

Methicillin-resistant *Staphylococcus aureus* from dental school clinic surfaces and students

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from frequently touched dental school clinic surfaces were compared with MRSA isolated nasal cultures of dental students.

Method: Sixty-one dental students and 95 environmental surfaces from 7 clinics were sampled using SANICULT (Starplex Scientific Inc, Etobicoke, Ontario, Canada) swabs. Antimicrobial susceptibility testing was performed, and pulsed-field gel electrophoresis analysis, the *mecA* gene, multilocus sequence type, and *SCCmec* type were determined by polymerase chain reaction and sequencing.

Results: Thirteen (21%) dental students and 8 (8.4%) surfaces were MRSA positive. Three MRSA strains were *SCCmec* type IV, whereas 3 were nontypeable isolates and Pantone-Valentine leukocidin positive (PVL+), and none were USA300. One surface and 1 student isolate shared the same multilocus sequence type ST 8 and were 75% related. Two groups of students carried the same MRSA strains.

Conclusion: The MRSA-positive samples were from 4 of 7 dental clinics. In addition, 21% of the dental students carried MRSA, which is > 10 times higher than the general public and twice as frequent as in other university students. This is the first study to characterize MRSA from dental clinic surfaces and dental students and suggests that both may be reservoirs for MRSA. Further studies are needed to verify this premise.

Key Words: MRSA; environmental; dental clinic surfaces; dental students.

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Staphylococcus aureus is a normal inhabitant of the nose, throat, and oral cavity. Methicillin-resistant *S aureus* (MRSA) infections have become a significant cause of morbidity and mortality in the hospital and community settings.¹ MRSA in health care settings are normally resistant to multiple classes of antibiotics,

whereas community-acquired MRSA isolates are susceptible to erythromycin, azithromycin, and tetracyclines, although the distinctions have been changing.¹ Studies have shown that rates of staphylococcal infection are higher in *S aureus* carriers than noncarriers, and data support the hypothesis that nasal carriage is a risk factor for development of staphylococcal disease.^{2,3}

Community-acquired MRSA infections are on the rise, with many patients previously healthy individuals with no health care exposure or apparent risk factors.^{1,4} A recently published surveillance study of ~10,000 noninstitutionalized United States residents determined that nasal colonization of MRSA had increased from 0.8% (2001-2002) to 1.5% (2003-2004).⁵ Another study found MRSA carriage of 7.4% among university students, whereas a third study found > 9% MRSA carriage among children attending clinics, suggesting that rates of MRSA carriage differs by populations.^{3,6} Similarly, MRSA colonization among health care professionals has varied (0%-30%) by specialty unit, hospital, type of patients in the unit, year the surveillance was done (MRSA carriage levels appear to be rising), and where the study was done.^{4,7}

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Carriage levels of MRSA among health care students have also varied. A recent study identified 26 MRSA-positive (32.5%) nasal cultures, from 80 people associated with one US dental school. The people cultured included dental assistants, students, staff, faculty, and patients,⁸ but the isolates from this study were not characterized. Previously, MRSA has been isolated from dental plaque of elderly patients,⁹ and a variety of coagulase-negative *Staphylococcus* spp have been isolated from the air in a UK dental clinic.¹⁰ These studies suggest that dental clinic surfaces and dental personnel could be potential reservoirs for MRSA.

The current study was undertaken to determine whether MRSA could be isolated from frequently touched dental operator surfaces and clinic floors in different dental clinics within the School of Dentistry. In addition, nasal cultures from dental students that used the clinics were taken, and all MRSA isolates were characterized.

