Efficacy of Alcohol-Based Hand Rinses under Frequent-Use Conditions

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Fifty volunteers randomly assigned to one of five hand washing agents (10 subjects per agent)—a nonantiseptic liquid soap (control), an antiseptic hand rinse containing 60% isopropyl alcohol (IPA) with emollients (Alc A), an antiseptic hand rinse containing 70% IPA and 0.5% chlorhexidine gluconate with emollients (Alc B), an antiseptic containing 4% chlorhexidine gluconate and 4% IPA (CHG), and 70% IPA—washed their hands 15 times per day for 5 days under supervision by using a standardized technique and measured amounts of test agent. Microbiologic samples of hand flora were obtained at base line and after hand washes 1 and 15 on test days 1 and 5. After the initial hand wash there were significant reductions over base line in aerobic and anaerobic log CFU among those using Alc A, CHG, and IPA. By the end of the first day of hand washing (15 washes), there were 2-log or greater reductions in aerobic counts among subjects using all antiseptics, but no significant reductions in controls. By the end of day 5, all agents produced significant reductions in aerobic (P = 0.0002) and anaerobic (P = 0.0002) counts over control soap. Subject assessment of effects of hand washing on the skin and overall satisfaction varied significantly by product (P = 0.04 and 0.05, respectively). We conclude that alcohol-based hand rinses are highly efficacious, and such products are recommended as a health care personnel hand wash, particularly when sink and running water are inaccessible.

The testing of health care personnel hand washing products occurs in several stages. In the laboratory, initial safety and efficacy data are obtained in vitro and then in vivo with volunteers. In vivo testing should include an evaluation of the effect of the product on selected stains of gram-positive and -negative bacteria after artificial inoculation onto the skin and an evaluation of the initial effects of the product on colonizing flora after a few hand washes, as well as an assessment of the sustained effect after frequent use of the product over an extended period. Recently, several alcohol-based hand washing products designed for use without water have become commercially available and are recommended by some as being at least as, if not more, effective than the more traditional agents used with sink and running water (3; B. Nystrom, Letter, Infect. Control [Thorofare] 5:211, 1984). Use of such agents has been shown to result in significant log reductions in normal flora of the hands when compared with other antiseptics in single-use trials (A. Morrison, J. Gratz, I. Cabezudo, and R. P. Wenzel, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 930, 1985). They have not been evaluated, however, under frequent-use conditions such as those practiced in the health care setting. In this paper, we report the results of in vivo tests of the antimicrobial efficacy of five hand washing products after extended use, 15 hand washes per day for 5 consecutive days.

MATERIALS AND METHODS

Subject selection and training. A convenience sample of subjects was selected from a pool of healthy adults, primarily employees and students in the study institution, who had no history of allergies, psoriasis, eczema, other skin diseases, or sensitivity to soap. All subjects gave informed consent and were remunerated for their participation.

Three days before the beginning of the testing period, subjects were provided with a mild, nonmedicated liquid soap (Safe 'n Sure; Calgon Corp., St. Louis, Mo.) for general bathing and hand washing and with disposable plastic gloves for use during shampooing, dishwashing, or hand contact with other soaps or lotions. They were instructed to use only this soap and to refrain from use of lotions or moisturizers for the 3-day weaning period and the entire test period (5 days). Subjects were instructed in a standardized 15-s wash technique. Each subject practiced this technique in the presence of an investigator until competence was demonstrated. Rings and nail polish were not worn during the study.

Hand washing regimen. Subjects were randomly assigned to one of five treatment groups: (i) liquid, nonmedicated soap (Safe 'n Sure; Calgon), (control), (ii) an antibacterial hand rinse containing 60% isopropyl alcohol in emollients (Cal Stat; Calgon), (Alc A), (iii) an antibacterial hand rinse containing 70% isopropyl alcohol and 0.3% (wt/wt) chlorhexidine gluconate in emollients (Hibistat; Stuart Pharmaceuticals, Wilmington, Del.) (Alc B), (iv) an antibacterial detergent containing 4% (wt/vol) chlorhexidine gluconate and 4% isopropyl alcohol (Hibicleans; Stuart) (CHG), and (v) 70% (wt/vol) isopropyl alcohol (IPA).

