Efficacy of sodium hypochlorite bleach and 'alternative' products in preventing transfer of bacteria to and from inanimate surfaces

Abstract
Advocates continue to promote the use of "environmentally friendly" products/mixtures as alternatives for Environmental Protection Agency (EPA) registered disinfectants even though information regarding the effectiveness of the "alternatives" is limited.

This study investigates the ability of sodium hypochlorite bleach at the dilution recommended for disinfection of nonporous surfaces, and several "alternatives" (ammonia, baking soda, borax, vinegar and a liquid dishwashing detergent) at concentrations in excess of normal recommendations for use, to kill and/or remove bacteria from surfaces. This study also explores the ability of these products to prevent the transfer of bacteria from one surface to another.

Results initial tests using a procedure required by the EPA for the determination of disinfectant efficacy of dilutable products, indicated that only bleach was effective against Staphylococcus aureus, Salmonella typhi, and Escherichia coli. These organisms represent the wide array of Gram positive and negative bacteria found on various surfaces. Although undiluted ammonia and vinegar also showed antimicrobial activity against the Gram negative...
organisms S. typhi and E. coli, none of the "alternatives" were effective against the Gram positive bacteria, S. aureus. As the identity of bacteria on any surface is unknown by the consumer, the use of a disinfectant proven to have a broad spectrum of antimicrobial activity would be more prudent than using a substance having limited or no efficacy.

A simulated use test using solutions of bleach, baking soda, borax, and the liquid detergent, as well as undiluted ammonia and vinegar against S. aureus and E. coli, also was performed. Antimicrobial activity, mechanical removal, and the transfer of organisms to a second surface during the simulated cleaning process were evaluated. The bleach exhibited the expected disinfectant efficacy by eliminating both test organisms from the original surface and the sponge, and preventing the transfer of the organisms to surrounding areas. Undiluted ammonia and vinegar were also effective, but only against E. coli. Again, none of the "alternatives" were effective against S. aureus.

Introduction

The use of environmentally "safe" products or "home mixtures" as alternatives for commercial products specifically manufactured for various home cleaning/disinfecting chores continues to be advocated in newspaper and magazine articles, in books, by environmental groups, and even by government agencies (Household Hazardous Waste Projects). By 1993, at least 35 states had issued guidebooks recommending the use of alternative products (1). Major reasons given by consumers for use of alternative products are: improving the environment, reducing hazards, reducing water pollution, and cost savings. At a recent environmental conference (October 1995), one participant, the author of a book recommending the use of alternative products, suggested that if one "uses fewer toxic chemicals," one's "lifestyle is healthier" and the use of "naturally sustainable ingredients" is better for the environment (2, 3).

However, unlike commercial products, "alternatives" and "home mixtures" are not controlled by federal safety regulations and have no precautionary labeling. They have no directions for use, nor for proper disposal. The preparations may be chemically unstable, and may degrade in the container in which they are stored. It is highly unlikely that the "alternatives" have been evaluated for environmental safety or compatibility, and they have not been registered by the Environmental Protection Agency as disinfectants.

Alternative products are usually recommended for cleaning kitchen and bathroom surfaces and glass, but some are suggested for disinfecting (4, 5, 6, 7), without mention of antimicrobial efficacy. The object of disinfection is to reduce the spread of infection and disease by eliminating the causal organisms. Mechanical removal of organisms from any surface is important; however, if removal is not combined with disinfection, or if the organisms are removed from one surface only to be deposited onto another, the process has not been successful. Several studies have shown the role of surfaces in households, day care centers, and various institutions in the transmission of bacterial and viral diseases (8, 9, 10, 11).
Two recent studies conducted to determine the antimicrobial activity of alternative products have shown that they may not be as effective as proclaimed by their advocates. A study at the University of Minnesota indicated the lack of antimicrobial efficacy of various alternative products on a plastic laminate surface in the presence of bathroom and kitchen soils (12). The "alternatives" used in these tests included those most commonly recommended for cleaning and for disinfection: ammonia, baking soda, borax, lemon juice, and vinegar. A commercial disinfectant containing bleach also was tested. The undiluted disinfectant and vinegar were most effective in reducing the number of organisms on the surfaces. The remaining alternative products showed little efficacy. No attempts were made to determine the number of viable organisms remaining on the sponges used to clean the surfaces.

