Effectiveness of two disinfectant solutions and microwave irradiation in disinfecting complete dentures contaminated with methicillin-resistant *Staphylococcus aureus*

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**ABSTRACT**

Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm on dentures can be aspirated, thus causing infections such as aspiration pneumonia. The authors evaluated the efficacy of two disinfectant solutions and microwave irradiation in disinfecting complete dentures contaminated with MRSA.

Methods. The authors contaminated 36 simulated complete dentures with MRSA and divided them into four equal groups: a positive control group consisting of dentures that were not disinfected; a group that soaked in 1 percent sodium hypochlorite for 10 minutes; a group that soaked in 2 percent chlorhexidine gluconate for 10 minutes; and a group that underwent microwave irradiation at 650 watts for three minutes. The authors quantified colony counts and evaluated the long-term effectiveness of disinfection.

Results. All dentures from the control group showed substantial microbial growth on the plates (6.24 log$_{10}$ colony-forming units per milliliter). The authors observed no evidence of microbial growth on plates of any disinfected dentures. After seven days' incubation, the authors observed broth turbidity in all beakers containing the dentures disinfected with 1 percent sodium hypochlorite.

Conclusions. Soaking in chlorhexidine gluconate solution and microwave irradiation resulted in complete disinfection of all dentures contaminated with MRSA in both the short and the long term. Soaking in sodium hypochlorite solution was effective only as a short-term disinfectant.

Clinical Implications. Microwave irradiation and 2 percent chlorhexidine gluconate may have a disinfective application in dental offices and institutions in which denture wearers are treated, thus improving the longevity and quality of life of patients and reducing the burden of disease caused by MRSA.

Key Words. Methicillin-resistant *Staphylococcus aureus* (MRSA); complete dentures; microwave irradiation; disinfection; disinfectants.
centrations of β-lactam antibiotics.1 Because it has been identified frequently in elderly or immunocompromised people, MRSA is of utmost concern.1 Approximately 52 percent of nosocomial infections in patients in intensive care units are due to MRSA,5 which is responsible for severe and virulent infections.6 Bacteremia caused by MRSA has been associated with increased mortality rates.7 MRSA strains, besides being disseminated systemically, have been isolated from the oral cavity.2 Some appliances within the mouth, such as removable dentures, may function as a reservoir of pathogens8 and render the patient more susceptible to oral colonization by MRSA.6,9 The biofilm on dentures can be released into the oral fluids and aspirated into the lower respiratory tract, thus causing infections such as aspiration pneumonia.10 Aspiration pneumonia has been considered one of the most clinically significant systemic infections caused by S. aureus11 and has been recognized as a predominant risk factor for mortality in elderly patients.12 Sumi and colleagues13 observed a high prevalence of respiratory pathogens on the surfaces of dentures, suggesting that poor denture hygiene may be related to the development of this systemic infection. Therefore, clinicians must consider carefully the role of a proper oral and denture hygiene program in preventing the spread and recurrence of local and systemic infections associated with MRSA. The results of a clinical study showed that the incidence of pneumonia and death was decreased in patients who underwent an intensive oral health care program.12 Such strategy must be adopted even more widely by hospitalized or institutionalized elderly patients and those with special needs. Patients who live in long-term care facilities or who have special needs usually have poor levels of oral and denture hygiene13 and oral health,14 both of which in turn create a favorable environment for Staphylococcus species and MRSA colonization.14,15

