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Efficacy of an Enzyme-based Floor Cleaner Containing N, N-bis (3-aminopropyl) Laurylamine against Foodborne Pathogens on Four Flooring Types Used in Foodservice Environments

ABSTRACT

The importance of contamination on non-food contact surfaces and the potential of transfer to food and food contact surfaces are often underestimated, although several studies have demonstrated the prevalence of foodborne pathogens on floors, floor drains, sinks, and milk crates and in dairy cases. In this study we investigated the antimicrobial efficacy of an enzyme-based floor cleaner with added sanitizer against spoilage and foodborne pathogen microorganisms on four different flooring types commonly used in food service and retail environments. After five minutes exposure time, all the organisms tested were reduced by 3 log CFU/cm^2 or greater. The two most resistant microorganisms, *Staphylococcus aureus* and *Enterobacter aerogenes*, were selected for further study in which the influence of microbial attachment time and surface morphology was investigated. Results showed that increased drying time on floor tiles did not influence antimicrobial activity performance of the solution; microscopy analysis indicated that on non-uniform surfaces, microorganisms were able to harbor in surface defects, resulting in decreased exposure to the antimicrobial solution. The results obtained in this research demonstrated the significance of floor surface characteristics and morphology on the effectiveness of the cleaning and sanitizing process as well as the importance of choosing the appropriate floor material for retail and food service environments.

INTRODUCTION

Cross-contamination, improper cleaning and sanitation, and incorrect time and temperature control for cooking and holding food are key risk factors contributing to contamination with, and spread and growth of, foodborne pathogens in food processing and/or food retail environments *(8, 10)*. Knowledge of the distribution, behavior,

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and transmission of foodborne pathogens in retail and food service environments is limited *(1, 6, 11)*. Park *(9)* observed bacteria harborage on non-food contact surfaces such as ceramic tiles, stainless steel, and glassware. The prevalence and distribution of *Listeria monocytogenes* in retail establishments has been investigated; improper hand washing and glove wearing, contact with contaminated equipment and utensils, and inadequate cleaning procedures were identified as the main contributors to contamination *(4, 5, 6)*. In this work, over 120 New York State retail deli establishments were included in a cross-sectional study that revealed a significantly greater presence of *L. monocytogenes* on non-food contact surfaces than on food contact surfaces. Prevalence was particularly high on floors, floor drains, sinks, dairy cases and milk crates. Three percent of 183 samples collected from slicers and 2% of 314 samples from bowls and/or cutting boards were *L. monocytogenes* positive. In contrast, 20% of the samples collected from non-food contact surfaces, e.g., floors and floor drains, tested positive *(5)*. These surfaces are generally harder to clean than food contact areas. Food service operations are usually less controlled than food processing environments and have fewer intervention opportunities in place *(6, 12)*. The control of pathogens on food contact surfaces and the prevention of cross-contamination of ready to eat (RTE) foods in retail and food-service environments are critical in the prevention of foodborne illness. However, the public health importance of contamination on non-food contact surfaces and the potential for transfer of food and food contact surfaces are less well studied and are likely to be underestimated as sources of foodborne illness outbreaks *(5, 6)*.

Approximately a decade ago, the use of enzyme-based floor cleaners was introduced into food service and retail environments as a means of addressing cleaning issues, particularly those associated with grease soils on floors *(3, 6)*. These solutions provide improved cleaning but do not provide antimicrobial activity against foodborne pathogens. To address this need, an antimicrobial agent was added to an enzyme-based floor cleaner and investigated for efficacy against different spoilage and foodborne microorganisms on four floor types commonly found in retail and food service environments. In addition, the influence of microbial attachment time and surface morphology on the efficacy of this cleaner was determined.

