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Shedding of bacteria and skin squames after handwashing

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SUMMARY

Particles released into the air by wringing the hands together were collected in a slit sampler before and after washing with bar soap, with three surgical scrubs, and after rubbing them with a spirit-based lotion. The particles were identified, their number estimated, those that bore bacteria counted, and the bacteria themselves classified. It was found that there was a significant increase, averaging 17-fold, in the number of particles carrying viable bacteria released after washing with soap. The increase in bacterial dissemination was suppressed if a surgical scrub was used in place of soap, or when the lotion was used without washing. The number of skin squames released increased by 18-fold or more after washing with soap or a surgical scrub, but not after using the lotion. This suggests that a surgical scrub should be used more widely in clinical practice, and that a spirit-based hand lotion might with advantage become a partial substitute for handwashing, particularly in areas where handwashing is frequent and iatrogenic coagulase-negative staphylococcal infection common.

INTRODUCTION

The air in inhabited places contains epithelial squames shed from the skin. Some of these carry micro-organisms originating from the surface flora of the occupants (Davies & Noble, 1962; Clark, 1974). An increase in the dispersal of skin bacteria has been reported after a showerbath (Speers *et al.* 1965; Bethune *et al.* 1965), and a preliminary observation has shown this also happens after washing the hands (Meers, 1976). Because of the possibility that the washing of hands by medical and paramedical staff contributes to hospital infection, the shedding of bacteria and squames was investigated in a group of volunteers before and after washing in turn with bar soap and three surgical scrubs, and after rubbing with a spirit-based lotion.

MATERIALS AND METHODS

Particles were collected with a Casella slit sampler, impinging 15 l of air in 30 s onto a Petri dish of agar while it rotated through 360°. For counting and differentiating particles, collection was onto 1·5% Noble agar (Difco) in normal saline; 10% horse-blood agar was used for enumerating the particles which carried bacteria. Observations were made in a room which was otherwise unused, in which

the number of occupants was restricted and movement kept to a minimum. A subject who had not washed her hands for at least an hour sat quietly in front of the sampler, which was placed just below head height, and the machine was switched on. After a period during which collection was made to estimate the background count, the subject began to rub her hands together with a wringing motion at a mean height of 15 cm above the slit of the sampler, continuing until the machine switched itself off when the rotation was complete. She then treated her hands as described, and the sampling process was repeated.

Hands were washed for 30 s using unmedicated bar soap or a surgical scrub and warm running water from a mixer-tap; they were dried on disposable paper towels. The three surgical scrubs used contained the disinfectants povidone-iodine (0·75% (w/v) available iodine, Disadine), chlorhexidine (4% (w/v) chlorhexidine gluconate, Hibiscrub), or 3% (w/w) hexachlorophane (Phisomed). In addition, the hands were sampled after smearing them lightly with glycerol following washing with soap, and after rubbing with approximately 2·5 ml of a spirit-based lotion for about 30 sec, until the alcohol had evaporated. This lotion, based on 68 over-proof industrial methylated spirit, contained 86% (v/v) alcohol, 1% (v/v) glycerol and 0·02% (w/v) chlorhexidine gluconate. Samples of the paper towels used in the experiments were tested by impression on the surface of blood agar plates. Repeated tests gave no growth of bacteria arising from the towels.

Two experiments were done. In the first, eight student nurses were selected for further study from among a group of 16 volunteers as being moderate, consistent disseminators of bacteria-carrying particles after washing their hands with soap and water. Each was examined as described, before and after washing her hands in turn with soap, the three surgical scrubs, and after applying glycerol or the lotion. Each pair of observations on each individual was separated from the next by at least 2 days, and counts before and after washing with soap were interposed between observations made with surgical scrubs or the lotion. Collection was on blood agar, the first 180° of the plate sampling to determine the background aerial contamination, the remainder receiving material desquamated from the hands. The plates were incubated overnight aerobically at 37 °C, followed by a further 24 h at room temperature, the latter to encourage development of pigment helpful in bacterial identification. The colonies were counted, and classed as staphylococci (coagulase positive or negative), streptococci, diphtheroids, neisseria, coliforms, aerobic spore-bearing bacilli, or others. This was done by subculture of representative colonial types, and their identification by standard methods, though with experience detailed examination was needed less often. The number of colonies developing from particles in the sample of 7·5 l of air collected on the first half of a plate, the background count, was subtracted from the count on the second half. The remainder was taken as the number of particles bearing bacteria capable of multiplying aerobically on blood agar released from the hands of a subject in 15 sec, collected from 7·5 l of air.