Several methods exist to reduce microbial contamination of dentures. Among them, chemical agents—such as sodium hypochlorite, chlorhexidine gluconate, chlorine dioxide and alcohol—have been used widely.16-18 Sodium hypochlorite can be useful as a denture cleanser because it inactivates bacterial plaque, removes stains and inhibits calculus formation on dentures.17,18 In a 2009 study, 2 percent sodium hypochlorite killed viable bacterial cells of MRSA in biofilms grown on denture surfaces after one minute of exposure.19 Chlorhexidine gluconate is another agent commonly recommended for denture disinfection. Evidence from a clinical study demonstrated the effectiveness of chlorhexidine gluconate in treating patients with denture stomatitis.20 This chemical agent possesses a broad spectrum of antimicrobial activity, being able to eliminate species of Candida, Streptococcus, Staphylococcus and Escherichia.17 Although chlorhexidine gluconate has been shown to be effective in eradicating planktonic cells of MRSA,18,22,23 it failed to eradicate MRSA biofilms.19 Despite their antimicrobial effect, these chemical solutions have given rise to some problems in use. Sodium hypochlorite frequently has been related to corrosion of the metal parts of dentures,19,24 bleaching of denture acrylic resin18,24 and changes in the flexural strength of denture base resins.25 Chlorhexidine gluconate solution has been responsible for other deleterious effects, such as discoloration of natural teeth26 and denture acrylic resin.19 Such disadvantages clearly are of concern, because a disinfection method should be effective without having any detrimental effect on denture materials.

To overcome the problems associated with chemical disinfection, several investigators have recommended physical methods of denture disinfection. Rossi and colleagues9 used heat to sterilize and eliminate biofilms from dentures belonging to patients with persistent oropharyngeal MRSA colonization. Another physical method that has received substantial attention for denture disinfection is microwave irradiation.21,27-31 In vitro studies27,28,30 have shown that using microwave irradiation for three minutes at 650 watts is an effective method of killing a wide variety of microorganisms, including the intrinsically resistant Candida glabrata, Candida dubliniensis, Candida krusei and S. aureus. In addition, in vivo study findings showed that this method of disinfection was effective in inactivating S. aureus in denture biofilms of 30 patients.29 However, the literature does not address its effectiveness against MRSA. In addition to being effective, the microwave disinfection regimen of three minutes at 650 W had no detrimental effect on the physical and mechanical properties of denture materials.32,33

We conducted a study to compare the efficacy of microwave irradiation, 2 percent chlorhexidine gluconate and 1 percent sodium hypochlorite.21

rite in disinfecting simulated complete dentures contaminated with MRSA.

METHODS
Production of simulated complete dentures. We made 36 simulated dentures according to the method described by Silva and colleagues. We duplicated a simulated maxillary complete denture by using a high-viscosity silicone mold (RTV 3120, D’Altomare, Santo Amaro, São Paulo, Brazil). We placed the acrylic artificial teeth (Dental Vipi, Pirassununga, São Paulo, Brazil) in the silicone mold, poured the melted wax, and fully seated a duplicate cast in the mold. After allowing the dentures to cool at room temperature for 30 minutes, we invested the wax-simulated dentures in metal dental flasks (Jon 5.5, Jon Produtos Odontológicos, São Paulo) with dental stone and condensation silicone with a putty consistency (Dental Vipi). We placed the flasks in boiling water to soften the baseplate wax and cleaned the stone and teeth with boiling water and liquid detergent (Ypê Clear, Química Amparo, Amparo, São Paulo, Brazil). We packed poly(methyl methacrylate) denture base resin (Lucitone 550, Dentsply International, York, Pa.) at dough stage into the molds, closed the flasks under pressure by using a hydraulic press (Dental Vipi) and placed them in an automatic polymerization tank (SOLAB Equipamentos Laboratoriais, Piracicaba, São Paulo) at 73°C for 90 minutes followed by 30 minutes in 100°C boiling water. We cooled the flasks at room temperature for 30 minutes and placed them in running tap water for 15 minutes. We opened the flasks, carefully recovered the dentures, trimmed the dentures by using a metal bur (Maxi-Cut, Dentsply-Maillefer, Ballaigues, Switzerland) and, finally, polished the dentures on a wet rag wheel by using a slurry of coarse pumice and then tin oxide. After polishing, we stored each set of dentures individually in a 200-mlitter beaker of distilled water at 37°C for 48 hours and sterilized it in an autoclave at 121°C for 20 minutes (AV 60, No. 6614, Phoenix Indústria e Comércio de Equipamentos Científicos, Araraquara, São Paulo, Brazil).

