

RESEARCH



Longevity of hand sanitisers on fingers

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ABSTRACT

Clinical relevance: Hand hygiene is important to reduce the spread of microbes in clinical settings. Hand sanitisers that last longer may be beneficial.

Background: Longevity of hand sanitisation products on fingers and hands may be important to help reduce microbial transmission. The current study evaluated the persistence of disinfection of three hand sanitisers.

Methods: Initially the minimum inhibitory concentrations of the hand sanitisers were determined using strains of *Staphylococcus epidermidis* and *S. aureus*. Then a cross-over study with participants randomly assigned to use three different hand sanitisers for 30 seconds was undertaken. The number of bacteria and fungi on fingers was assessed 10 and 20 minutes and 4 hours after use. The type of microbial inhibition of the capric acid sanitiser was studied by examining the effects of adding Tween 80 and lecithin to microbial agar.

Results: The minimum inhibitory concentration of an alcohol-based sanitiser (AS) was 10%, for the capric acid-based (CS) sanitiser was 70%, and for the quaternary ammonium-based (QS) sanitiser was < 10%. AS significantly reduced the number of microbes on fingers 10 minutes after hand washing (18.2 cfu/mL) compared to CS (59.7 cfu/mL; $p < 0.0001$) or QS (64.5 cfu/mL; $p < 0.0001$). Twenty minutes after use, microbes on fingers after AS (23 cfu/mL) or CS (16.7 cfu/mL) were significantly reduced compared to QS (72.2 cfu/mL; $p < 0.0001$) and the numbers on fingers after CS was significantly less than after AS ($p = 0.002$). Four hours after use of any hand sanitiser, the number of microbes increased to near baseline levels. The reduction in bacterial numbers was not affected by the use of neutralisers in agar ($48 \pm 28\%$ reduction with, $47 \pm 49\%$ reduction without; $p = 0.876$).

Conclusions: Hand sanitisers containing capric acid or alcohol out-performed one containing quaternary ammonium in the clinical trial and may help reduce the spread of microbes.

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Introduction

Contamination of hands can transmit diseases, either within healthcare settings or in the general community. Within healthcare settings, conventional surgical hand antisepsis often consists of using povidone iodine (PVP-I) or chlorhexidine-based detergents with water and scrubbing.¹ Within the community, people either use soap and water or hand sanitisers that may contain alcohol with or without the addition of disinfectants such as chlorhexidine or benzalkonium chloride.²⁻⁴

During the COVID-19 pandemic non-government organisations and government agencies have advocated for the frequent use of hand washing or hand sanitising as a way to minimise the spread of the SARS-CoV-2 virus. SARS-CoV-2 can survive on human skin explants for up to eight hours, considerably longer than the survival of influenza virus, but an alcohol-containing skin wash can inactivate both viruses within fifteen seconds.⁵ A review and meta-analysis of clinical trials of the effectiveness of hand sanitisers compared to the use of soap and water found a significant reduction in acute respiratory infections when using hand sanitisers.⁶ During the COVID-19 pandemic the use of hand sanitisers by the general public has increased, with a report from South Korea showing a six times increase in the use of hand sanitisers and a 10 times increase in carrying hand sanitisers.⁷

It has been recommended that people should 'clean their hands regularly' (https://www.who.int/gpsc/clean_hands_protection/en/). However, even within hospital emergency departments only 33% of the studies showed compliance of >50% with World Health Organisation hand washing guidelines.⁸ In a review of compliance of hand hygiene in different hospital settings in 1980 to 2000, the rate of compliance in intensive care units ranged from 12-81%.⁹ Furthermore, frequent use of soap and water or other hand sanitisers can cause skin cracking and irritation and may remove natural lipids on the skin that normally act to protect the skin.^{1,2}