In the second experiment, two volunteers repeated the first, but in addition the total of particles released from the hands in each test was estimated as well as counting those that carried bacteria. The volunteers, who were not included in

the first group, were chosen as being an average and a heavy disseminator. Particle collection was onto media in a divided Petri dish (Sterilin Ltd) half filled with blood agar, and half with Noble agar. The plate was positioned on the turntable of the slit sampler so that the first collection, on 110° of Noble agar surface, was of particles representing the background count. Hand-rubbing then commenced, and continued for 250°. This completed the observation, so that particle collection from the hands was on, in succession, 35° of Noble agar, 180° of blood agar, and finally another 35° of Noble agar. After sampling, three radially-oriented blocks, each about 7 mm wide and the full depth of the Noble agar were cut, lifted out, placed on a microscope slide, and a cover-slip put onto the surface bearing the particles. The blocks were cut so that the first sampled the centre of the background-count area, and each of the others was from an area bearing particles from the hands. Particles were identified and counted microscopically using a $\times 10$ objective and $\times 10$ eye-pieces, each field measuring 2·35 mm^2. Twenty-five fields were examined on each block, moving twice along the line of the radius of the original plate. The particles were classified as skin squames, fibres and other particles; non-fibrous particles smaller than 100 μm^2 were ignored. Counts of squames found in the background area were subtracted from counts in the two hand-rubbing areas, the results averaged and multiplied by a factor to make the count equivalent to that on half a plate, and so directly comparable with the number of bacterial colonies developing on blood agar in the same experiment. The bacterial count was made as has been described, after removal of the agar blocks. Background bacterial counts were established by air sampling onto blood agar plates immediately before hand sample counts were taken. The particle count on unexposed Noble agar was insignificant.

RESULTS

First experiment

An example of the results of colony counts and identifications from a single set of observations on one volunteer before and after washing her hands with soap were: background count before washing, 4; hand-rubbing count before washing, 7; background count after washing, 5; hand-rubbing count after washing, 49. The bacterial types involved in each of the observations were, respectively, coagulase-negative staphylococci, 4, 5, 5, 48; corynebacteria, 0, 1, 0, 1; *Bacillus* sp., 0, 1, 0, 0. These data were compressed as described for inclusion in Fig. 1 to give hand-rubbing bacterial counts before and after washing of 3 and 44. All the other points in Fig. 1 were derived in the same way. The median colony count for the pre- and post-washing background levels in the 48 observations made using soap were 3 and 5 respectively. The proportion of each of the varieties of bacteria isolated in all the observations in this experiment were, coagulase-negative staphylococci, 97·3%, corynebacteria, 1·0%, *Bacillus* spp., 0·8%, remainder, 0·9%; *Staphylococcus aureus* was not found.