Bacterial culture and suspension. We obtained a standard strain of MRSA from the American Type Culture Collection (ATCC 33591). We maintained the isolate in tryptic soy broth (TSB) medium (TSB, Acumedia Manufacturers, Baltimore) and froze it at −70°C until use. We adjusted the bacterial suspension to a density of 0.5 of the McFarland standard by using a turbidimeter (TurbidityMeter, Siemens Healthcare Diagnostics, West Sacramento, Calif.), which corresponds to 10^7 cells/mL in 10 mL of TSB.

Contamination and disinfection procedures. We transferred an aliquot of 100 microliters of bacterial suspension to each sterile beaker containing 200 mL of sterile TSB, and we aseptically placed the sterile dentures to be tested into the beakers, sealed them with foil and incubated them for 24 hours at 37°C in an orbital shaker at 75 revolutions per minute (Model Q816M20, Quimis Aparelhos Científicos, Diadema, São Paulo, Brazil).

We divided the inoculated dentures into four groups of nine each: one positive control and three experimental groups. In the positive control group, the dentures were not disinfected. After incubation, we immersed each contaminated denture in a beaker with 200 mL of sterile saline solution and immediately transferred it to another beaker with 200 mL of sterile saline solution. We vortexed these beakers vigorously in a shaker incubator (Model MA562, Marconi Equipamentos Laboratório, Piracicaba, São Paulo, Brazil) for one minute and allowed them to stand for nine minutes, followed by a short vortexing to resuspend any organisms present. To determine the number of microorganisms in the 10^−3, 10^−4, 10^−5 and 10^−6 dilutions, we transferred replicate specimens (25 µL) of the suspensions to plates of mannitol salt agar (Acumedia Manufacturers) and incubated the plates at 37°C for 48 hours. We conducted tests in duplicate for each denture.

In the experimental groups, we transferred the dentures to beakers and disinfected them by various means: we soaked one group in 1 percent sodium hypochlorite solution, we soaked a second group in 2 percent chlorhexidine gluconate solution and we irradiated a third group with microwaves. We individually soaked contaminated dentures from the sodium hypochlorite and chlorhexidine gluconate groups in a beaker containing 200 mL of 1 percent sodium hypochlorite (Labimpex Indústria e Comércio de Produtos para Laboratórios, Diadema, São Paulo, Brazil) and 2 percent chlorhexidine gluconate (Deg Importação de Produtos Químicos, São Paulo), respectively, for 10 minutes. In the microwave irradiation group, we transferred the contaminated dentures to a beaker containing 200 mL of sterile distilled water. We placed each beaker on the rotational plate in an unmodified domestic microwave oven (Sensor Crisp 38, Double Emission System, Brasemp, Manaus, Amazonas, Brazil) and irradiated it at 650 W for three minutes. After disinfection, we individ-

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ually placed all dentures in the experimental groups in sterile beakers containing 200 mL of saline solution, vortexed them as described previously, and made tenfold dilutions ($10^{-1}$, $10^{-2}$ and $10^{-3}$). To determine the number of microorganisms in each dilution, we performed the same procedures we had carried out for the positive control dentures.

After incubation for 48 hours, we quantified yeast colony counts of each plated denture by using a digital colony counter (CP 600 Plus, Phoenix Indústria e Comércio de Equipamentos Científicos) and then calculated the colony-forming units (CFUs/mL). To verify the long-term effectiveness of the three methods of disinfection, we transferred each disinfected denture to a sterile beaker containing 200 mL of TSB, which was incubated at 37°C for seven days. The presence of any visible increase in the culture medium cloudiness (turbidity) was indicative of bacteria’s remaining in the disinfected dentures.