MATERIALS AND METHODS

Microorganisms

Escherichia coli ATCC 11229, *Escherichia coli* O157:H7 ATCC 43895, *Staphylococcus aureus* ATCC 6538, *Enterobacter aerogenes* ATCC 13048, *Salmonella enterica* ser. Typhimurium ATCC 13311 and *Listeria monocytogenes* ATCC 49594 were used in this study. Cultures of *Escherichia coli* ATCC 11229, *E. coli* O157:H7 ATCC 43895, *S. aureus* ATCC 6538, and *E. aerogenes* ATCC 13048 were grown overnight in Nutrient Broth (Difco Laboratories, Spark, MD, USA) at 35°C; *S.* Typhimurium and *L. monocytogenes* were cultured in BHI broth (Brain Heart Infusion, Difco Laboratories, Spark, MD, USA). Cultures were transferred at least twice to reach a pre-determined initial population of 1.0×10^8 CFU/mL. The suspensions were then centrifuged for 10 min at 6149 × g (Sorvall Centrifuge, Thermo Scientific-Griesheim, Germany); the supernatant was decanted and the pellet was re-suspended in an equal volume of sterile phosphate buffer (pH 7). Five percent (5%) fetal bovine serum (Sigma-Aldrich, St Louis, MO, USA) was added to mimic soil load.

Floor selection and active solution preparation

Four surfaces commonly used in foodservice and retail kitchens were selected for these studies: quarry tile, poured epoxy tiles, poured methyl methacrylate (MMA) tiles, and sealed concrete tiles. A lipase floor cleaner containing the active ingredient N, N-bis (3-aminopropyl) laurylamine (antimicrobial solution) was used (Sanitizing Wash 'n Walk; Ecolab, St. Paul, MN, USA). The antimicrobial solution was prepared according to manufacturer's instructions, diluted to 1.38% in laboratory purified water, and applied at a rate of 0.02 ml/cm^2 for a total of 5 ml per tile. The final concentration of N, N-bis (3-aminopropyl) laurylamine in the solution used was 309 ppm.

Solution application and bacteria recovery

Common practice with an alkaline or neutral floor cleaner involves diluting the cleaner in hot water, applying the diluted solution to the floor using a mop and bucket, and rinsing the floor with clean water. In contrast, cleaning procedures for an enzyme-based floor cleaner typically involve diluting it in cold water, sloshing or slop mopping the diluted solution onto a floor, scrubbing the floor with a stiff bristled brush, and using a squeegee to remove excess or pooled cleaning solution to a floor drain.

In this study, the antimicrobial activity of the enzyme-based floor cleaner was evaluated without any additional mechanical action. Therefore, neither the brushing nor the squeegeeing step was performed.

Ceramic quarry tiles were spot-inoculated with 10 drops (10 µl each) of bacterial solution (population ~ 1.0×10^6 CFU/mL) and allowed to dry at room temperature for 15 min. The antimicrobial solution was then spread over the entire tile surface (225 cm^2). After 5 minutes, inoculated surfaces were swabbed with a sterile sponge, that had previously been soaked in 20 ml of Dey-Engley broth (Difco Laboratories, Spark, MD, USA). Decimal dilutions were plated on selective media, and surviving populations were enumerated. MacConkey Sorbitol Agar (Difco Laboratories, Spark, MD, USA) was used for *E. coli* and *E. coli* O157:H7; Mannitol Salt Agar (Difco Laboratories, Spark, MD, USA) for *S. aureus*; Eosin-Methylene Blue Agar (Difco Laboratories, Spark, MD, USA) for *E. aerogenes; Salmonella Shigella* Agar

(Difco Laboratories, Spark, MD, USA) for *S.* Typhimurium, and Modified Oxford Media (Difco Laboratories, Spark, MD, USA) for *L. monocytogenes*.

Extended attachment time experiment

The two microorganisms that showed the lowest log reduction were selected for further studies, and the influence of microbial attachment time and surface morphology was investigated. Microorganism attachment time was increased in order to simulate a worst case scenario of poor cleaning practices. After 4, 8, and 12 h culture drying times on tiles, the antimicrobial solution was applied as previously described. After a five-minute dwell time, the remaining population was enumerated following the protocol already described. Contaminated, but untreated, floor surfaces were used as controls.

Scanning electron microscopy analysis

The morphology of the different floor surfaces and possible interaction with *S. aureus* and *E. aerogenes* were investigated by scanning electron microscopy (SEM). Floor tiles were cut into 25 cm^2 pieces and inoculated. After 4 or 8 h of bacterial attachment, the samples were exposed to the antimicrobial solution for 5 min (using the protocol described in the "Solution application and bacterial recovery" section). Samples were mounted on specimen stubs and coated with a thin layer of gold using a Polaron E5100 sputter coater (Polaron Instruments, East Sussex, UK). The samples were examined with a Hitachi S-3400N scanning electron microscope (Hitachi High-Tech Inc., Northridge, CA, USA) at an accelerating voltage of 7 kV. Micrographs were collected at a dwell time of 20 s. Untreated samples (CONTROL) were used for comparison purposes.

