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Effectiveness of Sanitation with Quaternary Ammonium Compound or Chlorine on Stainless Steel and Other Domestic Food-Preparation Surfaces

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ABSTRACT

The relative ability of various materials used for domestic and/or food-service sinks and countertops to be sanitized was determined. Both smooth (unused) and abraded surfaces were tested by exposure to 200 mg of quaternary ammonium compound per liter or 200 mg of sodium hypochlorite per liter. Surface materials tested included mechanically polished (type 304, #4 finish) and electropolished stainless steel, polycarbonate, and mineral resin. Surfaces were prepared for testing by allowing attachment of a *Staphylococcus aureus* culture for 4 h to achieve an initial attached population of 10^4 to 10^5 CFU/cm^2. The test procedure involved immersion of the surface in sanitizer solution followed by wiping with a sanitizer-saturated cloth. Residual staphylococci were detected by overlaying agar directly on the treated surface. Results indicated that the stainless steels and the smooth polycarbonate, which had 0.5 log CFU/cm^2 or fewer of residual staphylococci, were more readily sanitized by quaternary ammonium compound than were either the mineral resin surfaces, which had nearly 2.0 log CFU/cm^2 of residual staphylococci, or the abraded polycarbonate which had nearly 1.0 log CFU/cm^2 of residual staphylococci. Chlorine was most effective on the mechanically polished stainless steel, the unabraded electropolished stainless steel, and the polycarbonate surfaces, reducing cell populations to less than 1.0 log CFU/cm^2. Chlorine was less effective on abraded electropolished stainless steel and mineral resin surfaces, where populations remained greater than 1.0 log CFU/cm^2. Sanitation with quaternary ammonium compound or chlorine reduced *S. aureus* populations more than 1,000-fold on all surfaces except unabraded mineral resin.

Key words: Quaternary ammonium compound, chlorine, stainless steel, *Staphylococcus aureus,* sanitizer, food-contact surface

Microorganisms attached to inert surfaces are less susceptible to the effect of chemical sanitizers than their free-living counterparts *(3, 7)*. Therefore, determination of sanitizer effectiveness should involve testing against adherent cells. Recent research using various materials including stainless steel, rubber, polyester and/or polyurethane and Teflon® demonstrated that the type of attachment surface influences sanitizer efficacy *(6, 11, 12)*. Results from these studies indicate that the effectiveness of sanitizing procedures must be determined using materials and methods as similar as possible to those found under conditions of actual use.

Holah and Thorpe *(5)* and Stevens and Holah *(13)* compared the cleanability of various domestic sink surfaces, including stainless steel, enameled stainless steel, mineral resin, and polycarbonate. They found that new materials were equally cleanable in regard to the ability of commercial dish-washing soap to remove attached microorganisms. However, abraded mineral resin and polycarbonate surfaces were more difficult to clean than abraded stainless steel. This result was attributed to the lower susceptibility of stainless steel to surface damage compared to mineral resin and polycarbonate. In this paper, we report the results of additional studies with an investigation comparing sanitation efficacy for new and abraded stainless steel, mineral resin, and polycarbonate surfaces using chlorine or quaternary ammonium compound sanitizers. We accomplished this objective by using a procedure that combines immersion in sanitizer solution and wiping with a sanitizer-saturated cloth-covered sponge.

MATERIALS AND METHODS

Surfaces

Surfaces tested in this study included stainless steel (type 304, mechanically polished to a #4 finish and electropolished), polycarbonate (Lexan, General Electric Structured Products, Mount Vernon, IN), and mineral resin (Ultrastone, UNR Home Products, Ruston, LA). Mineral resin was obtained from commercial sinks which were broken to obtain pieces of approximately 6 by 12 cm. The other surfaces were cut into pieces 7.5 by 11 cm. Surfaces were tested both new (smooth) and abraded. Abrasion was accomplished as described by Holah and Thorpe *(5)*, using 100- instead of 40-grit abrasive paper. New stainless-steel surfaces were cleaned first in acetone. All surfaces were scrubbed for 1 min with a 10 ml of cleaning solution (Micro™, International Products Corp, Burlington, VT) per liter of distilled water followed by two rinses in distilled water. All surfaces were sterilized by autoclaving before use.