We performed a log10 transformation of the CFUs/mL values among the control dentures (log10 CFUs/mL) to achieve a normal distribution. For a method of disinfection to be considered effective, it had to achieve a three-log reduction in CFUs/mL values from the experimental dentures in comparison with those obtained from the control dentures. We transferred the results from the positive control and experimental groups into a computer spreadsheet program (Excel 2007, Microsoft, Redmond, Wash.) and calculated the mean values and standard deviations.

RESULTS

All control dentures contaminated with MRSA showed substantial microbial growth on plates at 48 hours’ incubation (Figure 1). Disinfected dentures contaminated with MRSA showed no evidence of microbial growth at 48 hours on plates, regardless of the disinfection protocol (Figure 2). After seven days’ incubation, we observed no turbidity in TSB beakers containing the dentures disinfected by means of microwave irradiation for three minutes at 650 W or 10 minutes of immersion in 2 percent chlorhexidine gluconate solution (Figure 3). For dentures disinfected by 1 percent sodium hypochlorite, we observed turbidity in all broth beakers after seven days’ incubation (Figure 4). The table (page 275) presents the colony counts (log10 CFUs/mL) and standard deviations for all groups evaluated. The standard deviation was zero for all disinfected groups in which we observed no growth on plates.

DISCUSSION

MRSA has become one of the most clinically significant causes of nosocomial infection, and its worldwide emergence has been considered a global public health problem. Because this microorganism has been isolated from denture wearers, predominantly those who are elderly or are immunocompromised, in this study we compared the effectiveness of different methods of disinfecting complete dentures contaminated with MRSA.

In our study, all positive control dentures contaminated with MRSA produced microbial growth on the plates at 48 hours’ incubation. These results are in accordance with those of other studies in which this bacterium was able to adhere to and grow as biofilm on acrylic surfaces. Adhesion to a surface is the first step in the complex process of biofilm formation. Biofilms have been defined as a multicellular community surrounded by a self-produced polymeric matrix. The behavior and susceptibility of the microorganisms change throughout biofilm development, and this has important consequences for the management of patient care. Researchers in previous studies have demonstrated that although planktonic MRSA were killed rapidly by means of antimicrobial agents, the established MRSA biofilms were more resistant to killing by these agents. Researchers have suggested that bacterial cells in biofilms may have survived exposure to antimicrobial agents owing to their spatial arrangement and the physical protection of
the polymeric matrix. Therefore, microorganisms growing as biofilm, such as dental plaque, tend to be less susceptible to a range of antimicrobial agents. Investigators in one study reported the inability of denture cleansers to clear the contaminating MRSA on dentures successfully. In another study, investigators detected MRSA within potentially complex biofilms on the surface of dentures. These findings reinforce the importance of MRSA's biofilm development, which has been considered a key virulence factor of this microorganism and has a huge impact on both pathogenesis and the treatment of infections.

**Disinfection. Microwave irradiation.** We accepted the hypothesis that simulated complete dentures contaminated with MRSA could be disinfected by means of microwave irradiation, as well as by use of two disinfectant solutions (chlorhexidine gluconate and sodium hypochlorite). In this study, three minutes of microwave irradiation at 650 W completely disinfected all dentures contaminated with MRSA. This method of disinfection has been shown to be effective against several microorganisms, including some viruses, some species of *Candida*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*. In addition, its killing action against methicillin-susceptible *S. aureus* (MSSA) on dentures also has been demonstrated by researchers conducting in vitro and in vivo studies. Nevertheless, to our knowledge, we are the first investigators to report the effectiveness of this method in killing MRSA.

Although the lethal action of microwave irradiation is well established in the literature, its mechanism of destruction is not completely understood. Several reports in the literature attribute the lethal effects of such radiation solely to heat generated by the waves (thermal effect). However, precedent does exist for the assertion that temperature increases alone are insufficient in explaining the detrimental effects of microwaves. The mechanisms that have been suggested to elucidate the nature of such non-thermal radiation effects on microbial survival include molecular and mechanical effects or selective heating. Irrespective of the nature of the lethality, the results of a 2007 study demonstrated that microwave irradiation of a fungal suspension produced changes in structural integrity and permeability of cell membrane and cell metabolism that resulted in cell death. Probably, a combination of these mechanisms was responsible for the high susceptibility of MRSA to microwave irradiation, as demonstrated by the absence of microbial growth in both the short and the long term.