Bauer et al. also utilized a single test surface (Formica(R)), which after being inoculated with bacteria, was sprayed with the test product solutions and then wiped with dampened sponges (13). Ammonia, borax, baking soda, vinegar and one commercial antimicrobial spray product were evaluated. These tests showed that only the antimicrobial product significantly reduced the number of viable organisms on the Formica(R) surface and on the sponges. None of the "alternatives" showed efficacy in either the Association of Official Analytical Chemists (AOAC) Use Dilution Test (which is required by the EPA to verify efficacy of dilutable products as disinfectants) or the EPA Non-Food Contact Sanitizer Test (in which a 3 log reduction in the number of contaminating organisms demonstrates efficacy as a sanitizer).

The present study expands upon the simulated testing by Olson et al. by:

a) determining numbers of organisms on sponges used for cleaning, those transferred to surrounding areas, as well as those remaining on the initial contaminated surface;

b) using both ceramic and Formica(R), representative of the surfaces found in bathrooms and kitchens;

c) increasing the concentration of the alternative products to create a "best" situation;

d) using two bacteria: Escherichia coli and Staphylococcus aureus;

e) eliminating soil; and

f) decreasing the number of sponge strokes to reflect a more realistic cleaning process.

This study also differs from the simulated testing by Bauer et al. by:

a) determining numbers of organisms transferred to surrounding areas, as well as those remaining on the initial contaminated surfaces and the sponges;

b) using two surfaces: ceramic and Formica(R);

c) increasing the concentration of the alternative products to create a "best" situation; and
d) decreasing sponge strokes to reflect a more realistic cleaning process.

**Experimental Use Dilution Test Test Products**

All products were diluted in sterile distilled water, dilutions as noted in Table 1. These products included: Regular Clorox(R) Bleach [The Clorox Company, Oakland, CA]; Ammonia [Parsons(R) Clear Ammonia, The Dial Corp., Phoenix, AZ]; Baking Soda farm & Hammer(R), Church and Dwight, Princeton, NJ; Borax [20 Mule Team(R) Borax, The Dial Corp., Phoenix, AZ]; Vinegar, distilled white, 5% acetic acid [H.J. Heinz, Pittsburgh, PA]; Detergent [Dawn(R) Liquid, Procter & Gamble, Cincinnati, OH]. Sterile distilled water at 20 degrees C and 49 degrees C was used as a control.

**Bacterial Cultures**

Bacterial cultures used were Staphylococcus aureus, ATCC 6538; Escherichia coli (O157-H7), ATCC 35150; and Salmonella typhi, ATCC 6539. Lyophilized cultures were received from ATCC, reconstituted as directed, and propagated according to directions for the AOAC Use Dilution Method.

**Test procedure**

The products were tested according to the AOAC Use Dilution Method with modification as noted below (14). All products were tested in the presence of 5% serum to simulate a "soil" which might be present under actual conditions. The test organisms, which had been dried onto stainless steel cylinders, were exposed to diluted Clorox(R) Bleach for five minutes or to the alternative products or distilled water for 10 minutes. Sixty replicate carriers were tested for each test solution and each organism (15). The exposure temperature for most tests was the required 20 degrees C. A solution of borax and a distilled water control were run at 49 degrees C to determine the effect of the higher temperature on antimicrobial efficacy. After exposure to the test solution, each carrier was placed into a tube of Letheen Broth with 0.1 percent sodium thiosulfate to neutralize the activity of the test solution. The tubes were incubated at 33 +/- 2 degrees C for 48 hours. Turbidity of the media indicated growth of the test organisms and no efficacy of the test solution. To be considered effective, a disinfectant must kill the organisms on 59 of 60 carriers.

**Results Use Dilution Testing**

Results of the Use Dilution studies are shown in Table 1. The bleach was effective against all three organisms in five minutes. None of the "alternatives" were effective against the Gram positive bacteria, Staphylococcus aureus. Undiluted ammonia was effective against both Gram negative organisms, Salmonella typhi and Escherichia coli. but, when diluted 1 cup:gallon of water, was effective against neither. Undiluted vinegar showed efficacy against Salmonella typhi, but was only partially effective against Escherichia coli. Solutions of borax, baking soda, and Dawn detergent were not effective against either Gram negative organism. All alternative products were in contact with the organisms for 10 minutes.

