Epidem. Inf. (1987) **98**, 337–344
Printed in Great Britain

Comparative evaluation of the immediate and sustained antibacterial action of two regimens, based on triclosan- and chlorhexidine-containing handwash preparations, on volunteers

By C. A. BARTZOKAS*, J. E. CORKILL†, T. MAKIN† and E. PARRY‡

**Department of Microbiology, Clatterbridge Hospital, Bebington, Wirral L63 4JY; †University Department of Medical Microbiology, Duncan Building, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XW; ‡Department of Business Studies, Manchester Polytechnic, Aytoun Building, Aytoun Street, Manchester M1 3GH*

(*Accepted 30 December 1986*)

SUMMARY

The degerming effect of a 3 min handwash with 2% triclosan, or 4% chlorhexidine, in detergent and enhanced efficacy of either antiseptic in isopropyl alcohol, was evaluated in volunteers. Handwashing with either antiseptic preparation reduced the normal flora by a factor of 10; alcohol rubbing by approximately a factor of 1000. Both regimens eliminated *Micrococcus roseus*, artificially inoculated before every procedure. The sustained action of the same detergent preparations was further studied in gloved and ungloved hands by the Vinson's 'finger imprint test'. In the gloved hand both antiseptics inhibited *Staphylococcus epidermidis* for 4 h. In the ungloved hand however, triclosan remained active longer than chlorhexidine. Whilst the activity of chlorhexidine was short-lived against a clinical isolate of *S. aureus*, particularly in the ungloved hand, the sustained effect of triclosan against the same strain persisted for 4 h on either hand.

INTRODUCTION

Antiseptic handwash products are extensively used in hospitals; the choice is often empirical. Generally, they are selected according to a reduction of the normal or transient hand microflora after a single application. Following regular applications however, certain antiseptics can remain on the skin exhibiting sustained antimicrobial activity. Such remanent antimicrobial effect has been shown for hexachlorophane, chlorhexidine and triclosan but not for the commonly used iodophors or alcohols (Peterson, Rosenberg & Alatary, 1978; Bartzokas *et al.* 1983*a*; *b*).

A lasting remanent effect can enhance the value of surgical and hygienic hand disinfection. Since 1978, the Food and Drug Administration (USA) recommend that hand disinfectants possess a persistent (remanent) activity (Federal Register, 1974; 1978). Though, in theory, the significance of such antiseptic systems is recognized, their usefulness can be impaired by the limited understanding of the advantages which the remanent effect can confer in practice.

We report experiments on the comparative performance of antiseptic regimens, based on triclosan- and chlorhexidine-containing products, in which the protocols were designed to satisfy the requirements of surgical and hygienic hand disinfection.

MATERIALS AND METHODS

Experiment 1. Surgical skin disinfection

Materials

Test regimens. Triclosan: 2% w/v triclosan (Irgasan® DP 300, Ciba-Geigy AG) in an anionic/ampholytic detergent base (Aquasept®), followed by 0·5% w/v triclosan in 70% v/v isopropyl alcohol BP with emollients (Manusept®) supplied by Hough Hoseason & Co. Ltd. *Chlorhexidine*: 4% w/v chlorhexidine gluconate (Hibitane®) in a non-ionic detergent base (Hibiscrub®), followed by 0·5% w/v chlorhexidine gluconate in 70% w/w isopropyl alcohol BP with emollients (Hibisol®) supplied by ICI Pharmaceuticals plc.

Kneading fluid. 0·075 M phosphate buffer (pH 8), containing 0·1% Triton X-100, 0·3% sodium thiosulphate, 3% lecithin and 10% Tween 80.

Diluent. Tryptone water (Oxoid CM 87), containing 0·3% lecithin, 0·3% sodium thiosulphate and 3% Tween 80.

Recovery medium. Nutrient agar (Oxoid CM 3), containing 0·3% lecithin and 3% Tween 80.

Volunteers. Each regimen was evaluated on two separate groups of 15 hospital staff, allocated at random. All had short to normal length finger-nails, none had visible skin injuries, eczema or apparent skin disease. One week before the assessment volunteers were supplied with non-medicated bars of soap (Simple®, The Albion Soap Co. Ltd.) and instructed not to use any toiletries (e.g. medicated soaps, deodorants, anti-dandruff shampoos), which may contain antibacterial agents, or handle laboratory, or household disinfectants. Volunteers were also asked to refrain from swimming in chlorinated pools. During testing no rings or wrist watches were worn.

