Oral and rectal colonization of methicillin-resistant *Staphylococcus aureus* in long-term care facility residents and their association with clinical status

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Keywords
Staphylococcus aureus, MRSA, antibiotic resistance, oral cavity, long-term care facility

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Abbreviations

ABK, arbekacin; ABPC, ampicillin; ARB, antimicrobial-resistant bacteria; BZK, benzalkonium chloride; CEZ, cefazolin; CHX, chlorhexidine chloride; CLDM, clindamycin; CLSI, Clinical and laboratory standards institute; CMZ, cefmetazole; CPC, cetylpyridinium chloride; DDBJ, DNA Data Bank of Japan; ECOG, DAP, daptomcin; eastern cooperative oncology group; EM, erythromycin; GHSF, geriatric health service facility: GM, gentamicin; IPM, imipenem; iTOL, interactive tree of life; LCTF, long-term care facility; LVFX, levofloxacin; LZD, linezolid; MINO, minocycline; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MPIPC, oxacillin; MSSA, methicillin-sensitive *Staphylococcus aureus*; OHAT-J, Oral Health Assessment Tool–Japanese edition; PCG, penicillin G; PVPI, povidone iodide; SNP, single nucleotide polymorphism; ST, sequence type; TEIC, teicoplanin; VCM, vancomycin; WF, welfare facilities for the elderly requiring long-term care

ABSTRACT

Staphylococcus aureus is a commensal bacterium in humans, but it sometimes causes opportunistic infectious diseases such as suppurative skin disease, pneumonia and enteritis. Therefore, it is important to know the prevalence of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) in humans, especially older adults. In this study, we investigated the prevalence of *S. aureus* and MRSA in the oral cavity and feces of residents in long-term care facilities (LTCFs). *S. aureus* was isolated from the oral cavity of 61/178 (34.3%) participants, including 28 MRSA-positive participants (15.7%), and from the feces of 35/127 (27.6%) participants, including 16 MRSA-positive participants (12.6%). *S. aureus* and MRSA were isolated from both sites in 19/127 individuals (15.0%) and 10/127 individuals (7.9%), respectively. Among 19 participants with *S. aureus* isolation from both sites, 17 participants showed the same sequence type (ST) type. Then, we analyzed the correlation of *S. aureus* and MRSA in the oral cavity and rectum with the participant's condition. *S. aureus* and MRSA positivity in the oral cavity was significantly related to tube feeding, while there was no correlation of rectal *S. aureus*/MRSA with any factors. Our findings regarding the oral inhabitation of MRSA and its risk factors exhibit the importance of considering countermeasures against MRSA infection in LTCFs.

INTRODUCTION

Antimicrobial-resistant bacteria (ARB) are microorganisms that typically cause problems when treating infections with antibiotics [1–3]. Antibiotics are used to control bacterial and some fungal infections, but ARB have acquired resistance to some

antibiotics by internal mutation or the acquisition of external genes responsible for antibiotic resistance [4]. ARB can develop and spread by many factors, such as inadequate antibiotic use, antimicrobial abuse, and lack of infection control. The risk of the emergence of resistant bacteria increases when antibiotics are used extensively for nonmedical purposes in humans, such as in agriculture or livestock feed [5, 6]. The concern posed by antimicrobial-resistant organisms is that they make treatment of infections more difficult. Patients infected with ARB may require more potent treatments because regular antibiotics are not effective. The spread of ARB also poses a challenge to infection control and public health. In particular, in hospitals, there are many compromised hosts, so considerable countermeasures for infection control must be taken.

Recently, we reported that 3rd-generation cephalosporine-resistant or carbapenem-resistant gram-negative bacteria, including *Acinetobacter, Pseudomonas* and *Enterobacteriaceae,* were isolated from the oral cavity of residents in long-term care facilities [7]. ARB in the oral cavity are considered to spread the infection to other sites in individuals and to others through conversing, coughing and eating. In addition, it has been reported that oral bacteria sometimes cause bacteremia by tooth extraction or periodontitis, affecting various systemic diseases, such as endocarditis, diabetes, arteriosclerosis and so on[8–10]. Hence, ARB in the oral cavity may also be a cause of bacteremia. Therefore, we need to pay more attention to ARB in the oral cavity for not only infection control but also the prevention of systemic disease.

Staphylococcus aureus is indigenous to the human skin and nasal mucosa. This organism causes opportunistic infectious diseases such as suppurative skin disease, food

poisoning, and pneumonia[11–13]. In addition, the emergence of methicillin -resistant *S. aureus* (MRSA) has become more serious for chemotherapeutic treatment[14, 15]. *S. aureus,* including MRSA, is also isolated from the oral cavity[16, 17] and is a causative pathogen for aspiration pneumonia[18–20]. Aspiration pneumonia is caused by aspiration of food or saliva, which allows bacteria to enter the respiratory system and cause infection. Aspiration pneumonia mostly occurs in elderly populations. Antibiotics are commonly applied to cure this disease. However, MRSA makes it difficult to treat patients of pneumonia with chemotherapy. To prevent aspiration pneumonia, it is important to maintain oral hygiene in elderly populations, assist with eating and feeding, and assess and train for swallowing[21–23]. Hospitals and nursing homes should also implement thorough infection control measures and surgical hygiene. Disinfectants are sometimes used for oral care in the form of a mouthwash [24, 25]. The proper use of disinfectants can play an important role against resistant organisms such as *S. aureus* and MRSA. However, the susceptibility of *S. aureus*, including MRSA isolated in the oral cavity, to disinfectants has not been well studied.

In this study, we investigated the oral and rectal colonization of *S. aureus* in long-term care facility residents. Then, we performed whole-genome sequencing of all *S. aureus* isolates and investigated the relation of the susceptibility to antibiotics and disinfectants with resistance genes. Furthermore, we analyzed the relationship between *S. aureus*/MRSA existence and participant's status.

MATERIALS AND METHODS

112

113 **Study design and participants.**

114 We isolated *S. aureus,* including MRSA, from the oral cavities and rectums of participants
115 in Geriatric Health Service Facilities and Welfare Facilities for the Elderly Requiring
116 Long-term Care in Hiroshima, Japan, from 2019 to 2020. Oral and rectal samples were
117 obtained by swabbing. The demographic data of the participants were obtained from their
118 medical records and nursing care plans. The data included demographics (age, sex, and
119 unit of residence), Eastern cooperative oncology group (ECOG) performance status (0:
120 fully active, able to carry on all pre-disease performance without restriction, 1: restricted
121 in physically strenuous activity but ambulatory and able to carry out work of a light or
122 sedentary nature, e.g., light house work, office work, 2: ambulatory and capable of all
123 selfcare but unable to carry out any work activities; up and about more than 50% of
124 waking hours, 3: capable of only limited selfcare; confined to bed or chair more than 50%
125 of waking hours, 4: completely disabled; cannot carry on any selfcare; totally confined to
126 bed or chair), comorbidities, prior antibiotic use within 6 months before the sample
127 collection, enteral nutrition, length of stay in facilities in days, survival time in days (from
128 sample collection to participant discharge or the end of the follow-up period), and
129 mortality. The Oral Health Assessment Tool–Japanese edition (OHAT-J) was used to
130 assess oral health status [26]. OHAT-J can be performed by nursing and care-giving staff
131 to easily evaluate the oral condition of persons. This method is performed by visual
132 examination of the lips, tongue, gingiva, mucosa, saliva, remaining teeth, oral cleaning
133 status, toothache, and denture fracture and fit. Each item is rated on a scale of 0 to 2. High

scores indicate poor oral hygiene.