Statistical analysis

Experiments were run in triplicate for each microorganism and floor type. Control samples were included in the experimental design, with lab purified water used in lieu of the antimicrobial solution. During each test, two tiles were used as controls and four tiles were used for treatment. The remaining CFU/cm^2 and the log CFU/cm^2 reduction were determined. Results were presented as mean ± SD. ANOVA was performed and Tukey's test, implemented in Minitab 15 (Minitab Inc., State College, PA), was used to differentiate between treatments (with statistical significance set at $P < 0.05$).

RESULTS

Antimicrobial activity on different floor tiles

A minimum of 3.03 ± 0.30 log CFU/cm^2 reduction was observed after application of the antimicrobial solution (5 minute exposure) on floor tiles previously inoculated with bacteria. As shown in *Table 1*, log reductions differed depending on the microorganism and floor surface tested. The largest log reductions were obtained on quarry tile substrates where an average reduction of 4 log CFU/cm^2 was observed for all the bacteria tested. The log reduction observed on MMA tiles ranged from a minimum of 3 log CFU/cm^2 with *E. aerogenes* to a maximum of 4.5 log CFU/cm^2 with *L. monocytogenes*. Similar results were observed for sealed concrete and epoxy tiles. Overall, the data show

TABLE 1. Log reduction (means ± standard deviation) observed on quarry, MMA, sealed concrete and epoxy tiles for the six microorganisms tested

Microorganisms	LOG REDUCTION (Log CFU/cm^2)			
	QUARRY	MMA	SEALED CONCRETE	EPOXY
E. coli O157	4.44 ± 0.11^{A}	4.23 ± 0.09^{A}	$3.86 \pm 0.13^{A*}$	$3.03 \pm 0.30^{A*}$
L. monocytogenes	4.33 ± 0.15^{A}	4.47 ± 0.06^{A}	4.55 ± 0.20^{B}	4.13 ± 0.22^{B}
E. coli	4.29 ± 0.17^{A}	4.25 ± 0.15^{A}	$3.00 \pm 0.10^{A*}$	$3.46 \pm 0.34^{C*}$
S. Typhimurium	4.16 ± 0.13^{A}	4.36 ± 0.09^{A}	4.55 ± 0.12^{B}	$3.58 \pm 0.08^{C*}$
S. aureus	3.80 ± 0.19^{AB}	3.82 ± 0.06^{AB}	$4.19 \pm 0.12^{C*}$	3.64 ± 0.17^{C}
E. aerogenes	$3.80 \pm 0.17^{B*}$	3.07 ± 0.10^{B}	3.09 ± 0.22^{D}	3.21 ± 0.23^{C}

Values (means ± SD) with different letters in the same column and/or with asterisk in the same row are significantly different ($P < 0.05$).

that on flooring surfaces commonly found in foodservice and retail kitchens, the sanitizing floor cleaner tested reduced bacterial numbers by at least ≥ 3 log CFU/cm^2 for all organisms tested. These results met the requirement described in the "Product performance test guidelines for sanitizer used on hard surfaces," in which 99.9% reduction within 5 min is the criterion for success (2). *S. aureus* and *E. aerogenes* were the least sensitive bacteria to the action of the antimicrobial solution; therefore, these strains were selected for further experiments.