Hand sanitisers can be composed of different disinfectants, with the most common being alcohol (ethanol or isopropanol)¹⁰ followed by chlorhexidine, chloroxyphenol, triclosan and quaternary ammonium compounds.¹¹ Alcohol and triclosan work by denaturation of proteins in the membranes of microbes, chlorine products by halogenation/oxidation of cellular proteins, quaternary ammonium compounds may act by lowering surface tension, inactivating enzymes and degrading cellular proteins.¹¹

Producing a hand sanitiser with prolonged antimicrobial activity on the skin would be one way of obtaining the most benefit from hand hygiene and overcoming some of the issues with compliance.^{2,4} A study comparing an ethanol-based versus a benzalkonium chloride-based hand sanitiser

found that the hand sanitiser containing benzalkonium chloride produced 3.75 log₁₀ reduction in microbial numbers on hands four hours after use, whereas the ethanol-based hand sanitiser resulted in a non-significant reduction of 0.32 log₁₀ reduction in microbial numbers four hours after use.¹² Similarly, whilst chlorhexidine or triclosan-based hand sanitisers reduced numbers of microbes on hands by 2-2.8 log₁₀ 1.5 hours after use, propanol or mecetronium-alcohol-based sanitisers only produced 0.8-0.9 log₁₀ reduction 1.5 hours after use.⁴ The current study aimed to evaluate the persistence of disinfection of three hand sanitisers that contained different disinfectants and excipients.

Methods

Laboratory tests

The standard strain *Staphylococcus aureus* ATCC 6538 and two strains of *S. epidermidis* (strains 19 and 20) that had been isolated from fingers were used. The strains were grown overnight in tryptic soy broth (TSB; Becton Dickinson and Company, Sydney, NSW, Australia). Following incubation, bacterial cells were collected by centrifuging and were re-suspended Muller Hinton broth (MHB; Becton Dickinson and Company) to OD₆₆₀ 0.1 (approximately 1.0 × 10⁸ colony forming units (cfu/mL)). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the hand sanitisers were also measured using previously published methods.¹³⁻¹⁵

Briefly, each hand sanitiser was diluted from 100% concentration to 10% (v/v) in phosphate buffered saline (PBS; NaCl 8 g L⁻¹, KCl 0.2 g L⁻¹, Na₂HPO₄ 1.15 g L⁻¹, KH₂PO₄ 0.2 g L⁻¹; pH 7.2) and added to wells of a 96-well micro plates. Bacterial cells (20 µL) were added to achieve a final concentration of 5 × 10⁵ CFU/mL.¹⁶ The plates were incubated for 18 to 24 hours at 37°C after which growth turbidity was measured using a spectrophotometer (FLUOstar Omega, BMG LABTECH, Mornington VIC, Australia) at 660 nm. The MIC was the dilution of with no measurable difference in OD compared to controls of dilution of sanitisers in the absence of bacterial cells. The MBC was obtained by plating out cells from the wells containing the MIC and the next two lower dilutions on nutrient agar plates which were then incubated at 37°C for 18 to 24. The MBC was the concentration of sanitiser that gave 99.99% (3 log units) bacterial killing.

In addition, the strains of *S. epidermidis*, after resuspension in MHB, were streaked onto Muller Hinton agar (Becton Dickinson and Company) plates using a sterile glass rod. Aliquots (50 µL) of each dilution of each hand sanitiser were then dropped onto the agar plates, allowed to dry and then the plates were incubated for 18 to 24 hours at 37°C. After incubation the size of any zones of inhibition was measured and the lowest dilution giving a zone of inhibition recorded. Both these assays were repeated twice.

Clinical trial

This was a prospective, single-centre, randomised, open-label, cross-over study conducted at the School of Optometry and Vision Science, UNSW Sydney, Australia. The study was approved by the UNSW Sydney ethics committee and was conducted in accordance with the 2002 Declaration of Helsinki. Prior to commencing the trial each participant was

provided with a hard copy of the Participant Information Statement and Consent Form. The clinical trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN12621000462886). Once written informed consent was obtained, participants were enrolled in the study.