Isolation of *S. aureus.*

After obtaining oral and rectal swab samples, swab samples were inoculated on staphylococcal selective medium (Nissui Pharmaceutical, Tokyo, Japan). After 2 days of incubation at 37 °C under aerobic conditions, a yellow single colony (up to 4 colonies in each sample) was picked and replated on selective medium again. Small portions of bacterial cells were taken from each colony and suspended in 100 μl of CS buffer containing 10 μg of lysostaphin (Sigma–Aldrich, St. Louis, MO, USA). The bacterial suspension was incubated for 15 min at 37 °C. Then, the samples were heated at 95 °C for 10 min. After centrifugation at 10,000 x g for 5 min, the supernatant was used as template DNA for PCR. PCR was performed using specific primers for *S. aureus* identification. The obtained *S. aureus* isolates were stored in a freezer (-80 °C) before use. *S. aureus* was cultured in trypticase soy broth (TSB) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 37 °C under aerobic conditions. MRSA was defined by a positive *mecA* gene using genome data of each isolate.

Susceptibility test against antibacterial agents and disinfectants.

S. aureus susceptibility to various antibacterial agents was determined by MicroScan WalkAway (Beckman Coulter, Brea, CA, USA). The antibacterial agents were penicillin G (PCG), ampicillin (ABPC), oxacillin (MPIPC), cefazolin (CEZ), cefmetazole (CMZ), imipenem (IPM), gentamicin (GM), arbekacin (ABK), erythromycin (EM), clindamycin

(CLDM), minocycline (MINO), vancomycin (VCM), teicoplanin (TEIC), daptomycin (DAP), linezolid (LZD) and levofloxacin (LVFX). We defined the criteria based on Clinical and laboratory standards institute (CLSI) 2021 guidelines. To evaluate the susceptibility of disinfectants, MIC was determined using a microdilution assay described elsewhere [27]. The disinfectants used in this study were povidone iodine (PVPI: Mundipharma K. K., Tokyo, Japan), cetylpyridinium chloride (CPC: FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), benzalkonium chloride (BZK: FUJIFILM Wako Pure Chemical Corporation) and chlorhexidine chloride (CHX: FUJIFILM Wako Pure Chemical Corporation). For the disinfectants, we defined the criteria for resistance (R) as ≥MIC90.

Genome sequencing of *S. aureus* isolates.

DNA was isolated from *S. aureus* cells for DNA sequencing. Overnight cultures of *S. aureus* (1.5 ml) were centrifuged at 10,000 x g for 5 min, and then bacterial cells were suspended in 500 µl CS buffer (100mM Tris-HCl [pH 7.5], 150mM NaCl, 10mM EDTA) containing 1.5 µl of lysostaphin (5 mg/ml) and 1 µl of RNase (10 mg/ml). After incubation at 37 °C for 60 min, 50 µl of 10% SDS and 15 µl of proteinase K (5 mg/ml) were added and incubated for 3 h at 55 °C. Then, an equal volume of Tris-saturated phenol (pH 8.0) was added, and after immersion and centrifugation at 10,000 x g for 5 min, the supernatant was collected. Phenol–chloroform solution was added, and after immersion and centrifugation at 10,000 x g for 5 min, the supernatant was collected. Finally, DNA was precipitated with ethanol. After centrifugation and washing with 70% ethanol, DNA was

dissolved in 100 µl of TE buffer. Subsequently, DNA libraries were constructed as described previously [28], and paired-end sequencing (2 × 150 bp) was performed on the Illumina HiSeq X Five platform (Macrogen Japan Corporation, Tokyo, Japan). The Illumina read data of each isolate were used for *de novo* assembly using Shovill v1.1.0 (Seemann T. Shovill: faster SPAdes assembly of Illumina 2018 [Available from: https://github.com/tseemann/shovill]).

Genetic analysis.

The genes associated with resistance to antibacterial agents, including disinfectants, were analyzed with ResFinder (Center for Genomic Epidemiology: URL: https://cge.food.dtu.dk/services/ResFinder/). Phylogenetic trees of *S. aureus* isolates were generated based on whole-genome single nucleotide polymorphism (SNP) analysis using the CSI Phylogeny 1.4 pipeline available from the Center for Genomic Epidemiology (Lungby, Denmark). Then, the trees were annotated with Interactive Tree of Life (iTOL) software [29]. Multilocus sequence typing (MLST) analysis was performed by using MLST 2.0 from the Center for Genomic Epidemiology. The MLST alleles (*arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) and sequence type (ST) profiles that had not been previously described were submitted to pubMLST (https://pubmlst.org) to assign new designations. SCC*mec* typing was analyzed with SCCmecFinder 1.2 (Center for Genomic Epidemiology: URL: https://cge.food.dtu.dk/services/SCCmecFinder/).

Intra-falicilty transmission analysis

To examine the possible intra-facility transmission of MRSA and MSSA, the groups of isolates ($n \geq 5$) that had the same MLST and originated from the same facility were selected for pairwise distances analysis. If a pair of isolates were isolated from the same participant, no further review was performed on that pair. The isolate pairs with number of SNP differences below the threshold of 40 SNPs and originated from different individuals at the same facility were considered as the possible results of intra-facility transmission.

Statistical analysis.

Univariate analysis of the association between the participant's status and the presence of oral/rectal *S. aureus*/MRSA was analyzed by Fisher's exact test. Multiple logistic regression analysis was performed for factors with p values of less than 0.05 on univariate analysis. The results with a p value of less than 0.05 were considered significant in all statistics. All statistical analyses were conducted using JMP Pro version 16 (SAS Institute, Cary, NC, USA).

Ethics.

This study was approved by the ethics committees of the Hiroshima University Hospital review board (approval number E-1692) and the National Institute of Infectious Diseases Committee of Ethics (approval number 1017). This study was performed in accordance with the principles of the Declaration of Helsinki. All residents, except those who refused consent, admitted to facilities during the study period were eligible for inclusion. Written

222 informed consent was obtained from the participants prior to their enrollment in the study.
223 Additionally, we obtained written informed consent from the families of participants who
224 lacked the mental capacity to consent.
225
226 **Accession number.**
227 The genome data of *S. aureus* isolates used in this study have been deposited into the
228 DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA) accession number
229 DRA016928 under BioProject accession no. PRJDB16315.
230
231 **RESULTS**
232
233 **Characteristics of participants from two types of facilities.**
234 A total of 178 participants in 6 facilities, including 67 participants in 3 geriatric health
235 service facilities (GHSFs) and 111 participants in 3 Welfare Facilities for the Elderly
236 Requiring Long-term Care (WFs), were included in this study (Fig. 1). The residents in
237 GHSF requires rehabilitation, nursing or care to return home rather than hospital
238 treatment, and the residents in WF requires a long-term care and unable to live at home.
239 In 38 participants in one WF and 13 participants in one GHSF, *S. aureus* was only isolated
240 from the oral cavity (Table 1). In 127 participants (73 in WFs and 54 in GHSFs), *S. aureus*
241 was isolated from the oral cavity and rectum (Table 2). Participant's status for one GHSF
242 (32 participants) was not collected (Table 1). Participant's status between GHSFs and
243 WFs was compared previously (Supplemental Table 1) [7]. The average ages of all

participants, WF participants and GHSF participants were 87.0, 87.8 and 85.7 years, respectively. All participants from the WFs showed an ECOG performance status of 4.0, while 49, 9 and 9 participants from the GHSFs showed performance statuses of 4, 3 and 2, respectively. Twenty-three participants from the WFs had enteral nutrition, while no participants from the GHSFs had enteral nutrition. The isolation frequency of *S. aureus* from the oral cavity was 34.3%, including 34.2% in WF participants and 34.3% in GHSF participants, and the frequency of MRSA from the oral cavity was 15.7%, including 18.9% in WF participants and 10.4% in GHSF participants (Table 2). The isolation frequency of *S. aureus* from the rectum was 27.6%, including 27.4% in WF participants and 27.8% in GHSF participants, and the frequency of MRSA from the oral cavity was 12.6%, including 13.7% in WF participants and 11.1% in GHSF participants. The isolation ratio of MRSA in the oral cavities and rectums of WF participants was higher than that in the oral cavities and rectums of GHSF participants. However, there was no significant difference in *S. aureus* and MRSA isolation frequency between WF and GHSF participants.

