

Washing with contaminated bar soap is unlikely to transfer bacteria

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SUMMARY

Recent reports of the isolation of microorganisms from used soap bars have raised the concern that bacteria may be transferred from contaminated soap bars during handwashing. Since only one study addressing this question has been published, we developed an additional procedure to test this concern. In our new method prewashed and softened commercial deodorant soap bars (0.8% triclocarban) not active against Gram-negative bacteria were inoculated with *Escherichia coli* and *Pseudomonas aeruginosa* to give mean total survival levels of 4.4×10^5 c.f.u. per bar which was 70-fold higher than those reported on used soap bars. Sixteen panelists were instructed to wash with the inoculated bars using their normal handwashing procedure. After washing, none of the 16 panelists had detectable levels of either test bacterium on their hands. Thus, the results obtained using our new method were in complete agreement with those obtained with the previously published method even though the two methods differ in a number of procedural aspects. These findings, along with other published reports, show that little hazard exists in routine handwashing with previously used soap bars and support the frequent use of soap and water for handwashing to prevent the spread of disease.

INTRODUCTION

It is not surprising that microorganisms, which are ubiquitous in the environment, have been isolated from in-use soap bars (Bannan & Judge, 1965; Litsky & Litsky, 1967; Kabara & Brady, 1984; McBride, 1984). Such results, however, raise the question of whether or not bacteria can be transferred to hands from used soap bars during handwashing. The only study directly addressing this question was published over 20 years ago (Bannan & Judge, 1965). In this study 10 panelists, whose hands were inoculated with 5×10^9 c.f.u. *Serratia marcescens*, washed with a non-germicidal soap bar using their normal handwashing procedures. Ten additional panelists then washed with the 10 soap bars, which had an estimated mean level of 6.2×10^5 c.f.u. *S. marcescens* per bar, using their normal handwashing procedure. None of the second group of panelists had detectable (> 20) *S. marcescens* on their hands after washing. The results of this study indicated that transfer of bacteria to the hands does not occur during washing with deliberately contaminated soap bars.

Recently this study was criticized because no used soap bars or neutralizers were used in this study (Kabara & Brady, 1984; Kabara, 1985). A reply to this criticism has been published (Heinze, 1985) in which these points were discussed. It was stated that after the first panelist, with contaminated hands, washes with a new soap bar, the soap has become a used soap bar for the second panelist. Since anti-bacterial soap bars were not used, neutralizers were not necessary (Heinze, 1985, 1986). Because of the misunderstanding in the literature, we decided to re-examine the question of transfer of microorganisms to hands during washing with deliberately contaminated soap bars using a new procedure.

MATERIALS AND METHODS

Soap bars

The test soap bars used were commercial production deodorant bars (Dial®) containing 0.8% triclocarban (3,4,4'-trichlorocarbonyl chloride), which is bacteriostatic only against Gram-positive bacteria and is not active against the Gram-negative bacteria used in the test. Placebo soap bars containing no triclocarban or fragrance were produced in the Dial Technical Center pilot plant and were otherwise identical to the test bars.

Prior to inoculation the previously washed and air dried test soap bars were hand washed by rotating under running tap water ($\sim 100^\circ\text{F}$) for 30 s. The test soap bars were then softened by soaking for 30 min in individual soap dishes containing 10 ml of sterile water at ambient temperature. Each soaked test soap bar was then inverted and allowed to drain for 60 s prior to inoculation.

Bacterial cultures

Two Gram-negative bacteria were used for this test: *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 11229. These bacteria were maintained on Brain Heart Infusion agar (BBL) slants and were grown in Brain Heart Infusion broth (BBL). The third to seventh sequential 24 h transfer was used to inoculate the test soap bars. Aliquots of each culture were centrifuged 10 min at the highest setting using an IEC clinical table top centrifuge. The clear supernatant was carefully poured off and the cell pellet in each tube was resuspended by vortexing in a volume of sterile Ringer's solution equal to the original volume in the tubes. The cultures were further diluted with Ringer's solution and 0.1 ml aliquots were plated in duplicate on the following media to establish population densities for each culture: m-FC agar (Gibco Laboratories) (without rosolic acid) for *E. coli* and Pseudomonas Isolation agar (Difco) for *Ps. aeruginosa*.