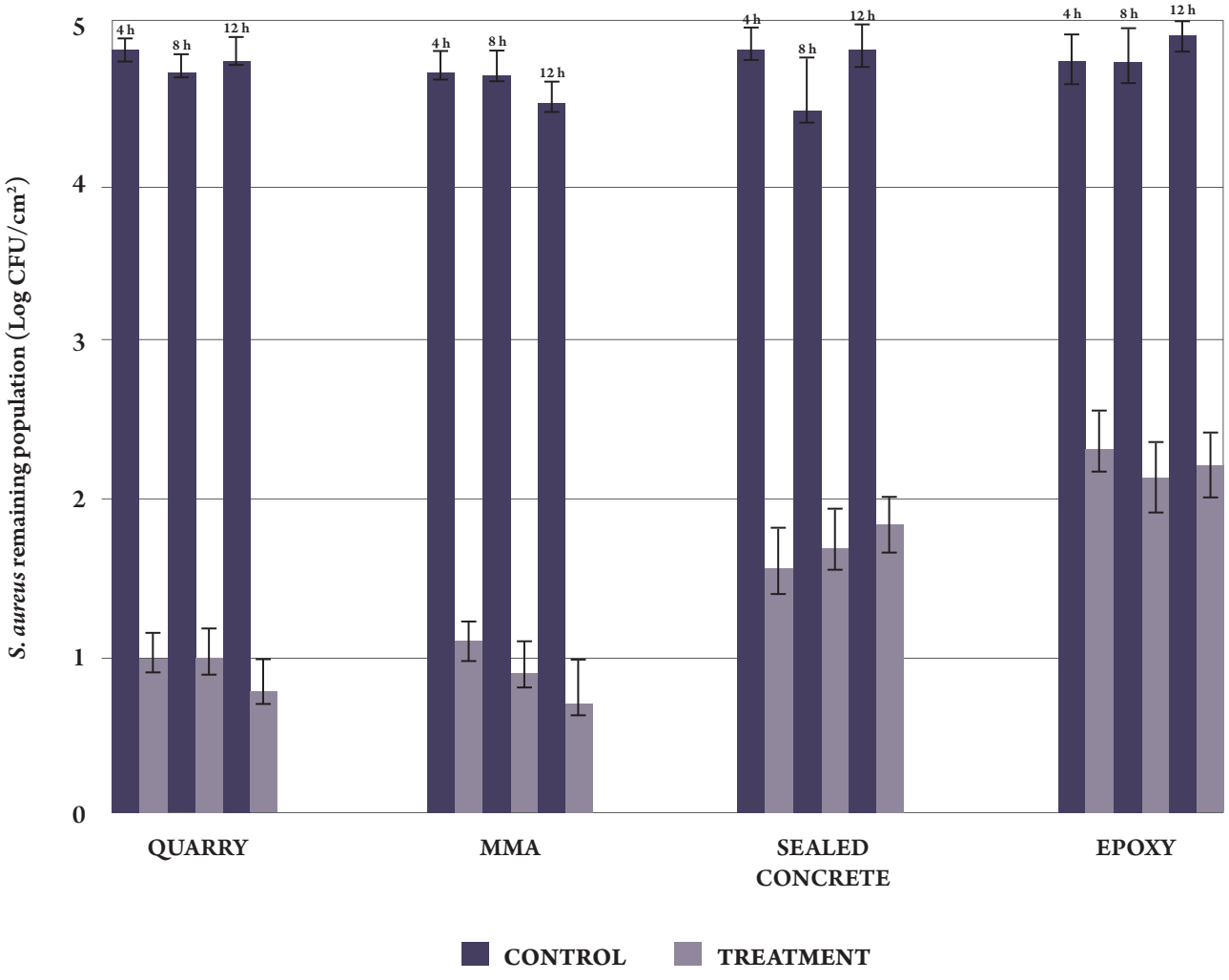
Effects of an extended attachment time

Remaining populations of *S. aureus* and *E. aerogenes* after attachment times of 4, 8, and 12 h are shown in *Fig. 1* and 2, respectively. On quarry tiles, *S aureus* reductions of 3.76 ± 0.14, 3.62 ± 0.17, and 3.78 ± 0.11 log CFU/cm^2 were found at 4, 8, and 12 hours, respectively, following 5 minutes exposure to the antimicrobial solution. When the attachment time was increased, no difference was observed among remaining populations on the same flooring type ($P > 0.05$). MMA, sealed concrete and epoxy tiles inoculated with either *S. aureus* or *E. aerogenes* gave similar results, with no differences seen in antimicrobial efficacy of the solution ($P > 0.05$). These data indicate that the parameter "bacterial attachment time" does not significantly affect the efficacy of the cleaning and sanitizing process under the conditions tested.

