Transient skin flora

Their removal by cleansing or disinfection in relation to their mode of deposition

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SUMMARY A suspension of *Staphylococcus aureus* deposited on the skin was much more effectively removed by soap-and-water washing when it had been spread and allowed to dry (mean survival 2%) than when it had been rubbed on to the skin (mean survival 29.9%); when antiseptics (70% ethyl alcohol, Hibiscrub without added water) were used, there was no difference between their action against bacteria dried on and bacteria rubbed on to the skin; both of these methods, and especially alcohol, were more effective than soap and water. When a detergent-disinfectant method (washing with Hibiscrub and water) was used, there was a significantly greater effect against rubbed-on than against dried-on bacteria; soap and water was slightly more effective than Hibiscrub and water against the latter. The need to reappraise methods of reducing transient skin flora in 'hygienic' hand cleansing and the tests used for this purpose are discussed.

Microorganisms that are deposited on the skin but do not colonise it are commonly called *transient flora* to distinguish them from the *resident skin flora* (Price, 1938; Lowbury *et al.*, 1960, 1964). The important medical distinction between resident and transient bacteria lies in the much greater ease of removal of the latter by washing with soap and water and by disinfection with antiseptics (Lowbury *et al.*, 1960, 1964). Unless or until they become established among the resident flora, bacteria deposited on the skin must be regarded as transients, and preventing the transfer of bacteria from one patient to another on the hands of nurses, doctors, physiotherapists, and others who handle patients is thus seen to depend mainly on the effective removal of such transients from the skin soon after they are acquired. The standard of cleansing or disinfection generally considered adequate for this purpose (*hygienic* skin cleansing and disinfection) is therefore less exacting than the standards required for members of the scrubbed surgical team, who are expected to use methods effective against the resident flora (*surgical* skin cleansing and disinfection).

Methods of skin cleansing and disinfection are commonly tested for their effectiveness against transient bacteria in the following way. Bacterial

suspensions are spread on the skin and allowed to dry, and the numbers of bacteria in such dried films before and after detergent cleansing or disinfection of the skin are compared (Gardner and Seddon, 1946; Story, 1952; Lowbury *et al.*, 1960, 1964; Rotter *et al.*, 1974). But the effectiveness of cleansing and disinfection against the transient flora is likely to vary with the pressure used and the length of time over which the bacteria were deposited. This view is supported by the fact that organisms that are sometimes present on healthy skin, though unlikely to multiply there (eg, *Clostridium welchii* and various Enterobacteria), have been found more difficult to remove by washing and disinfection than are those that have been deliberately spread and allowed to dry on the skin (Ayliffe and Lowbury, 1969; Ayliffe *et al.*, 1975).

In this paper we assess the effectiveness of different methods of cleansing and disinfection against bacteria deposited on the skin with friction ('rubbed on') compared with their effectiveness against the same bacteria spread and allowed to dry ('dried on').

Material and methods

Deposition of bacterial suspension

An overnight nutrient broth culture of *Staphylococcus aureus* (strain 77/6584), isolated from a burn, was centrifuged and resuspended in an equal volume

of sterile distilled water. The hands of four volunteers (members of the MRC unit staff) were thoroughly washed with soap and water and dried on a sterile towel. Drops of the bacterial suspension (0.02 ml from a standard dropping pipette) were deposited on the tips of all four fingers of the left hand. In one series of experiments a platinum loopful of the deposited suspension was transferred from each fingertip to the corresponding fingertip of the other hand; the suspension of both fingertips was then spread with the loop and allowed to dry. In another experiment, the bacterial suspension was deposited in the same way, but the fingertips of the two hands were then rubbed firmly together for about 30 seconds and left to dry for one minute.

Cleansing and disinfection

After deposition and drying of the bacterial suspension on the fingertips, the hands were given a standard detergent cleansing or disinfection or combined cleansing and disinfection. The following methods were used, each method being tested on each of the four volunteers:

- (1) a 30-second standard handwash (covering all surfaces, including fingertips) with unmedicated bar soap and running warm water from a tap;
- (2) a 30-second standard handwash with 4% chlorhexidine detergent solution (Hibiscrub) and running warm water;
- (3) a standard handwash with 70% ethyl alcohol, two successive lots of 5 ml being poured into the cupped hands and rubbed over all surfaces, including fingertips, until the hands were dry (Lowbury *et al.*, 1974; Ayliffe *et al.*, 1975); and
- (4) a standard 30-second handwash with Hibiscrub without the addition of water; this method was used to explain an apparent anomaly in the results of detergent disinfection with Hibiscrub and water (see below).

Assessments of cleansing and disinfection

In every experiment the volunteer rubbed his fingertips for three minutes against sterile glass beads in short flat-bottomed glass tubes (3 × 1 inch, 7.5 × 2.5 cm) containing 5 ml of sterile Ringer's solution with neutralisers—1% Tween 80, 1% Lubrol W, and 0.5% lecithin (Lowbury and Lilly, 1960). Viable counts of staphylococcal colonies on horse blood agar were obtained from these sampling fluids by the drop-counting method of Miles and Misra (1938).

In the analysis, counts from corresponding fingers of the two hands were added together. Separate experiments were made on fingers that had been and had not been disinfected after deposition of bacterial suspensions.