Inoculation of soap bars

Preliminary experiments were performed to determine the proper volume of each washed overnight culture needed to achieve surviving levels of 3.0×10^4 c.f.u. of each bacterium per bar. This volume was estimated from the observed survivors per bar and the actual volume of individual culture per bar. Linear extrapolation was used for four experiments in which four volumes per bar of each culture (in triplicate) were tested. A simple proportion was used for two experiments in which only one volume per bar of each culture (in triplicate) was tested. Soap bars were

prepared and inoculated with various mixtures of overnight culture in Ringer's solution. The individual volumes tested in the six experiments covered a range of 0.25–25 μl of *E. coli* overnight cultures and 0.75–25 μl of *Ps. aeruginosa* overnight cultures suspended in total volumes of 5–200 μl of Ringer's solution.

Based on the results of these experiments, the following bacterial suspension was used as an inoculum within 1 h of centrifugation: 1 μl *E. coli*, 3.0 μl *Ps. aeruginosa* and 96.0 μl Ringer's solution. The soap bars were tilted in a circular motion to spread the inoculum over the moist upper surface of the soap bar. After 60 s, the inoculated soap bars were issued to panelist for the test wash. Two inoculated soap bars not used by the panelists were sampled as described below to estimate the number of viable test bacteria on the soap bars.

Panelists

Sixteen panelists, 5 male and 11 female, 18 years of age or older and with normal skin, were recruited for the study. Individuals using antibiotics or steroids, either topically or orally, were not allowed to participate in the study. Informed consent was obtained from each panelist.

The panelists were issued non-germicidal soap bars, shampoo, and anti-perspirant to use exclusively during the 1 week washout period in which any antibacterial agent on the body was removed. In addition, the panelists were instructed to refrain from using perfumes, body lotions, and skin creams and from swimming during this period. They were also issued rubber gloves and instructed to wear the gloves for all household chores involving detergents, acids, alkalines, and solvents. On the test day the panelists reported to the laboratory and their hands were again examined. Panelists whose hands were free of cuts, scrapes, open wounds and skin disorders washed their hands *ad lib* with the placebo soap bar under warm running tap water and blotted the hands dry with disposable paper towels to reduce the numbers of transient bacteria on their hands. Both hands were then sampled as described below to ensure that none of the panelists were carriers of *E. coli* or *Ps. aeruginosa*.

Hand sampling

The panelists placed both hands in sterile gloves. Twenty ml of hand sampling solution (75 mM phosphate buffer + 5% Tween-80 + 0.5% lecithin + 0.1% Triton X100, pH 7.9 (Williamson & Kligman, 1965) were pipetted into each glove. The panelists then opened and closed their hands into tight fists repeatedly for 60s to dislodge any transient bacteria from the hands. One tenth ml of the hand sampling solution from each glove was added to triplicate plates of m-FC agar and *Pseudomonas* Isolation agar and spread with a sterile glass 'hockey stick'. The m-FC plates were incubated at 45 ± 0.5 °C for 24 h and the *Pseudomonas* Isolation agar plates were incubated at 35–37 °C for 48 h.

Wash test

After the placebo soap handwash and initial hand sampling, each panelist was issued an inoculated soap bar and instructed to wash their hands the way they normally did. Immediately after washing their hands, panelists' hands were sampled as described above, and the soap bars were sampled using the following

procedure: the soap bars were placed in sterile Whirl-pak® bags. One hundred ml of soap sampling solution (same as hand sampling solution except pH 7.0) were added to the bags. The bags were tied and shaken for 30 s. Triplicate 0.1 ml aliquots were spread on m-FC agar and Pseudomonas Isolation agar and incubated as described above. The panelists completed their participation in the test by washing their hands a final time with uninoculated test soap bars.

Calculation

The limit of detection for this experiment was 67 bacteria per hand for each panelist and bacterium. This number is determined by the use of a 20 ml sampling solution per hand and the plating of 0.1 ml aliquots in triplicate for each bacterium and hand. Thus if one bacterial colony were observed among the three plates made from one hand sampling solution, it would mean that only 67 bacteria were detected per hand according to the following formula:

$$\frac{1 \text{ bacterial colony} \times 20 \text{ ml/hand}}{3 \text{ plates} \times 0.1 \text{ ml/plate}} = 67 \text{ bacteria/hand.}$$

