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ORIGINAL ARTICLE

Phenotype, genotype, and antibiotic susceptibility of Swedish and Thai oral isolates of *Staphylococcus aureus*

Susanne Blomqvist[1], Åsa Leonhardt[1], Pratanporn Arirachakaran[2], Anette Carlen[1] and Gunnar Dahlén[1]*

[1]Department of Oral Microbiology and Immunology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [2]Oral Medicine Clinic, Dental Faculty, Chulalongkorn University, Bangkok, Thailand

Objective: The present study investigated phenotypes, virulence genotypes, and antibiotic susceptibility of oral *Staphylococcus aureus* strains in order to get more information on whether oral infections with this bacterium are associated with certain subtypes or related to an over-growth of the *S. aureus* variants normally found in the oral cavity of healthy carriers.

Materials and methods: A total number of 157 *S. aureus* strains were investigated. Sixty-two strains were isolated from Swedish adults with oral infections, 25 strains were from saliva of healthy Swedish dental students, and 45 strains were from tongue scrapings of HIV-positive subjects in Thailand, and 25 Thai strains from non-HIV controls. The isolates were tested for coagulase, nitrate, arginine, and hemolysin, and for the presence of the virulence genes: *hlg*, *clfA*, *can*, *sdrC*, *sdrD*, *sdrE*, *map/eap* (adhesins) and *sea*, *seb*, *sec*, *tst*, *eta*, *etb*, *pvl* (toxins). MIC_{90} and MIC_{50} were determined by E-test against penicillin V, oxacillin, amoxicillin, clindamycin, vancomycin, fusidic acid, and cefoxitin.

Results: While the hemolytic phenotype was significantly ($p < 0.001$) more common among the Thai strains compared to Swedish strains, the virulence genes were found in a similar frequency in the *S. aureus* strains isolated from all four subject groups. The Panton-Valentine leukocidin (PVL) genotype was found in 73–100% of the strains. More than 10% of the strains from Swedish oral infections and from Thai HIV-positives showed low antibiotic susceptibility, most commonly for clindamycin. Only three methicillin-resistant *S. aureus* (MRSA) strains were identified, two from oral infections and one from a Thai HIV patient.

Conclusions: *S. aureus* is occasionally occurring in the oral cavity in both health and disease in Sweden and Thailand. It is therefore most likely that *S. aureus* in opportunistic oral infections originate from the oral microbiota. *S. aureus* should be considered in case of oral infections and complaints and the antibiotic susceptibility (including MRSA) should regularly be checked. The frequent presence of *S. aureus*, although in low numbers among students and staff, emphasizes the importance of standard infection control precautions and of using diagnostic test in the dental clinic.

Keywords: *Staphylococcus aureus*; *oral infections*; *HIV-positive*; *virulence*; *antibiotic susceptibility*

*Correspondence to: Gunnar Dahlén, Department of Oral Microbiology and Immunology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Box 450, SE-40530 Göteborg, Sweden, Email: dahlen@odontologi.gu.se

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Staphylococcus aureus is a major human pathogen that occurs in many different types of infections of the human body. Infections caused by *S. aureus* differ in their severity and tissue tropisms, ranging from relatively mild conditions such as skin and soft tissue infections, to more severe diseases that include osteomyelitis, necrotizing pneumonia, surgical-site infection, and bacteremia/sepsis leading to endocarditis, septic shock, and septic arthritis (1). *S. aureus* is also the most common cause of nosocomial/hospital infections and opportunistic infections in immune-compromised individuals and individuals with implants (1). Infections with *S. aureus* have been increasingly difficult to handle due to the development of drug resistance, especially methicillin-resistant *S. aureus* (MRSA) (2).

S. aureus has two distinct lifestyles, one as a commensal asymptomatic and one as infectious, causing invasion and pathogenicity (3). The adult population is frequently colonized by *S. aureus* on the skin and anterior nares and more or less persistently acts as healthy carriers (4).

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The risk of infection is increased after nasal colonization (5), when a disruption in the skin or mucosal membrane occurs. The factors and mechanisms involved are, however, poorly understood. Host factors, like trauma, implants, and prosthesis, and systemic factors, such as diseases, for example, diabetes and cystic fibrosis or immunosuppression (cytostatic drugs, HIV), constitute risks for infection (6–9). In general, *S. aureus* infect by self-inoculation or by external contact. Genotypic variants, for example, Panton-Valentine leukocidin (PVL)-positive subtypes, are unevenly spread between individuals and among various populations of the world (10, 11). It is tempting to argue that infectious *S. aureus* strains have a different virulence arsenal than those colonizing healthy individuals.