Results

Table 1 shows the mean percentage survival on fingertips of the deposited bacteria after cleansing and disinfection, as judged by the mean counts of presumptive *Staph. aureus* in samplings taken after deposition of bacteria and, in separate experiments, in samplings taken after deposition of bacteria followed by cleansing or disinfection. With the soap and water wash, the mean survival was about 30% when the bacteria had been rubbed on to the skin, but only 2% when they had been allowed to dry on the surface. By contrast, there was no difference in the proportions of staphylococci deposited in these two ways that survived alcohol disinfection (0.3%), and these were considerably lower than the proportions that survived soap and water washing, whether they had been deposited by drying on or by rubbing on. Hibiscrub and water was more effective against bacteria rubbed on (mean survival 1.1%) than against bacteria dried on (mean survival 5.9%). This seemingly paradoxical result might have been due to dilution of the chlorhexidine by added water,

Table 1 *Effects of soap and water cleansing and of disinfection on transient skin bacteria applied in different ways*

Treatment (method of cleansing or disinfection)	Bacteria per ml washings recovered from fingers after cleansing or disinfection, expressed as percentage of numbers recovered in samples taken before treatment									
	Bacterial culture spread on fingertips and left to dry					Bacterial culture deposited on fingertips and rubbed on				
	Mean* 1st fingers	Mean 2nd fingers	Mean 3rd fingers	Mean 4th fingers	Mean all fingers	Mean 1st fingers	Mean 2nd fingers	Mean 3rd fingers	Mean 4th fingers	Mean all fingers
30-second wash with soap and water	1.7	3.2	1.4	1.5	2.0	35.7	24.9	31.1	26.7	29.6
70% ethyl alcohol rubbed on hands until dry (2 lots of 5 ml)	0.2	0.4	0.2	0.4	0.3	0.3	0.3	0.4	0.2	0.3
30-second wash with Hibiscrub and water	2.7	8.1	8.2	4.7	5.9	1.2	0.7	1.7	0.7	1.1
30-second wash with Hibiscrub; no added water	1.8	1.0	2.8	2.0	1.9	4.1	2.4	4.6	1.9	3.3

*Mean of combined counts from right and left fingers in 4 subjects; '1st fingers' = index fingers of both hands

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which was likely to have a greater effect when the deposited bacteria were lying on the surface than when they were rubbed into the horny layer. To test this hypothesis, we made an experiment using Hibiscrub without added water; the result, which showed a slightly greater survival of bacteria that had been rubbed on than of those that had been allowed to dry on the surface, was consistent with the hypothesis.

Table 2 shows the mean log reduction (that is, mean of log pre-treatment counts minus mean of log post-treatment counts) calculated from the counts of deposited bacteria recovered from fingertips. From these it was shown that the reduction of dried-on bacteria was significantly greater than that of rubbed-on bacteria when soap and water was used. With Hibiscrub and water, there was a significantly greater reduction of rubbed-on than of dried-on bacteria. When alcohol or Hibiscrub without added water was used, there was no significant difference in the reduction obtained on bacteria deposited by the two methods.

Table 3 shows the results of significance testing for differences between the different methods of cleansing and disinfection. There was no significant difference between soap and water and Hibiscrub with water when the bacteria were dried on, or between Hibiscrub with water and Hibiscrub with no added water when the bacteria were rubbed on. All the other comparisons showed significant differences.

Discussion

These studies showed that suspensions of *Staph. aureus* deposited on the skin were much more resistant to the effects of a soap-and-water wash when rubbed on to the skin than when spread on and allowed to dry. No such difference was detected on chemical disinfection of the artificially contaminated skin with 70% ethyl alcohol or with 4% chlorhexidine detergent solution (Hibiscrub) without addition of water during the treatment; with the addition of water to obtain detergent cleansing, Hibiscrub was less effective against dried-on than against rubbed-on bacteria. Hibiscrub with water was significantly less effective against dried-on bacteria than was a wash with soap and water; the result is in striking contrast with the good effects of Hibiscrub and the poor effects of soap and water against rubbed-on bacteria and against the resident skin flora (Lowbury and Lilly, 1973). In the use of a detergent disinfectant preparation, such as Hibiscrub, conditions which were optimal for detergent cleansing cancelled the effects of the disinfectant component against superficial transient bacteria.

In the assessment of methods for the removal of transient bacteria, the difference between results that might be expected with detergents, though not with antiseptics, against bacteria applied in different ways, must be borne in mind; so must the complex and, to some extent, incompatible effects of combined disinfectant-detergent action against these

Table 2 Mean log reduction factors (log pre-treatment count minus log post-treatment count)

Treatment (method of cleansing or disinfection)	Bacterial suspension		Significance of difference
	Dried on	Rubbed on	
Soap and water	1.76	0.53	t = 9.439 P < 0.001
70% ethyl alcohol	2.62	2.60	t = 0.188 P > 0.1 (NS)
Hibiscrub with water	1.35	2.07	t = 5.557 P < 0.001
Hibiscrub without added water	1.96	1.81	t = 1.174 P > 0.1 (NS)

Table 3 Comparison of effects of different treatment methods against dried-on and rubbed-on bacteria

Comparison of treatments	Significance of difference between effects against (t-tests)	
	Bacteria dried on	Bacteria rubbed on
Soap and water v alcohol	P < 0.001	P < 0.001
Soap and water v Hibiscrub with water	P < 0.01	P < 0.001
Soap and water v Hibiscrub with no added water	P > 0.1 (NS)	P < 0.001
Alcohol v Hibiscrub with water	P < 0.001	P < 0.001
Alcohol v Hibiscrub with no added water	P < 0.001	P < 0.001
Hibiscrub with water v Hibiscrub with no added water	P < 0.001	P < 0.1 > 0.05 (NS)

organisms, also the differences between different bacterial species and, probably, between different members of the same species (Ayliffe *et al.*, 1978). For this reason, it may be more useful to assess methods of disinfection and detergent cleansing against transient bacteria by their success in removing naturally occurring Gram-negative bacilli from the hands of nurses, as described by Ayliffe *et al.* (1975). If an artificial contaminant is required for a standard test of 'hygienic' hand cleansing and disinfection, both dried-on and rubbed-on inocula of two or more standard test strains should be used.

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