Isolation of *S. aureus* from oral and rectal cavities.

Among 178 participants from 6 facilities, *S. aureus* was isolated from the oral cavity of 61 participants (34.3%), including 28 participants (15.7%) with MRSA (Table 3). When the isolation frequency was compared between the oral cavity and rectum, we calculated each proportion among 127 participants from 4 facilities. Among 127 participants, *S. aureus* was isolated from the oral cavity of 46 participants (36.2%), including 20

participants (15.7%) with MRSA, while *S. aureus* was isolated from the rectum of 35 participants (27.6%), including 16 participants (12.6%) with MRSA (Table 3). Nineteen participants (15.0%) carried *S. aureus* from both sites. Among them, 10 participants (7.9%) showed MRSA isolations, either from both sites (9 participants) or only oral cavity (1 participant).

Susceptibility to antibacterial agents and disinfectants.

The MIC values of 52 methicillin-sensitive *S. aureus* (MSSA) and 44 MRSA strains against various antibacterial agents and disinfectants are shown in Tables 4 and 5. From the results of the MICs of antibacterial agents (Table 4), among 52 MSSA strains, all strains were susceptible to vancomycin (VCM), teicoplanin (TEIC), daptomycin (DAP), linezolid (LZD) and mupirocin (MUP). 19 MSSA strains showed both penicillin G (PCG) and ampicillin (ABPC) resistance, respectively, but all MSSA strains were oxacillin (MPIPC) susceptible. Among 44 MRSA strains, all strains showed resistance to PCG and ABPC, 43 strains showed resistance to MPIPC and DAP (1 strain: MPIPC: 2 μg/ml, DAP: 1 μg/ml), and all strains were susceptible to VCM, TEIC, MUP and LZD. Compared to MSSA strains, MRSA strains showed higher proportion of resistance to gentamicin (GM) (MR:40.9%, MS:32.7%), erythromycin (EM) (MR:81.8%, MS:23.0%), clindamycin (CLDM) (MR:9.1%, MS:1.9%), minocycline (MINO) (MR:6.8%, MS:0%) and levofloxacin (LVFX) (MR:97.7%, MS:26.9%). Then, we compared the proportion of resistance against seven antibiotics between WF and GHFS, MSSA and MRSA, or oral cavity and rectum (Table 5). We found that the proportions of ABPC, MPIPC, EM and

LVFX resistance in MRSA isolates were higher than those in MSSA strains. However, there were no significant differences between WF and GHFS or between oral cavity and rectal.

The MIC of disinfectants was also evaluated (Table 6). The MIC was variable for each disinfectant among the *S. aureus* strains. However, there was no significant difference in the MIC value for each disinfectant between MRSA and MSSA strains.

Genes responsible for resistance to antibacterial agents.

By using genomic data of *S. aureus* strains, resistance genes were identified (Table 7). For aminoglycoside resistance, *aac(6')-aph(2")* were found in 15 MRSA strains (34.1% of MRSA strains) and 13 MSSA strains (25.0% of MSSA strains), while *aad* and *bleO* genes were found in 8 MRSA strains and 1 MSSA strain (only *aad*). Among the 28 *aac(6')-aph(2")* positive strains, all strains showed GM resistance (≥16 μg/ml). For macrolide and lincosamide resistance, *erm(A)* was found in 9 MSSA strains (17.3%) and 35 MRSA strains (79.5%), and *erm(C)* was found in only 3 MSSA strains (5.8%). Among the 47 *erm(A)*- or *erm(C)*-positive strains, all strains showed EM resistance (≥8 μg/ml), but 5 strains showed CLDM resistance (≥4 μg/ml). For β-lactam resistance, the β-lactamase *blaZ* gene was found in 41 MRSA strains (93.2%) and 19 MSSA strains (36.5%). Among *blaZ*-positive MSSA strains, 14 of 19 MSSA strains showed ABPC resistance (≥4 μg/ml). All 44 *mecA*-positive strains (MRSA) showed resistance to MPIPC (≥4 μg/ml), while all 52 *mecA*-negative strains (MSSA) were susceptible to MPIPC. For quinolone resistance, the mutation of *gyrA* was found in 41 MRSA strains (93.2%) and

14 MSSA strains (26.9%), and *grlA/B* was found in 43 MRSA strains (97.7%) and 19 MSSA strains (36.5%). Among 55 *gyrA*-mutated strains, all strains showed resistance to LVFX (≥4 μg/ml), and among 62 *grl*-mutated strains, 57 strains showed resistance to LVFX, although these 57 strains showed *gyrA* mutations.

For resistance to quaternary ammonium compounds (QACs), *qacA* was found in 7 MRSA strains (15.9% of MRSA strains) and 7 MSSA strains (13.5% of MSSA strains), while the *qacB* gene was found in 1 MRSA strain. Among *qacA/B*-positive strains, only 2 of 14 strains showed an MIC value of 5 μg/ml with a higher MIC_{90} of BZK. The susceptibility of BZK showed no significant difference between *qacA/B*-positive and *qacA/B*-negative strains.

Comparison of the strains isolated from the oral and rectal regions of the same participant.

Among 127 participants with *S. aureus* isolated from the oral cavity or rectum, 19 participants showed *S. aureus* isolation from both sites (Table 8). Table 8 shows the ST type and presence of resistance genes of *S. aureus* strains. Among 19 participants, 17 participants showed the same ST type isolated from both sites, among which 16 participants had the same distribution of resistance genes, while the remaining 2 participants (swab no. 431 and 551) showed different ST type and different distributions of resistance genes. Oral isolate K079 and rectal isolate K003 isolated from the same individual showed the same ST type (ST8), but *blaZ* was detected only in K079. Among 19 participants, 8 participants had the same ST type of MRSA (ST1 [3 participants], ST8

[3 participants], ST8611 [1 participant], ST2725 [1 participant]).

Phylogenetic tree analysis.

Among 96 *S. aureus* isolates, 25 STs were observed, one of which had not been previously identified (ST8611) (Fig. 2). A new ST type, ST8611, was only isolated from one facility. However, there was no significant trend between STs and facilities. There were high proportions of ST8 (16 strains; 16.7%), ST1 (14 strains; 14.6%), ST8611 (11 strains 13.8%) and ST15 (11 strains 13.8%). Most MRSA strains belonged to ST1, ST8 and ST8611. The resistance gene profiles of the ST8611 and ST1 strains (except 2 isolates) were quite similar, showing additional resistance genes (*blaZ, erm(A), ant(9)-Ia,* and *mecA*) and mutations (*gyrA* and *grlA*). No significant trends in ST types were observed between oral and rectal isolates.

In addition, we investigated the relationship between ST type and SCC*mec* type among 44 MRSA strains (Suppl. Fig. 1). The combination types included ST1 with SCC*mec* type IV (ST1-IV) (12 strains, 27.3%), ST8611-IV (11 strains, 25.0%), ST8-I (7 strains, 15.9%), ST8-IV (6 strains, 13.6%), ST380-IV (3 strains, 6.8%), ST764-II (2 strains, 4.5%), ST2725-IV (2 strains, 4.5%) and ST5-II (7.8%).