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Microbial attachment

Staphylococcus aureus ATCC 12600 was grown in tryptic soy broth (TSB) at 35°C for 18 h. The test surfaces were submerged in 1.0 liter of this culture contained in a 1.5-liter beaker. Submersion for 4 h at 35°C was followed by rinsing the surface with sterile phosphate buffer (0.31 mM, pH 7.0) to remove unattached cells.

Surface-sanitation procedure

Sanitation procedures were designed to produce sufficient residual viable staphylococci to obtain a countable number of colonies, while simulating manual countertop cleaning. The procedure for quaternary ammonium compound (QAC) sanitization involved: (i) completely submerging the surface into a solution of 200 mg of *N*-alkyl dimethyl benzyl alkonium chloride per liter of distilled water (ZEP Manufacturing Co., Atlanta, GA) for 10 s at room temperature; (ii) wiping the surface for 5 s with a QAC-saturated weighted sponge (weighing 466 g with a 70 by 50 mm surface area) which was covered with a QAC-saturated fibrous paper towel (Super Pro wipes, Zep Manufacturing Co.); (iii) holding for 20 s at room temperature; (iv) neutralizing the QAC by submerging the surface in an aqueous solution of 5.3 g of lecithin (purified grade, Fisher Scientific Co., Fair Lawn, NJ), 37.5 g of Tween 80 (Fisher) and 0.5 g of K_2HPO_4 per liter (pH 7); and (v) rinsing with sterile phosphate buffer (0.31 mM, pH 7.0). The total time of exposure to QAC was 35 s. The procedure for chlorine sanitization was similar to that described for QAC treatment except that in step (i) submersion was for 20 s and in step (iv) neutralization was with 0.05% sodium thiosulfate-phosphate solution *(10)*. Chlorine sanitizer (200 mg/liter) was prepared from Fisher purified grade sodium hypochlorite (4 to 6%). Surfaces were exposed to chlorine sanitizer for a total of 45 s. Chlorine and QAC procedures differed in exposure time so that countable colonies would be obtained for each procedure. Controls included substituting sterile TSB for *S. aureus* culture during attachment, and substituting sterile phosphate buffer for sanitizer solution.

Determination of residual chlorine

Residual active chlorine in solution before and after treatment was determined by using a residual chlorine electrode (Model 97079, Orion, Boston, MA) calibrated as recommended by Orion in its *Residual Chlorine Electrode Instruction Manual.*

Analysis for attached staphylococci

Surviving staphylococci were determined by direct surface agar plating, a modification of the procedure of Angelotti and Foter *(1)*. Treated surfaces were allowed to dry for 1 h at room temperature. A 2 by 2 cm area (for mineral resin surfaces) or a 5.5 by 9.1 cm area (for all other surfaces) was coated with sterile plate count agar supplemented with 0.07% potassium tellurite solution (Difco Laboratories, Detroit, MI). The coated surfaces were incubated in sterile petri dishes for 48 h at 35°C. Black colonies were enumerated. This method was not used when surfaces were treated with buffer or when determining initial attachment levels, since in these cases too many colonies were present for enumeration via direct CFU enumeration. Rather, cells on these surfaces were fixed by submersion in 70% ethanol, and were then stained for direct epifluorescence microscopic enumeration *(4)*. Mineral resin was stained using a solution of 0.1 mg of acridine orange per ml (EM Science, Cherry Hill, NJ) and all other surfaces were stained using a solution of 50 mg of Hoescht 33258 (Aldrich Chemical Co, Milwaukee, WI) per ml. Hoescht stain produced superior contrast between cells and background on all surfaces except mineral resin, for which acridine orange was best. Stained surfaces were viewed with an epifluorescence microscope using a 405-nm excitation filter and a 515-nm emission filter. Cells were counted in a minimum of 10 fields per surface; more fields were counted when cell numbers were low. Mean cell numbers per field were converted to cell number per square centimeter.