Little is known about oral *S. aureus* in health and disease (for review see 12, 13). Oral *S. aureus* commonly occurs in local mucosal surface infections, such as angular cheilitis, denture stomatitis, and mucositis, or deep infections, such as osteomyelitis and parotitis. Especially troublesome for diagnosis and treatment are the classical opportunistic mucosal infections in oropharynx of systemically immune-compromised patients or patients with systemic diseases (9, 12, 13). In a study on oral mucosal infections and complaints, opportunistic microorganisms such as *Candida* spp., *S. aureus, Enterococcus faecalis*, enteric rods and *Pseudomonas* spp. were found in nearly 50% of the cases (14). *S. aureus* was present in high numbers in association with an infection in 12.5% of these cases. The amount (numbers) of opportunistic microorganisms found is seldom reported in the literature. A distinction between the occurrence of *S. aureus* in low numbers representing a carrier state and high numbers, indicating breakdown of the microbial homeostasis, is therefore difficult to make. When symptoms occur concomitantly with a high number of the opportunists, the term 'infection' is justified. Healthy carriers with *S. aureus* in skin and nostrils are reported to amount up to 50% of the population in some countries (1). An oral carriage rate of up to 48% among students is reported (12). In a recent study, *S. aureus* was found in high numbers in saliva (>1,000 CFU/ml) in 14% of highly-active antiretroviral therapy (HAART)-treated HIV-positive patients without any oral complaints. It was suggested that these immune-compromised patients could be at risk for oral infections if their medical conditions were worsened (15). Oral infections associated with *S. aureus* may be due to an imbalance of the host–parasite relationship favoring the growth of these opportunistic bacteria or could be due to the presence of certain subtypes of *S. aureus*.

In the current study, phenotypic and genotypic characteristics and antibiotic susceptibility were examined in a collection of Swedish *S. aureus* strains isolated from patients with oral infections and compared with oral strains isolated from non-infected controls. A Thai collection of *S. aureus* strains isolated from HIV-positive patients representing an immune-compromised population was similarly compared with strains from healthy non-HIV controls.

Material and methods

Bacterial strains

S. aureus isolates from Swedish patients

This group comprised 62 strains isolated during 3 years from all 1,050 oral microbiological samples, which were analyzed consecutively in the Department of Oral Microbiology, Institute of Odontology, Sahlgrenska Academy at Gothenburg University, Sweden (Table 1). Dentists in clinics in the western region of Sweden collected the samples. The majority came from clinics in, or close to, hospitals. Twenty-nine strains were isolated from oral mucosal infections, 18 from angular cheilitis, 10 from oral deep infections (abscess, bone infection, osteomyelitis), three from peri-implantitis, and two of unknown reason. Due to the character of the incoming samples, detailed information on the general medical conditions or oral status of all patients were not possible to obtain, but 27 of the 62 patients could be classified as medically compromised or multidiseased. The samples were taken as

Table 1. Number and amounts of *S. aureus* isolates in the four subject groups examined

Subjects	No. of sampled subjects	Sampling method	No. of strains identified as *S. aureus* (prevalence%)	No. of samples (%) with moderate growth or more
Swedish patients with oral infections	1,050	Scraping from infection site	62 (5.9)[a]	59 (95.2)
Swedish healthy controls	56	Saliva	25 (44.6)	0 (0)
Thai HIV-positive patients	221	Tongue scraping	45 (22.2)	35 (77.8)
Thai non-HIV controls	153[b]	Tongue scraping	25 (16.3)	12 (48.0)

[a]Twenty-nine strains were from oral mucosal lesions, 18 from angular cheilitis, 10 from deep oral infections, 3 from gingivitis/peri-implantitis patients, and 2 from unknown location.

[b]Seventy-four were dental staff from which seven *S. aureus* strains were isolated (prevalence 9.4%) and 79 were non-HIV patients from whom 18 strains were isolated (prevalence 22.8%).

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scrapings from the infection sites with an amalgam carver and transferred to transport medium viability medium gothenburg anaerobic III (VMGAIII) (16).