Analysis of intra-facility transmission of MRSA/MSSA

To examine the possible intra-facility transmission, we chose the facilities that had above five isolates with the same MLST for further investigation. Based on the results of the phylogenetic tree (Fig.2), isolates originated from facility No. 5 and belonged to ST8 (n

354 = 9), ST15 (n = 5), ST1 (n = 6), and ST8611 (n = 10) were selected for pairwise distances
355 analysis. Among these selected isolates, all ST8, ST1, and ST8611 strains were MRSA,
356 and all ST15 strains were MSSA. Results showed that there were two ST8 pairs, three
357 ST15 pairs, and one ST8611 pair that exhibited a pairwise distance below 40 SNPs [30–
358 32] (the isolate pairs collected from the same participant were not selected for reviewed)
359 (Fig. 3). Therefore, these six isolate pairs from different individual participants present
360 three plausible transmission chains within the facility No. 5.
361
362 **Relationship of *S. aureus* isolation status and participant conditions.**
363 We analyzed the correlation of *S. aureus*/MRSA in the oral cavity and rectum with the
364 participant's condition (Table 9, Supplemental Table 2a). In *S. aureus*/MRSA in the oral
365 cavity, we found that MRSA positivity was significantly related to tube feeding,
366 nasogastric tube feeding (p=0.0069) and gastrostomy and enterostomy (p=0.0417), and
367 OHAT-J: lip score (p=0.016). Additionally, we found that *S. aureus* positivity was
368 significantly related to nasogastric tubes feeding (p=0.0021) and OHAT-J: lip score
369 (p=0.03). However, we found no correlation with other factors, remaining teeth, PS scores
370 or the presence of comorbidities. We further performed multiple logistic regression
371 analysis of each item after adjusting for covariates, and we found that *S. aureus* and
372 MRSA existence in the oral cavity was associated with nasogastric tube feeding (*S.*
373 *aureus*; OR: 11.8, MRSA; OR: 8.17) and gastrostomy and enterostomy (MRSA; OR:
374 3.36) (Table 9, Supplemental Table 2a). In contrast, we found no correlation of *S.*
375 *aureus*/MRSA in rectal cavities with the participant's condition (Supplemental Table 2b

and 2c).

DISCUSSION

In this study, we demonstrated the prevalence of *S. aureus* (oral: 34.3%, rectal: 27.6%) and MRSA (oral: 15.7%, rectal: 12.6%) from oral and rectal cavities of the residents in the WFs and GHSFs. We first compared *S. aureus*/MRSA isolation frequency between WFs and GHSFs and observed a trend toward higher isolation rates of MRSA at WFs than at GHSFs. Previously, we investigated the prevalence of 3rd-generation cephalosporine-resistant gram-negative bacteria from oral and rectal specimens of the same residents in this study and found that the isolation rate of ESBL-producing Enterobacterales isolated from recta of WF residents was higher than that of GHSF residents, while oral isolation rate did not show a significant difference between WF and GHSF residents [7]. By comparison of participant's status between WF and GHSF residents, performance status and enteral nutrition showed significant differences (Supplemental Table 1). Furthermore, participants subjected to enteral nutrition had a significantly higher proportion of ESBL-producing *Enterobacterales* and *P. aeruginosa*. In addition, Le MN-T et al reported that the usage of percutaneous endoscopic gastronomy tube is the risk factor for antimicrobial resistant gram-negative bacteria in oral cavity [33]. Our results of *S. aureus*/MRSA isolation in the oral cavity exhibited a significant correlation with tube feeding, especially nasogastric tubes, while isolation in the rectum was not associated. Although the reason for this correlation remains unclear, we speculate that enteral nutrition might reduce the mastication activity followed by the

reduction of salivary secretion, promoting the *S. aureus*/MRSA colonization in oral cavity. Therefore, the isolation frequency of ARB from residents in WFs is higher than that of residents in GHSFs, and enteral nutrition is a critical factor for the localization of not only gram-negative drug-resistant bacteria but also gram-positive drug-resistant bacteria, MRSA, in the oral cavity.

Silva LP et al. reported the prevalence of *S. aureus* and MRSA in the nasal, oral and rectal cavities of 150 LTCF residents and 76 bedridden patients [34]. The prevalence of total *S. aureus* and MRSA was 33.6% (n = 76) and 8% (n = 18), respectively, and the prevalence of *S. aureus* and MRSA in 9 LTCFs was 16.6 to 85.7% and 13.3 to 25.5%, respectively. In this study, the prevalence of *S. aureus* and MRSA in the oral and rectal cavities was 23.8% to 57.7% and 4.8% to 30.8%, respectively (Table 1), showing a similar ratio of *S. aureus*/MRSA isolation frequency in LTCFs among the 2 experiments. In addition, there are several reports regarding *S. aureus* isolation from the oral cavity. Koukos G et al. isolated *S. aureus* from dental plaque, tongue and periodontal pockets (only periodontitis) of periodontally healthy, gingivitis and chronic periodontitis patients and found that the prevalence of *S. aureus* was 8% in healthy, 8% in gingivitis and 14% in periodontitis patients (average age: 46±8, 49±9 and 50±10 years) [16]. In another study, Campos J et al. reported the prevalence of *S. aureus* in the oral and nasal cavities, showing 13.9% from the nasal cavity, 12.0% from the oral cavity and 9.9% from both sites in healthy volunteers (average age: 21.83±3.53 years) [17]. Vanzato Palazzo IC et al. reported that the prevalence of *S. aureus* and MRSA was 47.6% and 4.1%, respectively, in the saliva of 340 health care workers [35]. Petti S et al. also reported that the prevalence of *S. aureus*

and MRSA was 8.9% and 1.9%, respectively, from oral swabs of 157 dental students[36]. Compared to these studies, the prevalence of *S. aureus* and especially MRSA in LTCFs shows a high frequency considering that older age- and elderly related clinical conditions may affect the increased ratio of *S. aureus* and MRSA colonization in the oral cavity. Additionally, we found three plausible transmission chains of *S. aureus* in one facility, suggesting that attention is needed to prevent intra-facility transmission of *S. aureus*.

In this study, we isolated 44 MRSA strains from the oral and rectal cavities of participants. Among 44 isolates, two combination types, ST1-SCC*mec*-IV and ST8611-SCC*mec*-IV, showed high proportion followed by ST8-SCC*mec*-IV and ST8-SCC*mec*-I. ST8611, which was newly designated in this study, was closely related to ST1 from phylogenetic tree analysis. Major MRSA clone isolated from Japanese hospitals was the New York/Japan clone (SCCmec type II/ST5). However, the prevalence of SCC*mec*-IV strains has been increasing recently [37–39]. Kaku N et al reported the major combination types were ST8-SCC*mec*-IV (30.7%) and ST1-SCCmec-IV (29.6%) among 270 MRSA strains detected in blood culture from 45 hospitals in Japan [40]. Based on these results, the major combination type of MRSA in this study is similar to recent trends.

Of 61 participants with *S. aureus* positivity, 19 participants (31.1%) showed *S. aureus* isolation from oral and rectal cavities, and 10 of 28 participants (35.7%) with MRSA positivity showed isolation from both sites. Among 17 sets of both *S. aureus* isolates, 16 participants showed the same ST and the same pattern of antibiotic resistance genes (except 1 set) (Table 8). Regarding the 3rd-generation cephalosporine-resistant gram-negative bacteria isolated from both oral and rectal cavities of the same individuals, we

previously compared the ST type and the susceptibility profiles of strains isolated from both sites of the same individuals and found that 6/9 individuals carried strains of the same ST type and similar tendency of the susceptibilities. [27]. Therefore, when drug resistant bacteria were localized in both sites of one individual, both sites generally harbor the same clone. However, 27 and 16 participants among 62 *S. aureus*-positive participants showed only oral and rectal isolation, respectively, so it may be difficult to localize in both sites because the oral and rectal environments are quite different. Microbiome analysis showed that gram-positive bacteria, especially streptococci, are dominant in the oral cavity, while gram-negative bacteria are dominant in the colon[41, 42]. Furthermore, the oral environment is quite different from the gut environment in terms of oxygen conditions (oral: aerobic, gut: anaerobic) and immune systems. Therefore, we considered that the characteristics of *S. aureus* may be different between oral and rectal isolates. However, our phylogenetic tree revealed that the cluster was not divided by the isolation site. Further studies need to determine the characteristics of oral- and rectal-derived *S. aureus*.

