A Meta-Analysis of the Published Literature on the Effectiveness of Antimicrobial Soaps

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ABSTRACT

The goal of this research was to conduct a systematic quantitative analysis of the existing data in the literature in order to determine if there is a difference between antimicrobial and nonantimicrobial soaps and to identify the methodological factors that might affect this difference. Data on hand washing efficacy and experimental conditions (sample size, wash duration, soap quantity, challenge organism, inoculum size, and neutralization method) from published studies were compiled and transferred to a relational database. A total of 25 publications, containing 374 observations, met the study selection criteria. The majority of the studies included fewer than 15 observations with each treatment and included a direct comparison between nonantimicrobial soap and antimicrobial soap. Although differences in efficacy between antimicrobial and nonantimicrobial soap were small (~0.5-log CFU reduction difference), antimicrobial soap produced consistently statistically significantly greater reductions. This difference was true for any of the antimicrobial compounds investigated where n was > 20 (chlorhexidine gluconate, iodophor, triclosan, or povidone). Average log reductions were statistically significantly greater (~2 log CFU) when either gram-positive or gram-negative transient organisms were deliberately added to hands compared with experiments done with resident hand flora (~0.5 log CFU). Our findings support the importance of using a high initial inoculum on the hands, well above the detection limit. The inherent variability in hand washing seen in the published literature underscores the importance of using a sufficiently large sample size to detect differences when they occur.

Proper hand washing with soap and water is accepted as one of the most effective ways to reduce the spread of disease in a variety of environments including the community, health care, and food-related contexts. Technically, soap is strictly defined as a salt of a fatty acid, saturated or unsaturated, containing at least eight carbon atoms, or a mixture of such salts (21), but for the purposes of this article, we will use the term “soap” to mean any hand washing compound, including soap. Numerous studies have compared the effectiveness of antimicrobial and nonantimicrobial soaps, using a variety of antimicrobial formations under a variety of conditions, with little consensus on whether antimicrobial soaps are more effective at reducing bacteria and preventing disease. This research was undertaken to examine this question through a systematic analysis of the published literature.

A quantitative risk assessment of hand washing was previously conducted by our research group with the objective of identifying the most important factors that influence hand washing efficacy (33). The analysis performed indicated that antimicrobial soaps, chlorhexidine gluconate (CHG) in particular, were more effective than nonantimicrobial soaps. It has been suggested that the particularly effective nature of CHG might have been due to the use of inappropriate neutralizer (44). Simulation results showed that soap type, hand drying method, and sanitizer use were the most important factors influencing microbial reduction on hands (33).

More recently, Aiello and others (1) analyzed the literature with the goal of conducting a qualitative risk-benefit analysis of triclosan-containing soaps in a community setting. The authors of this study specifically examined research conducted in community settings to focus on potential benefits and risks of everyday use of antimicrobials and their ability to reduce incidence of illness. They also specifically excluded research from health care settings from consideration. Based on their interpretation of available literature, they reached the conclusion that there is no clear benefit to using triclosan-containing soap rather than nonantimicrobial soap.

The goal of the research we present here was to conduct an expanded and systematic quantitative analysis of the existing hand washing data in the literature. Our objectives were to determine if washing with antimicrobial and nonantimicrobial soaps results in a difference in microbial reductions on the hands and to identify the methodological factors that might affect this difference. Such approaches, commonly referred to as meta-analyses, are increasingly seen as a useful tool for examining patterns that might not be apparent from a single or even a few studies. Such meta-analyses may be able to identify experimental design issues

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<th>Soap vol (ml or g)</th>
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*a* Obs, observations: number of data points presented in the manuscript and used in our analysis.

*b* Sample size for each data point.

*c* Rinse, sampling hand by rinsing and collecting sampling solution; vial, sampling fingertips only by pressing a vial containing sampling solution onto the fingertip and shaking; bead, sampling fingertips by rolling fingers on sterile beads and then mixing beads with sampling solution; glove juice, whole hand sampling by submerging the hand into a bag or glove filled with sampling solution and massaging the hand; kneading fluid, fingers kneaded in petri dishes containing kneading fluid; imprint with adjustment, sampling as with imprint method and performing a mathematical adjustment to estimate counts on the whole hand; imprint, sampling fingertips only by pressing fingertips directly onto an agar plate.

