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Surveillance of Methicillin-Resistant Staphylococcus aureus in a Periodontal Clinic

by

Yen-Tseng Lai

THESIS

Submitted in partial satisfaction of the requirements for the degree of

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ABSTRACT

Surveillance of Methicillin-Resistant *Staphylococcus aureus* in a Periodontal Clinic

Yen-Tseng Lai, DMD

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a staphylococcal bacterium that is resistant to β-lactam antibiotics. Today, MRSA accounts for 50-70% of the nosocomial *S. aureus* infections acquired in healthcare facilities. MRSA contaminated surfaces and MRSA colonized human carriers can act as reservoirs for transmission, and dental clinics may serve as a source of disease transmission in the community.

Objective: Survey an 18 chair university-based periodontal clinic for MRSA contamination among hard environmental surfaces, dental unit water lines (DUWLs), aerosols, and human carriers.

Methods: (1) Hard environmental surface contamination. 18 air-water syringes (AWS) from the assistant's rack were swabbed, incubated for 24 hours, and streaked onto a selective chromogenic medium that detects MRSA (CMRSAII). (2) DUWL contamination. The Millipore heterotrophic plate count sampler was used to sample water from the 18 DUWLs. Following a 48-hour incubation, grown colonies were inoculated on the CMRSAII plates. (3) Dental procedure aerosol contamination. The Millipore M Air T Tester was used to collect 1000L of aerosol during ultrasonic scaling procedures or surgical procedures using highspeed handpieces. After a 24-hour incubation, colonies morphologically consistent with *S. aureus* were picked, sub-cultured, and swabbed onto CMRSAII plates. (4) Human carriers. 30 healthy volunteers were recruited among the periodontal residents,

staff, and faculty (IRB#12-08736). Specimens were obtained from the anterior nares, incubated overnight, then triple streaked onto CMRSAII plates.

Results: (1) The AWS swabs yielded no MRSA colonies at 24 or 48 hours. (2) No MRSA were detected in the DUWLs. (3) 21 aerosol samples were taken during periodontal procedures using ultrasonics or dental handpieces. No MRSA were detected. (4) 30 samples from the anterior nares were collected and no MRSA were detected. Four individuals had Methicillin-resistant coagulase-negative *staphylococci*.

Conclusion: This study did not detect MRSA in the university-based periodontal clinic on environmental surfaces, DUWLs, aerosols, or human carriers. The literature so far, in conjunction with the data from this study, collectively suggests that the occupational risk of MRSA infection in a dental setting is probably minimal.

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1. INTRODUCTION

1.1 Methicillin-resistant *Staphylococcus aureus*

Staphylococcus aureus is one of the most devastating human pathogens, and is a leading cause of human bacterial infections worldwide. *S. aureus* was found to be the most prevalent cause of bloodstream infection, skin and soft tissue infection, and pneumonia in almost all geographic areas[1]. However, it is also frequently part of the normal human skin flora. About 30% of non-institutionalized individuals are colonized with *S. aureus* in the nares asymptomatically[2].

S. aureus is a facultative anaerobic, Gram-positive coccal bacterium that grows in "grapelike" clusters. It is primarily coagulase-positive (meaning it can produce the enzyme coagulase) that causes clot formation, whereas most other *Staphylococcus* species are coagulase-negative. *S. aureus* often produce a yellow carotenoid pigment, which has led to them being referred to as the "golden staph"[3]. Typically, nosocomial bacteremias are associated coagulase-negative staphylococci.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of staphylococcal bacteria that is resistant to treatment with β-lactam antibiotics, including methicillin, oxacillin, penicillin, and amoxicillin. Many strains are also resistant to erythromycin, clindamycin, gentamicin, tobramycin, and ciprofloxacin. MRSA was first described in England in the 1960s[4], soon after methicillin became available for clinical use. In the late 1970s, MRSA emerged as a pathogen causing healthcare-

associated infections in the US hospitals. Today, MRSA accounts for as many as 50-70% of the nosocomial *S. aureus* infections acquired in healthcare facilities. MRSA infections are of particular concern because treatment may require expensive antibiotics (usually given intravenously) and prolonged hospitalization, or may be associated with other complications and drug side effects, all of which contribute to the high cost of treating MRSA infections.

β-lactam antibiotics produce a bactericidal effect by binding to a penicillin-binding protein (PBP) found on *S. aureus* cell wall and inhibiting vital stages in the biosynthesis of the cell wall[3]. MRSA's resistance to β-lactam antibiotics results from the recombinase-mediated insertion of Staphylococcal Cassette Chromosome *mec* (SCC*mec*), the mobile genetic element carrying the *mecA* gene. There are regulatory sequences that produce a low-affinity PBP not present in typical *S. aureus*. Therefore, the production of an altered PBP confers resistance to all β-lactams and monobactams antimicrobial agents. Eight major SCC*mec* types (I-VIII) are described in MRSA[5-7]. SCC*mec* elements I-III encode molecules that provide resistance to several classes of antibiotics.

Some strains of MRSA also have genes encoding Panton-Valentin leukocidin (PVL). This toxin forms heptameric pores in phagocytic leukocytes' membranes and kills them. Therefore, at high concentrations, PVL causes lytic cell death. However, at sublytic concentrations, it seems to prime neutrophils for the enhanced production of reactive oxygen species, thereby causing an exaggerated inflammatory response

and injury to the host[8]. PVL-carrying strains can cause recurrent, chronic, and severe skin and soft tissue infections as well as fatal pneumonias.

Aside from the conventional method of developing resistance via genetic mutations and selection, certain resistance factors are actually self-transmissible[9]. It was found that bacteria can acquire additional genetic material in the form of extra-chromosomal or plasmid DNA[3]. Several mechanisms exist for the genetic exchange of resistance determinants between staphylococci. The horizontal gene transfer process includes the processes of transduction, transformation, conjugation, and phage-mediated conjugation[3]. Plasmids can recombine to form new entities and lead to further evolution of MRSA strains. This horizontal gene transfer is often much faster than adaptive evolution by accumulation of mutations. Therefore, resistance often emerges rapidly following the introduction of an antibiotic. This illustrates the ability of the bacteria to readily adapt to selective pressures in their environment.