MATERIALS AND METHODS

Clinic and students sample collection

Ninety-five frequently touched dental operator surfaces and the clinic floors were sampled using the SANICULT swab (Starplex Scientific Inc, Etobicoke, Ontario, Canada) from 7 dental clinics at the University of Washington as previously described.¹¹ One set of samples from each of the 7 clinics included 5 areas: (1) the uncovered areas of dental chair seat and arm rest, (2) the floor beneath the chair, (3) the sink next to the chair, (4) the towel dispenser next to the chair, and (5) the counter next to the chair. These surfaces were chosen as those most likely to accumulate bacteria. In clinics with 2 to 12 chairs, 2 chairs were randomly chosen, and the samples taken for each chair. In the clinic with 2 dental chairs, both were cultured. For the clinics with > 45 dental chairs, 4 or 5 chairs were randomly selected for culture. All clinic surface samples were taken in 1 afternoon after patients had left the clinics but before general cleaning and stocking for the next day was done.

Anterior nasal cultures were collected from 61 dental student volunteers (15 first year [6 males, 9 females] and 46 fourth year [28 males, 18 females]) from the University of Washington Dental School using sterile culture SANICULT swab as previously described.¹¹ The samples were taken over a 4-week period. Sex of the volunteers was the only demographic collected. The subjects' rights were protected, and the project had University of Washington Institutional Review Board approval. Informed consent was granted by each dental student, and they did not receive compensation.

Sample processing

Bacto m Staphylococcus broth (1.5×) (Difco Laboratories, Sparks, MD) supplemented with a final concentration of 75 mg/L polymyxin B and 0.01% potassium tellurite (Sigma Co, St. Louis, MO) were added to each swab and incubated in 5% CO₂ at 36.5°C until turbid (24-96 hours). The MRSA-positive samples were verified and further characterized as previously described.^{11,12} Multiple isolates from a sample were characterized (Table 1).

The enriched nasal samples were plated directly onto MRSA Select Screening Agar (Bio-Rad Laboratories, Hercules, CA). Isolates were screened, and verified as MRSA as previously described.¹¹ A single MRSA isolate from each student was characterized (Table 1). All students were confidentially informed of their results, and the MRSA-positive students were advised to see their own clinicians for more information and discussion of possible decolonization.

Detection of *mecA*, Panton-Valentine leukocidin gene, SCC*mec* typing, and multilocus sequence typing

S aureus and presumptive MRSA nasal isolates were tested for the presence of the *mecA* gene by a polymerase chain reaction (PCR) assay, and the presence of the Panton-Valentine leukocidin (PVL) gene and the SCC*mec* types I to V were determined using PCR assays as previously described.^{13,14} Those isolates that were not types I to V were labeled nontypeable (NT) for the current study. Positive and negative controls were used as previously described in all assays.^{11,12}

The multilocus sequence typing (MLST) PCR assays were performed using previously published primers and conditions.¹⁵ MLST sequence types were determined by the Web site (www.mlst.net). A USA300 isolate was used as the positive control.

Pulsed-field gel electrophoresis

MRSA isolates with the same MLST types were pulsed-field gel electrophoresis (PFGE) typed.¹² The different PFGE patterns were labeled A through G (Table 1) and the genetic relatedness of the isolates with the same ST type analyzed by Dice coefficient, unweighted pair group method with arithmetic mean (UPGMA) using the GelCompar II software according to the manufactures instructions (Applied Maths, Inc, Austin, TX). Strains that were ≥ 70% similar were classified as related.¹⁶

Detection of antibiotic resistance genes

Previously described PCR assays were used to detect the presence of kanamycin resistance gene *aadD*;