Distilled water tested at both 20 degrees C and 49 degrees C served as a control for these
studies. The water had no antibacterial effect at either temperature, indicating that the higher temperature was not a factor in this testing.

Simulated In-Use Test Test Products
Based on the results of the Use Dilution testing with these products, the test solutions for the simulated in-use study were prepared at the higher concentration (at least twice that normally recommended) or, in the case of ammonia and vinegar, undiluted. Hard water (100 ppm as Ca++) was used as the diluent to reflect the average hardness found in water in the United States (16). No organic soil was used.

**Bleach**
3/4 cup per gallon

**Ammonia**
Undiluted

**Baking Soda**
2 cups per gallon

**Borax**
1 cup per gallon

**Vinegar**
Undiluted

**Liquid Detergent**
1 cup per gallon

**Water**
sterile hard water, 100 ppm as Ca++

**Bacterial Cultures**
Bacterial cultures used were Staphylococcus aureus, ATCC 6538 and Escherichia coli, ATCC 11229. ATCC lyophilized cultures were reconstituted as directed and grown on Trypticase Soy Agar (TSA) (BBL) slants for 48 hours at 35 +/- 2 degrees C. For use, two transfers of the culture from the stock to fresh TSA slants were made and incubated at 35 +/- 2 degrees C for 20-24 hours. The suspensions were prepared in 0.1 percent tryptone/saline, adjusted to approximately 0.330 at O.D.451nm. These were further diluted 1:10 for testing.

**Test Surfaces**
Glazed ceramic tiles (2 x 2 inch) were washed with liquid detergent, rinsed several times in tap water, then in distilled water. The tiles were placed, glazed side up, into large pyrex dishes which were covered with aluminum foil, then sterilized by autoclaving for 25 minutes at 121 degrees C. The tiles were dried at room temperature for a least two days prior to use.

Formica(R) squares (2 x 2 inch) were washed in liquid detergent, rinsed thoroughly in tap and distilled water, then dried. Two to four days prior to use, the squares were soaked for five minutes in 95% ethanol, drained, and placed into a sterile large pyrex dish at room temperature.

**Plexiglas templates** (6 3/4 x 17 inch), cut to fit into the Gardner Abrasion Tester (Silver Spring, MD) were made with 2 x 2 inch depressions cut into the center so that the test tiles would fit tightly. The use of 2 x 2 inch squares, rather than an entire template, allowed easier manipulation and more complete retrieval of organisms from the test surfaces both prior to and after treatment with the various products. The entire test surface could be immersed into a liquid to remove the organisms. This design also allowed the determination of the spread of organisms from the original surface to the surrounding area during the cleaning process.
Sponges
Cellulose sponges (3 x 3 inch), washed, and without biocide treatment, were purchased from National Sponge Co., Brooklyn, NY. The sponges were rinsed in a Whirlpool Imperial Seventy washing machine at 140 degrees F without detergent, then dried in a large capacity Whirlpool clothes dryer set on HIGH. The sponges were dampened in sterile hard water, squeezed out by hand, and sterilized by autoclaving for 25 minutes.

Test Procedure
The study was designed to determine the ability of various products to kill/remove bacteria on/from the surfaces under study. It simulated the use of a diluted product applied to a sponge, and wiped across a surface to be cleaned/disinfected. As no visible soil was present, the surfaces were subjected to a "light duty" cleaning of 4 strokes with the sponge, after which the surfaces were exposed to the products for two minutes prior to being sampled for viable organisms.

Inoculation of surfaces
A single tile was placed into a sterile plastic Petri dish. The surface was inoculated with 100 μL of a bacterial suspension using the tip of the micropipet to spread the suspension. The tiles were dried for 30-35 minutes at 35 +/2 degrees C with the cover of the dish slightly ajar. At least 10^6 viable CFU were recoverable from the test surfaces.