In this study, we evaluated the susceptibility to antibiotics and compared these susceptibilities with the existence of resistance genes. Since clinical MRSA isolates have been reported to have multiple resistance to many antibiotics[43, 44], we confirmed this tendency, showing a higher proportion of erythromycin and levofloxacin resistance (Table 6). In addition, we investigated the susceptibility of ARB isolates to disinfectants because several disinfectants have been used daily for mouth rinse. We found variations in the susceptibility to these disinfectants, especially CPC, BZX and CHX. It has been reported

464 that *qac* genes encoding efflux pumps are involved in resistance to quaternary ammonium
465 compounds (QACs) and cationic biocides such as chlorhexidine in *S. aureus* [45–47].
466 These Qac efflux pumps are divided into two major protein families, the major facilitator
467 superfamily (MFS) belonging to QacA and QacB and the small multidrug resistance
468 (SMR) family (QacC, G, H and J). In this study, we found 14 *qacA*-positive isolates and
469 one *qacB*-positive isolate by ResFinder analysis. Compared to BZK susceptibility
470 between *qacA/B*-positive and negative isolates, we did not find a significant difference,
471 although the susceptibility of *qacA/B*-positive isolates (average MIC: 2.21 μg/ml) was
472 higher than that of *qacA/B*-negative isolates (average MIC: 1.65 μg/ml). In addition, the
473 concentration for oral administration is higher than that of the MIC value of each
474 disinfectant[27]. Therefore, oral disinfection can be effective for *S. aureus*. However,
475 routine oral care using mouthwashes containing disinfectants is sometimes performed for
476 residents in LTCFs. Careless use of mouthwashes may provide selective pressure toward
477 disinfectant resistance among bacteria, including *S. aureus*, leading to the emergence of
478 disinfectant-resistant *S. aureus* strains.
479 In conclusion, we showed the prevalence of *S. aureus*/MRSA in the oral and rectal
480 cavities of residents in elderly care facilities and found that tube feeding is a critical factor
481 for the colonization of *S. aureus*/MRSA in the oral cavity. Our findings indicate the
482 importance of considering countermeasures against MRSA infection in LTCFs.
483

484 **AUTHOR CONTRIBUTIONS**

485 HO, KT, MK-M, RN and HK developed the concept. AH, MY, TK and SK isolated the

strains. AH and SK performed the susceptibility test. TK, JH, AH and MS performed the identification of bacterial species and genome analysis. AH, ML, SK, MK-M and HK performed genetic analysis. SK, ML and MK-M created the figures and tables. AH, HO, KT MK-M, MS and HK was responsible for interpreting the results. SK and HK wrote the manuscript and RN, MM, ML, MS, KT and HO edited the manuscript. All authors read, sub-edited, and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

Hitoshi Komatsuzawa is an Editorial Board member of Microbiology and Immunology

and a co-author of this article. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure legends.

Fig. 1 Flowchart of the participant selection and sampling processes.

Fig. 2. Phylogenetic analysis and relationships between resistance genes and facilities. Red rectangles or the same red numbers indicate the oral and rectal isolates from the same individual.

Fig. 3. Genetic relatedness between *S. aureus* strains with same ST isolated from the same facility.

The numbers of SNP differences among *S. aureus* strains are shown in a red-yellow-green gradient. Alphabets (A-H) assigned to patients represent the strains from the same patient (oral and rectal isolates).

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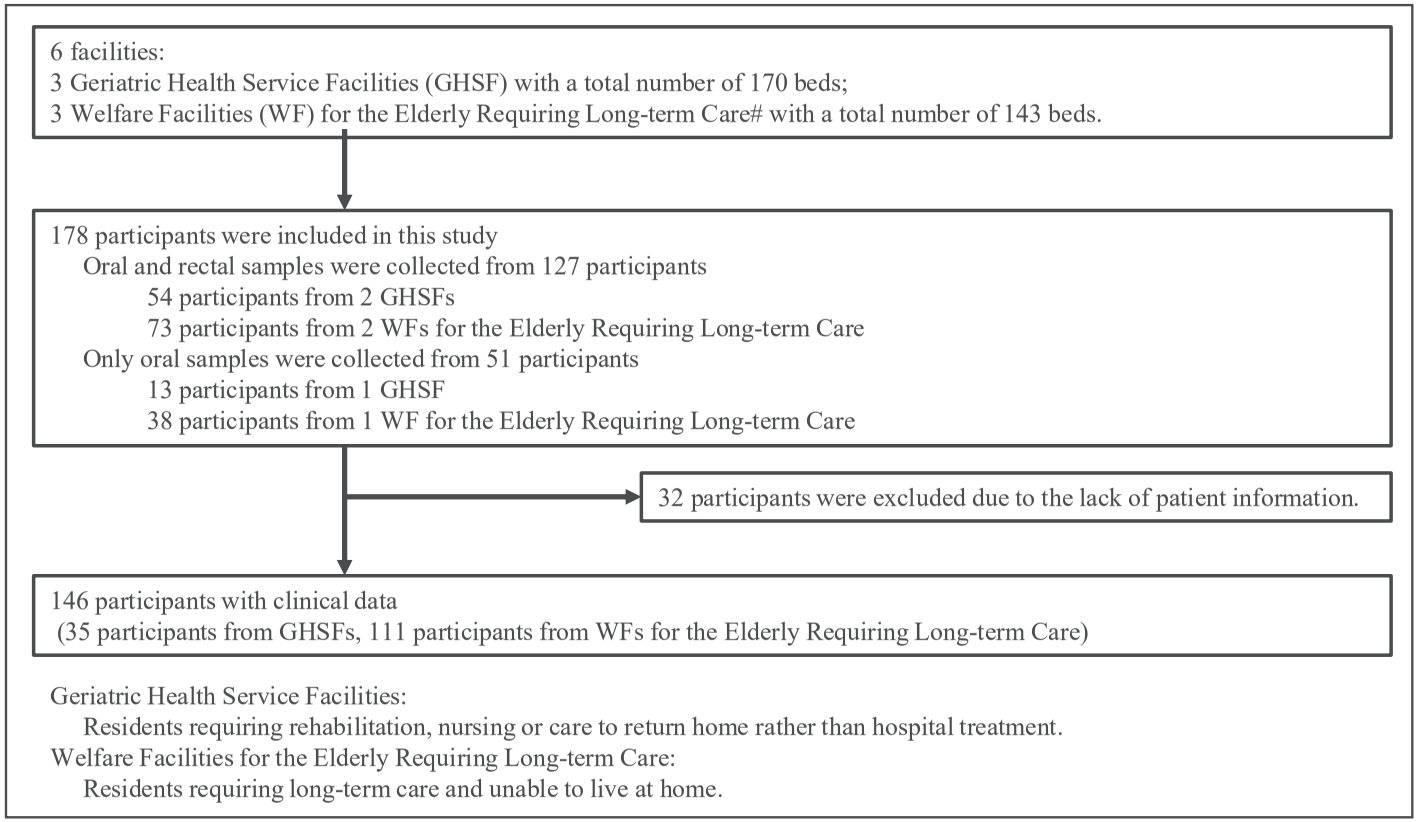
Fig. 1 Flowchart of the participant selection and the sampling process



