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Influence of Possible Disinfectant Transfer on *Staphylococcus aureus* Plate Counts After Agar Contact Sampling

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Asbestos-vinyl tile floor panels were mopped with three types of chemical disinfectant product, as well as after contact with the untreated control panel. were prepared according to the manufacturer's label instructions. Similar floor panels were inoculated artificially with *Staphylococcus aureus*. RODAC (replicate organism detection and counting) surface sampling plates were pressed to the disinfectant-treated panels or to the untreated control panel and then immediately pressed to sampling sites on the artificially inoculated floor panels. Plate counts were determined after contact with panels treated with each type of disinfectant, product, as well as after contact with the untreated control panel. Results indicate that disinfectant residues on environmental surfaces do not alter the average plate counts obtained by RODAC samplings.

Detection and enumeration of microorganisms on environmental surfaces have been the subject of many investigations by microbiologists in the public health field. These investigations were prompted by circumstantial evidence linking eating utensils to the spread of bacterial and viral infections (13) and by interest in microbial contamination of the inanimate environment of patient care facilities. As a result of these investigations, numerous techniques and methodologies were developed to accurately measure microbial populations of hard surfaces. Various approaches were suggested in an attempt to solve the major problems of simplicity, accuracy, and reproducibility, as well as economic feasibility relative to both personnel time and monetary investment.

Historically, three basic approaches to environmental sampling have been investigated: swab techniques, total rinse methods, and agar contact methods. Each of the above techniques has its own relative advantages and disadvantages, which are discussed at great length in a paper by Fincher (15). The development of a successful agar contact method was first reported in 1941 by Walter and Hucker (26). Further work with this method by Angelotti and Foter (5) showed that the method was capable of accurate quantitative detection of bacteria on surfaces. A study published concurrently by Angelotti et al. (6) indicated that agar contact methods yielded greater precision than swab rinse methods on surfaces with relatively low microbial densities.

The disposable RODAC (replicate organism detection and counting) plate reported by Rohde in 1963 (22) was utilized by Hall and Hartnett (17) in the development of the RODAC sampling method. This method was used extensively by the Committee on Microbial Contamination of Surfaces (4) to estimate the microbial populations of surfaces in hospital areas. An advantage of the RODAC plate is that it may be prepared and stored for weeks prior to use. A study by Bond et al. (9) on the shelf life of prepared RODAC plates detected no significant differences between fresh and stored (refrigerated) plates (4 weeks old). Additional advantages of the RODAC method include relatively low cost, consistent and precise recovery, effective use by personnel without extensive training, and the elimination of laboratory manipulation after sampling.

The control of microbial contamination in institutions such as hospitals is mandatory since large numbers of contaminating organisms are present that may serve as reservoirs of infection. Surveillance of microbial contamination has been directed to extensive horizontal areas (15), such as floors, in an effort to provide an index of sanitation for the general environment.

The use of chemical disinfectants on environmental surfaces to reduce microbial contamination is generally accepted in critical institutional areas (3, 18-20). The transmission of disease via contaminated environmental surfaces is generally less than by the animate route. However, the transfer of contamination from environmental surfaces by human contact can be an important route of infection. The elimination or substantial reduction of potential patho-

genic microorganisms from contaminated surfaces thus becomes an important consideration of the environmental sanitation program. Although microbiological sampling performed on a routine basis in the general hospital environment is not recommended (2, 3, 18, 19), efficacy evaluations for determining cleaning methods and choosing disinfectants is advisable (10).

The need to neutralize disinfectant residues has been recognized for many years. It is known that the presence of residual amounts of disinfectant chemicals may produce a bacteriostatic condition that would result in the suppression of the growth of viable organisms. Studies of neutralizers (21) have shown Letheen (lecithin) to accomplish the desired neutralization of phenols and quaternary ammonium compounds in liquid systems. However, some question has been raised concerning the adequate neutralization on agar contact surfaces (24). It has been alleged that highly bacteriostatic disinfectant chemical residues, such as quaternary ammonium compounds, found on treated surfaces are not adequately neutralized on agar contact surfaces. Some argue that this bacteriostatic pressure results in the production of "misleadingly low" population counts as determined by agar contact methods. Investigations to determine whether inadequate neutralization under in-use conditions actually exists have not been conducted.

This study was designed to investigate the adequacy of neutralization of disinfectant residues that may be transferred to surfaces of agar contact sampling plates. *Staphylococcus aureus* was utilized to determine whether bacteriostatic effects may occur due to the transfer of disinfectant residues to the surface of agar contact plates.

MATERIALS AND METHODS

Organism. A disinfectant-resistant strain of *S. aureus* ATCC 6835, grown as specified previously (7, 14), was utilized in this study. A 24-h broth culture was diluted to a concentration of approximately 10^5 viable organisms per ml as determined by serial plate counts.