Cleaning procedure
Using aseptic technique, a single tile was placed -- inoculated surface up -- into the depression in a Plexiglas template fitted into the Gardner Abrasion Tester. A sterile sponge wetted with the test solution was placed into the sponge holder of the Gardner tester, and passed for four strokes over the surface of the tile and the template. A timer was set for two minutes immediately after the fourth stroke to allow exposure of the test solution to the organisms remaining on the test surfaces.

Determination of viable organisms on sponges, tile surface, and template area
The sponge was aseptically removed from the holder, cut into several pieces and placed into 100 μL neutralizing solution (to neutralize the chlorine in the bleach, and other active ingredients in the alternative products), then blended (Lab Blender Stomacher 400, Seward, London, UK) for two minutes to release bacteria. Plate counts were performed to determine the numbers of viable organisms remaining on sponges. One and one-half minutes after cleaning, a sterile gauze pad dampened with sterile hard water was used to wipe the template on both sides of the tile along the path of the sponge to retrieve bacteria spread from the surface of the tile during sponging. Care was taken not to contact the tile surface during this procedure. The gauze was placed into 100 μL neutralizing solution and blended using the Stomacher apparatus. Plate counts were made to determine the numbers of viable organisms remaining on the template (removed by the gauze wipes).

After the two minute exposure period, the 2 x 2 inch tile was removed from the template and
placed -- surface down -- into a sterile beaker containing 3-4 mm glass beads and 50 μL neutralizing solution. The beaker was shaken for five minutes at 2500 rpm on a rotary shaker (New Brunswick, Edison, NJ) to release organisms remaining on the tile, and then placed into a sonicator (Benson 2200, Danbury, CT) for one minute, to break up any clumps of organisms. Plate counts were made to determine the numbers of viable organisms remaining on the surface of the tiles.

Dilutions, as required, were made in neutralizing solution. Pour plates were made in TSA (BBL) and plates were incubated at 35 ± 2 degrees C for 48 hours prior to counting. Ten replicates were performed for each surface, organism, and test treatment. For each 10 replicates, duplicate tiles were treated with sterile hard water as controls, and duplicate inoculated tiles without treatment were used to determine the number of viable organisms subjected to the various treatments. Templates were disinfected with a solution of bleach between uses.

Results Simulated In-Use Testing

Results demonstrate the effect of both antimicrobial and mechanical activity in the elimination/removal of organisms from the test surfaces.

The efficacy of the bleach against both S. aureus and E. coli is indicated in Figures 1-4. Organisms were eliminated from the ceramic and Formica(R) surfaces, the sponges, and from the surrounding template surfaces, indicating effective antibacterial activity.

Figures 1 and 2 also show that undiluted ammonia and vinegar were as effective as bleach in eliminating E. coli from the ceramic and Formica(R) surfaces, sponges, and surrounding template areas. None of the other "alternatives" showed significant antibacterial efficacy against this organism. Figures 3 and 4 indicate somewhat more antibacterial activity by vinegar against the S. aureus on Formica(R) than on the ceramic surface, but neither the ammonia nor the vinegar was effective in eliminating the S. aureus from the sponges or the surrounding areas.

Baking soda, borax and the detergent appear to be more effective in removing E. coli than S. aureus from both tile surfaces. However, no real differences in the numbers of either organism remaining on sponges or the surrounding template areas were detected regardless of the "alternative" solution tested. Overall, the activity of the "alternatives" against E. coli appears more effective than against S. aureus, although none were as effective as the bleach against both organisms.

Discussion

This study illustrates the importance of using EPA registered disinfectants/sanitizers such as sodium hypochlorite bleach rather than unproved "alternatives" or "home mixtures" to kill bacteria on household and other surfaces. We have shown that although organisms are removed from one contaminated surface, they can, if not killed by a product containing an
antimicrobial agent, be transferred to another surface by the sponge used to clean the primary surface. Commercial disinfectant products like bleach, used according to label directions, can eliminate these organisms. "Alternatives," in most cases, cannot.

The Use Dilution testing performed by Bauer et al. revealed that solutions of vinegar, baking soda, ammonia, and borax normally recommended for household use, were ineffective against Staphylococcus aureus and Salmonella choleraesuis (13). Both of these organisms are used for determining antimicrobial efficacy of products intended for EPA registration as broad spectrum disinfectants, and are representative of the many Gram positive and negative bacteria found on various surfaces. These results led us to determine whether increasing the concentrations of the "alternatives" would result in increased antibacterial efficacy.

