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Assessment of the remanent antibacterial effect of a 2% triclosan-detergent preparation on the skin

By C. A. BARTZOKAS*, J. E. CORKILL*, T. MAKIN* and D. C. PINDER†

* *University Department of Medical Microbiology, Duncan Building, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XW and*
† *Medical Department, Mersey Regional Health Authority, Wilberforce House, The Strand, Liverpool L2 7RW*

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SUMMARY

A method of quantifying the remanent antibacterial effect of a 2% triclosan preparation in detergent following three consecutive applications on the forearm of 20 volunteers over 2 h, is described with reference to its efficacy against a gentamicin- and multiply-resistant serotype of *Klebsiella aerogenes*. The relevance of the residual activity of triclosan and other skin antiseptics in surgical and hygienic hand disinfection are discussed.

INTRODUCTION

For over a century, Semmelweiss's postulate has remained timely and of increasing significance: handwashing remains the single most important factor in preventing infections. Antiseptic handwashing products are now recommended for hospital personnel who consistently need to reduce the numbers of micro-organisms on their hands: 'this occurs when the hands are possibly contaminated with virulent micro-organisms or whenever the resident skin flora of personnel are likely to cause disease after patient contact' (CDC Report, 1981). Antiseptic additives can facilitate the removal of 'transients' in hygienic hand disinfection and significantly reduce 'residents' in surgical hand disinfection (Price, 1938). However, a third category should have been included in Price's original concept of 'residents' and 'transients': the 'temporary residents' – micro-organisms which multiply and/or persist on the hands for a short period (Noble & Somerville, 1974). The efficacy of antiseptics against all three categories of the hand's microbial ecosystem should be evaluated not only for their immediate effect but also for their remanent effect. This is now a requirement in many official tests (Federal Register, 1978; Borneff *et al.* 1981).

Amongst the active antimicrobials currently preferred for handwashing, chlorhexidine and hexachlorophane but not the alcohols or iodophors have been shown to exhibit skin substantivity and remanent action (van der Hoeven & Hinton, 1968; Marples, 1969; Müntener, Schwarz & Reber, 1972; Lowbury & Lilly, 1973; Kundsin & Walter, 1973; Peterson, Rosenberg & Alatary, 1978). A model to quantify the remanent effect of triclosan, a new broad-spectrum antiseptic (Furia

& Schenkel, 1968; Regös & Hitz, 1974) in hygienic skin disinfection is described here, with particular reference to a serotype of *Klebsiella aerogenes* which is endemic in our hospital and a 'temporary resident' on the hands of personnel. The relevance of prolonged antibacterial activity of antiseptic handwashing products in hygienic and surgical hand disinfection, is also discussed.

MATERIALS AND METHODS

Materials

Test preparation. A liquid synthetic hand cleanser, based on monoalkalonamine lauryl sulphate, pH 7·3, containing 2% triclosan (supplied by Ciba-Geigy AG).

Control preparation. A liquid hand cleanser of an identical formulation as the test but without triclosan or any added preservative (supplied by Ciba-Geigy AG).

Inoculum. An overnight suspension of *K. aerogenes* (1×10^8 c.f.u./ml), serotype K39 (RLH 270) grown in Nutrient broth no. 2 (Oxoid CM67) (Hart, Gibson & Buckles, 1981).

Deactivator. 1% peptone water, 3% Tween 80, 0·04% sodium thioglycolate.

Recovery medium. MacConkey Agar, without salt, (Lab. M) containing 0·3% lecithin and 3% Tween 80.

Methods

Volunteers. Twenty volunteers with no visible skin injuries, eczema or other skin diseases were randomized. Washing and rinsing was under luke-warm Liverpool tap water (bacterial count < 1 c.f.u./ml). The forearms were dried with sterile paper towels.

Application of test and control formulations. The forearms were wetted, 1 ml aliquots of test and control soap were applied simultaneously on to opposite forearms by two attendants, massaged for 1 min and rinsed for 20 s. This procedure was repeated twice more. At the end of the third application and rinse, the forearms were dried separately.