Samples from the skin of the hands were obtained for bacterial culture at five intervals: before the first hand washing (base line), after the first and last hand wash on the first testing day, and after the first and last hand wash on the last testing day. The hands were rinsed thoroughly in running water before sampling. For 5 days, subjects washed their hands according to a standard protocol (15 sec each wash) 15 times per day by using a syringe to measure 5 ml per hand wash (2 ml per hand wash for IPA). A lesser amount of IPA was used to reduce the risk of skin damage as a result of exposure to alcohol without emollients. All washings were performed with the prescribed agent according to the directions of the manufacturer under supervision.
TABLE 1. CFUs from hands in five treatment groups

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Treatment group</th>
<th>Base line</th>
<th>Log₁₀ CFU (% reduction)¹</th>
<th>After hand wash 1</th>
<th>After last hand wash</th>
<th>After hand wash 1</th>
<th>After last hand wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobes</td>
<td>Control</td>
<td>7.00</td>
<td></td>
<td>6.41 (8.4)</td>
<td>6.19 (11.6)</td>
<td>6.67 (4.7)</td>
<td>6.31 (9.9)</td>
</tr>
<tr>
<td></td>
<td>Alc A</td>
<td>6.25</td>
<td></td>
<td>4.91 (21.4)</td>
<td>2.63 (57.9)</td>
<td>3.04 (51.4)</td>
<td>3.24 (48.2)</td>
</tr>
<tr>
<td></td>
<td>Alc B</td>
<td>6.91</td>
<td></td>
<td>6.11 (11.6)</td>
<td>3.47 (49.8)</td>
<td>3.89 (43.7)</td>
<td>3.03 (156.2)</td>
</tr>
<tr>
<td></td>
<td>CHG</td>
<td>7.02</td>
<td></td>
<td>5.93 (15.5)</td>
<td>4.29 (38.9)</td>
<td>3.89 (44.6)</td>
<td>2.68 (61.8)</td>
</tr>
<tr>
<td></td>
<td>IPA</td>
<td>7.09</td>
<td></td>
<td>5.89 (16.9)</td>
<td>4.26 (39.9)</td>
<td>5.11 (27.9)</td>
<td>3.92 (44.7)</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Control</td>
<td>6.88</td>
<td></td>
<td>6.38 (7.3)</td>
<td>5.96 (13.4)</td>
<td>6.26 (9.0)</td>
<td>6.18 (10.2)</td>
</tr>
<tr>
<td></td>
<td>Alc A</td>
<td>6.70</td>
<td></td>
<td>5.44 (18.8)</td>
<td>3.77 (43.7)</td>
<td>4.14 (38.2)</td>
<td>3.26 (51.3)</td>
</tr>
<tr>
<td></td>
<td>Alc B</td>
<td>6.24</td>
<td></td>
<td>6.13 (1.8)</td>
<td>3.43 (45.0)</td>
<td>4.97 (20.4)</td>
<td>2.79 (55.3)</td>
</tr>
<tr>
<td></td>
<td>CHG</td>
<td>6.66</td>
<td></td>
<td>5.64 (15.3)</td>
<td>4.49 (32.6)</td>
<td>5.17 (22.4)</td>
<td>3.83 (42.5)</td>
</tr>
<tr>
<td></td>
<td>IPA</td>
<td>7.07</td>
<td></td>
<td>6.05 (14.4)</td>
<td>5.62 (20.5)</td>
<td>5.31 (24.9)</td>
<td>4.49 (36.5)</td>
</tr>
</tbody>
</table>

¹ Percent reductions from base line calculated as [(base line count - test count)/base line count] × 100.

in a laboratory. For the control soap and CHG, hands were moistened with running water, washed, rinsed, and dried with a paper towel. For the alcohol-based products, no running water was used and hands were air dried.