The same reasoning applied to detecting bacteria left on test soap bars for each panelist after the *ad lib* washing. One hundred ml sampling solution was used per bar and 0.1 ml aliquots were plated in triplicate for each bacterium and panelist. This meant that the limit of detection was 330 bacteria per bar for each bacterium and panelist according to the following formula:

$$\frac{1 \text{ bacterial colony} \times 100 \text{ ml/bar}}{3 \text{ plates} \times 0.1 \text{ ml/plate}} = 330 \text{ bacteria/bar.}$$

Identification of recovered bacteria

After incubation the test plates were examined visually and suspect colonies were picked and inoculated on MacConkey agar (Difco) for *E. coli* or Pseudomonas Isolation agar for *Ps. aeruginosa*. The identity of suspect colonies was confirmed using the API 20E test kit.

RESULTS

The results of six experiments to determine the volume of the overnight cultures needed to obtain the target survival level (3.0×10^4 per bar) are shown in Table 1. The inoculation volumes estimated to achieve target survival levels varied over almost a 20-fold range. The geometric mean volumes determined from these experiments were used to inoculate the test bars used by the panelists (Tables 2 and 3).

Actual survival levels of bacteria observed on two inoculated but unused test bars are shown in Table 2. The mean survival level for *E. coli* was 4.3×10^5 per bar. The mean survival level for *Ps. aeruginosa* was 5.2×10^3 per bar.

None of the 16 panelists picked up detectable levels of either test bacterium on their hands after washing with the inoculated soap bars (Table 3). *E. coli* was recovered from two of the used soap bars and *Ps. aeruginosa* was also recovered from one of these two bars.

Table 1. *Volumes of overnight cultures of E. coli and Ps. aeruginosa to give 3 × 10⁴ survivors each on inoculated test soap bars*

	μl per bar	
	<i>E. coli</i>	<i>Ps. aeruginosa</i>
Range observed in six experiments	0.25–4.8	1.1–7.1
Geometric mean volume	1.1	3.1
95% confidence interval	0.096–13.7	0.65–13.9

Table 2. *Survival of test bacteria on soap bars*

Culture	Inoculum (c.f.u./bar)	Survival levels c.f.u./bar			Geometric mean recovery as a percent of the inoculum
		Bar A	Bar B	Geometric mean	
<i>E. coli</i>	8.9×10^5	3.1×10^5	5.9×10^5	4.3×10^5	48
<i>Ps. aeruginosa</i>	1.1×10^5	6.7×10^2	4.0×10^4	5.2×10^3	4.7
Total bacteria	1.0×10^6	3.1×10^5	6.3×10^5	4.4×10^5	44

Table 3. *Recovery of test bacteria from inoculated soap bars after panelist handwashing*

Inoculum	Recovery from hands† (c.f.u./hand)	Recovery from bars after washing*		
		No. positive per no. tested	Mean c.f.u. per positive bar	Mean (%) recovery
<i>E. coli</i>	< 67	2/16	3000	0.70
<i>Ps. aeruginosa</i>	< 67	1/16	330	6.3

* Minimum level of detection was 330 c.f.u. per bar.

† Minimum level of detection was 67 c.f.u. per hand.

DISCUSSION

A new procedure has been developed to test for the transfer of microorganisms to the hands during washing with inoculated soap bars. This new method differs in a number of aspects from the original method (Bannan & Judge, 1965). Unlike the original method, we employed a direct and more controlled inoculation procedure. Phosphate buffer and neutralizers were used in the recovery media (Williamson & Kligman, 1965). An improved method for sampling the soap bars was developed. A number of refinements and controls were added including confirmatory bacterial identification tests, a larger number of panelists and explicit restrictions on panelists' use of antimicrobials prior to the test. However, the principal difference between the two methods was the inoculation procedure used in our new method.

Because we wanted to use a direct inoculation procedure rather than relying on

panelists washing with contaminated hands to inoculate the soap (Bannan & Judge, 1965), it was necessary to precondition the soap bars (Kabara & Brady, 1984). We chose to prewash and water soften previously used tests bars immediately prior to inoculation and panelists usage. Our assumption was that prewashing and soaking would improve the survival of the test bacteria on the bars and facilitate any transfer of the bacteria to panelists' hands. While the assumption of better survival on wet bars seems reasonable (McBride, 1984), we are unaware of any data which directly support it. Consequently, the results obtained using our procedure do not address the possibility of the transfer of bacteria from dry soap bars.