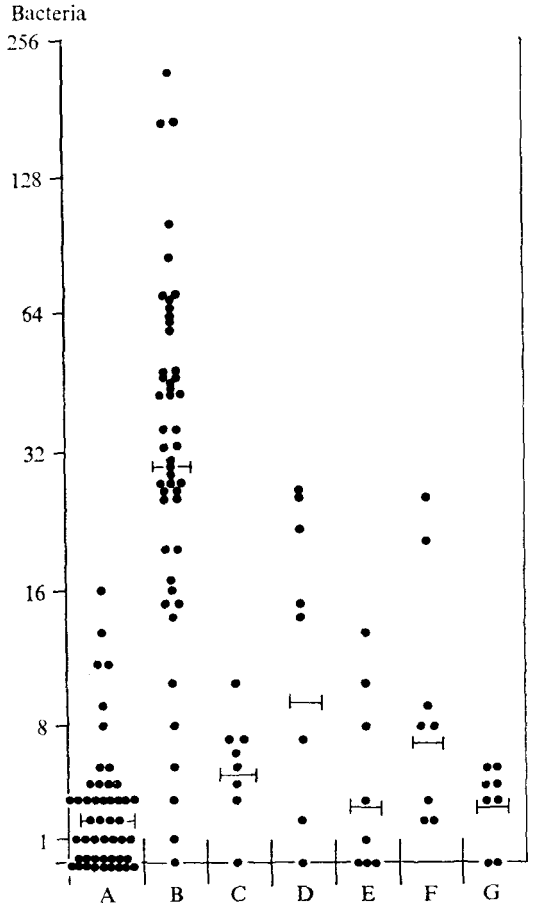

Fig. 1. Counts of particles carrying viable bacteria from eight volunteers collected by wringing together the hands for 15 s over a slit-sampler, before and after various treatments. Bars represent geometric means. (A) Immediately before treatment B. (B) After washing with bar soap and water. (C) As B, but with a smear of glycerol afterwards. (D) After washing with povidone-iodine surgical scrub. (E) After washing with chlorhexidine surgical scrub. (F) After washing with hexachlorophane surgical scrub. (G) After rubbing with a spirit-based lotion.

Second experiment

Bacterial counts derived as described in the first experiment are recorded in Fig. 2, together with the related counts of skin squames. A typical series of particle counts from an experiment on a volunteer before and after washing her hands with soap were: background count before washing, 385 squames, 0 fibres, 256 other particles; hand-rubbing count before washing, 684 squames, 86 fibres, 107 other particles; background count after washing, 470 squames, 86 fibres, 299 other particles; hand-rubbing count after washing, 4658 squames, 321 fibres, 855 other particles. In Fig. 2 these become 299 and 4188 squames before and after washing. In the course of this experiment, the median corrected number of bacteria-carrying particles collected before hand-washing was 2 for the first volunteer and

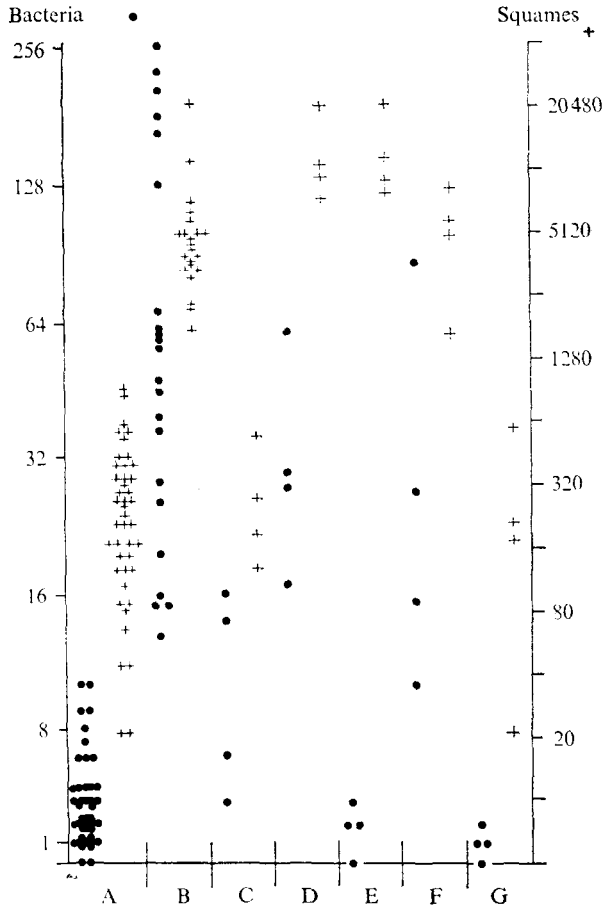

Fig. 2. Counts of particles bearing viable bacteria from the hands of two volunteers derived as in Fig. 1, together with counts of skin squames released at the same time. The letters A–G signify the treatments listed in the footnote to Fig. 1. The dots and crosses in each column are the counts of bacteria and squames, respectively.