Statistical analysis

Data were analyzed using the general linear models procedures of the Statistical Analysis System (SAS Institute, Cary, NC). A significance level of $P \leq 0.05$ was employed. Duncan's multiple range test was used to determine differences among means when significant effects were observed. All experiments were replicated five times.

RESULTS

S. aureus attached equally (no statistically significant differences) to all surfaces in the range of 10^4 to 10^5 CFU/cm^2. Abrading the surfaces did not consistently increase cell attachment. Figure 1 shows data on the presence of *S. aureus* on the surfaces after they were wiped with buffer. All surfaces demonstrated at least a 0.5-log unit reduction in the level of *S. aureus* after wiping with buffer only. The greatest reduction was observed for the unabraded electropolished stainless steel. Wiping with buffer achieved a 2-log CFU/cm^2 reduction on the electropolished stainless steel; however, this value decreased to a 1-log CFU/cm^2 reduction if the surface was abraded. However, abrasion made no significant difference in the ability of wiping with buffer to remove cells from any of the other surfaces.

The level of *S. aureus* remaining on each surface after treatment with QAC is presented in Figure 2. Abraded and smooth mineral resin surfaces had significantly more residual viable cells than did the other surfaces, with nearly 100 CFU/cm^2 remaining on these surfaces after sanitizing. Abraded polycarbonate also had significantly more residual staphylococci than did the stainless steels and the smooth polycarbonate. The abraded electropolished stainless steel

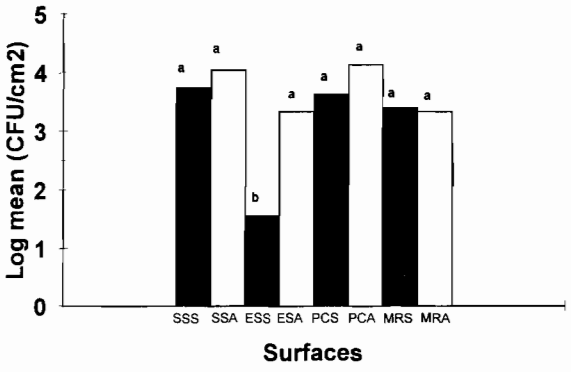

FIGURE 1. Staphylococcus aureus *(log CFU/cm^2) remaining on various surfaces after wiping with sterile phosphate buffer. SSS, mechanically polished stainless steel; SSA, abraded mechanically polished stainless steel; ESS, electropolished stainless steel; ESA, abraded electropolished stainless steel; PCS, polycarbonate; PCA, abraded polycarbonate; MRS, mineral resin; MRA, abraded mineral resin. Bars marked with the same letter represent data that are not significantly different (P > 0.05).*

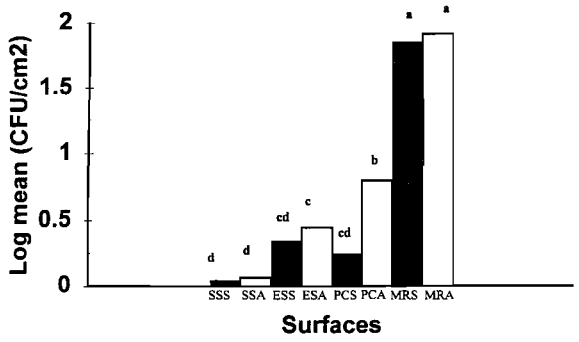

FIGURE 2. Staphylococcus aureus *(log CFU/cm²) remaining on various surfaces after treatment with quaternary ammonium compound. SSS, mechanically polished stainless steel; SSA, abraded mechanically polished stainless steel: ESS, electropolished stainless steel; ESA, abraded electropolished stainless steel: PCS, polycarbonate; PCA, abraded polycarbonate; MRS, mineral resin; MRA, abraded mineral resin. Bars marked with the same letter represent data that are not significantly different (P > 0.05).*

actually had more residual viable cells per unit area than did the mechanically polished stainless steels, but levels were low on all these surfaces. Abrasion significantly reduced the ability of polycarbonate to be sanitized by QAC, but had no significant effect on the ability of the other surfaces to be sanitized.