*d* CHG, chlorhexidine gluconate; PCMX, parachlorometaxylenol; TCC, trichlorocarbanilide bar soap; BCT, 1.0 wt% benzalkonium cetylphosphate and 0.2 wt% triclocarban. The form of the wash agent is always liquid, unless bar soap is indicated.

*e* Y, antimicrobial neutralizer was used postsampling; N, antimicrobial neutralizer was not used postsampling.

*f* Bland means nonantimicrobial.

*g* NA, not available, not reported in methods.
and quantify sources of variability between studies, which is possible only by looking at large quantities of data comprehensively. While meta-analysis is not without its limitations (including the effects of publication bias and the complexity of the statistical analysis) (51), this approach allows patterns to be identified and interpreted in the larger context of the published literature.

**MATERIALS AND METHODS**

An extensive search of the hand washing literature was conducted, initially using references cited in Aiello et al. (1) and Montville et al. (33) and proceeding through citations of relevant articles, to include as many original published data on the effects of hand washing on bacteria as possible. Since our focus was strictly on washing hands with soap, data were excluded from the analysis if they examined the efficacy of soaps on anything other than bare hands (e.g., washing of forearms (8) or fingernails (31)); also excluded were data from studies that used only alcohol-based sanitizing solutions (16, 23). Data on hand sanitizers (2) and on surgical-type hand wash with a contact time of greater than 3 min and/or use of nail brushes or automatic hand washing machines were also excluded (20, 40, 43, 56). Since our analysis is quantitative, studies that provided only limited quantitative information on elimination of bacteria could not be included (10, 11, 13, 15, 22, 24, 30, 38, 39, 49, 50, 53).

Data on hand washing efficacy and experimental conditions (sample size, wash duration, soap quantity, challenge organism, inoculum size, and neutralization method) from included studies were compiled in Excel (Microsoft, Redmond, WA) and transferred to a relational database in Access (Microsoft).

Where necessary, the percent bacterial reduction was converted to log bacterial reduction. The comparative efficacies of antimicrobial soap and nonantimicrobial soap within a study were calculated by subtracting for each replicate individually, where possible. Comparison of factors was conducted by using Access queries and data analysis functions of Excel. Frequency distributions were created to show the number of times a particular value was observed in the data set. Relative frequency distributions, which show the fraction or percentage of times a value was observed relative to the total number of observations, were also created. Relative frequency is a useful measure when comparing two or more variables that have a different number of total observations because it shows the percentage of times a value was observed, so the two variables can be compared on the same scale.

Linear regression analysis was performed using Microsoft Excel; unpaired t tests and P value and confidence interval determinations were performed with GraphPad QuickCalcs Online Calculator for Scientists (http://graphpad.com/quickcalcs).

**RESULTS**

A total of 25 publications, containing 374 observations, met the selection criteria outlined above and are summarized in Table 1. The majority of the studies included fewer than 15 observations with each treatment and included a direct comparison between nonantimicrobial soap and antimicrobial soap. Wash time ranged from 10 to 180 s, and soap quantity (measured by volume or weight) ranged from 0.5 to 7 ml or g. Ten different organisms were used as test strains, and 11 studies looked at the effect of hand washing on resident flora on hands. A variety of inoculation and sampling procedures were used including the glove juice method, pressing fingertips to an agar plate and predicting the amount expected on the hand, rinsing the hand, and sampling only the fingertips using agar, liquid, or glass beads. The selected literature included data on efficacy of 37 different soap formulations and a plain water wash. The efficacies of nonantimicrobial and antimicrobial soap were compared by plotting the relative frequency of log reductions from each treatment (Fig. 1), where relative frequency is the proportion of times a particular log reduction was observed of the total number of observations. Note that all other factors (type of antimicrobial, duration of wash, soap quantity, and organism used) were not considered in constructing the figure. The result of this comparison suggests that soap containing an antimicrobial agent causes a reduction of bacteria similar to that of one that does not. As shown by the error bars at the top of Figure 1, the mean reduction for antimicrobial soap is slightly higher (1.56 ± 1.13) than for nonantimicrobial soap (1.31 ± 1.01), but this difference is less than 0.5 log, and both reductions are accompanied by large standard deviations. The large variation seen with this analytical technique led us to explore other, more refined methods.