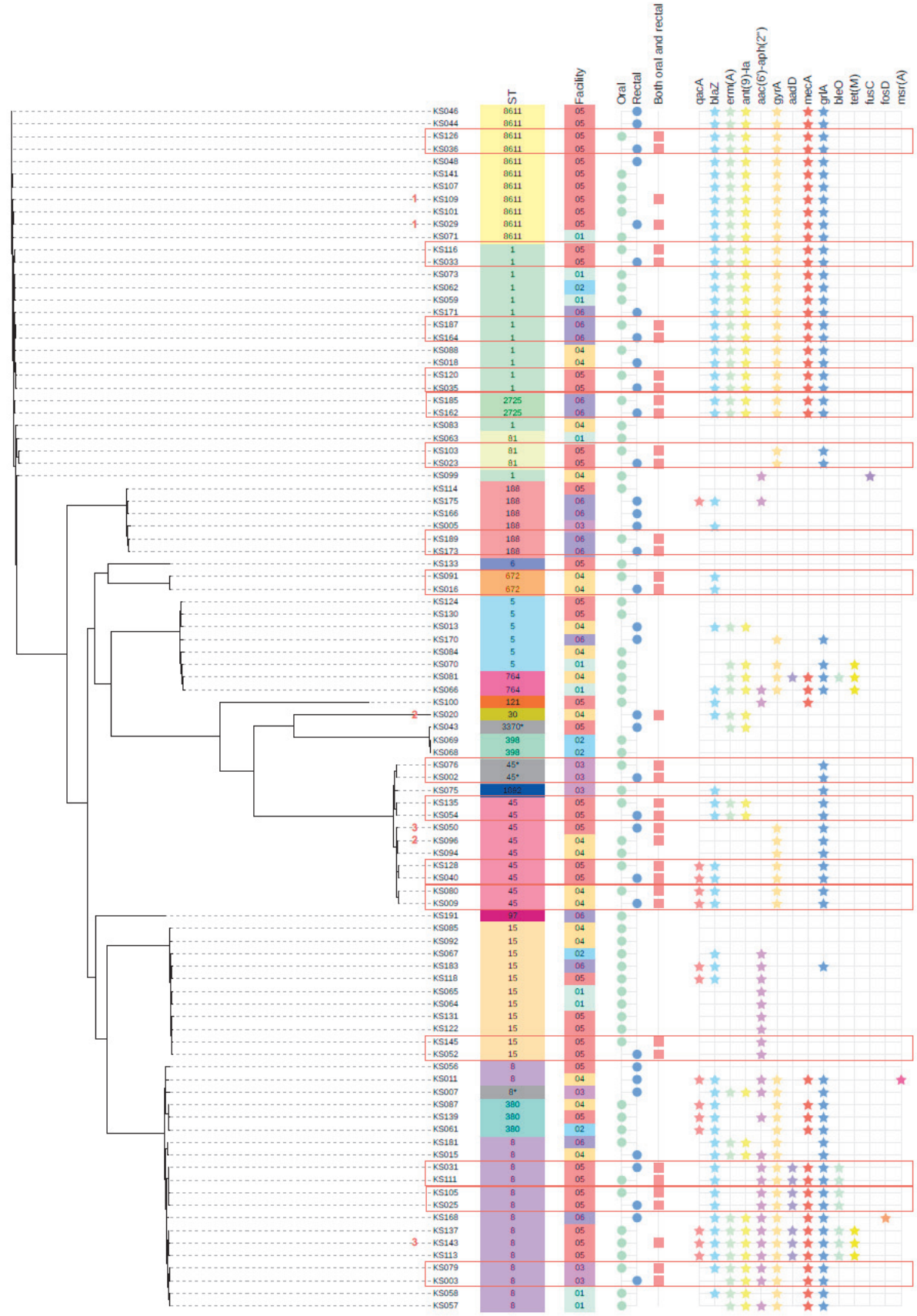


Fig. 2. Phylogenetic analysis and relationships between resistance genes and facilities. In the MLST analysis results, the background color indicates the strain ST.

ST8

Facility No.	Patient	MRSA/ MSSA	Strain	KS113	KS143	KS137	KS025	KS105	KS111	KS031	KS139	KS056
5		MRSA	KS113	0	11	50	363	363	363	379	779	777
5		MRSA	KS143	11	0	61	374	374	374	390	790	788
5		MRSA	KS137	50	61	0	373	373	373	389	789	787
5	A	MRSA	KS025	363	374	373	0	0	2	18	760	761
5	A	MRSA	KS105	363	374	373	0	0	2	18	760	761
5	B	MRSA	KS111	363	374	373	2	2	0	18	760	761
5	B	MRSA	KS031	379	390	389	18	18	18	0	776	777
5		MRSA	KS139	779	790	789	760	760	760	776	0	628
5		MRSA	KS056	777	788	787	761	761	761	777	628	0

ST15

Facility No.	Patient	MRSA/ MSSA	Strain	KS052	KS145	KS122	KS131	KS118
5	C	MSSA	KS052	0	13	22	30	198
5	C	MSSA	KS145	13	0	23	27	193
5		MSSA	KS122	22	23	0	26	200
5		MSSA	KS131	30	27	26	0	204
5		MSSA	KS118	198	193	200	204	0

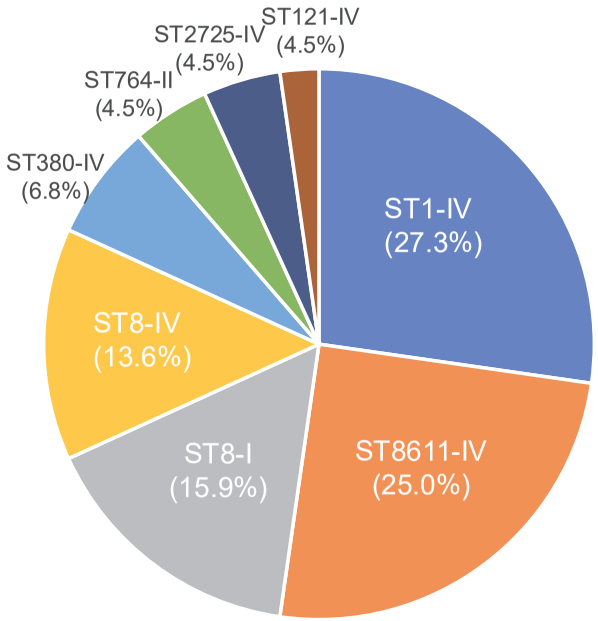
ST1

Facility No.	Patient	MRSA/ MSSA	Strain	KS120	KS035	KS116	KS033	KS103	KS023
5	D	MRSA	KS120	0	9	226	235	649	665
5	D	MRSA	KS035	9	0	231	236	654	670
5	E	MRSA	KS116	226	231	0	15	551	567
5	E	MRSA	KS033	235	236	15	0	554	570
5	F	MRSA	KS103	649	654	551	554	0	18
5	F	MRSA	KS023	665	670	567	570	18	0

ST8611

Facility No.	Patient	MRSA/ MSSA	Strain	KS029	KS109	KS101	KS048	KS141	KS107	KS126	KS036	KS044	KS046
5	G	MRSA	KS029	0	45	47	95	102	104	105	116	109	125
5	G	MRSA	KS109	45	0	18	64	69	69	72	83	76	94
5		MRSA	KS101	47	18	0	60	71	67	72	79	72	86
5		MRSA	KS048	95	64	60	0	51	53	48	55	42	58
5		MRSA	KS141	102	69	71	51	0	56	67	78	71	81
5		MRSA	KS107	104	69	67	53	56	0	69	74	65	87
5	H	MRSA	KS126	105	72	72	48	67	69	0	19	58	74
5	H	MRSA	KS036	116	83	79	55	78	74	19	0	67	87
5		MRSA	KS044	109	76	72	42	71	65	58	67	0	60
5		MRSA	KS046	125	94	86	58	81	87	74	87	60	0

Fig. 3. Genetic relatedness between *S. aureus* strains with same ST isolated from same facility.