S. aureus isolates from Swedish healthy controls

Twenty-five strains were isolated from saliva samples of 56 Swedish dental students with healthy oral conditions. The samples were transferred after collection directly into transport medium VMGAIIS (16). The students were informed about the study and participated voluntarily after giving their informed consent.

S. aureus isolates from Thai HIV subjects

Forty-five *S. aureus* strains were isolated from HIV-positive patients attending the Clinic of Infectious Diseases at the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (Table 1). In all 221 patients (including 148 undergoing HAART therapy, 53 vertically transmitted and undergoing HAART, and 20 HIV non-HAART subjects) were consecutively recruited during 2011–2012 for a microbiological investigation of oral mucosal samples (15). Sampling was performed by scraping the tongue and in some cases the gingiva. The samples were transferred to transport-medium VMGAIII (16).

S. aureus isolates from Thai non-HIV controls

Twenty-five strains were isolated by tongue scrapings from 153 non-HIV subjects among 74 staff members and 79 patients at the Clinic of Oral medicine at Chulalongkorn University (Table 1) and who were at a similar age as the HIV HAART group. The Ethics Committee at the Chulalongkorn University approved the study protocols and all the participants signed an informed consent agreement. The samples were transferred to transport-medium VMGAIII (16).

Microbiological processing

The samples reached the laboratory within 24 hours. The sample bottles were warmed to 37°C and shaken with a whirly mixer for 20 seconds. A volume of 0.1 ml of the medium was placed and stroked in a standardized fashion on media that are used for all incoming samples for routine diagnostic purpose as previously reported (14). In the present study we focused on isolation of *S. aureus* and their prevalence in relation to the total number of the facultative oral microbiota (alpha-hemolytic streptococci). For that purpose the following media were used: blood agar (4% Blood Agar Base No. 2 CM 271, Oxoid, Basingstoke, UK) with 5% defibrinated horse blood and mannitol-agar (Difco Staphylococcus Medium 110, Becton, Dickinson and Company, Sparks, MD) incubated in air with 10% CO_2 at 37°C for 2–3 days. The plates were examined for typical colony morphology and special attention was paid to colonies with the appearance of staphylococci and alpha-hemolytic streptococci, which were semi-quantified according to a scale previously published (14). Very sparse growth was used for colonies <10, sparse growth for 10–100, moderate growth for 100–1,000, heavy growth for 1,000–10,000, and very heavy growth for $>10{,}000$ colonies. Moderate growth or more indicated a colonized state of *S. aureus* in the oral cavity of the subjects and less numbers indicated a non-established (occasional) presence. Only one strain from each subject was isolated.

Species identification and phenotypic characterization

Suspected staphylococcal colonies were selected from the blood-agar plate, pure cultured, and stored on beads. All isolates were checked for growth on mannitol-salt agar and differentiation between *S. aureus* and other staphylococci was done by the DNase test (DNase test agar, BD Diagnostics). *S. aureus* identity was further confirmed by the coagulase test (17). All strains were tested for hemolysis, arginine, urease, and nitrate activity. Hemolysin activity was recorded after growth on the blood agar plate as a clear halo around each colony. Arginine, urease, and nitrite activity were examined using routine laboratory tests (17).

Genotypic characterization

All strains were tested for genotype variation using primers and conditions in the polymerase chain reaction assays as described in detail by Campell et al. (11). The following virulence genes were identified: *clf A* (clumping factor A, adhesin), *cna* (collagen binding antigen gene, adhesin), *map/eap* (adhesin), *sdrC*, *sdrD*, *sdrE* (serine-aspartate repeat proteins for adhesion), *eta* and *etb* (exfoliative toxins), *sea* and *seb* (staphylococcal enterotoxins), *tst* (toxin), *hlg* (toxin), and PVL.

Antibiotic susceptibility

Minimal inhibitory concentration (MIC) determinations were performed using the E-test method (bioMerieux, Marcy-l × Etoile, France) against penicillin V, oxacillin, amoxicillin, cefoxitin, clindamycin, vancomycin, and fusidic acid. After incubation, the MICs were read from the intercept where the ellipse inhibition zone intersected with the scale. The MICs including 90 and 50% of the strains were calculated. Strains that showed resistance for oxacillin and cefoxitin were tested for the *mecA* gene with primers and condition as described by Siripornmongcolchai et al. (18) to confirm the identity of MRSA strains.