Interaction between floor and microbial cells

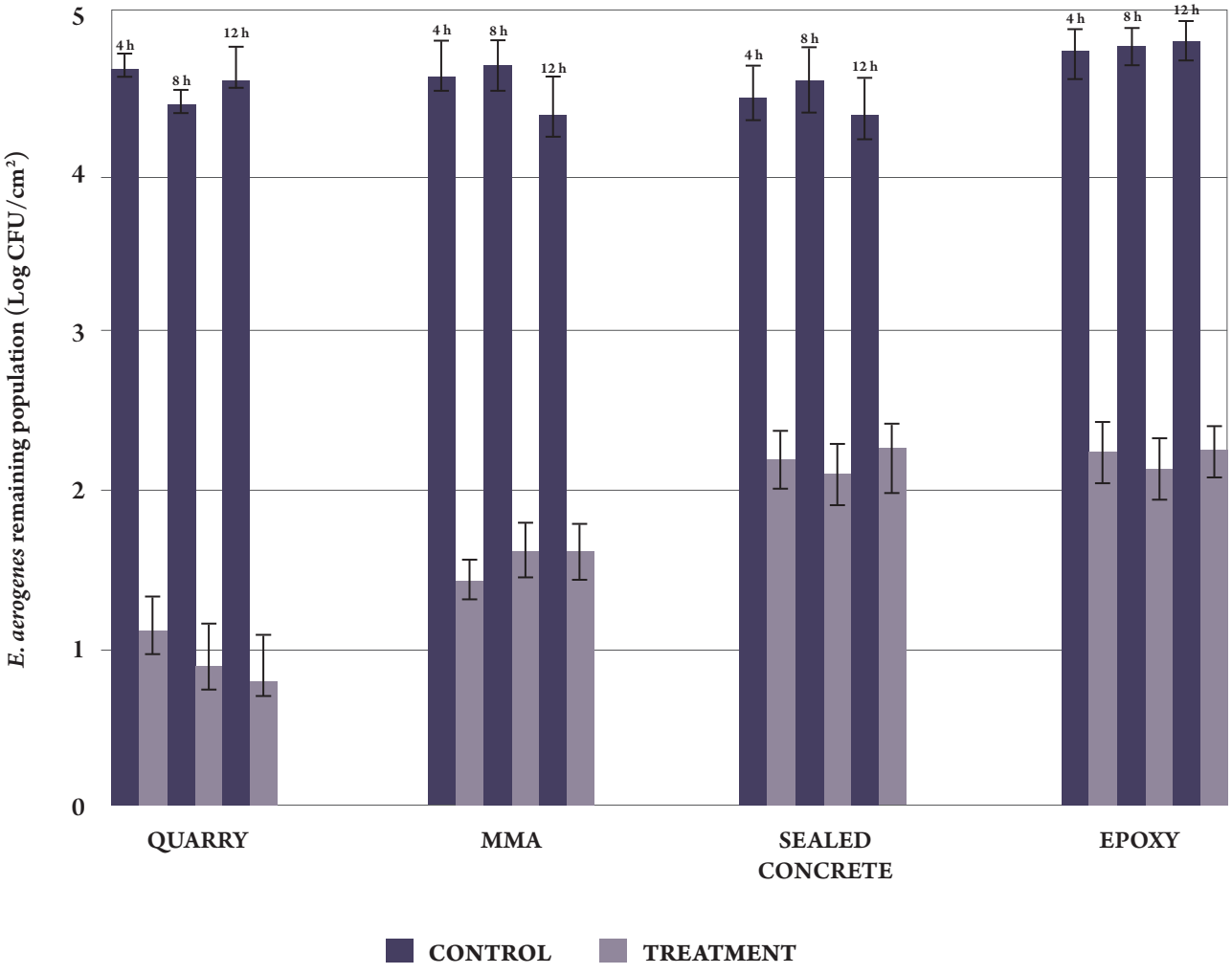
SEM images of *S. aureus* and *E. aerogenes* on quarry, MMA, sealed concrete and epoxy tiles after 4 and 8 h

FIGURE 1. Remaining population of S. aureus *at attachment times of 4, 8 and 12 h; microbial counts on control (no treatment) and treated tiles for 5 minutes*



Detection limit of the test was 0.5 log CFU/cm^2. No significant differences ($P < 0.05$) among treatment samples of the same floor type were observed.