In addition, subjects assessed the appearance, intactness, moisture content, and sensation on the skin of the hands at base line and again after the 5 days of hand washing. An ordinal scale with a maximum of 28 points (7 points on a Likert-type scale for each of the four factors assessed) was used; the lower the score, the worse the condition of the skin. At the end of the test week, subjects also rated the acceptability of their assigned hand washing product on a similar 7-point scale from "I hate this product; I would never choose it" (1 point) to "I love this product; I would always choose it" (7 points).

Microbiology. The sterile bag technique of Larson et al. (8) was used to assay microorganisms on the hand. The subject inserted the dominant hand into a sterile polyethylene bag containing 50 ml of sampling solution (sterile distilled water containing, per liter: lecithin, 20 g; sodium thiосulfate, 6 g; sodium oleate, 6 g; protease peptone, 1 g; tryptone, 1 g; and Tween 80, 50 ml; pH 7.2 to 7.4). In preliminary studies, we determined that there was no significant increase or decrease in CFUs in the solution within the first 2 h after sampling.

The entire hand surface was rubbed vigorously through the wall of the bag for 3 min. All specimens were plated within 2 h of sampling. A 0.1-ml volume of serial dilutions up to 10⁻⁷ was placed on three media. For aerobic incubation, Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing yeast extract, 5 g/liter; Tween 80, 1 ml/liter; and MacConkey agar (BBL) were used. For anaerobic incubation, prereduced brain heart infusion agar (BBL) containing 7% sheep blood and yeast extract, 5 g/liter, was used. Plates were incubated at 37°C aerobically for 48 h and anaerobically for 5 days. CFUs were counted.

Statistical analysis. Analysis of covariance, controlling for base line counts, was used to test the significance of differences in bacterial counts (counted as log₁₀ CFU) between the five treatment groups. The nonparametric Kruskal-Wallis analysis of variance was used to test the significance of differences between subject assessment of their skin and of product acceptability among the treatment groups. In cases where multiple comparisons between groups were done, the Tukey technique was employed (13). A probability of P < 0.05 was considered to be statistically significant. A sample size of 10 subjects per treatment group would allow one to detect a statistically significant difference in bacterial counts of 0.70 log or greater with 80% power (14).

RESULTS

Fifty individuals were recruited for the study, and all completed the entire testing period. There were 15 (30%) men and 35 (70%) women ranging in age from 19 to 50 years (mean, 29.5 ± 6.8). Ages and sexes were evenly distributed throughout the five treatment groups.

Microbiology. There were minimal reductions in log CFU over the test period among subjects using the control soap (Table 1). After the first wash, there were significant reductions over base line in counts among those using Alc A, CHG, and IPA (P = 0.002). By the end of day 1 (15 hand washes) all antiseptics resulted in a 2-log or greater reduction (P < 0.001), whereas a less than 1-log reduction in counts was apparent in the control group (P = 0.18). This was the case for both aerobic and anaerobic counts. There were significant reductions in aerobic counts after the first hand wash on day 5 as compared with base line counts for Alc A, Alc B, and CHG (P = 0.0001). By the end of the test period, all agents produced significant reductions in both aerobic and anaerobic counts when compared with control soap (P = 0.0002 and 0.002, respectively) but were not significantly different from one another. Some subjects using control soap or IPA became colonized with gram-negative bacteria, whereas gram-negative bacteria were rarely detected after a week of washing with Alc A, Alc B, or CHG (Table 2).

Subject assessment. In four of the five treatment groups, mean subject ratings of the condition of the skin of their hands worsened. That is, subjects felt that their skin was adversely affected by the week of frequent hand washing.