If the number of staphylococci remaining after sanitizer treatment (Figure 2) is subtracted from the number of staphylococci remaining after treatment with buffer (Figure 1), an estimate of the amount of cell inactivation due to only sanitizer (as opposed to sanitizer inactivation combined with physical removal) is obtained. These data are presented in Figure 3A. These corrected data confirm that QAC is least effective on smooth and abraded mineral resin. This calculation also shows a low log-unit reduction for the electropolished stainless steel, but this low level is due to the relatively large reduction which resulted from the buffer treatment alone (Figure 1). Figure 3B presents data showing the total reduction in cell numbers obtained after applying QAC treatment to each surface (obtained by subtracting residual levels from levels of initial attachment). These data represent cell decreases due to both physical removal (as was determined by buffer treatment) and chemical inactivation. Mechanically polished stainless steel exhibited a reduction of 10^5 log CFU/cm², which is slightly higher than that on the electropolished stainless steel. Smooth polycarbonate showed reductions in populations similar to the stainless steels. Mineral resin (smooth and abraded) and abraded polycarbonate exhibited reductions of only 100- to 1,000-fold, significantly less than reductions achieved with mechanically polished stainless steel. Smooth electropolished stainless steel exhibited a greater reduction of staphylococci than did mechanically polished stainless steel. This may be due to the lower initial attachment of staphylococci to the electropolished surface, since there was no significant difference between residual levels of *S. aureus* on unabraded electropolished stainless steel and the mechanically polished surface (Figure 2).

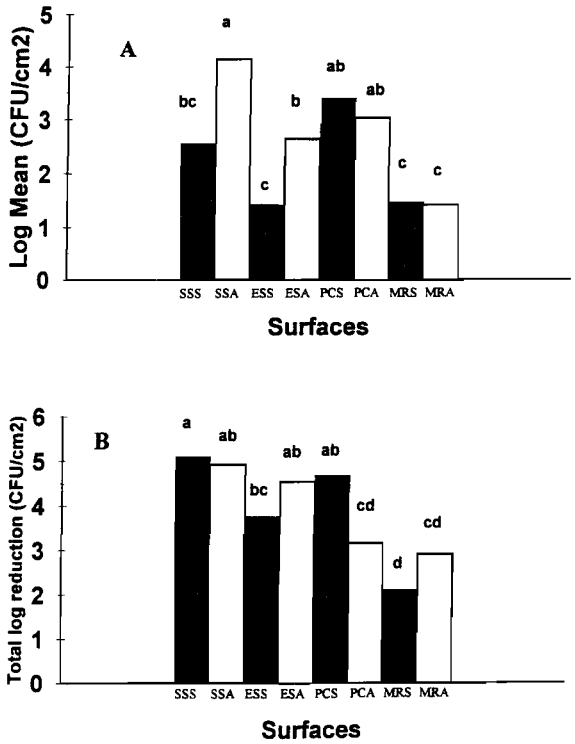

FIGURE 3. *A. Log-unit reduction in* Staphylococcus aureus *(CFU/cm²) on various surfaces due to treatment with quaternary ammonium compound not including reduction accounted for by treatment with phosphate buffer. B. Total log-unit reduction in* Staphylococcus aureus *(CFU/cm²) on various surfaces due to treatment with quaternary ammonium compound. SSS, mechanically polished stainless steel; SSA, abraded mechanically polished stainless steel; ESS, electropolished stainless steel; ESA, abraded electropolished stainless steel; PCS, polycarbonate; PCA, abraded polycarbonate; MRS, mineral resin; MRA, abraded mineral resin. Bars marked with the same letter represent data that are not significantly different (P > 0.05).*