Rinsing was performed under running lukewarm tap water: pH 7·4, total chlorine $< 0{\cdot}1$ p.p.m., hardness 92·1 mg/l $CaCO_3$, average colony count < 3 per ml.

Methods

Initial cleansing. Volunteers washed hands and lower third of forearms with Simple® soap for 30 s and rinsed for 30 s.

First contamination. Immediately after (without drying), two 2·5 ml aliquots of *Micrococcus roseus* (NCTC 7523) at 10^8 c.f.u./ml were dispensed on to cupped hands and volunteers rubbed over hands and lower third of forearms for 45 s. Whilst the inoculum was allowed to air-dry (in *c.* 3 min), hands were rotated uppermost and fingers flexed continuously to avoid droplet formation on the finger-tips.

Baseline flora and inoculum sampling. Immediately after the inoculum air-dried, hands were placed simultaneously over two Petri dishes, each containing 10 ml kneading fluid (without deactivators), and fingers kneaded continuously for 1 min.

Deactivators were not added to this kneading fluid, since any such residues on the skin could potentially interfere with the efficacy of the antiseptics subsequently applied. The kneading fluids from the right and left hand were pooled and 0·1 ml aliquots of serial tenfold dilutions from 10^0 to 10^{-5} were spread over the surface of duplicate Petri dishes containing recovery medium. Cultures were incubated at 30 °C for 72 h and the colony-forming units (c.f.u.) in plates containing 30–300 were differentially enumerated. For each volunteer, the c.f.u./ml recovered from this and all subsequent samplings were transformed to $\log_{10}$ values. Subsequent post-disinfection samplings were similarly performed.

Second contamination. Hands were re-contaminated as described above.

Handwash procedure and sampling. Immediately after the inoculum air-dried, a 5 ml aliquot of tap water was dispensed on to cupped hands and volunteers rubbed over hands and lower third of forearms for 5 s. Then a 5 ml aliquot of a test detergent preparation (i.e. Aquasept® or Hibiscrub®) was similarly dispensed: hands and forearms were washed energetically in a standard manner for 75 s. This procedure was then repeated. After a total of 3 min handwash, volunteers rinsed hands and forearms for 30 s and towel-dried with sterile Kleenex® paper towels. Volunteers kneaded fingers in fluid containing deactivators, rinsed for 30 s and towel-dried.

Third contamination. Hands were re-contaminated.

Alcoholic handrub procedure and sampling. Immediately after the inoculum air-dried, a 5 ml aliquot of a test alcoholic preparation was dispensed on to cupped hands and volunteers rubbed over hands and lower third of the forearms energetically to dryness (in *c.* 75–90 s). This procedure was then repeated. Volunteers kneaded fingers in fluid containing deactivators.

Experiment 2. Remanent skin antibacterial effect

Materials

Test preparations. Two per cent w/v triclosan in detergent (Aquasept®) and 4% w/v chlorhexidine gluconate in detergent base (Hibiscrub®), as in Experiment 1.

Volunteers. Each preparation was separately evaluated in 12 volunteers, selected as previously described.

Finger imprint plates. One per cent dilution of 24 h cultures of either methicillin and multiply-resistant *Staphylococcus aureus* (RLH No. 4779), or *S. epidermidis* (NCTC 7944) strains in nutrient broth (10^8 c.f.u./ml) were incorporated in 25 ml of nutrient agar (Oxoid CM 1) and solidified in 121 mm square plates.

Methods

Pre-handwash control. Before an initial 30 s hand and forearm cleansing with Simple® soap, the pulps of the right and left fore fingers were simultaneously applied on the surface of two plates, overlayed with either *S. aureus* or *S. epidermidis*, for 30 s.

Handwash procedure. Immediately after a 5 ml aliquot of a test preparation was dispensed on to cupped hands. Volunteers rubbed over hands and lower third of forearms energetically in a standard manner, for 75 s. This procedure was then repeated. After 3 min handwash, volunteers rinsed hands and forearms for 30 s and towel-dried.