The difference between the efficacy of antimicrobial soap and that of nonantimicrobial soap was also calculated for individual observations or calculated means within a study when possible (a total of 249 differences), and this comparison is shown in Figure 2. Note that if the authors did not explicitly indicate a difference, one was determined in our analysis. A negative difference (e.g., −0.5-log reduction) means that the nonantimicrobial soap was more effective than antimicrobial soap, and a positive difference shows the opposite. A difference of zero indicates that the two treatments have the same efficacy. The range of differences was −1.66 to 2.75 log CFU, with a mean of 0.45 and a standard deviation of 0.62. In this case, the mean difference is statistically significantly different from zero (P < 0.0001), which suggests that, within a study, when other factors are held constant, antimicrobial soaps do produce a greater reduction than
nonantimicrobial soap, a reduction that is highly statistically significant. This comparison produces a more refined result than what is shown in Figure 1, since it controls for any errors introduced by differences between experimental designs.

The data shown in Figure 2 can be further analyzed by separating the data by antimicrobial compound as shown in Figure 3. Here, the relative frequency of the within-study difference between antimicrobial soap and nonantimicrobial soap for different antimicrobial agents (where $n \geq 20$) is shown. Each of the various antimicrobial compounds appears to be similarly effective compared with nonantimicrobial soap, with all showing a pattern similar to that seen in Figure 2. The mean log reduction is statistically significantly different from zero for each of the antimicrobial agents shown in Figure 3 (CHG, $P < 0.0001$; iodophor, $P < 0.0001$; triclosan, $P = 0.0037$; povidone, $P = 0.0028$). A number of other antimicrobials showed similar trends, but with only a limited number of observations ($n < 10$) a statistically significant difference was not observed.

A comparison of the relative frequency of microbial reduction by a challenge organism showed the organism used had a statistically significant effect on log reduction of bacteria (Fig. 4). Studies that used resident flora on hands as an indicator of hand washing efficacy showed an average log reduction of $0.31 \pm 0.34$, a considerably smaller effect than those that used either gram-negative (1.93 ± 0.91) or gram-positive (1.91 ± 1.03) inoculated transient bacteria. There was no statistically significant difference in hand washing effectiveness between gram-negative and gram-positive transient bacteria ($P < 0.0001$), although the log reductions for both the transient organisms and the resident flora were all significantly different from zero ($P < 0.0001$).

An analysis comparing a variety of experimental design factors and log bacterial reduction showed a number of factors that had either no or limited influence on hand washing efficacy. Nonantimicrobial soaps in bar and liquid forms were similarly effective (analysis not shown). The publication year of the research also had no effect on hand washing efficacy (analysis not shown). There were no strong interactions between volume of soap and effectiveness of antimicrobial and nonantimicrobial soap (Fig. 5A). The data suggest that a very small volume of nonantimicrobial soap (<1 ml) is less effective than a slightly larger volume and that there may be some benefit to using a larger volume (>1 ml) of antimicrobial soap.

The relationship between the duration of hand washing and the effectiveness of antimicrobial and nonantimicrobial soap is shown in Figure 5B. The data show that wash times of 120 s have statistically significantly ($P = 0.0002$) higher log reductions for antimicrobial soap (0.94 ± 0.69) versus nonantimicrobial soap (0.17 ± 0.44). This trend was also statistically significant at 30 s ($P = 0.0005$), where antimicrobial soap showed a 2.42 ± 0.88 log reduction versus 1.91 ± 0.75 for nonantimicrobial soap. Clearly, more studies are needed to understand the importance of wash time in hand washing effectiveness.
Inoculum size had an effect on the reported efficacy of soaps (Fig. 6). The linear regression line for the relationship between inoculum size and log reduction is shown in Figure 6. Although the $R^2$ value is low (0.42) because of the scatter seen in the data, it is highly significant ($P < 0.0001$). The $P$ value in the case of linear regression is used to test if the slope of the line is significantly different from zero. If the slope were zero in this case, it would mean that no relationship exists between inoculum size and log reduction, which is clearly not the case in this analysis.

All cases in which hand washing was shown to increase bacteria on hands (i.e., log reduction was negative) occurred when the inoculum size was below 4 log CFU, with the most observations appearing when the inoculum size was close to the limit of detection (∼2 log CFU). The largest reductions were observed at the highest inoculum levels. This is a logical result because, for example, one cannot measure a 3-log CFU reduction if the initial level was only 2 log CFU.