TABLE 2. Effect of hand washing products on isolation of gram-negative bacteria

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of subjects with gram-negative bacteria isolated from hands</th>
<th>Base line</th>
<th>After 1 wk</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>4</td>
<td>+4</td>
<td></td>
</tr>
<tr>
<td>Alc A</td>
<td>3</td>
<td>0</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>Alc B</td>
<td>2</td>
<td>0</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>CHG</td>
<td>4</td>
<td>1</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>IPA</td>
<td>1</td>
<td>4</td>
<td>+3</td>
<td></td>
</tr>
</tbody>
</table>
The mean changes in ratings from days 1 to 5 were controls, \(-1.9\); Alc A, \(-4.5\); Alc B, \(-2.5\); CHG, +0.7; and IPA, \(-1.3\) \((P = 0.04\) for differences between groups). Ratings by the subjects of overall satisfaction with the products (total possible = 7) were controls, 4.6; Alc A, 3.6; Alc B, 3.9; CHG, 5.9; IPA, 3.7 \((P = 0.05)\).

**DISCUSSION**

To our knowledge, this is the first frequent-use trial of several alcohol-based health care personnel hand rinses designed for use without running water and the first trial in which these products are compared during frequent-use conditions with a product containing CHG. Previous comparisons have been primarily between CHG-containing products and products containing povidone-iodine (2, 4, 7, 12). It should be noted that this protocol did not mimic the situation on wards in that the hand washing practices of subjects were controlled, and subjects were not working in clinical settings in which they would have frequent or prolonged physical contact with patients. The two alcohol-based products designed to be applied without the use of water and air dried resulted in the most rapid immediate reduction in flora. The reductions in bacterial counts among those using CHG were initially less dramatic, but more consistent than those seen with the alcohol-based products. This was probably a result of the substantivity of CHG, a characteristic which results in the continuous release on the skin of chemical activity for several hours after application of the product. Substantivity is a desirable characteristic when prolonged antibacterial activity is important, such as during procedures during which gloves are worn.

At all test times for reducing counts of aerobes, anaerobes, and gram-negative bacteria, Alc A, Alc B, and CHG appeared to be superior to IPA. This finding is consistent with what others have also described (1). This superiority may be in part to the substantivity which is known to occur with CHG, to the presence of emollients in the hand rinses which increase the time in which the active ingredient contacts the skin by delaying drying time, and to the fact that a larger amount of Alc A and B than IPA was used (5 versus 2 ml).

The efficacy of hand washing products is generally tested against aerobes only, since anaerobes likely to be transmitted from the hands seem to be of little clinical importance. It is reassuring to note, however, that the trends and patterns of the test agents noted against aerobes were similar for anaerobes. Hence, the testing of products against aerobes alone seems to be an accurate reflection of their effectiveness.

In this study, we did not identify species of organisms. In a previous study (7) we found that types of organisms isolated after frequent hand washing did not change substantially. However, an increased risk of harboring gram-negative bacteria on the hands has been associated with skin trauma (6), and this probably explains why gram-negative bacteria were isolated more frequently from individuals using the nonmedicated control soap, or alcohol, which contained no emollients to protect the skin.

The utility of a hand washing product is related not only to its antimicrobial effectiveness, but also to its user acceptability (5, 10). An effective product is of no benefit if it is not used. Users in this study judged the two alcohol-based rinses as having little more acceptability than plain IPA. As a matter of fact, Alc A was judged to cause significantly more skin irritation than any of the other products. This was probably because subjects were required to use 5 ml per hand wash, whereas the manufacturer recommends 1 to 2 ml per hand wash. Although only subjective measures of product acceptability were used, the results were consistent with previously reported findings based on objective measurements of skin trauma (7). That is, CHG was evaluated as the mildest and most preferred preparation, even when compared with control soap.

For some time, alcohol-based hand washing products have been successfully used in Europe (9, 11; Nystrom, Letter, Infect. Control [Thorofare] 5:211, 1985), but have come into some disfavor and disuse in the United States. Based on our data, we conclude that such disuse is unjustified. Alcohol hand rinses are highly efficacious, and their use is recommended in situations where an antisepctic health care personnel hand wash is indicated, particularly when traditional hand washing facilities (sink and running water) may be inaccessible or inconvenient.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**