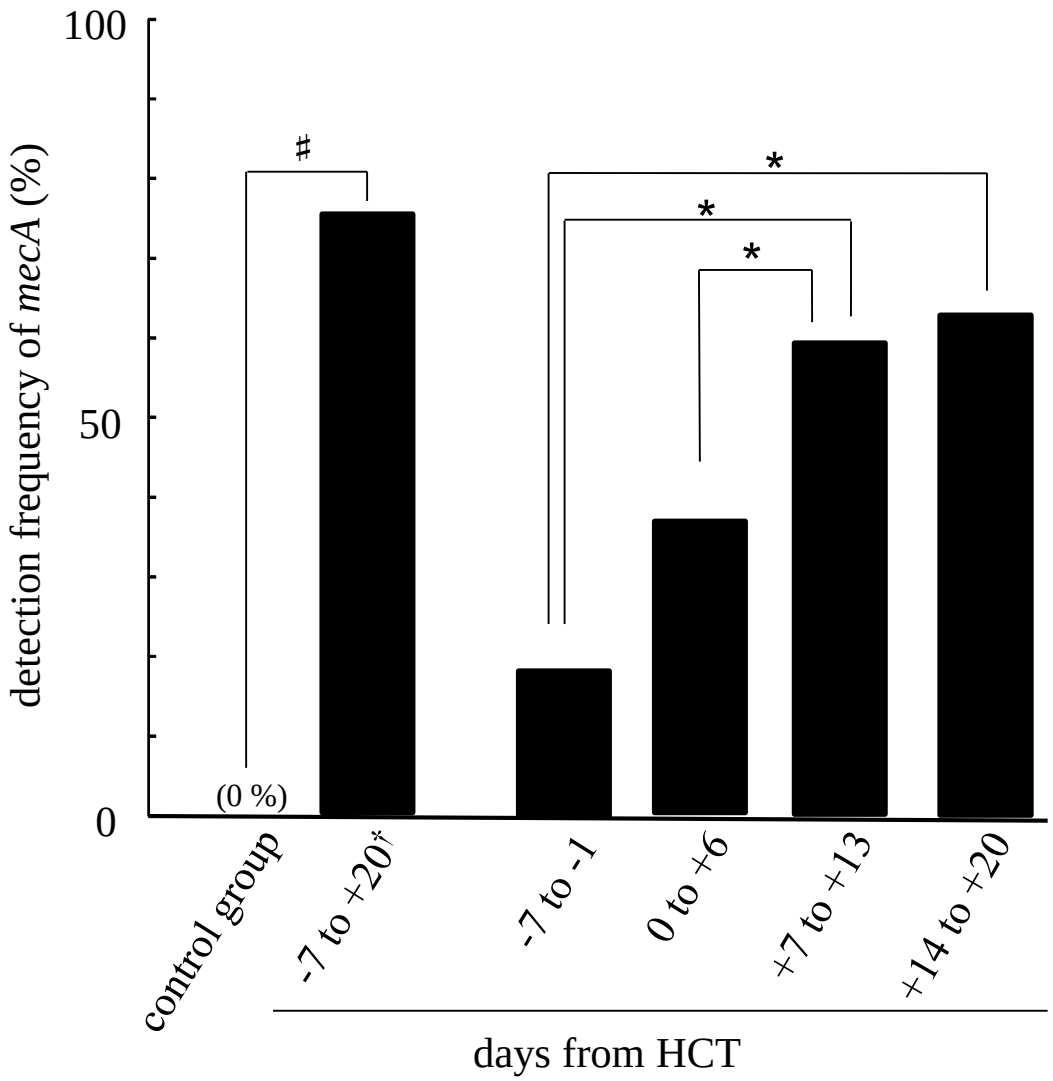
(*****$P < 0.01$, Fisher's exact test)

Table 3. Transition of the frequency of *mecA* carriers in HCT patients

Days from HCT	*mec A*		total
	+	–	
-7 to -1	10 (19.2 %)	42 (80.8 %)	52
0 to +6	20 (36.4 %)	35 (63.6 %)	55
+7 to +13	28 (60.9 %)*	18 (39.1 %)	46
+14 to +20	24 (63.2 %)*	14 (36.8 %)	38

(******P* < 0.01, ANOVA, compared with day -7 to -1)

Figure 1



(#P<0.01, Fisher's exact test)
(*P<0.01, ANOVA)