MRSA has been shown to be a strongly healthcare-associated infection control problem. The National Nosocomial Infection Surveillance survey found that the prevalence of methicillin resistance among nosocomial *S. aureus* isolates increased from 2.1% to 35% from 1991 to 1997[10]. In the United States, 2001 data indicate that 35-40% of *S. aureus* isolates from US hospitals are now methicillin-resistant[1]. In 2005, reports of invasive MRSA were investigated at 9 sites participating in the Active Bacterial Core surveillance/Emerging Infections Program Network. It was

found that the standardized incidence rate of invasive MRSA was 31.8 per 100,000 and the standardized mortality rate was 6.3 per 100,000. About 85% were healthcare-associated infections[11]. In Oct and Nov 2006, the Association for Professionals in Infection Control and Epidemiology (APIC) conducted a national MRSA prevalence study. Data shows that 46 out of every 1000 in-patients of US healthcare facilities in the study were either infected or colonized with MRSA[12]. This rate is 8-11x higher than previous estimates[13]. By 2010, the APIC found that the overall MRSA prevalence rate had increased to 66.4 per 1,000 in-patients[14].

MRSA can be healthcare-associated (HA) or community-associated (CA). Healthcare-associated MRSA can have a hospital-onset or a community-onset. HA-MRSA isolates are normally multi-drug resistant and are frequent causes of healthcare-associated bloodstream and catheter-related infections. Usually these infections occur in patients with predisposing risk factors or illnesses. On the other hand, CA-MRSA are acquired by persons who have no other established healthcare risk factors for MRSA. CA-MRSA can occur in otherwise healthy individuals[15] suggesting that these strains may have greater virulence than traditional HA-MRSA strains. CA-MRSA isolates are often only resistant to β-lactam agents and erythromycin, but they are an emerging cause of community-associated infections, especially skin and soft tissue infections and necrotizing pneumonias. In general, HA-MRSA is multidrug resistant, PVL negative, SCC*mec* I-III whereas CA-MRSA is β-lactam resistant, PVL positive, SCC*mec* IV, V[5 6]. In a population-based survey of MRSA in San Francisco, it was found that the annual incidence of CA-MRSA actually surpassed that of HA-

MRSA[16]. Globally, it has also been shown that the proportion of infections caused by CA-MRSA has increased[17 18].

It is apparent that MRSA is a major public health problem primarily related to health care but no longer confined to intensive care units or acute care hospitals. A surveillance study of 10,000 non-institutionalized Americans showed that nasal colonization of MRSA increased from 0.8% to 1.5% between 2001 and 2004[2]. It is possible that ambulatory patients seeking dental care could be carriers or have active MRSA infections. Carriers typically have *S. aureus* in their anterior nares and in their saliva. Dental students and staff have been found to be carriers as well[19 20]. The primary mode of transmission of *S. aureus* is by direct contact, usually skin-to-skin contact, but contact with contaminated objects and surfaces may also have a role. Therefore, MRSA contaminated surfaces and MRSA colonized human carriers can act as reservoirs for transmission. Dental clinics have special infection control challenges due to the dust-raising foot-traffic of patients and staff, the repeated, rapid usage of operatories, and the inability to sterilize dental units and chairs. Therefore, it is possible that dental clinics serve as a source of disease transmission in the community and should be of concern for infection control.

The aim of this study was to survey for MRSA in an 18 chair university-based periodontal clinic. Different routes of possible MRSA contamination were examined including hard environmental surfaces, dental unit water lines, aerosols, and human carriers.

1.2 Environmental Surface Contamination in a Dental Clinic

The ability of MRSA to contaminate a large variety of hospital surfaces and items (e.g. computer keyboards, door handles, bed rails, bedside tables, telephones) have been demonstrated in many studies[21 22]. An important consideration is the fact that most of these items are frequently touched, and can thus be considered "high-touch" surfaces. In the dental operatory, such "high-touch" surfaces can include light handles, air-water syringes, switches, drawer handles, etc. These surfaces are likely to have higher frequency of contamination than other sites[23]. Furthermore, it has also been shown that MRSA is able to survive for many months over a wide array of temperatures, humidity, and sunlight exposures[21 23 24]. Therefore, these "high-touch" surfaces can potentially become contaminated, harbor MRSA for a period of time, then be capable of transferring the bacteria to other dental care providers, patients, or instruments.

In 1995, a Japanese dental school tried to isolate Methicillin-resistant staphylococci in the dental operatory. They detected Methicillin-resistant coagulase-negative staphylococci on dental surfaces such as chairs, brackets, cabinets, and floors. However, MRSA was not detected[25]. Another Japanese study in 2006 surveyed for MRSA contamination on dental operatory surfaces in a hospital-based clinic. MRSA was found on the surfaces of dental operatory including the air-water syringe (4 colonies) and reclining chair (1 colony)[26]. In 2011, a study from the University of Washington surveyed for MRSA isolates from frequently touched dental school

clinic surfaces. Ninety-five surfaces deemed most likely to accumulate bacteria from 7 different clinics and the clinic floors were sampled. They found that 4 of the 7 clinics had a positive MRSA surface sample. These surfaces included the uncovered parts of the dental chair seat and armrest, the floor beneath the chair, and the counter, sink and towel dispenser next to the chair. The MRSA positive samples came from dental chairs and floor samples[19]. More recently, Stony Brook University School of Dental Medicine swabbed environmental surfaces for *S. aureus* and found that none of the strains were methicillin-resistant[27]. These results show that although infrequent, it is possible for dental operatories to become contaminated and serve as potential reservoirs for disease transmission.

The current infection control practices at the University of California San Francisco Postgraduate Periodontal clinic for hard surface include:

1) Removing debris from the floor and counter tops

2) Disinfecting the following surfaces by spraying with an isopropyl alcohol and quaternary ammonium chloride compound (CaviCide Spray: Metrex, Orange, CA) and wiping with a paper towel saturated with disinfectant: handles, receptacles, brackets and valves of saliva ejector, high speed evacuator, counter surfaces, patient and operator chairs, air-water syringe handles, sinks and faucet handle

3) Using large plastic barrier covers for patient chair, dental chair tray, and moveable surgical tray

4) After patient dismissal, instruments are scrubbed in the sink to remove blood and debris. All covering are removed and surfaces are sprayed and wiped with

disinfectant.

It was decided to sample a "high touch" surface in this study. Although it was found that the floors and dental chairs were highly likely to accumulate bacteria, it would be unusual for the operator or patient to touch the floor at any given visit. Also, as per current infection control practices, chairs are covered with single-use plastic barriers for all patient visits. It was determined that air-water syringes (AWS) would be an appropriate "high touch" surface to sample as they may commonly be overlooked during routine infection control procedures. In particular, the crevices under the buttons on the top surfaces of the AWS are highly suspicious sites for bacterial contamination. Since previous studies have reported positive MRSA sampling at the dental patient chairs [19 26], it was decided to sample the AWS nearest to the patient chair – i.e. AWS on assistant's rack.