Our Use Dilution testing indicated that sodium hypochlorite bleach, diluted as recommended for disinfection, was effective against Staphylococcus aureus, as well as two gram negative organisms, Salmonella typhi and Escherichia coli, with a five minute contact time. Given a 10 minute contact time, undiluted ammonia and vinegar, although ineffective against Staphylococcus aureus, did show antimicrobial activity against both Salmonella typhi and Escherichia coli. The solutions of ammonia, baking soda, borax and liquid detergent were not effective against any of the organisms tested.

The results of the simulated testing essentially mirrored the results of the Use Dilution testing. Bleach, diluted according to label instructions, eliminated both E. coli and S. aureus from the ceramic and Formica(R) tiles, the sponges, and the surrounding template after four cleaning strokes with a sponge wet with the test product and a two minute exposure time of the product to the organisms. By using this EPA registered disinfectant, the chance for dissemination of the organisms to other surfaces is eliminated. Undiluted vinegar and ammonia were as effective as the bleach, but only against E. coli; no significant decrease in the number of S. aureus on the tiles, sponges or surrounding areas was found.

As part of the risk assessment process for determining whether to use a commercial product or an "alternative" for disinfection, the consumer would need to know the type(s) of organisms on the surfaces to be cleaned or disinfected. As this is impossible, the use of vinegar or ammonia would not be a good choice for disinfecting any household surface. As none of the remaining three "alternatives" (borax, baking soda, liquid detergent) significantly reduced the numbers of organisms from the surfaces, sponges or surrounding template areas, the use of these solutions for disinfection would also be imprudent. If pathogenic organisms are present, the use of the "alternatives" may not decrease the chance of disease transmission, and could possibly increase the chances by spreading pathogens to a far wider area than originally contaminated.

Retrieval of bacteria from the sponges in the study by Bauer et al. showed that large numbers of bacteria are transferred to the sponges from the contaminated surface during the cleaning process (13). However, the application of bacteria to the entire midsection (3 x 17 inch) of
the Formica(R) surface did not confirm that the organisms were actually transferred from the sponge as it passed back and forth over the surface. Use of the smaller 2 x 2 inch area of initial contamination in the middle of the large template allowed us to demonstrate the spread of the bacteria from this small area to the 3 x 6 inch template surfaces on both sides of the tile as the sponge passed back and forth along the cleaning path.

All three sites analyzed in this testing must be considered in order to determine whether or not the product is truly effective in killing the organisms and reducing the possibility of disease by preventing the spread of organisms. Viable organisms remaining on one site present the chance for transfer of organisms from one surface to another, from surfaces to hands, etc. Except for the efficacy of undiluted ammonia and vinegar against E. coli, the organisms left on the flies, sponges, and on the surrounding template areas when cleaning with the "alternatives" indicate indeed that unless killed, the organisms will be spread -in this case -- by the sponge.

Microorganisms have been shown to persist on surfaces from hours to weeks, under various conditions (17, 18, 19, 20, 21, 22). Gastrointestinal organisms in toilets can be aerosolized and settle on various surfaces in bathrooms when toilets are flushed (23). It is also well known that food poisoning is caused by organisms transmitted by hands to food, surfaces and utensils; and from food to surfaces, utensils, and hands. Sponges and cloths used to clean various kitchen surfaces provide excellent growth conditions and can spread microorganisms during the cleaning process (9, 24).

The study by Olson et al. incorporated simulated bathroom and kitchen soils in conjunction with the microorganisms (13). The efficacy of the commercial disinfectant containing hypochlorite bleach to remove organisms from the surfaces did not seem to be affected by either soil. However, as no counts were made to determine surviving organisms on the sponges, the organisms may merely have been removed, but not killed. It is not clear whether the inability of the "alternatives" to significantly decrease the numbers of organisms on the surfaces was due to the presence of the soils. Presence of substances such as food and fecal material are known to aid in survival of organisms on surfaces (17, 18, 22). No soil was used in the present study in order to determine the effect of the test products in the absence of interfering substances. Results show that, in the absence of soil, the "alternatives" have little antimicrobial activity. It seems likely that the presence of food, fecal material, or other soils would serve to further decrease any antimicrobial activity of the products used as substitutes for commercial disinfectants.