Vinson's finger imprint test (Vinson *et al.* 1961), *modified*. Immediately after, the pulps of the right and left hand fore fingers of six volunteers were simultaneously applied on two plates, overlayed with either *S. aureus* or *S. epidermidis* (time 0). Following 30 s contact, two attendants disinfected the fore fingers with 70% isopropyl alcohol and donned the non-dominant hand with a glove (Dispos-a-glove®, Surgikos Ltd.) which was sealed around the wrist with Sellotape®. The dominant hand remained unoccluded. Volunteers refrained from handwashing or any contact with chemicals for 4 h. At 1, 2, 3 and 4 h after the imprint of the fore fingers, the digital pulps of the middle, ring, little fingers and thumbs – in that order – were similarly imprinted and disinfected. Gloves were not removed from the non-dominant hands, but were amputated around the middle phalanx of the appropriate finger. After imprinting and disinfection, the cut end was sealed with Sellotape®.

Plates were incubated at 37 °C for 24 h. The zones of growth inhibition were rated 4–0 according to the criteria originally described by Vinson *et al.* (1961), as follows

Rating	Activity	Growth inhibition characteristics
4	Excellent	Clear area of no growth, with sharp periphery.
3	Good	Clear area of no growth with hazy periphery.
2	Fair	Partial growth.
1	Slight	Growth almost equal to surrounding agar.
0	None	Confluent growth equal to surrounding agar.

RESULTS AND STATISTICAL ANALYSIS

Experiment 1

After the baseline flora and artifical inoculum sampling, the mean $\log_{10}$ values of *M. roseus*, recovered after the triclosan regimen was 4·66; after the chlorhexidine regimen, 4·68. This marker organism was never recovered from any subsequent samplings.

The mean $\log_{10}$ value for the baseline resident flora (A) recovered from the 15 volunteers during the evaluation of triclosan was 5·88 with a standard deviation (S.D.) of 0·38. Values obtained following the Aquasept® handwash (B) was 4·85 (S.D. 0·18) and the Manusept® handrub (C) 3·09 (S.D. 0·49). The A–B and A–C reduction factors were 1·03 (S.D. 0·30) and 2·79 (S.D. 0·68) respectively. Similarly, the $\log_{10}$ values for the resident flora following the chlorhexidine regimen (A) was 5·82 (S.D. 0·62). Values obtained following the Hibiscrub® handwash (B) was 4·79 (S.D. 0·40) and the Hibisol® handrub (C) was 2·86 (S.D. 0·55). The A–B and A–C reduction factors were 1·03 (S.D. 0·55) and 2·96 (S.D. 0·38) respectively. Since the mean A–B reduction factors obtained with both regimens are virtually identical, a statistical test is unnecessary. However, as the mean A–C reduction factors appeared different, the Mann–Whitney U test (Siegel, 1956) was applied. There was no statistically significant difference between the A–C reduction factors ($U = 83$, $P \geqslant 0{\cdot}05$).

Experiment 2

The Vinson's growth inhibition ratings (4–0) of *S. epidermidis* are presented in Table 1. The Aquasept® ratings obtained at a given time (0–4 h) were compared

Table 1. *Vinson's finger imprint ratings over 4 h, following 3 min handwash with Aquasept® or Hibiscrub®*, versus Staphylococcus epidermidis

	Gloved hand (h)					Ungloved hand (h)				
Vol.	0	1	2	3	4	0	1	2	3	4
					(*a*) Aquasept®					
1	4	4	4	4	3	4	4	4	4	3
2	4	4	4	4	4	4	4	4	4	3
3	4	4	4	3	3	4	4	4	3	3
4	4	4	4	4	3	4	4	4	3	3
5	4	4	4	3	3	4	4	4	3	3
6	4	4	4	4	4	4	4	4	4	3
A	0	0	0	2	4	0	0	0	0*	0*
B	6	6	6	4	2	6	6	6	6*	6*
					(*b*) Hibiscrub®					
1	4	4	4	3	3	4	2	1	1	1
2	4	4	4	4	4	4	3	2	1	1
3	4	4	4	4	4	4	3	1	1	1
4	4	4	4	4	3	4	2	1	1	0
5	4	4	4	4	3	4	3	2	1	1
6	4	4	4	4	4	4	3	2	2	2
C	0	0	0	1	3	0	6	6	6*	6*
D	6	6	6	5	3	6	0	0	0*	0*
$D_{0\cdot05}$	2	2	2	0	6	2	2	2	2	2
Sign.	No	No	No	No	No	No	Yes	Yes	Yes	Yes