Table 1. MRSA isolates from dental school clinic sites and dental students

Isolate No.	PFGE	SCCmec type	PVL	ST	tet		erm		msr(A)	aadD
					M	K	A	C		
Dental students, sex*										
1-19 Female		IV	—	ST1159	+	—	—	+	+	—
4-91 Female	A	NT†	—	ST8	+	—	—	—	—	—
4-73 Male	B	NT	—	ST30	+	—	+	—	—	—
4-105 Male	B	NT	+	ST30	+	—	—	—	—	—
4-84 Female	B‡	NT	+	ST30	+	—	—	—	—	—
4-95 Male		NT	—	ST39	+	—	—	—	+	—
4-50 Male		NT	—	ST45	+	—	—	+	—	—
4-63 Female		NT	—	ST101	+	—	—	+	+	—
4-71 Male		NT	—	ST109	+	—	+	—	—	—
4-94 Male	C	NT	—	ST256	+	—	—	—	—	—
4-96 Female	C	NT	—	ST256	+	—	—	—	+	—
4-64 Female		NT	—	ST1474§	+	—	—	—	—	—
4-90 Female		NT	—	ST1475§	+	+	—	+	—	+
Dental clinic surfaces location										
317-81 Chair	A	NT	+	ST8	+	—	—	—	—	—
O5-22-1 floor	E	NT	—	ST97	+	—	—	—	—	—
O5-22-2 floor	E	NT	—	ST97	+	—	—	—	—	—
O5-22-5 floor	E	NT	—	ST97	+	—	—	—	—	—
D2-6-1 chair	F	IV	—	ST1461§	—	+	—	—	+	+
D2-6-2 chair	F	IV	—	ST1461§	—	+	—	—	+	+
D2-6E chair	G	IV	—	ST1461§	+	+	—	+	+	+
D2-6G chair	G	NT	—	ST1461§	+	—	—	—	—	—

NOTE. PVL+ = carried the PVL gene; PVL- = no gene; ST = MLST type.

MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; PVL, Pantan-Valentine leukocidin.

*1-19 is from a first-year student; the others are fourth-year students.

[†]NT = not SCCmec types I-V.[‡]Greater than 70% related to isolates 4-73 and 4-105, which are 100% related.[§]Represent new ST types.^{||}Environmental 317-81 was 75% related by PFGE to student 4-91 isolate.

macrolide resistance genes *erm(A)*, *erm(C)*, and *msr(A)*, and tetracycline resistance genes *tet(M)* and *tet(K)*.¹² The PCR products were verified as described previously.¹² Plasmids with cloned *erm*, *msr(A)*, and *tet* genes were used as positive controls.¹² The MRSA clinical strain MS361 was used as the positive control for the *aadD* PCR assay. *Enterococcus faecalis* JH2-2 was used as a negative control.¹²

RESULTS

Detection of MRSA from dental clinic surfaces and students

Four (57%) of the 7 clinics had a positive MRSA surface sample. The MRSA positive samples included 2 (10.5%) of the 19 dental chairs and 2 (10.5%) of 19 floor samples from 2 different small clinics and both large clinics. In contrast, no sink, counter, or towel cultured surface was MRSA positive. The MRSA isolate from 1 of the 4 samples was lost on subculture. Eight MRSA isolates from 3 of the 4 clinics were further characterized (Table 1).

Of the 61 students, 13 (21%) of the nasal cultures were MRSA positive and included 1 first-year and 6 fourth-year female and 6 fourth-year male students (Table 1). Two other nasal cultures were presumptively identified as MRSA positive based on the dark red colony color on the chromogenic agar; however, biochemical tests identified these isolates as methicillin-resistant coagulase-negative *Staphylococcus* spp and not MRSA.

Genotypic characterization of clinic and student MRSA isolates

Student isolate 4-84 (ST30) and 4-105 (ST30) and surface isolate 317-81 (ST8) were PVL positive (PVL⁺), whereas the remaining MRSA isolates were PVL negative (PVL⁻). One student isolate, 1-19 ST1159, and 3 surface isolates, D2-6-1 (ST1461), D2-6-1 (ST1461), and D2-6-1 (ST1461), were SCCmec type IV. The remaining isolates were not SCCmec types I to V [NT]. None of the isolates were USA300. All MRSA isolates were multidrug resistant. Seventeen carried the *tet(M)* gene (12 students, 5 surfaces), 2 carried both the

tet(M) and *tet*(K) genes (1 student, 1 surface), and 2 surface isolates carried the *tet*(K) gene. Eleven MRSA isolates carried 1 ($n = 8$) or 2 ($n = 3$) macrolide resistance gene(s), with the *msr*(A) gene the most common (2 students, 2 surfaces), followed by *erm*(C) plus *msr*(A) genes (2 students, 1 surface) and then *erm*(A) gene (2 students) and *erm*(C) gene (2 students) (Table 1). The *aadD* gene was found in a single student and 3 surface MRSA isolates (Table 1).