1.3 Dental Unit Waterline Contamination

Dental unit waterlines (DUWL) are defined as the narrow-bore plastic tubing that carries water to the high-speed handpiece, air-water syringe, and ultrasonic scaler. The waterline can be an open water system, where the source of the water is municipal tap water, or a closed water system, where the tubing is connected to a container. Many studies have shown that dental unit waterlines can become colonized with microorganisms including bacteria, fungi, and protozoa[28-32]. Counts as high as 1,000,000 colony-forming units per milliliter (CFU/mL) have been

reported in the literature[31 33]. Protected by a polysaccharide glycocalyx, these microorganisms colonize and replicate on the interior surfaces of the waterline tubing and form a biofilm. It has been reported that the biofilm can form within 8 hours of the DUWL being connected to the main water supply, and can develop to reach a climax community by 6 days[34]. The narrow diameter tubing and water stagnation creates a favorable environment for the bacteria to grow[35]. Bacteria can be shed more or less continuously from the biofilm into the DUWL. Detachment of the surface microorganisms from the biofilm results in planktonic microorganims that can be flushed into the mouths of patients and subsequently become airborne as aerosols or droplets of splatter.

In 1995, the American Dental Association set a goal to reduce the level of bacterial burden in the DUWL to ≤200 CFU/mL by the year 2000[36]. In 2003, the Centers for Disease Control (CDC) issued their guidelines and recommendations for DUWLs. At this time, it is within the standard of practice to use drinking water meeting EPA regulatory standards (i.e., ≤500 CFU/mL of heterotrophic water bacteria) in the DUWL for routine non-surgical dental treatment[37].

The organisms in the DUWL may originate from local drinking water but oral organisms have also been recovered. This suggests that there may be an issue of fluid retraction/siphonage. Oral flora could be retracted into DUWL as a consequence of the transient negative pressure that occurs. Recently manufactured dental units are equipped with integrated anti-retraction devices to prevent the

backflow of fluids. However, many studies have shown that patients' oral fluids were still being retracted into the DUWL during patient treatment[35 38 39]. This enhances the potential for disease transmission. The 2003 CDC guidelines advocate that dental devices connected to the DUWL should be flushed of air and water for at least 2 minutes at the beginning of the day and for 20-30 seconds after each patient[37]. The flushing can reduce the level of planktonic bacterial contamination but unfortunately cannot eliminate it[40], and the sessile, attached biofilm remains.

Although oral flora and human pathogens such as *Pseudomonas aeruginosa,* Legionella species, nontuberculous Mycobacterium species[28 41 42] have been isolated from DUWLs, the majority of organisms recovered from dental waterlines have been common heterotrophic water bacteria that exhibit limited pathogenic potential for healthy immunocompetent individuals[31]. The number of published cases of infection or respiratory symptoms resulting from exposure to water from contaminated DUWL is limited. However, exposing patients or other dental healthcare personnel to water of uncertain microbiological quality, despite the lack of documented adverse health effects[43], is inconsistent with accepted infection-control principles.

At the UCSF Postgraduate Periodontal clinic, the DUWL is a closed water system. The tubes are connected to a water reservoir container attached to the dental chair. On the operator's rack, the DUWL is connected to the AWS, the highspeed handpiece, and the ultrasonic scaler. The handpiece is used for periodontal surgery

to remove or recontour bone, and the ultrasonic scaler is used for most hygiene appointments. During periodontal surgery, in addition to the DUWL, sterile saline supplied from a single-use sterile syringe is used for additional irrigation. Similarly, for dental implant procedures, or procedures using piezoelectric devices, a special handpiece is cooled and irrigated using only sterile saline in a system independent of the DUWL. The university's current infection control practices stipulate that all the lines are flushed for a full 2 minutes in the sink prior to the first patient of the day, and again for 30 seconds in between patients. At the end of each day, the water reservoir container should be emptied and allowed to air dry. Drying has not shown an effect on biofilm in DUWL. Currently, there is no formal monitoring of the individual operator's practices or adherence to university policies regarding DUWLs.

At this time, although *Staphylococcus aureus* has been isolated from dental units[32] [42], there has been no documented evidence of MRSA isolated from DUWL. This portion of our pilot study aims to survey for MRSA in the DUWL.

1.4 Dental Procedure Aerosol Contamination

Dental procedures can generate potentially hazardous splatters and aerosols from highspeed dental handpieces, air-water syringes, polishing units, sonic and ultrasonic scaling devices, etc[44]. By definition, splatter droplets are >50 μm in diameter, visible to the naked eye, travel in a ballistic trajectory, and settle quickly

on nearby surfaces. On the other hand, aerosol droplets are < 50 μm and can remain suspended in the air for considerable periods of time and can be transported with air flows at long distances[45-47]. Depending on the humidity in the environment, the water in the aerosol droplet can evaporate, leaving behind very minute particles. Aerosols generated in a dental clinic can contain blood, saliva, tooth fragments, dental plaque, calculus, restorative debris, microorganisms etc. Larger aerosol particles are mostly captured in the upper airways, but particles 0.5-5 μm can be small enough to penetrate the alveoli of the lungs[48], thereby posing a risk for nosocomial infection for all persons in the clinic. Humidity, temperature, particle size, ventilation can all influence the spread of the aerosols. The most intensive aerosol and splatter emission occur while using an ultrasonic scaler or a highspeed handpiece[44 49 50]. Over 99% of the aerosol particles generated by highspeed handpieces are < 5μm[45]. Many studies have shown that during highspeed instrumentation or ultrasonic scaler use, aerosol contamination can spread >1m from the patient's mouth[51-54]. This means that blood-contaminated aerosols can be suspended in the air, even at 1m from behind the patient's head[55]. The maximum levels of air contamination occur during active dental treatment. However, it has been shown that the CFU counts in air sample remain very high even 30 minutes following active dental treatment[56 57].