**Chlorhexidine gluconate.** As observed for microwave irradiation, 10 minutes of immersion in 2 percent chlorhexidine gluconate solution resulted in complete disinfection of all dentures contaminated with MRSA in both the short and the long term. These findings are in agreement with those of other investigators, who demonstrated the efficacy of chlorhexidine gluconate against several microorganisms, such as *C. albicans* and a wide range of bacteria (*Streptococcus*, *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Escherichia* and *Staphylococcus*, including MRSA). The protocol we used in our study was based on those of previous investigators who demonstrated successful use of chlorhexidine gluconate solution as a disinfection agent. Smith and Hunter established a disinfection protocol in which 24 hours of immersion in a solution of 4 percent concentration was effective against planktonic cells of MRSA. However, researchers have shown that 4 percent chlorhexidine gluconate solution has a negative effect on the hardness and roughness of acrylic resins. Considering that chlorhexidine’s deleterious effects on acrylic resins are affected by...
concentration and time of exposure, investigators undertook studies to test lower concentrations and immersion periods. Immersion in 2 percent chlorhexidine gluconate during short periods (three and 10 minutes) was effective in eradicating planktonic cells of MRSA23 and MSSA biofilm,17 respectively. These findings agree with those of our study, in which 2 percent chlorhexidine gluconate produced consistent disinfection of dentures contaminated with MRSA.

Unlike microwave irradiation, chlorhexidine gluconate solution is a chemical means of disinfection. The positively charged molecules of chlorhexidine gluconate adhere to the negatively charged cell wall of the bacteria, mainly to phosphate groups in lipopolysaccharides and carboxyl groups; this causes selective protein precipitation from the cell wall, cytoplasm coagulation and the breakdown of low-molecular-weight intracellular components.43 These mechanisms allow chlorhexidine gluconate to act as a bacteriostatic agent at low concentrations and a bactericide at high concentrations.44 Despite chlorhexidine gluconate’s killing action against MRSA, researchers have observed an association between the intensity of chlorhexidine gluconate and the higher prevalence of MRSA and have shown that strains resistant to antiseptics also could emerge.45 Resistance of MRSA to antiseptics, including chlorhexidine gluconate, is conferred by two major gene families, qacA and qacB.45 The qacA and B genes encode a proton motive force-dependent multidrug efflux protein,46 which confers a high-level resistance to antiseptics on some strains. Accordingly, investigators have reported a significant number of MRSA strains carrying chlorhexidine-resistant loci qacA/B genes.45 Thus, one must be cautious when generalizing the results of 2 percent chlorhexidine gluconate use that we observed in this study.

**Sodium hypochlorite.** Sodium hypochlorite solution has been used extensively in dentistry as a denture cleanser because, besides being bactericidal and fungicidal,16,17,20,47,48 it dissolves mucin and other organic substances.19 It is believed that its mechanism of action is related to a direct action of the solution on the organic matrix of the biofilm, causing dissolution of the polymer structure.49 Researchers have shown that planktonic cells of MSSA can be killed rapidly—within four minutes—by immersion in sodium hypochlorite at concentrations of 0.5 percent.16 In susceptibility tests of MRSA, sodium hypochlorite had a minimum inhibitory concentration of 0.03 percent.20 Nevertheless, S. aureus biofilms were less susceptible to this disinfectant than were their planktonic counterparts, so that higher concentrations or time of exposure is required to fully inactivate these microorganisms. In our study, 10 minutes of immersion in 1 percent sodium hypochlorite solution was effective for a short-term disinfection of dentures contaminated with MRSA.