Rather than inoculation with *S. marcescens* as previously done (Bannan & Judge, 1965), we chose to use two other Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. Gram negatives were used since the antimicrobial agent in the test soap, triclocarban, is not active against these bacteria (MIC greater than 1000 p.p.m., unpublished data) and because we found in preliminary experiments that they survive better on soap bars than do Gram-positive bacteria, confirming published results with inoculated bars (Kabara & Brady, 1984). *E. coli* and *Pseudomonas* spp. have been frequently isolated from nosocomial infections (Bennett, 1979) and from moist soap dishes (Jarvis *et al.* 1979) although Gram-positive bacteria, especially coagulase-negative *Staphylococcus* spp., have been more frequently isolated from used soap bars (Kabara & Brady, 1984; McBride, 1984). Thus, our results do not address the possibility of the transfer of Gram-positive bacteria from soap bars.

For our direct inoculation procedure, overnight cultures of *E. coli* and *Ps. aeruginosa* were resuspended in Ringer's solution, and aliquots in a volume of 100 μ l were evenly spread over the top surface of the moistened soap bars. We chose as our target for each bacterium a level of 3.0×10^4 survivors per bar. Using this inoculation procedure, the actual mean survival levels achieved on the test bars used by the panelists were 14-fold higher than the target level for *E. coli* and 5.8-fold lower than the target level for *Ps. aeruginosa* (Table 2). The *E. coli* level appears to be higher than expected due to an exceptionally high survival rate on the bars while the *Ps. aeruginosa* level appears to be lower than expected due to the exceptionally low viable cell number in the overnight culture used for the inoculum. Nonetheless, these survival levels were within the expected range since a 20-fold range in survival levels had been observed in the six experiments conducted to determine the volume of the overnight cultures needed to obtain the target survival level (Table 1). Moreover, this inoculation procedure appears to be more reproducible than that used in the original method (Bannan & Judge, 1965) where the actual survival levels on 10 inoculated bars varied over a 10^3 range.

The target survival levels were chosen to give 6.0×10^4 total bacteria per bar, approximately 10 times the highest levels reported on used soap bars (Kabara & Brady, 1984; McBride, 1984). The actual mean survival level achieved on the test bars used by the panelists was 4.4×10^5 total bacteria per bar, a level similar to that used in the original procedure (Bannan & Judge, 1965), and about 70-fold higher than the highest reported in-use levels. Nonetheless, these results do not address the possibility of the transfer of bacteria to hands from bars contaminated with higher levels of bacteria or using recovery methods having a detection limit lower than 67 c.f.u. per hand.

Despite the differences in the two methods, the results obtained with the new method are completely consistent with those reported using the original method (Bannan & Judge, 1965), namely that bacteria are not transferred to the hands during washing with soap bars contaminated with much higher levels of microorganisms than those found on in-use bars (Kabara & Brady, 1984; McBride, 1984). The finding in the new study, of low levels of test bacteria on 2 of the 16 test bars after panelists' use, is also consistent with the results of Bannan & Judge, who recovered inoculated bacteria from 1 of 10 soap bars. These results indicate that routine washing of soap bars greatly reduces the level of, but may not completely eliminate, contaminating bacteria on soap bars. These results help explain the low levels and the transient nature of the bacteria found on continually used soap bars (McBride, 1984).

The results obtained with the new procedure strongly support the major conclusion made from the older method that there is little, if any, risk of cross contamination from washing with previously used soap bars. Moreover, there is no evidence that, even if bacteria were transferred during handwashing, this would necessarily cause infection. On the contrary, soap bars have not been implicated in the spread of any disease, even nosocomial infections. Indeed, washing with soap and water is still recommended in the USA as the single most important procedure for preventing nosocomial infection (Steere & Mallison, 1975; Hastings, 1983, Garner & Favero, 1985). Moreover, a review of the history of commercial soaps and detergents suggests that the increased use of soap for washing has had a very positive impact on American public health (Greene, 1984).

Additional experiments using other microorganisms and inoculation procedures could be developed to further test the hypothesis that bacteria on soap bars can be transferred during routine handwashing. However, results of experiments described in this paper and those reported previously provided evidence that, contrary to the transfer hypothesis, frequent washing with soap and water helps to prevent the spread of disease (Steere & Mallison, 1975; Hastings, 1983; Garner & Favero, 1985).

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