$D_{0\cdot05}$, Critical frequency value for significance at 0·05.
* Based on a $< 3/ > 3$ classification of the 2×2 contingency table.

with the reciprocal ratings obtained with Hibiscrub® by a 2×2 contingency table (Finney, 1948), as follows

	Vinson's ratings		
	< 4	4	
Aquasept®	A	B	6
Hibiscrub®	C	D	6
			12

where A, B, C, and D are rating frequencies. Since these frequencies are too small for a χ^2 test, the Fisher–Yates test of significance was applied. In the gloved hand no statistically significant difference between the Aquasept® and Hibiscrub® samples was detected. In the ungloved hand, apart from the ratings obtained immediately after the antiseptic handwash procedure (time 0), the two regimens exhibited Vinson's ratings at 1, 2, 3 and 4 h which, statistically, are significantly different.

The Vinson's growth inhibition ratings (4–0) of *S. aureus* after 3 min handwash with either Aquasept® or Hibiscrub® were similarly analysed and presented in Table 2. In the gloved hand, apart from the ratings obtained at time 0 and 4, at 1, 2 and 3 h the two regimens exhibited ratings which differed statistically significantly. In the ungloved hand the ratings differed significantly at each time period.

https://doi.org/10.1017/S0950268800062099 Published online by Cambridge University Press

Table 2. *Vinson's finger imprint ratings over 4 h, following 3 min handwash with Aquasept® or Hibiscrub®*, versus Staphylococcus aureus

	Gloved hand (h)					Ungloved hand (h)				
Vol.	0	1	2	3	4	0	1	2	3	4
					(*a*) Aquasept®					
1	4	4	4	4	4	4	4	4	4	4
2	4	4	4	4	4	4	4	4	4	4
3	4	4	4	4	3	4	4	4	4	3
4	4	4	4	3	3	4	4	4	4	3
5	4	4	4	3	3	4	4	4	4	3
6	4	4	4	4	4	4	4	4	4	4
A	0	0	0	2	0*	0	0	0	0	0*
B	6	6	6	4	6*	6	6	6	6	6*
					(*b*) Hibiscrub®					
1	3	3	2	2	2	3	1	0	0	0
2	3	3	3	3	3	3	1	0	0	0
3	3	3	3	3	3	3	1	0	0	0
4	4	3	3	3	3	3	1	0	0	0
5	4	4	3	3	3	3	1	0	0	0
6	4	3	3	3	3	3	1	1	0	0
C	3	5	6	6	1*	6	6	6	6	6*
D	3	1	0	0	5*	0	0	0	0	0*
$D_{0\cdot05}$	2	2	2	0	2	2	2	2	2	2
Sign.	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

$D_{0\cdot05}$, Critical frequency value for significance at 0·05.
* Based on a $< 3/ > 3$ classification of the 2×2 contingency table.

DISCUSSION

Since bacterial growth is promoted under the occlusion of gloves and perforated gloves unnoticed during surgery (Lowbury & Lilly, 1960; Walter & Kundsin, 1969; Church & Sanderson, 1980) have been implicated in post-operative sepsis, a long lasting antiseptic activity seems a useful feature of surgical hand disinfection (Lowbury & Lilly, 1973; Reber *et al.* 1975, Bartzokas *et al.* 1983*a*). The antiseptic regimens studied in Experiment 1 appeared equally efficient when tested immediately after use: a 3 min energetic handwash reduced the normal skin flora by one $\log_{10}$. A further two applications of the same antiseptics, formulated in isopropyl alcohol, achieved an almost three $\log_{10}$ reduction. A reduction of about one $\log_{10}$ in the resident flora appears to be the norm for skin disinfection with cleansing preparations. A 2·5–3 $\log_{10}$ reduction in Europe is a prerequisite for efficient surgical disinfection with alcohol-based rubs (Rotter, Koller & Wewalka, 1981).