Data showing the number of *S. aureus* remaining on each surface after treatment with chlorine are presented in Figure 4. Abraded electropolished stainless steel and the mineral resin (smooth and abraded) had significantly higher levels of residual viable cells after chlorine treatment (all > 10 CFU/cm²) than the other surfaces (all < 10 CFU/cm²). Data showing the reduction in numbers due to sanitizer inactivation exclusive of that reduced by buffer treatment (Figure 5A) indicate that chlorine produced the highest reduction on the mechanically polished stainless steels and the polycarbonate. Data in Figure 5B show the overall reduction of *S. aureus*. These data indicate that only the smooth mineral resin has a significantly reduced ability to be sanitized when compared to the unabraded mechanically polished stainless steel and unabraded polycarbonate.

There were no significant reductions in residual active chlorine during the surface treatments (data not shown), indicating that excess active chlorine was available for cell inactivation throughout the whole exposure time.

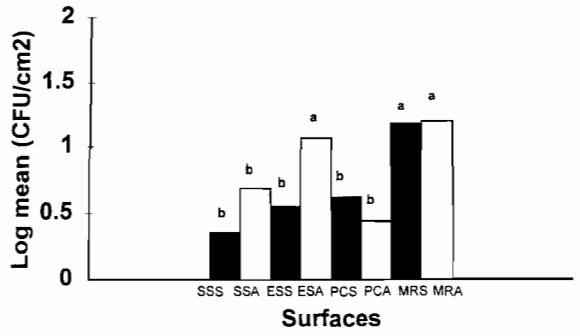

FIGURE 4. Staphylococcus aureus *(log CFU/cm²) remaining on various surfaces after treatment with chlorine. SSS, mechanically polished stainless steel; SSA, abraded mechanically polished stainless steel; ESS, electropolished stainless steel; ESA, abraded electropolished stainless steel; PCS, polycarbonate; PCA, abraded polycarbonate; MRS, mineral resin; MRA, abraded mineral resin. Bars marked with the same letter represent data that are not significantly different* ($P > 0.05$).

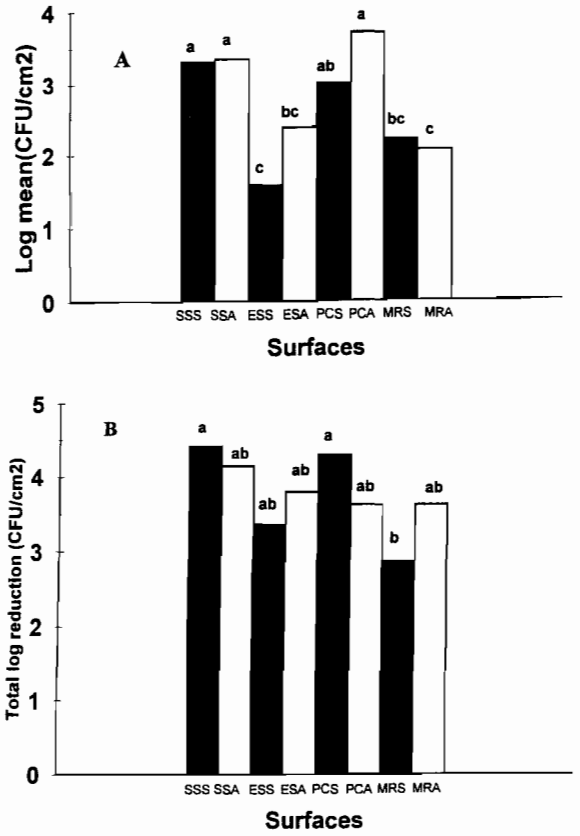

FIGURE 5. *A. Log-unit reduction in* Staphylococcus aureus *(CFU/cm²) on various surfaces due to treatment with chlorine not including reduction accounted for by treatment with phosphate buffer. B. Total log-unit reduction in* Staphylococcus aureus *on various surfaces due to treatment with chlorine. SSS, mechanically polished stainless steel; SSA, abraded mechanically polished stainless steel; ESS, electropolished stainless steel; ESA, abraded electropolished stainless steel; PCS, polycarbonate; PCA, abraded polycarbonate; MRS, mineral resin; MRA, abraded mineral resin. Bars marked with the same letter represent data that are not significantly different* ($P > 0.05$).