Artifical contamination. Sterile plastic templates were used to mark five (six on two volunteers) 22 mm diameter positions on each forearm; 25 μl aliquots ($2{\cdot}5 \times 10^6$ c.f.u.) of inoculum were applied to each of the marked positions, evenly spread by means of a sterile glass rod and left to air dry (in approximately 6–8 min).

Determination of remanent effect. Ten min after the artificial contamination, the first two samples from the test and control positions were collected as follows: a 22 mm diameter sterile plastic cylinder was placed firmly over one of the previously marked (and inoculated) positions and 1 ml of deactivator solution was introduced to elutriate surviving klebsiellae by pipetting up and down for 15 s. The eluate was serially diluted in deactivator and 0·1 ml aliquots of undiluted and 1:10, 1:100, 1:1000, 1:10000 and 1:100000 dilutions were spread over the surface of Petri dishes containing recovery medium. An identical procedure was followed at 30, 60, 90 and 120 min (180 min in two volunteers). After incubation at 37 °C for 48 h, the total colony count in plates containing 30–300 c.f.u. were enumerated. Results

Table 1. $\log_{10}$ (*ratios*) = $\log_{10}$ (*blank soap*) − $\log_{10}$ (*test soap*)

	Time in minutes					
Volunteer	10	30	60	90	120	180
1	0·3010	−0·1249	0·8539	2·4771	1·1549	—
2	1·0969	1·0414	1·3188	1·7648	1·5110	—
3	0·3010	0·7959	1·7782	2·1761	2·4771	—
4	1·3010	1·9031	1·3010	2·5740	3·0000	—
5	0·7404	0·6990	0·9853	1·0512	1·7202	2·0884
6	0·6410	0·8603	1·6232	1·8239	1·9208	—
7	0·8717	0·7634	1·2218	1·1383	1·1597	—
8	1·0371	1·0185	1·0500	1·8558	2·2596	—
9	1·1024	0·3118	1·1280	2·0696	1·6628	—
10	0·7270	0·8893	0·9445	1·6676	2·6021	—
11	0·9031	0·7659	1·4914	1·7570	1·7132	—
12	1·6021	1·9274	1·4030	1·0000	1·2139	1·3979
13	1·4137	1·8381	2·7634	3·6021	3·3424	—
14	1·4318	2·3010	3·2430	3·7782	3·5051	—
15	1·2788	1·4393	1·6990	2·0512	2·1249	—
16	1·0458	0·9878	1·3010	1·9700	2·0378	—
17	0·2455	0·3979	1·0512	1·5051	1·7570	—
18	0·8573	1·0580	1·1576	1·7447	2·4929	—
19	0·9686	1·0835	1·2041	1·1139	1·3680	—
20	1·0969	1·3010	1·1171	1·7782	2·0000	—
Mean	0·9482	1·0629	1·4318	1·9449	2·0512	1·7432

Table 2. *Analysis of variance*

Source of variation in log ratios	D.F.	Corrected sum of squares	Mean square
Between times			
Regression	1	17·6010	17·6010
Deviation from regression	4	2·5521	0·6380
Within times	96	35·6686	0·3715
Total	101	55·8217	—

Test for linear relationship: F(4, 96) = 0·6380/0·3715 = 1·72, $P > 5\%$.

were expressed as the $\log_{10}$ of c.f.u. surviving on the skin over specified time periods.

RESULTS AND STATISTICAL ANALYSIS

The forearms of each volunteer were treated as a matched pair, and the differences (control soap minus test soap) in $\log_{10}$ bacterial counts between the forearms, over six points of time, are presented in Table 1.