Control of airborne transmission of infectious diseases is especially important in indoor medical environments. Many studies have shown that MRSA is present in the oral cavity[58]. *S. aureus* has been isolated from 24-36% of healthy oral cavities, and

the incidence has been reported to rise to 48% in patients that wear dentures[59 60]. Since the early 1990s, MRSA was detected in dental plaque from a group of intensive care unit patients undergoing antibiotic treatment[61]. It was subsequently detected in dental plaque of many hospitalized patients who were in general poor condition or had mental disabilities[62-64]. A British study provided evidence that airborne transmission of staphylococci does occur[65]. It can be speculated that dentists and dental staff could have a higher respiratory risk than the general population due to their prolonged and chronic exposure. Dentists do indeed have higher rates of seropositivity titer to Legionella[29]. These aerosols may cause various infections or sensitizations leading to illnesses such as asthma, rhinitis, allergic alveolitis[54]. Dental aerosols containing opportunistic pathogens could also transmit serious nosocomial infections to immunosuppressed patients. Shiomori et al demonstrated that MRSA carried on dust particles was capable of being aerosolized and was present in the respiratory-penetrable size range[66]. The dispersal of blood and blood-tinged saliva by dental procedure aerosols raises a specter of blood-borne disease transmission, but this present project is specifically focused on MRSA surveillance.

Not only can patients disperse airborne bacteria during active dental treatment, healthcare workers can also disperse bacteria into the air. Up to 10% of healthy *S. aureus* nasal carriers disperse the organism into the air[67]. Besides having the organisms in their nose and oropharynx, carriers could also have the bacteria colonizing their skin. The skin sloughs millions of squamous cells from the epithelium each day, and a sloughed “squame” could have a few bacteria attached.

The squames can fluff out in air currents from the neck, sleeves, and hems of the carrier's garments, and add to the contamination of the air. This phenomenon of airborne dispersal of *S. aureus* is called the "cloud" phenomenon[68]. In 1996, an outbreak of MRSA pneumonia occurred in an intensive care unit due to a "cloud adult" – a physician with a prolonged upper respiratory infection[69].

There is considerable evidence that staphylococci can commonly survive a wide range of environments[21 23 24]. It is thus not surprising that *S. aureus* has been identified in dental clinic aerosols[32 70]. Many bacterial isolates in aerosols have been found to demonstrate resistance to one or more antibiotics, especially the β-lactam family of antibiotics[70]. However, at this time, Methicillin-resistant staphylococci have yet to be isolated in dental clinic aerosols[70]. This portion of the study aims to survey for MRSA in dental aerosols generated during routine periodontal non-surgical therapy using ultrasonic scalers, as well as during periodontal surgical therapy using highspeed instruments to contour bone.

1.5 Human Carriers in a Dental Clinic

S. aureus is ubiquitous and may be present as part of the natural human flora. It colonizes many sites on the human body, of which the anterior nares is the most common. It is present in the nasal vestibule of at least 30% of individuals in the normal population[2 71]. Three patterns of carriage was described: those who always carry a strain, those who carry the organism intermittently with changing strains,

and those who never carry *S. aureus*[72]. Persistent carriage is more common in children than in adults. Nasal carriages may be divided into persistent carriers with high risk of infection, and intermittent or non-carriers with low risk of infection[73]. Interestingly, CA-MRSA is less often found in the anterior nares than are methicillin-sensitive *S. aureus* and HA-MRSA[74 75]. Rather CA-MRSA colonizes the skin, especially in the perineal area and the rectum[76]. It is possible that some people can be MRSA carriers unknowingly. In 2005, a surveillance study done at the Emory University School of Medicine in Atlanta, GA suggested that about 7% of adults are MRSA nasal carriers at the time of patient admission[77]. In a systematic review on the colonization of healthcare professionals by MRSA from 1980 to 2006, it was noted that the average prevalence of MRSA was 4.6% among the 33,318 healthcare professionals assessed[78].

Earlier papers investigating the carriage of staphylococci in the dental setting have demonstrated the isolation of *S. aureus* from the nasal cavity in 30-40% of dental students[79 80]. Horiba's 1995 study investigated MRSA contamination by collecting nasal swab samples from personnel in a dental school setting. Methicillin-resistant coagulase-negative Staphylococci, especially *S. epidermidis*, were detected in 8 of 39 dental staff, but MRSA was not detected in that study[25]. Numerous subsequent studies surveying for MRSA in the dental school settings around the world failed to detect any MRSA[80-82]. More recent studies have found nasal cultures positive for MRSA in patients[26 83] and students[19] at university-based dental clinics. Zimmerli's study at the University of Basel, Switzerland, found 2 MRSA strains among 500

dental patients and concluded that the potential of MRSA carriage among dental patients existed but was low[83]. It's also been shown that older age was a risk factor for carriage of MRSA[84]. Since the patient population of periodontal clinics tends to be older, it is important to consider the possibility that some of our patients may be MRSA carriers. To our knowledge, there is only one documented case of transmission of MRSA from a dentist who was a nasal carrier to two patients in England[85]. However, that occurred prior to the institution of standard infection control protocols and the practitioner was not using gloves routinely. The remaining cases of demonstrated or suspected MRSA colonization following oral or maxillofacial surgery occurred in hospitalized patients in poor general condition, such as cancer patients[86]. The transmission of MRSA cannot be directly linked to the dental procedure as these patients may have acquired it during hospitalization.

Controversy exists as to which body sites are the most effective for MRSA surveillance[87]. The European MRSA Consensus Conference in 2007 stated that the nose is the most important screening site[88]. Nasal screening has an 80% sensitivity. Nasal swabs also tend to provide the largest number of overall MRSA isolates, as well as the largest number of MRSA isolates from a single site[89]. Studies show that obtaining screening samples from additional body sites can increase the sensitivity to >90%[90 91]. However, the additional screening sites can include perianal regions, axillae, open wounds, etc, that would be inconvenient and unrealistic in a dental clinic setting.

In general, the frequency of nasal carriage of MRSA among dental patients and personnel has been shown to be lower than hospital workers and were more similar to the general population rates[86]. However, screening for MRSA in healthy carriers can help to reduce potential transmission/contain outbreaks, prevent the development of infection, and prevent spread of clones. Studies have indicated a positive correlation between having a positive nasal screening and a subsequent development of infection[92]. The aim of this section was to survey the MRSA carrier rate in the anterior nares in the Postgraduate Periodontology Clinic among the residents, staff, and faculty.

2. PURPOSE

The aim of this observational study was to survey for MRSA in an 18 chair university-based periodontal clinic. Different routes of possible MRSA contamination were examined including 1. hard environmental surfaces, 2. dental unit water lines, 3. aerosols, and 4. human carriers.