The inclusion criteria for participants were that they had to be 18 years or older, the exclusion criteria were any active respiratory illness, current use of systemic or topical antibiotics, current use of systemic or topical steroids, no history of allergy to alcohol or household disinfectants by self-report.

Participants were asked to place the fingers of their dominant hand on two sterile agar plates, firstly one composed of chocolate blood agar for bacterial growth and secondly Sabouraud's dextrose agar (Oxoid, Basingstoke, Hampshire, UK) for fungal growth. Incubation was performed in 5% CO₂ for 48 hours at 37°C for bacterial growth and 7 days at 25°C for fungal growth. After incubation of the agar plates, the number of bacterial and fungal colonies was counted. In addition, the bacterial colonies were identified using standard techniques¹⁷ such as the use VITEK 2 GP rapid identification (BioMerieux, North Ryde, NSW, Australia).

A randomisation table was generated for the participants in Excel. The participants were then randomised to use one of three hand sanitisers to disinfect both hands for the recommended disinfection time - minimum 30 seconds. The disinfectants were Dettol (Reckitt Benckiser, Sydney, NSW, Australia), Zoono (Zoono Group, Sydney, NSW, Australia) and Doxall (Wintermute Biomedical, Bundoora, Vic, Australia). Table 1 shows the main ingredients in each of these products, and the products will be referred to by their main disinfecting agent from now on, i.e. alcohol, quaternary ammonium or capric acid.

Finger cultures were collected from the dominant hand of each participant 10 and 20 minutes and 4 hours after hand sanitiser use. Cultures after the use of the hand sanitisers were performed as for the baseline pre-sanitiser use and the participants did not wipe their fingers after collection of samples. During the period between collection of finger cultures, the participants were instructed not to use any hand sanitiser but could resume their normal daily work habits. After each participant had used their initial randomly allocated hand sanitiser they were instructed to come back to the facility to use the second hand sanitiser at least 3 days after their first visit and again at least three days after the second visit to use the third hand sanitiser.

The study also determined whether Tween 80 and lecithin in the chocolate agar affected the numbers of microbes collected from fingers or the effect of time on the number of microbes. This part of the study used the capric acid-based sanitiser only to investigate whether the reduction in bacterial numbers on fingers caused by this microbicide was bactericidal or bacteriostatic as the mode of action of the

Table 1. Hand sanitisers used in this study.

Sanitiser	Manufacturer	Listed ingredients
Dettol	Reckitt Benckiser	Alcohol Denat. (70%), water, PEG/PPG-17/6 copolymer, propylene glycol, acrylates/C10-30 alkyl acrylate cross-polymer, tetrahydroxypropyl ethylenediamine, fragrance limonene
Zoono	Zoono Group	Quaternary ammonium compound (0.6%), deionised water, 'emulsifier' and 'fragrance'
Doxall	Wintermute Biomedical	Capric acid (Decanoic acid; 1.5%), L-arginine, fragrance, almond oil, coconut oil and water

disinfectants in the other hand sanitisers has been well documented.^{11,18,19} The ten participants had the numbers of bacteria on their dominant hands measured as described above except that chocolate blood agar with or without the addition of 0.5% w/v Tween 80 (polysorbate 80; Sigma Aldrich, Sydney, NSW Australia and 0.7% w/v lecithin (Sigma Aldrich) was used.

Participants were randomly assigned to use chocolate agar only or chocolate agar plus Tween 80 and lecithin, and then crossed over to use the alternate agar for finger culture. The fingers of participants were sampled as described above prior to use of the capric acid-based sanitiser, and then 10 and 20 minutes after use. Numbers of bacteria were counted after culture, as described above.