Statistical calculation

Pearson's Chi square test was used for testing differences between isolates from infected and non-infected carriers. *P*-value below 0.05 was considered significant.

Results

S. aureus *isolates and phenotypic characteristics*

The frequency of *S. aureus* isolates recovered from the four subject groups are shown in Table 1. Isolates from

oral infections occurred as could be expected as heavy growth and were predominant in comparison with alpha-hemolytic streptococci. None of the students had more than 100 CFU of *S. aureus* per ml saliva. Thirty-five (77.8%) of the 45 HIV-infected Thai subjects showed *S. aureus* as moderate growth or more and it occurred as sparse in the other subjects. Twelve (48.0%) of the 25 *S. aureus*-positive non-HIV subjects yielded *S. aureus* as moderate and heavy growth (>1,000 CFU/ml saliva).

All strains were coagulase and nitrate positive, 91.1–100% were arginine positive, and 82.2–96.0% were urease positive. Only three Swedish strains (4.8%) isolated from infections were hemolytic compared to nine (36.0%) from the healthy individuals. There were no difference in the phenotypic characteristics of the 10 *S. aureus* strains isolated from oral deep infections compared to those isolated from mucosal infections. Among the Thai isolates 74.3% were hemolytic, which was significantly ($p < 0.001$) more than among the Swedish isolates (13.8%). The frequency of hemolytic strains from the HIV patients (73.3%) did not differ significantly from that of the non-HIV controls (76.0%).

Genotypic variation of S. aureus isolates

Table 2 shows the genotypic variation between *S. aureus* strains from Swedish and Thai patients compared with controls. A generally high rate of genes for adhesins and toxins were found in the Swedish strains from both infections and healthy controls as well as in strains from the Thai HIV-positive patients and non-HIV controls. The exception was *etb*, which was not detected in any isolate, and *seb* that was only sporadically found. A high rate of the PVL gene (73.3–100%) among all four subject groups was noted and the 100% rate among isolates from non-HIV controls was significantly ($p < 0.05$) higher than the isolates from the HIV patients (73.3%). A significant difference of genotypes between the infected Swedes and the controls was found for *eta* only, with 72.0% noted for the control strains and 43.5% among strains from infections. Among Thai strains, the genes for toxins (*sea, sec, tst*) and for adhesins (*cna* and *map/eap*) were significantly ($p < 0.05$) more often found in isolates from non-HIV compared to those from HIV patients. Only the gene *seb* was more often found in isolates from the HIV patients ($p < 0.05$).

Plasmids were detected in a similar rate (40.0–48.4%) in the isolates from the four groups. Commonly, 1–2 plasmids/strain and sometimes even more were found. Two strains harbored six different plasmids.

Antibiotypes

The antibiotic susceptibility (MIC_{90}) of strains from infected Swedish and from the Thai HIV patients was generally higher than in strains from the controls (Table 3). Antibiotic resistance was most frequently (>10%) found for clindamycin (MIC > 256 μg/ml) in strains from Swedish infections (seven strains) and strains from Thai HIV-positive subjects (six strains). In the latter group, resistance was sporadically (1–2 strains) found for oxacillin and for amoxicillin, while three MRSA strains

Table 2. The distribution of plasmids and virulence genes encoding for toxins and adhesins among *S. aureus* isolates from Swedish and Thai patients and healthy controls

Virulence type	Genotype	Isolates from Swedish oral infections $n = 62$ (%)	Isolates from Swedish healthy controls $n = 25$ (%)	Isolates from Thai HIV patients $n = 45$ (%)	Isolates from Thai non-HIV controls $n = 25$ (%)
Toxins	*sea*	25 (40.3)	9 (36.0)	15 (33.3)	15 (60.0)[a]
	seb	4 (6.5)	3 (12.0)	8 (17.8)	0 (0)[a]
	sec	35 (56.5)	11 (44.0)	6 (13.3)	15 (60.0)[a]
	tst	45 (72.6)	15 (60.0)	0 (0)	8 (32.0)[a]
	eta	27 (43.5)	18 (72.0)[a]	17 (37.8)	14 (56.0)
	etb	0 (0)	0 (0)	0 (0)	0 (0)
	PVL	50 (80.6)	22 (88.0)	33 (73.3)	25 (100)[a]
Adhesins	*hlg*	61 (98.4)	24 (96.0)	44 (97.8)	24 (96.0)
	clfA	60 (96.8)	24 (96.0)	39 (86.7)	24 (96.0)
	cna	54 (87.1)	17 (68.0)	19 (42.2)	21 (84.0)[a]
	sdrC	45 (72.6)	16 (64.0)	32 (71.1)	16 (64.0)
	sdrD	53 (85.5)	25 (100)	34 (75.6)	23 (42.0)
	sdrE	48 (77.4)	19 (76.0)	26 (57.8)	20 (80.0)
	map/eap	41 (66.1)	11 (44.0)	25 (55.6)	20 (80.0)[a]
Plasmids		30 (48.4.)	10 (40.0)	21 (46.7)	12 (48.0)