3. MATERIALS AND METHODS

3.1 Environmental Surface Contamination in a Dental Clinic

No faculty, residents, or staff members were notified or alerted prior to our sampling procedures. Wearing sterile gloves and following an aseptic technique, the AWS from the assistant's rack was lifted from its holder. The handle area and top surfaces of the valve buttons and the crevices under and around the buttons were swabbed using BBL Culture Swabs (BD Diagnostics, Sparks, MD) pre-moistened with the sterile nonselective trypticase soybroth with 6.5% NaCl (BBL Prepared Culture Media; BD Diagnostics). The swabs were immediately placed in the tubes of the enrichment broth to inoculate it, and then removed. The closed tubes were incubated at 35°C for 24 hours. During this time, BBL CHROMagar MRSA II (CMRSAII: BD Diagnostics) agar plates were allowed to warm to room temperature in a dark room. Subsequently, the enrichment broth was inoculated on another swab and underwent a 3-streak dilution method on the agar plates. The plates were then incubated in an inverted position in the dark at 35°C for 24 hours. Any mauve colonies morphologically consistent with *S. aureus* were confirmed as *S. aureus* with a positive coagulase test (Fluka Coagulase Disc: Sigma-Aldrich Chemie GmbH, Buchs, Switzerland).

The coagulase test was performed by placing mauve colonies on a glass microscope slide with 1 drop of distilled water. The test strain was emulsified to obtain a homogenous thick suspension. One coagulase disc was added and rubbed about in the suspension, using the tip of a wire loop. A second drop of distilled water was

added and mixed. Visible macroscopic clumping within 30 seconds indicated a positive coagulase test

3.2 Dental Unit Waterline Contamination

Wearing sterile gloves and following an aseptic technique, the AWS from the assistant rack was flushed for 2 minutes. A clean AWS tip was inserted and the paddle removed from the Millipore container. Using the AWS, the Millipore HPC Sampler container was filled with water from the dental unit waterline to the 2cc mark. Sterile saline was then added to the 20cc mark. The Millipore paddle was replaced firmly in the case with the water sample and the entire container was placed horizontally with the filter side down for 30 seconds. Subsequently, the paddle was removed and the excess water shaken off. All liquid was emptied from the sampler container. The paddle was replaced firmly and the unit incubated at room temperature, filter side down. Following 48-hour incubation, the paddle filters were examined under the microscope. Colonies appear as spots on the filter and can vary in size and color. Only the colonies lying on the full squares were counted; those on partial squares at the periphery of the paddle were not counted. Normally, the Millipore sampler is made to draw in 1mL of sample into the paddle, so the count obtained is equivalent to the colony-forming units per milliliter (CFU/mL). However, due to the sterile water dilution, this number would be multiplied by 10 to obtain the true CFU/mL. Grown colonies were inoculated on the CMRSAII plates following methods previously described. Any mauve colonies were subjected to a subsequent coagulase test.

3.3 Dental Procedure Aerosol Contamination

Each patient was seated and reclined in the dental chair. Aerosol collection occurred while practitioners were using ultrasonic scalers for scaling procedures or highspeed handpieces for surgical osseous recontouring procedures. No faculty, resident, student, or staff members were alerted prior to the sampling procedure.

The orifice of the sampler was located 25-30 inches from the patient's mouth, on a tray behind the patient, to minimize direct splatter. The ultrasonic scaler or highspeed handpiece on each dental unit was connected to the closed water system reservoir. Seven-minute air samples (1000L) were obtained using an aerosol-sampling monitor (M Air T Air Tester, Millipore Corp., Bedford, MA). Temperature and humidity were recorded during the sampling process.

Following a 35C, 24-hour incubation on the trypticase-soy agar plates, the colonies were counted for total viable count (TVC) and those with the color and morphology consistent with *S. aureus* were picked with a sterile loop and sub-cultured in salt enrichment broth (BBL Prepared Culture Media enrichment broth). These tubes were incubated at 35C for 24 hours. Subsequently, the culture media were inoculated with swabs on the CMRSAII agar plates following methods previously described. Any mauve colonies were subjected to a subsequent coagulase test.

3.4 Human Carriers in a Dental Clinic

In July 2012, 30 healthy volunteers were recruited among the residents, staff, and faculty of the University of California San Francisco Postgraduate Periodontology Clinic. The volunteers included those involved in direct patient contact, and were in good systemic health with no known present or past MRSA carrier status.

The subjects were informed on the study verbally and in writing and provided verbal informed consents. The study had been approved by the Committee on Human Research (IRB#12-08736). Subjects were informed of the risks of MRSA carriage. Culture results were confidential. Identified MRSA carriers were notified and advised to seek appropriate decolonization treatments with their primary care provider.

Specimens were obtained from the anterior nares with sterile fiber-tipped swabs (BBL Culture Swabs, BD Diagnostics) moistened with sterile saline. The swab was inserted 1cm and rolled 3 times in each nostril while the subject pressed the side of their nostril closed. Immediately after, the swabs were inoculated into a non-selective TSA enrichment broth (BBL Prepared Culture Media; BD Diagnostics). The samples were labeled and coded with a random number list prepared using a random number generator. After incubation at 35°C overnight, the broth was triple streaked onto a chromogenic agar selective for *S. aureus* (BBL CHROMAaagar MRSA II; BD Diagnostics). Following another 24 hour incubation at 35°C, any mauve

colonies subsequently underwent a coagulase test. The code sheet was destroyed at the end of the project.

4. RESULTS

4.1 Environmental Surface Contamination

Air-water syringe surfaces from 18 chairs in the Periodontology clinic were sampled in December 2011. Upon a 24-hour incubation period in the enrichment broth, the specimens were inoculated on the CMRSAII agar plates. At 24 hours, no mauve colonies or any other colonies were observed on any of the plates. At 48 hours, no mauve colonies or any other colonies were observed macroscopically or at 10.5x magnification under the microscope. There were no MRSA on these frequently touched surfaces.

4.2 Dental Unit Waterline Contamination

The water from the AWS line from 18 chairs in the Periodontology clinic were sampled in February 2012. These units had iodine-releasing devices (DentaPure) installed a year previously. Table 1 shows the count from the diluted HPC sampler and the final CFU/mL.

Table 1. Total viable counts from AWS water samples on HPC Sampler plates (1:10 Diluted HPC Sampler Count)

Unit	Count*	CFU/mL
1	2.7	27
2	731	7310
3	20	200
4	21	210
5	210	2100

6	400	4000
7	172.3	1723
8	38.3	383
9	87. 7	877
10	14.3	143
11	86.7	867
12	458.3	4583
13	28.7	287
14	311.3	3113
15	72.3	723
16	1.3	13
17	0	0
18	63.3	633

*Note- The count represents the average value of 3 separate counts performed by 2 investigators

Colonies grown on the HPC sampler were inoculated on the CMRSAII plates and the plates were examined at 24 and 48 hours. Zero mauve colonies were observed at either of the observation times but maroon colored, rounded, elevated colonies/slime were noticed on certain plates and are designated by a * in Table 2. Two of the plates with a large amount of these maroon colonies underwent a coagulase test and both tested negative. Unit 2 underwent a Gram stain. No cocci were seen at 150X or 600X.