Statistical analyses

Statistical analyses were performed in SPSS (Version 26; IBM). The sample size was calculated based upon data from two previous publications, one of which used an alcohol-based hand sanitiser and found that the number of microbial colonies increased from $3.4 \pm 0.5 \log_{10}$ cfu/mL immediately after hand sanitiser use to $4.2 \pm 0.8 \log_{10}$ cfu/mL 3 hours after hand sanitiser use.²⁰ The second study demonstrated that an alcohol-based hand rub could reduce the microbial load on hands from $3.28 \log_{10}$ cfu/mL (95% confidence interval 3.11-3.38) to $2.58 \log_{10}$ cfu/mL (95% confidence interval 2.08-2.93).²¹ Using either of these studies, a sample size of 9 participants was required to demonstrate these magnitudes of differences in the current study with 80% power and 0.05 type I error rate.

A more recent paper compared antimicrobial efficacy of one hand sanitiser over time and compared its effect to that of washing hands in water.¹⁵ That study found that washing in water resulted in a $23.18 \pm 9.75\%$ reduction in microbial contamination of the thumbs of volunteers, whereas washing in a hand sanitiser composed of herbal extracts in isopropyl alcohol resulted in $94.30 \pm 7.73\%$ reduction in microbial contamination of thumbs.¹⁵ Using this data only four volunteers were required. Based upon these analyses and to account for any potential drop out during the study, ten participants were recruited.

A generalised linear mixed model was used to examine the change over time and by handwash in number of bacteria. For the data examining the effects of chocolate agar with and without Tween 80 and lecithin, a similar generalised linear model was performed examining the change over time and by agar type. Fixed effects of time, handwash or agar and the interaction were estimated, as well as a random intercept to account for correlation due to repeated measures. To account for overdispersion, robust standard errors were used. Pairwise handwash or agar differences at each time, and time differences within a handwash or agar, with adjusted p-values (via sequential Bonferroni) were obtained.

Results

Determination of MIC and MBC

The MICs and MBC for the hand sanitisers are given in Table 2. The MIC for Dettol was 10% v/v dilution for all strains, the MIC for Zoono was 10% for the two strains of *S. epidermidis* and only 1.7% for *S. aureus* ATCC 6538. The MIC of Doxall was 70%

Table 2. MIC and MBC of hand sanitisers against staphylococci.

Bacterial strain	Hand sanitiser (MIC/MBC) – percent of hand sanitiser		
	Dettol	Zoono	Doxall
<i>S. epidermidis</i> 19	10/30	10/10	70/80
<i>S. epidermidis</i> 20	10/30	10/10	70/80
<i>S. aureus</i> ATCC 6538	10/20	1.7/2.5	70/80

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

for all strains. The alternative way of measuring growth inhibition, by growing strains on agar plates and overlaying with dilutions of each hand sanitiser gave slightly different results. In this assay, the lowest dilutions that produced clear zones of inhibition (2-3 mm) for the *S. epidermidis* strains were: Zoono - 10%; Doxall - 70%; Dettol - 100%.

Clinical trial of hand sanitisers

The CONSORT flowchart of participants is given in Supplementary Figure 1. Demographic data on the participants is given in Table 3. All participants used all three hand sanitisers. None of the participants reported any adverse effects of any of the hand sanitisers.

The number of bacteria on fingers was variable, as can be seen in Table 4. There was no statistically significant difference in the numbers of microbes isolated from fingers after use of a particular hand sanitiser on the different cross-over occasions, and therefore the data for each hand sanitiser is provided. The effect of each hand sanitiser over time on the number of bacteria isolated from the fingers of the participants is given in Figure 1. All the bacteria that were isolated were identified as coagulase negative *staphylococci*. All participants were university staff or students, and resumed their normal day-to-day activities after use of the hand wash which consisted mostly (90% of participants) of computer use. As there were too few fungi isolated from fingers for a meaningful statistical analysis, all microbial data were combined. All p values presented are Bonferroni corrected.