Artificially applied transient bacteria vary in their ability to survive on the skin: most Gram-negative bacilli die rapidly. Ayliffe, Babb & Lilly (1981) suggested that 'an organism which can be distinguished from the normal skin flora, such as a pigmented strain of coagulase-negative micrococcus, would be ideal if its resistance to skin disinfectants is similar to that of *Staphylococcus aureus*'. The artificially inoculated *M. roseus* was completely removed from the skin. Despite

https://doi.org/10.1017/S0950268800062099 Published online by Cambridge University Press

two further inocula, this marker was not recovered from any post-treatment samplings. This may have been due to its susceptibility to the antiseptics tested, or perhaps an altered adherence on the skin.

In Experiment 2, gloved hands simulated the moist, warm environment created during surgery; ungloved hands, the skin condition between hygienic hand disinfections. To assess remanent effect, *S. epidermidis* representing normal flora, and methicillin-resistant *S. aureus* as a member of the transient flora, were used. When either antiseptic was challenged with *S. epidermidis*, no statistically significant different remanent effect was demonstrated on the gloved hand over 4 h. In the ungloved hand, however, the remanent effect of Aquasept® remained unaltered for up to 2 h, diminishing only slightly over the following 2 h, whereas the effect of Hibiscrub® was progressively reduced from time 0 (Table 1). When triclosan and chlorhexidine gluconate were challenged with *S. aureus*, their different remanent effects were amplified: in the ungloved hand the remanent effect of triclosan was clearly superior to that of chlorhexidine, even immediately after a 3 min handwash (time 0). The difference of remanent effect between triclosan and chlorhexidine gluconate, depending on whether gloves were worn, has not been previously described. The possible enhancement of this effect, in relation to the occluded skin, is being investigated.

The remanent effect of chlorhexidine gluconate against resident flora has previously been studied up to 6 h following hand disinfection (Aly & Maibach, 1979; Werner & Borneff, 1980; La Rocca & La Rocca, 1982), but in occluded hands only; its effect in unoccluded hands has not been evaluated longer than 1 h post-application (Lowbury, Lilly & Ayliffe, 1974; Peterson, Rosenberg & Alatary, 1978; Aly & Maibach, 1980). In this study the concentration of triclosan, deposited on the skin, was sufficient to inhibit 10^6 c.f.u./ml of *S. aureus* and *S. epidermidis* after 30 s contact. In a previous study (Bartzokas *et al.* 1983*a*) another triclosan preparation, similar to Aquasept®, reduced by 350–fold a $2{\cdot}5 \times 10^6$ forearm inoculum of an antibiotic-resistant *Klebsiella aerogenes* in 2 h.

Prolonged activity against hand-mediated hospital pathogens can have practical applications. Unlike chlorhexidine (Brumfitt, Dixon & Hamilton-Miller, 1985), triclosan remains active against all *S. aureus* strains tested in this laboratory, regardless of their susceptibility to antibiotics. The rapid, sustained bacterial attrition exhibited by triclosan against important skin transients can, therefore, counteract inevitable lapses in frequency and procedure in handwashing by hospital staff (Taylor, 1978). The sustained activity of hand disinfectants, cosmetically appealing to staff and patients (Slade, Williams & Bartzokas, 1986), can afford simple and inexpensive prevention of hand-mediated sepsis. However it is their judicious application and the continuous education of clinical staff, rather than their remanent effect *per se*, that can effectively prevent hospital infections.

REFERENCES

Aly, R. & Maibach, H. I. (1979). Comparative study on the antimicrobial effect of 0·5% chlorhexidine gluconate and 70% isopropyl alcohol on the normal flora of hands. *Applied and Environmental Microbiology* **37**, 610–613.

Aly, R. & Maibach, H. I. (1980). A comparison of the antimicrobial effect of 0·5% chlorhexidine ('Hibistat') and 70% isopropyl alcohol on hands contaminated with *Serratia marcescens*. *Clinical Experimental Dermatology* **5**, 197–201.

AYLIFFE, G. A. J., BABB, J. R. & LILLY, H. A. (1981). Tests for Hand Disinfection. In *Disinfectants: their use and evaluation of effectiveness* (ed. C. H. Collins, M. C. Allwood, S. F. Bloomfield and A. Fox), pp. 37–59. London: Academic Press.