Surface characteristics and sanitizer efficacy

Scanning electron micrographs of new and abraded mechanically polished stainless steel, polycarbonate, and mineral resin have been published by Holah and Thorpe *(5)*. They reported that abraded mineral resin appears to be the most porous of these surfaces, and unabraded polycarbonate the most smooth. Polycarbonate and mineral resin surfaces are more dramatically affected by abrasion than is stainless steel. If surface roughness was an important factor in determining sanitizer efficacy, then one would expect that the abraded mineral resin and polycarbonate would maintain greater levels of residual staphylococci after sanitizer treatment than their smooth counterparts. However, this was the case only for the polycarbonate when sanitized with QAC. Evidence that surface roughness of stainless steel may slightly decrease sanitizer efficacy is found when comparing the number of residual cells on the smooth electropolished surface with the number of cells on the abraded electropolished surface after chlorine treatment. The observed decrease was about 0.5 log cycle, a difference that may not have practical significance.

DISCUSSION

Mosteller and Bishop *(11)* proposed that a 3-log unit reduction in population of surface-adherent cells is a reasonable goal for effective sanitation. Using this criterion, only the nonabraded mineral resin was not effectively sanitized by the procedure we employed. QAC treatment achieved 5-log unit reductions in staphylococcal populations on three of the four stainless-steel surfaces and the smooth polycarbonate. Results of this study further demonstrate that materials have inherently different abilities to be sanitized. Krysinski et al. *(6)* arrived at the same conclusion studying inactivation of *Listeria monocytogenes* attached to stainless steel and polyester and/or polyurethane. Sanitation effectiveness for a given surface is not simply a result of surface roughness or porosity. For example, abrasion appears to dramatically increase the porosity of the mineral resin by removing the smooth polymer coating *(5)*; however, abrasion was not associated with a decreased ability to sanitize this surface. The surface finish of stainless steel appears to influence sanitizer efficacy. It is not apparent why abraded electropolished stainless steel maintained higher residual levels of viable cells after QAC and chlorine treatments than did mechanically polished stainless steel. In contrast, abrasion of the mechanically polished stainless steel had no adverse effect on sanitizer efficacy. Additional research on the relationship between surface roughness and stainless-steel cleaning and sanitizing is needed to clarify these observations.

The 4-h cell attachment period used in this study was selected to allow attachment but to limit microcolony development. Surface microcolonies are associated with increased resistance to chlorine *(9)* and QAC *(2)*. Chlorine has only limited ability to penetrate glycocalyx material surrounding biofilm cells *(2)*.

Studies of Holah and Thorpe *(5)* and Stevens and Holah *(13)* indicate that abrasion significantly reduces the ability of mineral resin and polycarbonate surfaces to be cleaned.

Leclercq-Perlat and Lalande *(8)* observed that ease of soil removal during cleaning is related to the surface finish of stainless steel, with a rougher finish being more difficult to clean. Although our results indicate that abrasion (and therefore increasing surface roughness) does not always reduce the effectiveness of sanitizing on clean surfaces, if adherent soil is not removed, microbial survival could be enhanced. Since effective sanitation is dependent on effective cleaning, surface roughness and resistance to abrasion may be more important characteristics for sanitary design than the data reported here indicate. Therefore, the conclusion reached by Holah and Thorpe *(5)* that the inherent resistance to surface damage exhibited by stainless steel makes it a superior material when effective cleaning and sanitizing are a high priority during its use remains valid.

ACKNOWLEDGMENTS

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