FIGURE 2. Remaining population of **E. aerogenes** *at attachment times of 4, 8 and 12 h; microbial counts on control (no treatment) and treated tiles for 5 minutes are reported for each floor type tested*



Detection limit of the test was 0.5 log CFU/cm². No significant differences ($P < 0.05$) among treatment samples of the same floor type were observed.

bacterial attachment time are shown in *Fig. 3* and *4*, respectively. Bacterial populations were uniformly distributed on control surfaces. SEM analysis of these surfaces following exposure to the test floor cleaner confirmed the results reported previously by plate enumeration *(Figures 1 and 2)*. Remaining populations on quarry and MMA tiles were significantly reduced ($P < 0.05$) after treatment, and reductions were independent of attachment duration. As shown in the images, fewer microorganisms were present on the tile after treatment than on the control tiles. Less microbial reduction was observed on sealed concrete and epoxy tile substrates. In fact, as shown by the SEM images, non-uniform surfaces such as sealed concrete and epoxy tiles microorganisms were able to harbor bacteria in surface defects. Therefore, higher microbial counts were observed on these surfaces than on the surfaces of the more uniform materials (e.g., quarry and MMA).

DISCUSSION

Identifying the vectors that contribute to contamination in retail and food service environments and tracking the potential microbial path is essential for reducing risk of cross- contamination. Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) are key programs that address food safety concerns, however validated control measures are limited *(6, 11)*. The possibility of cross-contamination from floor to food has been investigated, with floor drains being identified as additional places where bacteria can survive and grow *(7, 8)*. The same research reported that habits such as failing to change gloves after

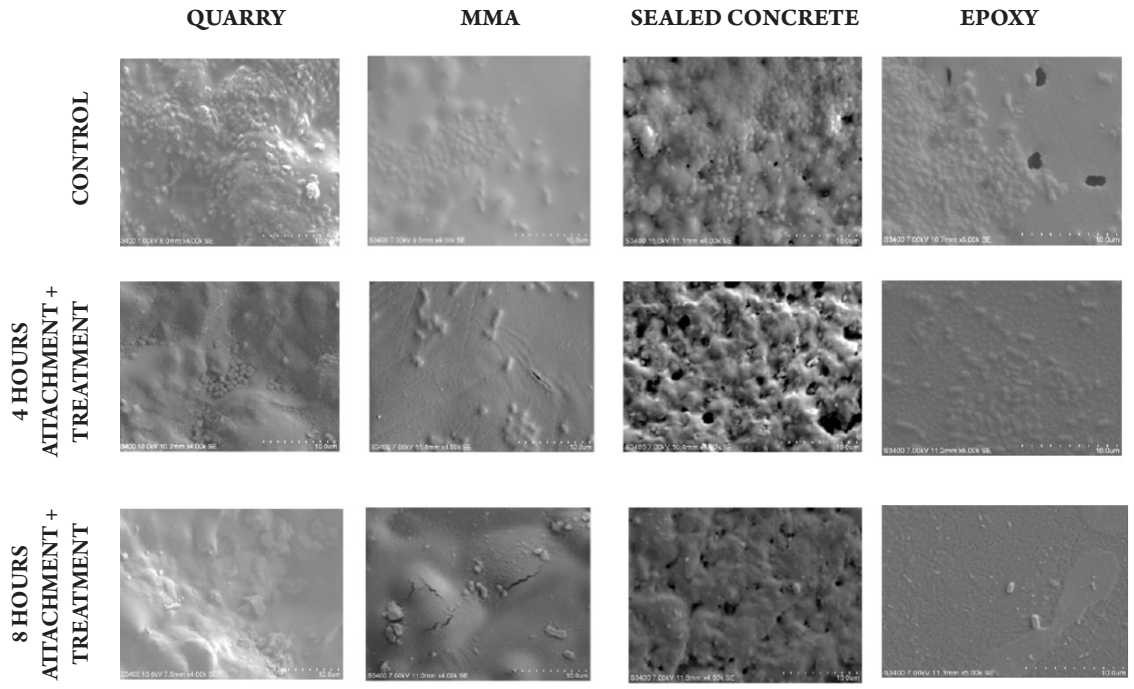


FIGURE 3. SEM images of **S. aureus** *on Quarry, MMA, Sealed Concrete and Epoxy tiles untreated (CONTROL) and treated after attachment times of 4 and 8 h.*