![Image](http://jada.ada.org)

**TABLE**

Mean log₁₀ colony-forming units per milliliter (CFUs/mL) of methicillin-resistant *Staphylococcus aureus* on dentures and turbidity of disinfected dentures in broth beakers.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>LOG₁₀ CFUs/mL (SD*) AFTER 48 HOURS’ INCUBATION</th>
<th>TURBIDITY AFTER SEVEN DAYS’ INCUBATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>6.24 (0.24)</td>
<td>NA†</td>
</tr>
<tr>
<td>1% Sodium Hypochlorite Immersion</td>
<td>0.00 (0.00)</td>
<td>Positive</td>
</tr>
<tr>
<td>2% Chlorhexidine Gluconate Immersion</td>
<td>0.00 (0.00)</td>
<td>Negative</td>
</tr>
<tr>
<td>Microwave Irradiation</td>
<td>0.00 (0.00)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* SD: Standard deviation. † NA: Not applicable.

These results are consistent with data from another report in which some viable S. aureus cells remained—although in low numbers—on acrylic surfaces after immersion in 1 percent sodium hypochlorite for 10 minutes.17 The clinical relevance of this is that surviving MRSA cells have the ability to resuscitate the biofilm, sustain a source of contamination and, consequently, create a persistent infection.9,18 Thus,
higher concentrations probably would be needed to completely eliminate the possibility of regrowth of *S. aureus*. For MRSA biofilm inactivation, a lower immersion time (one minute) was highly effective when the concentration of sodium hypochlorite was increased to 2 percent.\(^20\) However, besides the fact that the long-term effectiveness of such a protocol has not yet been demonstrated, investigators observed a decrease in hardness and an increase in surface roughness after immersing acrylic materials in 2 percent sodium hypochlorite solution for five minutes.\(^41\) Thus, it could be of interest to evaluate whether immersing denture materials in 2 percent sodium hypochlorite for one minute has any detrimental effect.

**The use of disinfectant solutions versus microwave irradiation.** Despite being effective, the soaking of dentures in the disinfectant solutions used in our study has been discouraged because of their possible detrimental effects on the denture material.\(^{17,24,25,41,42,47,50}\) Chlorhexidine gluconate solution has been associated with discoloration of artificial teeth,\(^26\) and sodium hypochlorite has been shown to cause bleaching of denture acrylic resin, corroding of metal denture components,\(^{19,24}\) and alterations of the flexural strength\(^22\) and hardness\(^41,42\) of denture base resins. Other inconveniences of these chemical solutions are related to their side effects, which have implications for compliance. Besides its unpleasant taste, chlorhexidine gluconate solution may cause a brown staining of tissue and alter taste sensation in some patients.\(^51,52\) Furthermore, overuse of this antiseptic agent has resulted in the emergence of antiseptic-resistant strains of MRSA,\(^54\) which is a major disadvantage. The odor and aftertaste of sodium hypochlorite also are objectionable to many patients\(^53\) and, in a more concentrated solution, this agent is known to be cytotoxic.\(^54\) Thus, microwave irradiation may be an attractive alternative to the inconveniences and detrimental effects of these disinfectant solutions. Although we did not evaluate the properties of denture materials in this study, investigators already have demonstrated that the mechanical properties of acrylic resins are not detrimentally affected by microwave irradiation for three minutes at 650 W.\(^{29,32,33}\) The results of a 2007 study also showed that this microwave regimen produced no harmful effect in the adaptation of denture bases to the associated tissues.\(^55\) Moreover, given that microwave irradiation is a physical method of disinfection, its use could prevent the emergence of resistant microorganisms. Although all methods of disinfection we tested were effective in reducing MRSA colonization of the dentures, microwave disinfection may provide further advantages over disinfectant solutions.

**CONCLUSION**

The results of our study demonstrated that microwave irradiation and 2 percent chlorhexidine gluconate solution can provide long-lasting disinfection of complete dentures contaminated with MRSA. The protocols adopted in our study may be used in private dental offices and institutions or hospitals in which patients wearing dentures are treated, thus improving the longevity and quality of life of these patients and reducing the burden of disease caused by MRSA.

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