[a]Denotes the statistical difference ($p < 0.05$) between Swedish oral infections versus healthy controls or between Thai HIV patients versus non-HIV controls.

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Table 3. Antibiotic susceptibility (MIC determination using E-test, μg/ml) for 157 Swedish and Thai *S. aureus* strains isolated from the oral cavity

	Swedish strains				Thai strains			
	Oral infections ($n=62$)		Healthy controls ($n=25$)		HIV patients ($n=45$)		Non-HIV controls ($n=25$)	
Antibiotics	MIC90	MIC 50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50
Penicillin V	32	2	16	1	24	4	4	4
Oxacillin	4	0.25	0.5	0.25	0.75[a]	0.32	0.32	0.32
Ampicillin	12	2	8	1	16[a]	2	2	2
Clindamycin	256	0.064	0.064	0.64	256	0.94	0.64	0.64
Vancomycin	2	2	3	3	3	2	3	2
Fusidic acid	16	0.75	1	0.75	1.5	1	1	1
Cefoxitin	3[b]	2	3	2	3[b]	2	2	2

[a]One strain had MIC > 256 μg/ml for oxacillin; one strain had MIC > 256 μg/ml and one has MIC > 128 μg/ml for ampicillin.
[b]Including two MRSA strains from Swedish oral infections, and one from a Thai HIV patient.

(resistant against cefoxitin MIC > 256 μg/ml and presence of the *mecA* gene) were identified from two Swedish oral infections and one Thai HIV-positive patient.

Discussion

This study failed to show that *S. aureus* strains isolated from oral infections and non-infected controls represent different subgroups with respect to phenotypic and genotypic characteristics. The oral infections with *S. aureus* is suggested to be a result of a compromised condition either generally due to systemic diseases and medication or locally by disruption of the epithelial lining (angular cheilitis) or mucosal membrane (mucositis, ulcers, trauma, dental constructions such as dentures and implants). It is therefore suggested that oral infections with *S. aureus* are mainly classical opportunistic infections that develop due to an imbalance within the host–parasite relation. Treatment of such infections using antibiotics has a limited effect as long as the compromised condition prevails. Consequently, it is likely that the high number of *S. aureus* in the oral cavity of the HIV patients was associated with a change in the microbial homeostasis due to the immune-compromised condition or frequent antibiotic treatments. Although it has been reported that oral opportunistic infections have declined after introduction of HAART (19), *S. aureus* was common in the oral cavity and often at high numbers in Thai HIV-HAART patients. Still, the patients did not complain about mucosal lesions and may not be considered 'infected' but persistent carriers, at risk for oral infections if the HIV conditions should worsen.

The commensal asymptomatic character of *S. aureus* is illustrated by the high rate of healthy carriers in different populations and age groups (20). In this referred study (20), including a number of European countries, the persistent carrier rate among Swedish patients (nasal swabs) was found to be higher (29.4%) than in most other countries (mean 21.6%), but of the same magnitude (29.7%) as reported for Thai medical students (21). The most common location for *S. aureus* carriage is the nostrils, where the environment seems to be ideal for colonization and the competition with other bacteria is low. The carrier rate in the oral cavity is not consistent and a range from 17 to 48% within a student population has been reported (22). Our finding of 44.6% carriers among Swedish dental students is well in-line with these figures. It should be noted that the bacteria were found in low numbers, while the predominating streptococci usually reached 10^6 CFU/ml saliva. The prevalence of *S. aureus* in saliva among Swedish dental students seems to be higher compared to that of tongue scrapings from the Thai non-HIV controls (16.3%), but this difference may be due to different sampling methods. The significantly more frequent presence of a hemolytic phenotype among the Thai HIV patients and controls indicate that they often carry a more virulent phenotype than the Swedish patients or dental students (1). The fact that *S. aureus* is frequently present in the oral cavity of patients, students and staff both in Sweden and Thailand shows that it should be of increasing concern in cross-infection control in the dental clinic (23). However, we think that the risk for cross-infection between the subjects included in the present study is low. The students were first semester students and had been together for only a few months and had not yet been exposed to patients.