Suppl. Fig. 1. Proportion of the combination type (ST and SCC*mec*) among 44 MRSA isolates.

Table 1. Number of participants with *S. aureus*/MRSA positive

Facilities		*S. aurues*				MRSA				No. of participants	Participant's data
		total	Oral only	Rectal only	both	total	Oral only	Rectal only	both		
WF[a]	1	10 (26.3%)	10 (26.3%)	-[c]	-	6 (15.8%)	6 (15.8%)	-	-	38	+
	3	5 (23.8%)	1 (4.8%)	2 (9.5%)	2 (9.5%)	1 (4.8%)	0 (0%)	0 (0%)	1 (4.8%)	21	+
	5	30 (57.7%)	14 (26.9%)	5 (9.6%)	11 (21.2%)	16 (30.8%)	7 (13.5%)	2 (3.8%)	7 (13.5%)	52	+
GHSF[b]	2	5 (38.5%)	5 (38.5%)	-	-	2 (15.4%)	2 (15.4%)	-	-	13	+
	4	16 (50.0%)	9 (28.1%)	4 (12.5%)	3 (9.4%)	5 (15.6%)	3 (9.4%)	2 (6.3%)	0 (0%)	32	-
	6	11 (50.0%)	3 (13.6%)	5 (22.7%)	3 (13.6%)	4 (18.2%)	0 (0%)	2 (9.1%)	2 (9.1%)	22	+

[a]Welfare Facilities for the Elderly Requiring Long-term Care, [b]Geriatric Health Service Facilities

[c]*S. aureus*/MRSA isolation was not performed.

Table 2. Isolation frequencies of *S. aureus* and MRSA from participants in two facilities

	Total	Welfare Facility	Geriatric Health Services Facility	*p* value[a]
Oral cavity (participants)	178	111	67	
S. aureus	61 (34.3%)	38 (34.2%)	23 (34.3%)	1.0000
MRSA	28 (15.7%)	21 (18.9%)	7 (10.4%)	0.1441
Rectal (participants)	127	73	54	
S. aureus	35 (27.6%)	20 (27.4%)	15 (27.8%)	1.0000
MRSA	16 (12.6%)	10 (13.7%)	6 (11.1%)	0.7895

[a] comparison of the isolation rate between welfare facility and geriatric health services facility

Table 3. Number of participants with *S. aureus* and MRSA from oral cavity and rectal

	Number of participants						
	178 participants (%)	127 participants (%)					
	Oral	Oral	Oral only	Rectal	Rectal only	Oral + Rectal	total
S. aureus	61 (34.3%)	46 (36.2%)	27 (21.3%)	35 (27.6%)	16 (12.6%)	19 (15.0%)	62/127 (48.8%)
MRSA	28 (15.7%)	20 (15.7%)	10 (7.9%)	16 (12.6%)	6 (4.7%)	10 (7.9%)	26/127 (20.5%)

Table 4. Antibiotics susceptibility among *S. aureus* isolates

		PCG		ABPC		MPIPC		VCM		TEIC	
	No. of strains	MIC range (μg/ml)	No. of Resi.(%)*	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)
S. aureus	96	≤0.06 - >8	63(65.6)	1≤ - >8	63(65.6)	≤0.25 - >4	43(44.8)	0.5 - 1	0(0)	≤1 - 2	0(0)
MSSA	52	≤0.06 - >8	19(36.5)	1≤ - >8	19(36.5)	0.25 - 0.5	0(0)	0.5 - 1	0(0)	≤1 - 2	0(0)
MRSA	44	4 - >8	44(100.0)	≥8	44(100.0)	2 - >4	43(97.7)	0.5 - 1	0(0)	≤1	0(0)

		GM		EM		CLDM		MINO		LVFX	
	No. of strains	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)
S. aureus	96	≤1 - >8	35(36.5)	≤0.25 - >4	48(50.0)	≤0.25 - >2	5(5.2)	≤1 - >8	3(3.1)	≤0.5 - >4	57(59.4)
MSSA	52	≤1 - >8	17(32.7)	≤0.25 - >4	12(23.0)	≤0.25 - >2	1(1.9)	≤1 - 8	0(0)	≤0.5 - >4	14(26.9)
MRSA	44	≤1 - >8	18(40.9)	≤0.25 - >4	36(81.8)	≤0.25 - >2	4(9.1)	≤1 ->8	3(6.8)	≤0.5 - >4	43(97.7)

		DAP		LZD		MUP	
	No. of strains	MIC range (μg/ml)	No. of Resi.(%)*	MIC range (μg/ml)	No. of Resi. (%)	MIC range (μg/ml)	No. of Resi. (%)
S. aureus	96	≤0.25 - 1	1(1.0)	1 - 4	0(0)	0. 25 - 16	0(0)
MSSA	52	≤0.25 - 0.5	0(0)	1 - 4	0(0)	0. 25 - 16	0(0)
MRSA	44	≤0.25 - 1	1(2.3)	1 - 4	0(0)	0. 25 - 16	0(0)

*Criteria for resistance (Resi.): PCG: ≥0.25 μg/ml, ABPC: ≥2 μg/ml, MPIPC : ≥4 μg/ml, VCM : ≥16 μg/ml, TEIC : ≥32 μg/ml, GM: ≥16 μg/ml, EM: ≥8 μg/ml, CLDM: ≥4 μg/ml, MINO: ≥16 μg/ml, LVFX : ≥4 μg/ml, DAP : ≥1 μg/ml, LZD : ≥8 μg/ml, MUP : ≥512 μg/ml

Table 5. Proportion of antibiotic resistance in different condition

	WF	GHFS	*P* value[b]	MSSA	MRSA	*P* value[c]	Oral	Rectal	*P* value[d]
Total isolates	58	38		52	44		61	35	
ABPC	40[a]	23	0.6537	19	44	<0.0001	37	26	0.6455
MPIPC	30	13	0.0993	0	43	<0.0001	27	16	0.0565
GM	25	10	0.1293	17	18	0.5236	21	14	0.6615
EM	31	17	0.5315	12	36	<0.0001	27	21	0.6718
CLDM	2	3	0.3814	1	4	0.1758	3	2	1.0000
MINO	2	1	1.0000	0	3	0.0927	3	0	0.2977
LVFX	37	20	0.2958	14	43	<0.0001	34	23	0.3920

[a]number of isolates showing a resistance to antibiotics

[b] comparison of the isolation rate between WF and GHSF,

[c] comparison of the isolation rate between MSSA and MRSA

[d] comparison of the isolation rate between oral and rectal.