Table 2. Mauve-colored colony counts on CMRSAII plates inoculated from HPC Samplers of dental unit AWS water

Unit	Count at 24H	Count at 48H	Coagulase test
1	0	0	N/A
2	2*	13*	Negative
3	0	0	N/A
4	0	0	N/A
5	0	0	N/A
6	0	2*	N/A
7	0	0	N/A
8	0	0	N/A
9	0	0	N/A

10	0	1*	N/A
11	17*	14*	Negative
12	0	2*	N/A
13	0	0	N/A
14	0	1*	N/A
15	0	0	N/A
16	0	0	N/A
17	N/A	N/A	N/A
18	0	0	N/A

* = maroon colored, rounded, elevated colonies/slime
N/A = not applicable

There were no MRSA bacteria detected in HPC samplers of DUWL samples.

4.3 Dental Procedure Aerosol Contamination

21 aerosol samples were taken during periodontal procedures using ultrasonics or dental handpieces between April to June 2012. The following Table 3 summarizes the sampling conditions. The procedure types were either osseous surgery (OS) using highspeed handpieces or ultrasonic scaling (US) using Cavitron devices. Suction methods were either via surgical tip evacuator (ST), saliva ejector (SE), or highspeed evacuator (HE).

Table 3. Periodontal clinic aerosol samples and sampling conditions

Sample	Procedure	Temperature (°F)	Relative Humidity (%)	Pre-operative Chlorhexidine rinse	Suction method
1	OS	73.9	37	Y	ST, SE
2	US	72.5	44	N	SE
3	US	71.6	45	N	SE
4	US	72.3	47	N	SE
5	US	73.2	45	N	SE

6	US	71.4	52	N	SE
7	US	76.2	42	N	SE
8	US	73.4	44	N	SE
9	OS	72.8	45	Y	ST, SE
10	US	73	46	N	SE
11	US	72.5	51	N	SE
12	US	75	46	N	SE
13	US	75.5	39	N	SE
14	US	74	41	N	SE
15	US	72.1	43	N	SE
16	US	71.6	49	N	SE
17	US	72	50	N	SE
18	US	75.3	44	N	SE
19	US	72.1	50	N	SE
20	US	73	50	N	SE
21	US	72.3	51	N	SE

OS- osseous surgery
US- ultrasonic scaling
Y/N – yes/no
SE- saliva ejector
ST – surgical tip evacuator

After sampling, the TSA plates were incubated for 48-hours and the total viable counts were made (Table 4). Most of the colonies observed were about 1 to 5mm in diameter, domed-shaped, milky white, and smooth. There were also a number of large densely white colonies which were examined and found to be Gram-positive bacilli.

Table 4. Aerosol sample results. Golden-colored colonies on TSA plates suspicious of Staphylococcus aureus were re-inoculated on CMRSAII plates

Unit	*TVC/m^3	Golden colonies	Mauve colonies on CMRSAII
1	39.3	0	N/A
2	18	1	0
3	3	0	N/A
4	36.7	0	N/A
5	15	1	0

6	12.5	0	N/A
7	7	1	0
8	13	1	0
9	11	0	N/A
10	38	0	N/A
11	42	0	N/A
12	33	0	N/A
13	19	0	N/A
14	28	0	N/A
15	32	1	0
16	19	0	N/A
17	17	0	N/A
18	13	0	N/A
19	12	0	N/A
20	22	0	N/A
21	39	0	N/A

* TVC/m^3 values represent an average of 3 separate counts performed by 2 investigators of the total viable count per cubic meter air sample.
N/A = not applicable

There were no *S. aureus* or MRSA detected in any periodontal procedure aerosols.

4.4 Human Carriers

Between July to November 2012, 30 samples were collected from the anterior nares of the volunteers. This included the periodontal residents, staff, and faculty. The following Table 5 summarizes our findings.

Table 5. Anterior nares samples of Periodontal Clinic students, faculty, and staff enriched in liquid media and inoculated on CMRSAII plates.

Sample	Mauve colonies at 24-hour	Mauve colonies at 48-hour	Coagulase test
1	N	N	N/A
2	Y	Y	Negative
3	Y	Y	Negative

4	N	N	N/A
5	N	N	N/A
6	N	N	N/A
7	N	N	N/A
8	N	Y	Negative
9	N	N	N/A
10	N	N	N/A
11	N	N	N/A
12	N	N	N/A
13	N	N	N/A
14	N	N	N/A
15	N	N	N/A
16	N	N	N/A
17	N	N	N/A
18	Y	Y	Negative
19	N	N	N/A
20	N	N	N/A
21	N	N	N/A
22	N	N	N/A
23	N	N	N/A
24	N	N	N/A
25	N	N	N/A
26	N	N	N/A
27	N	N	N/A
28	N	N	N/A
29	N	N	N/A
30	N	N	N/A

Y, N = yes, no
N/A = not applicable

The coagulase-negative mauve-colored colonies detected in 4 persons were not MRSA. These were coagulase-negative staphylococci (CoNS).

5. DISCUSSION

This study did not detect any MRSA in the university-based periodontal clinic on environmental surfaces, waterlines, aerosols, or human carriers. These findings confirm other studies in dental clinics that have shown similar results.

A. Routes of Contamination

Environmental surfaces

There has been some evidence in literature that the dental clinic environment can harbor MRSA[19 26]. Meanwhile, other studies have shown results similar to ours and have failed to detect MRSA in the dental clinic[25 27]. To our knowledge, there have been no reports of patients getting MRSA infections from a contaminated surface in a dental clinic. Therefore, the overall risk that the dental clinic environment poses to endemic MRSA transmission is very minor.

It would appear as though the current infection control protocol employed at UCSF for hard surfaces are adequate as long as they are properly executed. The Cavicide manufacturer's website (http://www.metrex.com/surface-Cavicide) claims that the spray is effective in killing MRSA in 2 minutes. The student body and the staff in the clinic should follow the manufacturer's directions for usage. Developing an understanding of which sites are more likely to be contaminated with pathogens ("high touch surfaces") can also help to guide infection control practices and direct

new innovation. Due to the fact that there is evidence to show that MRSA can survive for long periods of time on contaminated surfaces, the possibility of disease transmission, although minor, still remains[23 93].