One student 4-91 and 1 surface isolate 317-81 were ST8 type and, by PFGE patterns, were $> 75\%$ related to each other. Two student isolates 4-94 and 4-96 were ST256 and by PFGE 100% related. Two student isolates 4-73 and 4-105 had the same ST30 and were $> 95\%$ related, whereas a third student isolate 4-84 had the same ST30 and was 70% related to these isolates. Three isolates from clinic O5-22 were ST97 *SCCmec* type NT and had indistinguishable PFGE patterns (100% related), suggesting they represented a single MRSA strain (Table 1). The remaining 4 isolates from dental clinic D2 all had a novel ST1461 type—3 were *SCCmec* type IV and 1 NT—but represented 2 distinct strains (Table 1). The remaining 7 students' MRSA isolates had 7 different ST types, including 2 new ST types, ST1474 and ST1475, and by PFGE patterns represented distinct strains (Table 1).

DISCUSSION

This is the first isolation and characterization of MRSA strains from dental clinic surfaces not associated with hospital setting, although a 1995 study found methicillin-resistant, coagulase-negative *Staphylococcus* spp from some of the same dental clinic surfaces as the current study.¹⁷ A more recent study indicates that dental operatories within the hospital may also become contaminated and be a potential reservoir for a nosocomial multidrug-resistant MRSA outbreak in hospitalized patients.¹⁸

In the current study, 1 clinic had *SCCmec* type IV isolates with novel ST types, and a second clinic had type NT ST97, which is commonly found in animals. The third clinic had type NT PVL⁺ ST8, which is most commonly associated with human isolates. This isolate shared $> 75\%$ homology with the student isolated 4-91 MRSA isolate, suggesting that there may have been a common source for the 2 isolates.^{16,17,19} All surface MRSA isolates were resistant to multiple classes of antibiotics suggestive of hospital-like strains. More recently, we cultured the same high-touch surfaces in a large community/migrant dental health center in the Pacific Northwest and found that 2 (12.5%) of the 16 samples were MRSA positive. These isolates did not carry *SCCmec* types I to V, suggesting that the results from the dental school study were not unique.

Thirteen dental students—1 (6.6%) of the 15 first-year and 12 (26%) of the 46 fourth-year dental students—were MRSA positive. The difference in student groups could be due to the small number of first-year students sampled as compared with fourth-year students, or it could suggest that, as dental students have more exposure to patients, they are more likely to become MRSA carriers. The dental student MRSA carriage rate was > 10 times higher than the MRSA carriage rate found in the general US population⁵ and higher than a 2004 study that found MRSA carriage in 1% to 5% of the medical fellows, medical students, and the nursing students.²⁰ In contrast, the 21% carriage rate in dental students from the current study was lower than the 32% carriage rate found in 22 dental students from a Buffalo study.⁸ The student MRSA isolates were multidrug resistant, suggestive of hospital-like MRSA, and 1 isolate was *SCCmec* type IV. Two student isolates, 4-94 and 4-96, were 100% related, whereas student isolates 4-73 and 4-105 were $> 95\%$ related to each other. This suggests there may have been common sources for these isolates, but we do not know the potential sources of the students' MRSA strains.

Two other students' nasal cultures had colonies consistent with presumptive MRSA on the chromogenic agar but were biochemically confirmed as methicillin-resistant, coagulase-negative *Staphylococcus* spp and not MRSA or *S aureus*. False MRSA positive nasal cultures as well as environmental cultures using chromogenic media have been previously been identified suggesting that presumptive MRSA-positive isolates from chromogenic media must be confirmed as *S aureus* and MRSA.^{21,22}

This study suggests that MRSA contaminated dental surfaces and MRSA colonized dental students may be reservoirs for transmission to other students, personnel, patients, and/or clinic surfaces, although the level of risk is unknown.^{20,23} These results are unlikely to be unique to the Pacific Northwest, and further studies are needed from more dental clinic surfaces, dental patients, dental personnel, and students from diverse geographic locations.