Many organisms are found in both dry (floors, clothing) and wet (sinks and baths, damp washcloths, dishcloths, sponges) areas in homes; E. coli and S. aureus are among the organisms identified (9, 24, 25, 26, 27). Gram negative bacteria, such as E. coli, Salmonella and Shigella spp. are generally accepted as being more sensitive than Gram positive cocci, such as S. aureus, to disinfectants (28). Although no differences were noted in experiments
with bleach, results indicate that the sensitivity of E. coli to both undiluted vinegar and ammonia is greater than that of S. aureus to these solutions. The extreme pH values of both these products undoubtedly altered the hydrogen ion concentration around the cells and may have inactivated surface elements of the E. coli more readily than the S. aureus. The actual sites affected by the highly acidic and basic products were not assessed here. Viruses also differ in their relative resistance to biocidal chemicals as do bacteria. Influenza and Herpes are generally more sensitive to disinfectants than are Rhinoviruses, Rotavirus, and Hepatitis A, and thus are easier to kill. These viruses are also easier to kill than parasites such as Giardia. We have no information regarding the resistance of any virus or parasite to the "alternatives" recommended for disinfection.

Concerns about the role the type of surface might play in the efficacy of the products led to the use of a glazed surface (ceramic) as well as laminate (Formica(R)) in this study. As previously stated, these are representative of surfaces found in bathrooms and kitchens. We saw no consistent differences in the reduction/removal of organisms from either surface with the alternative solutions. When strong bactericidal activity was evident, such as with the bleach, the surfaces certainly did not play a role. The detergent removed both organisms somewhat more readily from the ceramic than from the Formica(R), but the bacteria were merely transferred to the sponges and onto the surrounding areas.

Estimates of total foodborne diseases in U.S. homes are between 6.3 and 80 million (29). Recent documentation shows how microorganisms can be transmitted to and from foods, surfaces, and people (30). Hands, utensils and surfaces not properly cleaned and sanitized or disinfected spread organisms throughout the kitchen. Microorganisms are also found on food preparation surfaces, in diaper changing areas, on toys, and on the hands of children and attendant adults in day care facilities. Evidence is increasing that respiratory and enteric communicable diseases are being brought home by the children and undoubtedly transmitted to family members via the hands and from various surfaces in the home. As the number of children in day care increases, so does the incidence of adults with diarrheal diseases (caused by Rotavirus, Giardia, Shigella spp.), respiratory diseases, Hepatitis A, and Cytomegalovirus (19, 22, 31, 32). The need to promote improved hygiene in the home must be stressed due to the occurrence of increased incidences of foodborne disease and other enteric illnesses (33).

The use of sodium hypochlorite bleach to kill microorganisms on surfaces, thus preventing their spread from one surface to another, and potentially limiting the spread of disease, has been demonstrated. The study also has illustrated that "environmentally friendly" mixtures recommended for this purpose do not possess antimicrobial activity against both Gram positive and Gram negative bacteria. There is sufficient proof that diseases can be spread by organisms which can survive for extended periods of time on environmental surfaces. As one has little, if any, idea about the types, numbers or conditions of the organisms present on any given surface, it is prudent to use a product with proven efficacy against a wider range of
microorganisms.

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TABLE 1. Use Dilution Test -- Results

Legend for Chart:
A - Product
B - Usage[a]
C - Temp
D - S. aureus[1]
E - S. typhi[2]
F - E. coli[3]
G - exp. time
H - organic load

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</tr>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td></td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

a dilutions in distilled water

b number carriers positive for growth/number of carriers tested

Staphylococcus aureus, ATCC 6538
Salmonella typhi, ATCC 6539
Escherichia coli (O157:H7), ATCC 35150

GRAPH: FIGURE 2. E. coli -- Formica(R)

GRAPH: FIGURE 3. S. aureus -- Ceramic

GRAPH: FIGURE 4. S. aureus -- Formica(R)

REFERENCES
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