Analysis of variance was performed on the data from Table 1 to test for linear relationship between the $\log_{10}$ ratios and time. The calculations (Table 2) show that the deviation from a linear regression is not significantly greater than the within-times variation ($F(4, 96) = 1·72$, $P < 5\%$). Having demonstrated a

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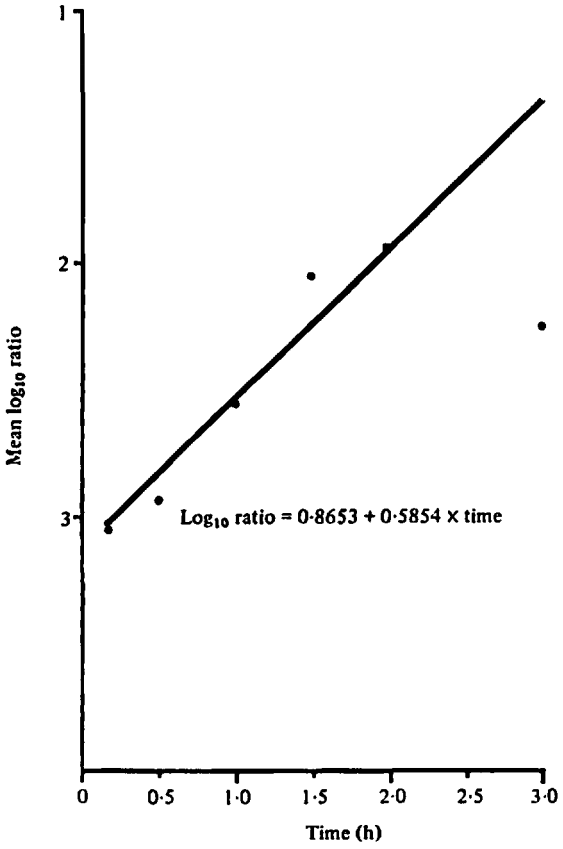

Fig. 1. Plot of $\log_{10}$ ratios (blank soap:test soap) against time.

satisfactory linear relationship between the $\log_{10}$ ratios and time, the regression equation was calculated as follows:

$$\log_{10} \text{ ratio} = x(a+b).$$

$a = 0{\cdot}8653$ (S.E. 0·1109).

$b = 0{\cdot}5854$ (S.E. 0·0863) h.

$x =$ time in hours.

The mean $\log_{10}$ ratios from Table 1 and the fitted regression line are plotted in Fig. 1. Both regression coefficients are significantly greater than zero. As the inocula applied to the forearms were equal at time 0, the positive intercept of the regression line indicates that there was a very rapid bacterial attrition on the test forearm relative to the control, in the first 10 min, whilst the inoculum air dried. In addition, the positive slope of the line indicates a continuing higher attrition rate on the test forearm, for at least 2 h afterwards.

DISCUSSION

Hands have consistently proved to be an important vector in transmitting antibiotic-susceptible *Staphylococcus aureus* and other Gram positive bacteria (Frappier-Davignon, Frappier & St Pierre, 1959; Rammelkamp, Mortimer & Wolinsky, 1964; Mortimer, Wolinsky & Rammelkamp, 1965) and intestinal Gram

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negative bacteria (Fleck & Klein, 1959; Casewell & Phillips, 1977; Parry *et al.* 1980). In the 1970s the spread of gentamicin- and multiply-resistant enterobacteria (Hable *et al.* 1972; Pollack *et al.* 1972; Casewell *et al.* 1977; Curie *et al.* 1978; Gordon, 1980; Hart, 1982), and methicillin-and multiply-resistant *S. aureus* (Crossley, Landesman & Zaske, 1979; Crossley *et al.* 1979; Price, Brain & Dickson, 1980; Peacock *et al.* 1981) was shown to be hand-mediated. In 1981 Hart, Gibson & Buckles determined the mean survival of a 1×10^3 inoculum of *K. aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii* and *Escherichia coli* on the unwashed forearm to be 70, 45, 10 and 13 min respectively. The durable klebsiella in these studies was a typical gentamicin-and multiply-resistant strain, being endemic at the Royal Liverpool Hospital since 1979 and having affected over 500 patients to date. In our experiments, a regimen of three consecutive 1 min applications of triclosan-detergent was arbitrarily selected; and following the application of a $2{\cdot}5 \times 10^6$ inoculum of the same endemic serotype to the forearm previously washed thrice with an unmediated soap, $4{\cdot}4 \times 10^5$ klebsiellae survived, even after 90 min. At the same time, the inoculum was reduced by 350-fold to $7{\cdot}1 \times 10^3$ on the triclosan-treated forearm. With such high inocula surviving on the skin after periods as long as 2 h, a long-lasting antibacterial effect would confer significant advantages. Within the first 10 min of contact there was only a 3-fold reduction with the control but an 18-fold reduction with the test: this rapid bacterial attrition on the triclosan-treated forearm is probably due to enhanced action on a drying bacterial suspension. However, the continuing antibacterial activity on the test forearm after the inoculum dried – always higher than in the control – must be due to triclosan remaining on the skin. Such immediate and continuing antisepsis illustrates the remanent effect of a 2% triclosan-detergent preparation in our model.