Dental unit water lines (DUWL)

There has been no documented evidence of MRSA isolated from DUWL. This study also failed to detect any MRSA in the periodontal clinic waterlines.

In a study comparing five commercially available assay systems for microbial testing in hemodialysis fluids, the HPC sampler showed the most consistency in obtaining the highest viable counts[94]. When compared to the gold standard of the R2A and HPC agars, the HPC sampler showed an accuracy of 92.6%, sensitivity of ~98%, and specificity of ~77%[95]. The HPC sampler has been criticized because it cannot grow all types of bacteria, and tends to underestimate colony counts[96]. However, no single media, temperature, or incubation time can provide the ideal conditions for the recovery of all microorganisms. The HPC samplers are advantageous since they are readily available, and provide an acceptable balance between microbial recovery and required sampling time, equipment, and expertise[95 97].

In 2003, the CDC recommended that sterile solutions such as sterile saline or water should be used for irrigation in the performance of oral surgery procedures. These procedures carry a greater opportunity for the entry of microorganisms into the vascular system. This in turn increases the potential for localized or systemic

infections[37]. While the UCSF periodontal clinic uses sterile saline when using piezoelectric devices or placing dental implants, most periodontal surgery procedures are performed using handpieces connected to the DUWL. Due to the high bacterial counts found in the DUWLs, ClO2 was introduced into the periodontal clinic's waterlines at this point. All the water reservoirs in the periodontal clinic were filled with a phosphate buffer-stabilized ClO2. It has been shown that the use of a ClO2 mouthrinse as the lavage during ultrasonic scaling can safely and significantly reduce the bacterial count[45]. However, evaluating the effectiveness of using the ClO2 was beyond the scope of this study, and further studies are required to evaluate whether a change in university protocol would be necessary.

Aerosols

No MRSA were detected in the periodontal procedure aerosols. However, many large white colonies were seen on the TSA plates. These were identified to be Bacillus colonies, often found in dust from foot traffic. These were found in dust samples grown on TSA plates, samples from base of dental chair, and in random air samples without any aerosol. For this reason, floor cleaners and the housekeeping staff should be an integral part of the infection control team.

Human carriers

No MRSA were detected in the anterior nares samples collected from the 30 periodontal residents, staff, or faculty. However, in 4 of the volunteers (~13% of

total sample), Methicillin-resistant coagulase-negative staphylococci (MR-CoNS) were found.

CoNS are a heterogeneous group of organisms, distinguished from *S. aureus* by their inability to clot blood plasma. There are 32 CoNS species[98]. About 15 of them are indigenous in humans, while the rest are nonhuman pathogens. Most of the staphylococci isolated from humans belong to the *S. saprophyticus* or *S. epidermidis* group. *S. epidermidis* is the predominant human species and comprises 65-90% of all staphylococci recovered from human sources[99]. It can be isolated from moist habitats such as mucous membranes, or dry exposed skin surfaces. A normal individual can harbor up to 24 different *S. epidermidis* strains. Most newborns are colonized with multiple strains of CoNS within the first week of life[100].

Virulence factors in CoNS are not as clearly established as they are in *S. aureus*. CoNS are the most common cause of bacteremia related to indwelling devices[98]. They also cause important infections such as central nervous system shunt infections, native or prosthetic valve endocarditis, urinary tract infections, and endophthalmitis. However, CoNS are the most frequent blood culture contaminant and it is sometimes difficult to distinguish between infection and contamination.

Penicillins are often the first-line agents for the treatment of staphylococcal infections. Therefore, it is not surprising that many strains of CoNS are now resistant to Methicillin. In a study investigating nasal carriage of MR-CoNS in 291

patients at hospital admission in France, the carriage rate was found to be 19.2%[100]. This is not too different from the rate found in our small study.

SCC*mec* displays more polymorphous structure in MR-CoNS, with frequent recombinase-encoding gene complex combinations not described in MRSA[101 102]. Several reports suggest that there is a potential for horizontal gene transfer from MR-CoNS to Methicillin-susceptible *S. aureus*[102]. Although the exact mechanism is unknown, this entails that MR-CoNS, although not highly pathogenic on its own, can act as a source of SCC*mec* for MRSA.

Hand hygiene is the single most beneficial intervention in the control of MRSA and MR-CoNS, among many other pathogens. Contaminated hands have been shown to be the chief mode of transmission for most patients who acquire a hospital infection[103]. Although this study did not take nasal samples from patients, the general public should also consider their own attitude to hygiene. Increasing numbers of community strains of MRSA have been associated with hygiene issues and frequent antibiotics use[104]. Since MR-CoNS can transfer antibiotic resistant genes to *S. aureus*, this issue requires urgent appraisal.

B. Collection and Culturing Methods

Salt Enrichment

Culture techniques sometimes involve the use of salt enrichment broth to enhance the growth of staphylococci. It has been shown that specimen enrichment in salt-containing trypticase soybroth increased yield by 9%[105]. Broth enrichment allows detection of small numbers of MRSA, thus enhancing sensitivity of detection but requires an extra 24 hour of incubation. Therefore the broth enrichment technique was utilized in parts of this study.

Culture Plates

Traditionally, MRSA were detected using culture techniques on conventional agar plates containing different types of antibiotics. In recent years, chromogenic mediums are often used as they provide a similar level of sensitivity to conventional culture with the advantage of obtaining results more rapidly. CMRSAII is a selective and differential chromogenic medium that incorporates cefoxitin (5.2mg/L) for the qualitative direct detection of MRSA. Cefoxitin is a second-generation cephalosporin, and is considered a strong β-lactamase inducer. The incorporation of cefoxitin in culture media has proven to be superior to the incorporation of ciprofloxacin, oxacillin, and methicillin in culture media in the detection of MRSA[106].

MRSA strains will grow in the presence of cefoxitin and produce mauve-colored colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of Gram-negative organisms,

yeast, and some Gram-positive cocci. On this medium, MRSA are visualized as mauve colonies. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in the growth of colonies that are not mauve in color. Studies on CMRSAII have reported 24hour sensitivity of 84.6-95.3%. At 48 hours, sensitivity increased to >95% but with a decrease in specificity[107-109]. It was thought that despite moderate increases in sensitivity at 48hours, prolonged incubation should be avoided due to an important reduction in specificity. Occasional strains of coagulase-negative staphylococci may produce mauve colored colonies, requiring a confirmatory coagulase test for confirmation of MRSA.