Table 6. Disinfectants susceptibility among *S. aureus* isolates

		PVPI			CPC			BZK			CHX		
	No. of strains	MIC range (μg/ml)	MIC_{90} (μg/ml)	No. of resi.[a]	MIC range (μg/ml)	MIC_{90} (μg/ml)	No. of resi.	MIC range (μg/ml)	MIC_{90} (μg/ml)	No. of resi.	MIC range (μg/ml)	MIC_{90} (μg/ml)	No. of resi.
S. aureus	96	547～4375	2188	8	0.08～2.5	2.5	0	0.63～5	2.5	3	1.56～25	12.5	3
MSSA	52	1094～4375	2188	4	0.16～2.5	2.5	0	0.63～5	2.5	2	1.56～25	12.5	0
MRSA	44	546～4375	2188	4	0.08～2.5	2.5	0	0.63～5	2.5	1	1.56～25	12.5	3

[a]number of resistant isolates showing above MIC_{90}

Table 7. Antibiotic resistant genes from *S.aureus* and MRSA

Strains	number	Acquired antibiotic resistant genes											Point mutation	
		QAC		Aminoglycosides				MLS		β-lactam		Tet	Quinolone	
		qacA	*qacB*	*ant(9)-Ia*	*aac(6')-aph (2'')*	*aad*	*bleO*	*erm(A)*	*erm(C)*	*blaZ*	*mecA*	*Tet(M)*	*gyrA*	*grlA/B*
S. aureus	96	14	1	44	28	9	8	44	3	60	44	6	55	62
MSSA	52	7	0	9	13	1	0	9	3	19	0	1	14	19
oral	33	4	0	3	9	0	0	3	2	9	0	1	7	11
rectal	19	3	0	6	4	1	0	6	1	10	0	0	7	8
MRSA	44	7	1	35	15	8	8	35	0	41	44	5	41	43
oral	28	6	0	22	10	6	6	22	0	26	28	5	26	27
rectal	16	1	1	13	5	2	2	13	0	15	16	0	15	16

QAC, quaternary ammonium compound; MLS, macrolides-lincosamide-streptogramin; Tet, tetracycline

Table 8. Characterization comparison of isolates from the same participant

Swab No.	strain	site[a]	ST	Antibiotic resistant gene										
				mecA	*qacA*	*blaZ*	*erm(A)*	*ant*[b]	*aac*[c]	*aad*	*bleO*	*tet(M)*	*gyrA*	*grlA*
312	K076	O	8615	-	-	-	-	-	-	-	-	-	-	+
	K002	R	8615	-	-	-	-	-	-	-	-	-	-	+
313	K079	O	8	+	-	+	+	+	+	-	-	-	+	+
	K003	R	8	+	-	-	+	+	+	-	-	-	+	+
402	K080	O	45	-	+	+	-	-	-	-	-	-	+	+
	K009	R	45	-	+	+	-	-	-	-	-	-	+	+
418	K091	O	672	-	-	+	-	-	-	-	-	-	-	-
	K016	R	672	-	-	+	-	-	-	-	-	-	-	-
431	K096	O	45	-	-	-	-	-	-	-	-	-	+	+
	K020	R	30	-	-	+	+	+	-	-	-	-	-	-
504	K103	O	81	-	-	-	-	-	-	-	-	-	+	+
	K023	R	81	-	-	-	-	-	-	-	-	-	+	+
511	K105	O	8	+	-	+	-	-	+	+	+	-	+	+
	K025	R	8	+	-	+	-	-	+	+	+	-	+	+
513	K109	O	8611	+	-	+	+	+	-	-	-	-	+	+
	K029	R	8611	+	-	+	+	+	-	-	-	-	+	+
514	K111	O	8	+	-	+	-	-	+	+	+	-	+	+
	K031	R	8	+	-	+	-	-	+	+	+	-	+	+
517	K116	O	1	+	-	+	+	+	-	-	-	-	+	+
	K033	R	1	+	-	+	+	+	-	-	-	-	+	+
519	K120	O	1	+	-	+	+	+	-	-	-	-	+	+

	K035	R	1	+	-	+	+	+	-	-	-	-	+	+
527	K126	O	8611	+	-	+	+	+	-	-	-	-	+	+
	K036	R	8611	+	-	+	+	+	-	-	-	-	+	+
530	K128	O	45	-	+	+	-	-	-	-	-	-	+	+
	K040	R	45	-	+	+	-	-	-	-	-	-	+	+
538	K135	O	45	-	-	+	+	+	-	-	-	-	-	+
	K054	R	45	-	-	+	+	+	-	-	-	-	-	+
551	K143	O	8	+	+	+	+	+	+	+	+	+	+	+
	K050	R	45	-	-	-	-	-	-	-	-	-	+	+
552	K145	O	15	-	-	-	-	-	+	-	-	-	-	-
	K052	R	15	-	-	-	-	-	+	-	-	-	-	-
605	K185	O	2725	+	-	+	+	+	-	-	-	-	+	+
	K162	R	2725	+	-	+	+	+	-	-	-	-	+	+
607	K187	O	1	+	-	+	+	+	-	-	-	-	+	+
	K164	R	1	+	-	+	+	+	-	-	-	-	+	+
618	K189	O	188	-	-	-	-	-	-	-	-	-	-	-
	K173	R	188	-	-	-	-	-	-	-	-	-	-	-

[a] O:oral, R:rectal

[b] *ant(9)-Ia*

[c] *aac(6')-aph(2'')*

Table 9. Clinical characteristics of patients and risk factors associated with oral MRSA isolates

	MRSA positive	MRSA negative	Univariate analysis [a]			Multivariate analysis [b]		
	(n=28)	(n=118)	OR	95% CI	*p* value	OR	95% CI	*p* value
Male sex, n (%)	7 (25.0)	18 (15.3)	1.85	0.69-4.99	0.26			
Age average, years (SD)	13 (46.4)	58 (49.2)	0.90	0.39-2.05	0.84			
OHAT-J								
Lip score=1 n, (%)	13 (46.4)	26 (22.0)	3.07	1.30-7.25	0.016*	1.97	0.72-5.39	0.19
Tongue score=1 n, (%)	11 (39.3)	60 (50.8)	0.63	0.27-1.45	0.30			
Gum and mucosa score=1 n, (%)	7 (25.0)	34 (28.8)	0.82	0.32-2.12	0.82			
Saliva score=1 n, (%)	7 (25.0)	32 (27.1)	0.90	0.35-2.31	1			
Natural teeth score=1 n, (%)	12 (42.9)	51 (43.2)	0.99	0.43-2.26	1			
Denture score=1 n, (%)	1 (3.6)	9 (7.6)	0.45	0.05-3.69	0.69			
Oral cleanliness score=1 n, (%)	13 (46.4)	63 (53.4)	0.76	0.33-1.73	0.53			
Tooth pain score=1 n, (%)	0 (0.0)	1 (0.8)	0	-	1			
With remaining teeth, n (%)	20 (71.4)	84 (71.2)	1.01	0.41-2.52	1			
Edentulous ridge, n (%)	8 (28.6)	34 (28.8)	0.99	0.40-2.46	1			
PS= 4n (%)	27 (96.4)	111 (94.1)	1.70	0.20-14.4	1			
Tube feeding, n (%)	11 (39.3)	12 (10.2)						
Nasogastric tube, n (%)	5 (17.9)	3 (2.5)	8.33	1.86-37.3	0.0069**	8.17	1.55-43.2	0.013*
Gastrostomy and enterostomy, n (%)	6 (21.4)	9 (7.6)	3.30	1.07-10.2	0.0417*	3.36	0.99-11.5	0.053
Presence of co-morbidities								
Thigh bone fracture n, (%)	7 (25.0)	36 (30.5)	0.76	0.30-1.95	0.65			
Strokes n, (%)	15 (53.6)	45 (38.1)	1.87	0.82-4.29	0.14			
Cardiovascular disease n, (%)	8 (28.6)	33 (28.0)	1.27	0.52-3.10	0.64			
Diabetes n, (%)	3 (10.7)	15 (12.7)	0.82	0.22-3.07	1			
Tumor-bearing n, (%)	2 (7.1)	12 (10.2)	0.68	0.14-3.22	1			
Dementia n, (%)	22 (78.6)	89 (75.4)	1.19	0.44-3.23	0.81			
Clinical outcome n, (%)								
Alive	22 (78.6)	88 (74.6)	0.8	0.30-2.16	0.81			
Dead	6 (21.4)	30 (25.4)	0.8	0.30-2.16	0.81			

[a]Fisher's Exact Test, [b]Multiple logistic regression analysis. **P* value of ≤0.05; ***P* value of ≤0.01.