We were not able to find any clear difference in virulence genes between the isolates from infected and from non-infected carriers. Passariello et al. (24) found a higher prevalence of exotoxin encoding genes (*seb, tst, eta, etb*) but not of those encoding for adhesion, in isolates from

periodontitis patients compared with isolates from healthy individuals. It was claimed that the gingival conditions in the patients select for strains possessing a significantly wider armamentarium of virulence. We were not able to see any difference in virulence genes between the strains isolated from deep infections versus those from mucosal lesions. Therefore, our data do not support the suggestion by Passariello et al. (24) of more toxinogenic strains in the absence of adhesins in deep infection sites. It may be more likely that the environment regulates the expression of genes than that *S. aureus* strains with different virulence genotype pattern are present in infection and in health, respectively. Not even the leukotoxic gene (PVL) was found to be related to the type of host from which it was isolated. PVL-positive strains have been associated with severe *S. aureus* infections, whereas less PVL-positive strains were found in healthy carriers (25). Our findings suggest that the oral strains, whether they come from infections or healthy sites, are community acquired and of similar virulence genotypes.

Antibiotic resistance has been a major concern for *S. aureus* and especially for MRSA. The increase of community-acquired MRSA (CA-MRSA) has gained special attention (26, 27). An increasing prevalence of MRSA in healthy carriers amounting up to 26% in the nasopharynx of a population is reported (28). The carriage rate of MRSA among dental students was found to be 21% in one US study (29), while lower frequencies were reported for dentists and dental hygienists (4.2 and 1.5% respectively) in another (23). The prevalence of MRSA in the oral cavity is less known although MRSA has been found in oral swab or rinse specimens in some reports (22). No MRSA were detected among our controls. It is known that the prevalence of MRSA among health care workers in Sweden as well as in Thailand is higher than in the general population (20, 30, 31). The prevalence of CA-MRSA is increasing and outbreaks of MRSA infections happen intermittently (32). The HIV-positives have been considered to be a risk group for an increasing prevalence of MRSA and opportunistic infection if the HIV status should worsen (33). Only two of the 62 *S. aureus* isolates from oral infections investigated in this study and only one isolate from a HIV-positive Thai subject were found to be MRSA. Although the MRSA frequency seems to be very low in the oral cavity of patients in the dental clinic, the frequency may increase in the future and dentists should be aware that multiresistant bacteria might appear among dental patients, both in oral infections and in healthy carriers.

An important finding in this study was the significantly higher rate of clindamycin resistant strains isolated from oral infections and from the tongue of HIV-positive subjects compared to the control isolates (34). This is of deep concern since clindamycin is widely used in dentistry and many clinics have substituted common penicillins (oxacillin and methicillin) with clindamycin in the treatment of deep and of mucosal oral infections (35). Clindamycin is prescribed in case of allergy to beta-lactams but also generally due to the opinion that clindamycin reaches a higher concentration and efficiency in bone infections. Notably, seven of the 11 clindamycin resistant strains in this study came from deep infections and from patients under antibiotic treatment. Unfortunately, we had no information on the antibiotics used for this group of patients.

This study shows that *S. aureus* occur in high numbers in oral mucosal deep infections and should be considered as a source of systemic infection such as septicemia. This study was based on clinical samples coming to our laboratory for microbiological analyses and we have limited knowledge about the infections, the treatment strategy, and outcome of the infections. Dentists, in general, do not have a tradition of taking microbiological samples for diagnosis and for antibiotic susceptibility testing (22, 36). The clinical relevance and conclusion of this study is that the clinicians (oral surgeons, doctors in oral medicine, and dentists in general) should be more aware of the increasing problem with opportunistic *S. aureus* infections. They should consider the risk of spread of these microorganisms in the dental setting, and microbiological samples should be taken in all suspected opportunistic infections for diagnosis and for evaluation of antibiotic susceptibility if antibiotics are to be prescribed.

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Conflict of interest and funding

There is no conflict of interest in the present study for any of the authors.

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