BD Diagnostics' internal testing determined that there was potential cross-reactivity of non-Staphylococcus organisms with CMRSAII. In their unpublished study, strains of *Chryseobacterium meningosepticum, Corynebacterium jeikeium, Enterococcus faecalis, Rhodococcus equi, Bacillus cereus, Staphylococcus simulans, S. epidermidis*, and MRSA produced mauve-colored colonies. Therefore, to increase the sensitivity of our methods, a coagulase test was used to confirm the presence of *S. aureus*.

Coagulase Test

The coagulase test is used for the detection of coagulase-positive organisms. The test discs are made from rabbit plasma. *S. aureus* produces free and bound coagulase enzymes. Bound coagulase, or clumping factor, is localized on the surface of the cell wall and reacts with plasma fibrinogen to form a coagulate. With the slide coagulase test only the bound form of coagulase is measured[110]. A positive coagulase

test is confirmed when macroscopic clumping occurs. The slide coagulase test has a sensitivity of 93-97% and was as accurate as other commercially available coagulase method *S. aureus* identification kits[111-113].

Water Testing

There are a variety of commercially available bacteriologic samplers designed for water testing. The Heterotrophic Plate Count (HPC) sampler (Millipore Corp., Bedford, MA) has been used to analyze water samples simply and quickly in the pharmaceutical, food, and beverage industries for over 15 years. It is a sterile, disposable, self-contained testing device. It consists of a 20cc plastic vial into which a plastic paddle with an inner layer of nutrient medium covered with a 0.45μm pore membrane filter is inserted. The filter is ruled with squares to aid counting. It is designed to grow heterotrophic mesophilic bacteria, such as *Staphylococcus aureus*, that make up the main population of aquatic biofilm. In turn this can provide an adequate indication of active biofilm colonization.

Aerosol Sampling

Microbial air sampling was done using the Millipore M Air T Tester in this study. The M Air T is a lightweight, battery-powered, portable system for testing the microbial quality of air in clean rooms, barrier environments, and critical filling areas. It has been used in the pharmaceuticals, food and beverage, hospital, and electronic industries. The air sampler consists of a rechargeable battery, timer, turbine, housing for culture plates, and a removable sterilizable sieve through which air is

circulated to the culture plate. The device pulls $1m^3$ air samples through the sieve in 7 minutes and deposits particles on a sterile plate of trypticase-soy agar (with ruled cross lines on the bottom for ease of counting). M Air T cassettes have a consistent filling level and flat surface, which helps to ensure that the same reproducible volume of air is sampled during every test. The sieve has about 1000 micro-perforations that evenly distribute the air over the agar surface, thereby minimizing colony overlap.

C. Study Limitations

There are a number of limitations with this study. To begin with, this is a small observational study. It was not hypothesis driven which one could test for statistical significance. Next, although much effort was put into determining "high-touch" surfaces, it is possible that the actual areas sampled may not have been the best surfaces for bacterial colonization. Furthermore, we were limited in our sampling techniques – bacterial loads could have been too low for our methods of detection (versus using DNA hybridization or other highly sensitive techniques). We were often at the mercy of our materials and instruments for detection. For example, BD Diagnostics' internal testing determined that there was potential cross-reactivity of non-Staphylococcus organisms with CMRSAII. For this reason, to increase the sensitivity of our methods, a coagulase test was used to confirm the presence of *S. aureus*. However, even with a positive coagulase test, there is a small possibility that mauve colonies could still be Methicillin-sensitive *S. aureus*. The

coagulase test itself is also not error-free. Certain strains of *S. aureus* may not produce bound coagulase (identified by slide coagulase test) and may need to be identified via tube coagulase testing. Lastly, since the samples were only taken once, there is no way of determining whether the MR-CoNS carriers were transient or permanent carriers.

MRSA was not detected in this study. However, if MRSA was found in this study, the following issue needs to be considered: What size staphylococcus inoculum is required to initiate infection? It has been shown that an inoculum of anything from ten to several million CFUs could potentially cause an infection in a patient[21]. A few viable units from the environment could cause an infection in a vulnerable patient at a vulnerable site. It is possible that these patient-related factors are the chief determinants of whether or not a person succumbs to infection. Therefore it is important to bear in mind that the presence of a pathogen does not necessarily represent a risk of infection, but does represent a risk for transmission[23]. While the potential for transmission is higher in certain settings, such as intensive care units, there are no ascertained cases of transmission in a dental healthcare setting[86].

In 2010, JAMA published encouraging results from a CDC study. The study showed that from 2005 through 2008, incidence rate of hospital-onset invasive MRSA infections decreased 9.4% per year and the incidence of health care-associated community-onset infections decreased 5.7% per year[114]. Even though the 2010 APIC MRSA prevalence survey has found that the US MRSA prevalence rate was higher

than in 2006, the relative proportion of MRSA-infected to MRSA-colonized patients has reversed.[12 14]. The decrease in MRSA infections appears to be consistent with other national or multicenter reports using different populations and methods[11 114 115]. While HA-MRSA continues to be an important public health concern, the downward trend in incidence of infection remains encouraging and optimistic for the future. However, there is now a changing paradigm of MRSA – prevalence of disease in the community now surpasses that in the hospital[16]. Theoretically, the MR-CoNS carriers could disseminate the antibiotic resistant gene to Methicillin-sensitive *S. aureus* in the community. It remains to be determined whether these bacteria can be transferred to patients and cause clinically relevant infections.

6. CONCLUSION

The literature so far, in conjunction with the data from our study, collectively suggests that the occupational risk of MRSA infection in a dental setting is probably minimal.

In dentistry, antibiotics are often prescribed to prevent or treat infections. It is crucial for the dentist to only prescribe antibiotics when necessary and to prescribe the precise amount necessary. Indiscriminate antibiotics usage encourages the selection for resistant organisms and leads to bacteria with a broad range of antibiotic resistance.

Reducing patient and provider's exposure to MRSA as well as other nosocomial infections should continue to be high priorities for health care professionals. MRSA infection transmission is a serious public dental health issue and a cause for concern. Further research is needed to improve the knowledge and awareness regarding the true risk of MRSA infection.

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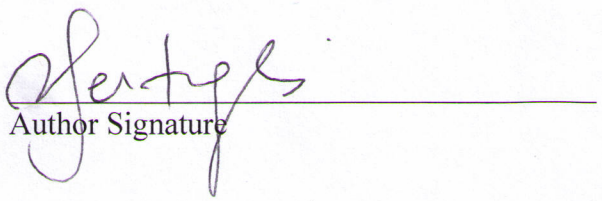

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