References

1. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007;298:1763-71.
2. Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of *Staphylococcus aureus*? *Trends Microbiol* 2001;9:605-10.
3. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;5:751-62.
4. Eady EA, Cove JH. Staphylococcal resistance revisited: community-acquired methicillin resistant *Staphylococcus aureus*—an emerging problem for the management of skin and soft tissue infections. *Curr Opin Infect Dis* 2003;16:103-24.

5. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, et al. Changes in *Staphylococcus aureus* nasal colonization in the United States, 2001-2004. *J Infect Dis* 2008;197:1226-34.
6. Rohde RE, Denham R, Brannon A. Methicillin-resistant *Staphylococcus aureus*: carriage rates and characterization of students in a Texas university. *Clin Lab Sci* 2009;22:176-84.
7. Eveillard M, Martin Y, Hidri N, Boussougant Y, Joly-Guillou ML. Carriage of methicillin-resistant *Staphylococcus aureus* among hospital employees: prevalence, duration, and transmission to households. *Infect Control Hosp Epidemiol* 2004;25:114-20.
8. Haraszthy VI, Gerber DS, Clark B, Shah C, Zambon JJ. Methicillin-resistant *Staphylococcus aureus* in a dental school population. Abstract No. 3423. Abstracts of the Eighty-seventh International Association for Dental Research, April 1-4, 2009, Miami, FL.
9. Tada A, Senpuku H, Motozawa Y, Yoshihara A, Hanada N, Tanzawa H. Association between commensal bacteria and opportunistic pathogens in the dental plaque of elderly individuals. *Clin Microbiol Infect* 2006;12:776-81.
10. Decraene V, Ready D, Pratten J, Wilson M. Air-borne microbial contamination of surfaces in a UK dental clinic. *J Gen Appl Microbiol* 2008;54:195-203.
11. Roberts MC, Soge OO, No DB, Beck NK, Meschke JS. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from fire stations in two northwest fire districts. *Am J Infect Control* 2011;39:382-9.
12. Soge OO, Meschke JS, No DB, Roberts MC. Characterization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp isolated from US West Coast public marine beaches. *J Antimicrob Chemother* 2009;64:1148-55.
13. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;29:1128-32.
14. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005;43:5026-33.
15. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;38:1008-15.
16. Cespedes C, Miller M, Quagliarello B, Vavagiakis P, Klein RS, Lowy FD. Differences between *Staphylococcus aureus* isolates from medical and nonmedical hospital personnel. *J Clin Microbiol* 2002;40:2594-7.
17. Horiba N, Yoshida T, Suzuki K, Maekawa Y, Ito M, Matsumoto T, et al. Isolation of methicillin-resistant *Staphylococci* in the dental operatory. *J Endo* 1995;21:21-5.
18. Kurita H, Kurashina K, Honda T. Nosocomial transmission of methicillin-resistant *Staphylococcus aureus* via the surfaces of the dental operatory. *Br Dent J* 2006;201:297-300.
19. Sung JM-L, Lloyd DH, Lindsay JA. *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. *Microbiology* 2008;154:1949-59.
20. Datta R, Huang SS. Risk of infection and death due to methicillin-resistant *Staphylococcus aureus* in long-term carriers. *Clin Infect Dis* 2008;47:176-81.
21. Paule SM, Mehta M, Hacek DM, Gonzales TM, Robicsek A, Peterson LR. Chromogenic media vs real-time PCR for nasal surveillance of methicillin-resistant *Staphylococcus aureus*. *Am J Clin Pathol* 2009;131:532-9.
22. Roberts MC, Meschke JS, Soge OO, Reynolds KA. Comment on MRSA studies in high school wrestling and athletic training facilities. *J Environ Health* 2010;72:48-9.
23. Safdar N, Bradley EA. The risk of infection after nasal colonization with *Staphylococcus aureus*. *Am J Med* 2008;121:310-5.