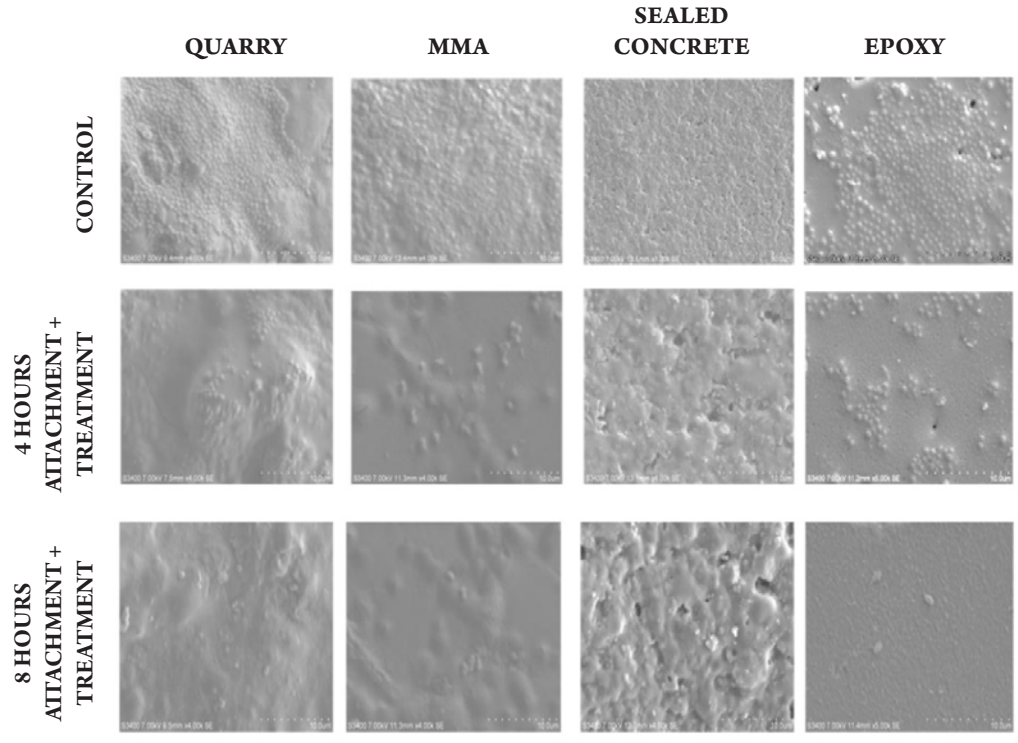


FIGURE 4. SEM images of **E. aerogenes** *on Quarry, MMA, Sealed Concrete and Epoxy tiles untreated (CONTROL) and treated after attachment times of 4 and 8 h.*

picking items up off the floors or using hands to crouch down to get items out of the cooler or from lower shelf, were all possible contamination pathways from floor to food contact surfaces *(8, 11)*.

The present research indicates that an enzyme-based floor cleaner with added sanitizer may be effective for control of foodborne pathogens and spoilage microorganisms on floor surfaces typically used in foodservice environments. Significant microbial reductions were observed against all the microorganisms tested and on all the floor tiles analyzed.

CONCLUSIONS

Results obtained in this study demonstrate the efficacy of an antimicrobial solution against foodborne pathogens and spoilage microorganisms on different floor surfaces. On flooring commonly found in foodservice and retail kitchens, the sanitizing floor cleaner reduced all organisms tested by at least 3 log CFU/cm^2. Organisms least affected by the test treatment were *S. aureus* and *E. aerogenes*. Prolonged attachment time on floor tiles was used to mimic conditions where there could be prolonged time between contamination events and cleaning and sanitizing. Enumeration of bacteria and microscopy analysis demonstrated the significance of the characteristics and morphology of floor surfaces on the effectiveness of the cleaning and sanitizing process, and consequently the importance of choosing the appropriate floor material for retail and food service environments. Further experiments are necessary to expand the investigation to other types of floor materials and other types of enzyme-based products.

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