BARTZOKAS, C. A., CORKILL, J. E., MAKIN, T. & PINDER, D. C. (1983*a*). Assessment of the remanent antibacterial effect of a 2% triclosan-detergent preparation on the skin. *Journal of Hygiene* **91**, 521–528.

BARTZOKAS, C. A., GIBSON, M. F., GRAHAM, R. & PINDER, D. C. (1983*b*). A comparison of triclosan and chlorhexidine preparations with 60 per cent isopropyl alcohol for hygienic hand disinfection. *Journal of Hospital Infection* **4**, 245–255.

BRUMFITT, W., DIXON, S. & HAMILTON-MILLER, J. M. T. (1985). Resistance to antiseptics in methicillin and gentamicin resistant *Staphylococcus aureus*. *Lancet* i, 1442–1443.

CHURCH, J. & SANDERSON, P. (1980). Surgical glove punctures. *Journal of Hospital Infection* **1**, 84.

FEDERAL REGISTER (1974). OTC Topical antimicrobial products. *Federal Register* **39**(179), 33140.

FEDERAL REGISTER (1978). OTC Topical Antimicrobial Products. *Federal Register* **43**(4), 1215.

FINNEY, D. J. (1948). The Fisher–Yates test of significance in 2 × 2 contingency tables. *Biometrika* **35**, 145–156.

LA ROCCA, M. A. & LA ROCCA, P. T. (1982). An evaluation of the antimicrobial effect of a hand sponge-brush impregnated with 4% chlorhexidine gluconate (Hibiclens). *Developments in Industrial Microbiology* **23**, 543–546.

LOWBURY, E. J. L. & LILLY, H. A. (1960). Disinfection of the hands of surgeons and nurses. *British Medical Journal* **1**, 1445–1450.

LOWBURY, E. J. L. & LILLY, H. A. (1973). Use of 4% chlorhexidine detergent solution (Hibiscrub) and other methods of skin disinfection. *British Medical Journal* **1**, 510–515.

LOWBURY, E. J. L., LILLY, H. A. & AYLIFFE, G. A. J. (1974). Preoperative disinfection of surgeons' hands: use of alcoholic solutions and effects of gloves on skin flora. *British Medical Journal* **4**, 370–372.

PETERSON, A. F., ROSENBERG, A. & ALATARY, S. D. (1978). Comparative evaluation of surgical scrub preparation. *Surgery, Gynaecology and Obstetrics* **146**, 63–65.

REBER, H., MÜNTENER, M., NECK, K. & LIPS, U. (1975). Zur prufmethodik der chirurgeschen handedesinfektion. *Zentralblatt für Bacteriologie und Hygiene im Abteilung Originale* **160**, 601–627.

ROTTER, M., KOLLER, W. & WEWALKA, G. (1981). Eignung von chlorhexidinglukonat- und PVP-Jod-haltigen präparationen zur händedesinfektion. *Hygiene und Medizin* **6**, 425–430.

SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company.

SLADE, P. D., WILLIAMS, E. & BARTZOKAS, C. A. (1986). Psychological contributions to the control of hospital infection. In *Current Issues in Clinical Psychology* (ed. N. Eisenberg and D. Glasgow). London: Gower.

TAYLOR, L. J. (1978). An evaluation of hand-washing techniques. *Nursing Times* **74**, 54–55, 108–110.

VINSON, L. J., AMBYE, E. L., BENNETT, A. G., SCHNEIDER, W. C. & TRAVERS, J. J. (1961). *In vitro* tests for measuring antibacterial activity of toilet soap and detergent bars. *Journal of Pharmaceutical Sciences* **50**, 827–830.

WALTER, C. W. & KUNDSIN, R. B. (1969). The bacteriologic study of surgical gloves from 250 operations. *Surgery, Gynaecology and Obstetrics* **129**, 949–952.

WERNER, H.-P. & BORNEFF, J. (1980). Die charakterisierung der desinfizierenden wirksamkeit von händedesinfektionsverfahren am beispiel von 0·5% chlorhexidingluconat in 70% isopropylalkohol. *Hygiene und Medizin* **5**, 61–70.