**DISCUSSION**

A difference in the effectiveness of antimicrobial and nonantimicrobial soaps appears to exist and is repeatedly observed through a variety of analyses; antimicrobial soap is consistently and statistically significantly always more effective than nonantimicrobial soap. The magnitude of the difference may be small (∼0.5 log CFU), especially when the experimental design uses only the resident (naturally occurring) microflora to measure the effect. Methodological factors appear to play a significant role in determining whether this effect can be observed. Key methodological factors identified in this study include within-study comparison of antimicrobial versus nonantimicrobial efficacy, use of inoculated transient organisms rather than resident flora, and a sufficiently high inoculum level. Our analysis also suggests that other factors such as soap volume and formulation may also be important in some cases.

Our analysis of the literature showed that initial bacterial levels on hands have a substantial effect on the measured effectiveness of soaps. Experiments that use a high inoculum size (>6 log CFU) make it possible to determine the full capacity of soap to reduce bacterial populations on the hands. This also better mimics the microbial load of unwashed hands, as in research done by De Wit (14), who showed that the average microbial load on food workers’ hands ranged from 5.63 to 7.3 log CFU. Inoculum levels in this range not only are best able to show efficacy and match levels found on food workers’ hands but also are relatively easy to achieve in a laboratory setting. Experiments that use levels of >8 log CFU per hand may require more extensive laboratory preparation, including concentration of cells, and are thus less practical for routine use.

In what may be a related result, the challenge organism used also had a significant effect on the measured effectiveness of soaps. Studies that used resident flora showed a statistically significantly smaller log reduction than those that used inoculated transient gram-negative or gram-positive bacteria (see Table 1 for a listing of studies), although the choice of gram positive or negative
itself appeared to have little effect. There are two possible explanations for this result: this effect could be due strictly to the effect of inoculum size because studies that used resident flora tended to also have a lower initial microbial load (on average about 3.6 log CFU lower), or it may be that resident and transient flora have different attachment characteristics, and the resident flora are more difficult to remove. If the latter is true, using artificial inoculation rather than resident microflora may be preferable because most foodborne pathogens are not native to the skin. Our analysis also indicates that since antimicrobial soaps are significantly less effective at removing resident flora, concerns about the effects that these soaps have on the skin’s resident flora merit some skepticism.

This analysis suggests that there is some connection between the volume of soap used and microbial reduction. This result is supported by Fuls and others (18), who found that increasing the volume of antimicrobial soap from 1.5 to 3 g increased microbial reduction by >0.5 log CFU. Larson and others (26) found a similar result when comparing 1 ml to 3 ml of antimicrobial soap but found no effect with nonantimicrobial soap. It appears that a very large volume of soap (>5 ml) might be less effective; however, this result is potentially misleading because all data on a large volume of soap came from only two publications that used the same methods, so the apparent reduced effectiveness could be caused by other confounding factors specific to those studies (27, 28).

It is also possible that there are additional factors not included in this analysis that influence the effectiveness of soaps. Soap formulation may play a role in efficacy as shown by Taylor et al. (54). These investigators examined the effect of percent saturation of triclosan and the role of surfactants in formulations. They found that saturated solutions of triclosan had greater and faster bacterial reductions than less saturated formulas. They also noted that increasing surfactant concentration in a triclosan formulation decreases efficacy.

While care must always be taken in the interpretation of results from meta-analysis (51), the results of our analysis emphasize both the importance of careful experimental design for hand washing studies and the dangers of sweeping generalizations based on limited data. Although differences in efficacy between antimicrobial and nonantimicrobial soap may be relatively small, they do exist, and small but significant differences in pathogen levels on hands can have a significant effect on public health (17). Our findings also support the importance of using a relative high inoculum on the hands, well above the detection limit, preferably with inoculation methods that mimic natural contamination. As we have shown in other work, inoculum size can have a significant effect on measured bacterial cross-contamination rates between surfaces (34), although the method by which observations below the detectable limit are incorporated in the data analysis can impact any statistical analyses (35). Finally, the inherent variability in hand washing seen in the published literature (12) as well as our analysis here underscores the importance of using a sufficiently large sample size both to characterize the variability associated with these processes and to detect differences when they occur.

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