Of the antimicrobials currently incorporated into hand disinfectants, little is known of their remanent effect against skin 'transients' and important 'temporary residents' such as endemic hospital strains. 'Hibiscrub' (4% chlorhexidine gluconate in a detergent base) leaves an antibacterial residue on the skin, active against *S. aureus* and *E. coli*, whereas hexachlorophane residues probably exert an effect against Gram positive organisms only (Lowbury & Lilly, 1973). The activity of 'Hibiclens' (U.S. equivalent of 'Hibiscrub') against *Serratia marsescens* with regular handwashing may be in part due to an accumulation of chlorhexidine on the skin: no such phenomenon was noted following usage of povidone-iodine or hexachlorophane-containing preparations (Peterson, Rosenberg & Alatary, 1978).

In a modified Peterson glove-rinse test assessing surgical hand disinfection, the immediate and remanent effects of 0·5% alcoholic triclosan, ('Manusept') and 0·5% alcoholic chlorhexidine ('Hibisol') were found to be similar (T. A. McAllister, personal communication). Four other commercially available liquid products containing 0·3–2·0% triclosan, evaluated in a hygienic hand disinfection model in volunteers, achieved an immediate reduction of an artificially inoculated *E. coli*, which was similar to that of 60% isopropyl alcohol (Bartzokas *et al.* 1983). Thus a single handwash with triclosan in detergent offers not only ordinary cleansing but confers hygienic disinfection comparable to an alcoholic standard. Following successive handwashings the remanent action of triclosan or chlorhexidine should rapidly eliminate transients before they are established to a 'temporary residency' status.

Hospital personnel frequently fail to wash their hands when they should (Casewell & Phillips, 1977). When they do wash their hands, the procedure rarely exceeds 20 s. and it is generally a rinse rather than a thorough and energetic process (Taylor, 1978). Failure of compliance with such well-established control of infection practice involves psychological factors which have only recently begun to be appreciated (Bromley, 1983), but this remains an intractable problem. The long-lasting antibacterial action against a devastating hospital pathogen which has been demonstrated here can off-set the consequences of both infrequent and of regular, but inadequate, handwashing practices.

A prolonged antibacterial effect is of particular relevance in surgical hand disinfection. Gloves alone do not provide an adequate safeguard against per-operative contamination. Bacterial growth is promoted under the occlusion of gloves relative to ungloved hands (Price, 1938) and unnoticed glove punctures or tears do frequently occur (Devenish & Miles, 1939; Shouldice & Martin, 1959; Lowbury & Lilly, 1960; Walter & Kundsin, 1969; Church & Sanderson, 1980). Perforated gloves have been unequivocally associated with post-operative bacterial wound infections (Devenish & Miles, 1939; Cruse & Foord, 1973) and probably transmission of Hepatitis B virus (Collaborative Report, 1980). All these factors must become proportionally more important during the prolonged surgical and obstetric procedures of today.

The use of a long-lasting antiseptic handwash product is particularly desirable when nursing patients in special care units, containment isolation (especially when the patient is infected by multiply-resistant bacteria) and in the control of epidemics of nosocomial pathogens known to be transmitted by direct contact (CDC Report, 1981). Although it has been recognized that the remanent activity of such antiseptics is of at least as much importance as their immediate effect, very few published data are available on the characterization and quantification of this property. Our results show that it is possible to obtain useful quantitative data from small numbers of volunteers using a fairly simple protocol. This could provide the basis for future standardized comparative evaluations of remanent effects of antiseptics on the skin.

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