3 for the second. These were associated with the median dissemination of 299 and 171 squames, respectively. After handwashing with soap, the figures were 129 bacteria-carrying particles for the first volunteer and 26 for the second; median counts of squames were 5000 and 3563 respectively. The ratios of bacteria-carrying particles to the totals of squames released derived from these figures are 1:150 and 1:57 before washing, and 1:39 and 1:137 afterwards.

DISCUSSION

This report confirms the earlier observation that washing the hands with unmedicated soap makes it easier for skin squames carrying viable bacteria to escape into the air. Eight of sixteen volunteers chosen for this study had an average 17-fold increase in the dispersal of bacteria-carrying particles after washing

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(Fig. 1). A second experiment with two volunteers showed that this was paralleled by an increase in the release of squames, the average in this case being 18-fold (Fig. 2). The lack of a clean air enclosure in which to make the observations forced us to accept a rather high background count of particles, including squames. However, the increase in the number of squames released after handwashing was so large as to make the background count insignificant. In addition the technique described grossly underestimated the coryneform organisms present on, and presumably released from, the skin. For this reason the number of bacteria-carrying particles and their ratios to total particles calculated from the data presented are not absolute, nor should such ratios be compared with those from other parts of the body, because of the specialized nature of the palmar skin.

As newly washed, ungloved hands are used for a great variety of medical procedures, including donning gloves, these facts must have some relevance. Apart from gravitational settling onto clean or sterile surfaces, particles released in this way are attracted by, and adhere to, an object bearing a static electrical charge such as develops readily on plastic apparatus, for instance the piercing cannula of an intravenous giving set. Because the release of these particles is suppressed by a thin smear of glycerol, the process is probably a simple physical one, due to the dislodgement of squames loosened by washing. The dissemination of viable bacteria is reduced almost to the level before washing if an antiseptic surgical scrub is used instead of soap, but the release of squames is increased still further by two of these preparations (Fig. 2), perhaps because of stronger detergent action. The two concerned are those containing chlorhexidine and povidone-iodine, these produced on average a 45-fold increase in the release of squames. The dissemination of squames following the use of the hexachlorophane scrub was similar to that found after washing with soap. The comparative effectiveness of the three surgical scrubs as antibacterial agents measured by the method reported is the same as that demonstrated by Lowbury & Lilly (1973) using a different technique, chlorhexidine again proving most active. However, the lotion was at least as effective as the best surgical scrub, and it did not cause the prolific increase in the release of squames which accompanied the use of soap or a detergent. The lotion described is similar to one that has been proposed for use in operating theatres by Lowbury, Lilly & Ayliffe (1974) and Lowbury & Lilly (1975), and in some wards by Kurtz & Boxall (1976). Its use to replace at least some handwashing deserves encouragement as this will lead to a saving of time and money (Kurtz & Boxall, 1976), and in areas where hands are washed very frequently, perhaps to real bacteriological benefit as well (Ojajärvi, Mäkelä & Rantasalo, 1977). The amount of chlorhexidine in the lotion proposed is lower than the 0·5 % used by the authors quoted, to take account of the increased concentration of chlorhexidine as alcohol evaporates on the skin, especially if the lotion is used on two or three occasions consecutively.

Coagulase-negative staphylococci were by far the most numerous bacteria found in the experiments recorded. Organisms in this group are found increasingly as pathogens in iatrogenic infections following intravenous therapy or other intravascular manipulation, the insertion of surgical prostheses, and operations on the urinary tract or in immunosuppressed patients. The importance of the skin of a

patient compared with that of his attendants as the source of organisms causing infections remains to be determined, but it must be assumed that each plays a part, so a reduction in infection will follow the application of measures designed to reduce the spread to patients of coagulase-negative staphylococci from medical and paramedical staff. For this as well as other reasons, an effective antiseptic hand scrub should be substituted for soap, where this has not already happened, in those areas where iatrogenic coagulase-negative staphylococcal infections are more common. The areas concerned are intensive therapy units, and wards where intravenous infusions are commonplace or where patients are nursed after surgery on the urinary tract, as well as in operating theatres.

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