Sampling plates. RODAC plates (Falcon Plastics) filled with approximately 15.5 to 16.5 ml of nutrient Letheen agar were prepared. The Letheen agar contained 0.5% beef extract (Difco), 0.5% NaCl, 1.0% peptone (Anatone), 1.6% agar, 0.5% sorbitan monooleate (Polysorbate 80), and 0.07% lecithin (Azolectin).

Disinfectants utilized. Three major types of disinfectant products were used in this evaluation: a phenolic preparation (One-Stroke Vesphene, Vestal Labs, St. Louis, Mo.), a quaternary ammonium chloride formulation (A-33 Liquid, Airkem, Carlstadt, N.J.), and a nonionic-iodine complex (Wescodyne, West Chemical, Long Island, N.Y.). The composition of the formulations was as follows: One-Stroke Vesphene, 10.0% *o*-phenylphenol, 8.5% *o*-benzyl-*p*-chlorophenol, and 2.0% *p*-tertiary-amylphenol; Wescodyne, 9.10% polyethoxy polypropoxy polyethoxy ethanol-iodine complex and 8.74% nonylphenoxy polyethylene oxyethanol-iodine complex (provides a minimum of 1.6% titratable iodine); A-33 Liquid, 3.0% *n*-alkyl(60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chlorides, 0.7% essential oils, and 0.143% tetrasodium ethylenediaminetetraacetate. All products were diluted with sterile distilled water to the manufacturer's recommended concentration for hard-surface disinfection.

Mop heads. Miniature versions of commercial cotton mop heads were constructed by cutting approximately 100 12-inch (ca. 30.5-cm) strands from a new cotton mop head, folding them in half, and binding them at the folded end. Mop heads were placed individually into empty 4,000-ml beakers, covered loosely with foil, and sterilized in a steam autoclave at 121°C for 30 min just prior to use.

Construction of simulated floor panels. Eight artificial floor panels were prepared, each consisting of four 1-foot (ca. 30.5-cm) squares of asbestos-vinyl floor tiles mounted on 0.75-inch (ca. 1.9-cm) thick plywood. Sixty-four sampling sites were identified on each floor panel by using water-insoluble enamel spots and an X-Y coordinate grid system.

Preparation of simulated floor panels prior to testing. Eight simulated floor panels were scrubbed with a warm nonionic detergent solution, rinsed with warm tap water, and finally rinsed with sterile distilled water. Floor panels were placed in a "clean area" to prevent airborne contamination. Isopropyl alcohol (70%) was poured onto the panels and dispersed by means of sterile paper towels to remove any foreign residues as well as contaminating microorganisms. The alcohol solution was allowed to air dry.

Each of three test panels was mopped with one of the disinfectant test solutions and allowed to air dry. Based on the actual in-use manufacturer's (Airkem) recommended application rate of 1 gallon (ca. 3.8 liters) per 500 square feet (ca. 46.5 m^2) of area mopped, a minimum of 30 ml of disinfectant solution was applied to each treated floor panel. Four of the remaining panels were inoculated artificially by dispersing 2.0 ml of the *S. aureus* solution (10^5 organisms per ml) over each panel. Dispersion was accomplished by using a sterilized sheet of lens paper, which was drawn across the entire surface of the floor panel. Inoculated panels were also allowed to air dry for approximately 15 min. One floor panel was left untreated.

Test conditions. All experiments were conducted under ambient room conditions. Temperatures ranged between 68 and 72°F (ca. 20 and 22.2°C). The relative humidity ranged between 64 and 68%.

Test procedure. Individual RODAC plates were pressed with moderate vertical pressure (8) to each of the 64 sampling sites on the three disinfectant-treated floor panels. Immediately thereafter, the same plates were touched to corresponding sites on three of the artificially inoculated floor panels. In

addition to the three disinfectant-treated floor panels, an untreated floor panel (which served as a control) was sampled with 64 RODAC plates, which were subsequently touched to the fourth artificially inoculated panel, according to the procedure employed for the disinfectant-treated panels. A total of 64 plates were then derived for each disinfectant-treated floor panel as well as for the untreated control panel. This experiment was repeated on three different occasions.

All RODAC plates were incubated for 24 h at 35°C. Plate counts were then obtained for each plate with a Quebec colony counter. Individual counts for the 64 plates from each floor panel were totaled to obtain an average plate count.

RESULTS

An average plate count was calculated for each disinfectant-treated panel, as well as for the control (Table 1). A final average was computed on the basis of three independent experiments. Each final average represents a total of 192 individual plate counts. The frequency distribution pattern of the data indicated a normal distribution curve. Since more than two-thirds of the individual counts fell within one standard deviation of the mean, the mean presented in conjunction with the standard deviation is felt to represent the data adequately (24). The final averages indicate that plate counts were generally higher from the disinfectant-treated panel compared with the untreated control. The greatest difference was noted with respect to the quaternary formulation. Some of the possible explanations for this phenomenon will be expanded upon below.

DISCUSSION

A number of approaches were investigated in an attempt to design the most appropriate protocol to clarify the current ambiguity concerning the adequacy of disinfectant residue neutralization on agar contact surfaces. My prime objectives were directed towards the simulation of conditions most likely to be encountered under commonly used RODAC surface sampling procedures.

The quantity of various disinfectant residues removed by contact from the surface of disinfectant-treated floor surfaces has not been reported. Attempts to extract and accurately measure these residues from the floor were unsuccessful. It was decided that, although these figures would be of interest, it was more important to determine whether the unknown quantity of residue extracted from a treated surface was, in fact, sufficient to depress the number of colony-forming units picked up by the RODAC plate. Various authorities in the disinfectant field, such as Engley and Dey (12) and Walter and Foris (25), have investigated the possible inadequacy of standard inactivators or neutralizers under conditions not simulating actual surface sampling procedures. In essence, what I hoped to examine was whether residues picked up on agar surfaces as a result of commonly used RODAC surface sampling procedures would reduce significantly the colony count on the RODAC plate after subsequent incubation.

TABLE 1. *Results of three experimental trials*

Panel	Avg no. of colonies for 64 plates		
	Expt 1	Expt 2	Expt 3
Untreated control	53 ± 15.4	176 ± 60.7	60 ± 39.9
Phenolic	60 ± 18.0	188 ± 46.5	50 ± 18.8
Quaternary	75 ± 22.5	191 ± 47.1	58 ± 23.1
Iodophor	71 ± 23.1	160 ± 42.1	63 ± 20.3

To determine this, several important considerations were taken into account. First, the disinfectant residues had to be implanted in a manner identical to that encountered under normal conditions. Second, a procedure had to be developed that would supply a consistent number of the proper organisms, which would then be distributed evenly across the agar surface. Third, a direct application of disinfectant to organism or organism to disinfectant would result in the ultimate reduction of viable organisms to a level too low to be useful; therefore contact between the disinfectant residue and the organism had to be made under "dry" conditions.

The first consideration was handled by constructing actual floor panels from plywood covered with floor tiles. These artificial floor panels could be treated with various disinfectant solutions and subsequently used to supply the residues by placing sterile RODAC plates in contact with the treated panels.

The second consideration was the most difficult since the proper organism had to be chosen and that organism had to be distributed evenly to assure relatively consistent plate counts. Since quaternary ammonium compounds have been highlighted because of their exceptionally high bacteriostatic activity (11), it was logical to select an organism that would be susceptible to this bacteriostatic pressure. One of the reasons why *S. aureus* was chosen as the test organism is its high sensitivity to low concentrations of quaternaries (23). In addition, *Staphylococcus* is unusually resistant to desiccation. Trials utilizing gram-negative organisms such as *Escherichia coli* and *Klebsiella pneumonia* were unsuccessful because viable cells were not recoverable after a drying period

at levels high enough to allow proper interpretation of this study.

Once the appropriate organism was chosen, another consideration was the method of dispersal onto the floor panels to assure relatively uniform distribution. Initially, it was thought that a spray technique would be adequate. Three types of aerosolization were examined, including nitrogen pressurization, the DeVilblis technique, and the Preval technique. These methods, discarded after numerous trials, indicated that dispersion was not uniform. Further difficulties arose when large quantities of the diluted inoculum were lost as I was attempting to spray the edges of the floor panels. Also, the desiccation factor from aerosolization was very high, causing a significant reduction in the numbers of surviving organisms.

The next approach turned out to be the most consistent. This method involved the use of a sterile lens paper to draw the inoculum across the panel. A series of experiments was run to determine the concentration of inoculum necessary to produce consistent counts between 30 and 150 colonies. Once a method for consistent inoculum dispersion was established, subsequent developments progressed satisfactorily.

To produce a "dry" contact between the disinfectant residue and the "dry" test organism, the RODAC plate was utilized by first contacting the panel treated with disinfectant solution (and allowed to dry) and immediately thereafter touching the same RODAC plate to the inoculated panel (also allowed to dry).

The panel treated with a quaternary compound produced, on the average, higher counts than colony counts obtained in conjunction with the other two types of disinfectant products, as well as the control. This is probably a result of the detergent properties of the formulations which cause a greater dispersion of clustered organisms. Macroscopic colonies may be formed as a result of one organism or many organisms in a cluster. The physical breaking up of clusters could cause an increase in colony numbers without a corresponding increase in viable organisms.

Extrapolation of any simulated in-use test results to actual in-use test results must be made very cautiously. However, the methods and procedures utilized in this study were designed to approach, as closely as possible, conditions existing on actual environmental surfaces. Therefore, based on the results accumulated over a number of experimental trials, it is concluded that disinfectant residues that are picked up on the surface of RODAC plates containing the neutralizers lecithin and Polysorbate 80, collectively known as Letheen, are neutralized adequately and in fact do not alter the validity of RODAC sampling.

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