There was a significant difference at baseline between the numbers of microbes on fingers of those people that were going to use the quaternary ammonium-based sanitiser (average $76.6 \text{ cfu} \pm 7.6$ standard error or the mean [SEM]) or the alcohol-based sanitiser ($62.2 \text{ cfu} \pm 8$ SEM; $p = 0.001$) and the capric acid-based ($81.2 \text{ cfu} \pm 11$ SEM; Figure 1). Also, there was a significant difference in the numbers of microbes on fingers at baseline of those people going to use the alcohol-based sanitiser and the capric acid-based sanitiser ($p < 0.0001$; Figure 1).

Ten minutes after hand washing, the numbers of microbes recovered from fingers after the use of the alcohol-based sanitiser ($18.2 \text{ cfu} \pm 1.4$ SEM) were significantly reduced compared to the quaternary ammonium-based ($64.5 \text{ cfu} \pm 7.9$ SEM; $p < 0.0001$) or the capric acid-based ($59.7 \text{ cfu} \pm 3.9$ SEM; $p < 0.0001$) sanitiser, with numbers recovered from fingers after use of the alcohol-based sanitiser being 3.5 times less than with the quaternary ammonium-based and 3.3 times less than with the capric acid-based sanitisers (Figure 1).

Twenty minutes after hand washing, the numbers of microbes recovered from fingers after the use of the capric acid-based sanitiser ($16.7 \text{ cfu} \pm 2.3$ SEM) was significantly reduced compared to the quaternary ammonium-based ($72.2 \text{ cfu} \pm 6.5$ SEM; $p < 0.0001$) or alcohol-based ($23.0 \text{ cfu} \pm 2.0$ SEM; $p = 0.002$) sanitiser, with numbers recovered from

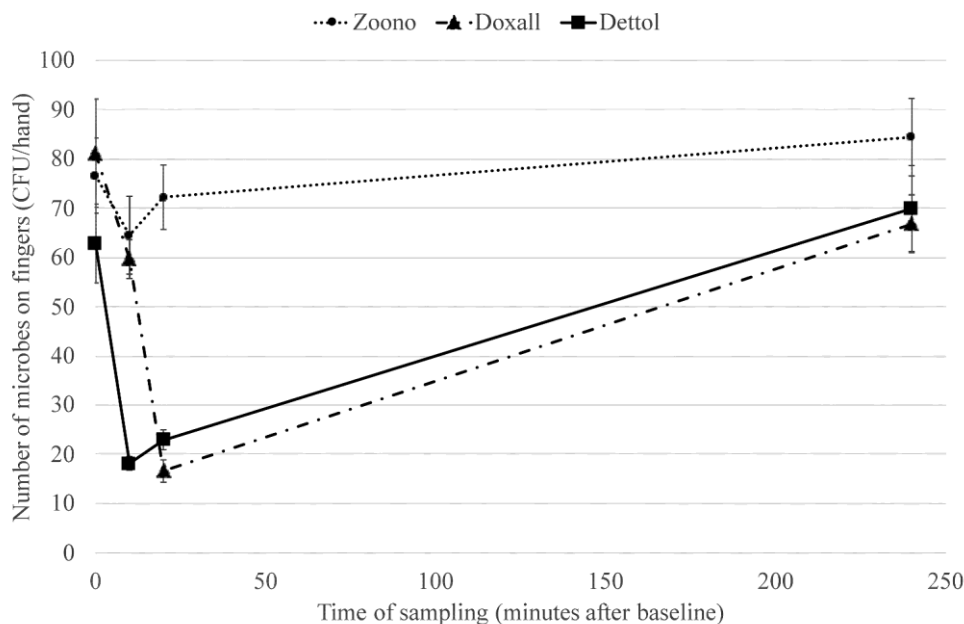


Figure 1. Number of bacteria cultured from the fingers of participants at baseline and after use of each hand sanitiser. Data are for mean \pm Standard Error of the mean.

Table 3. Demographic data of the participants.

Age (years; mean (SD))	39.7 (8.7)
Sex (female; n (%))	8 (80%)
Occupation:	
Student (n; %)	4 (40%)
Staff (n; %)	6 (60%)

Table 4. The number of microbial colonies cultured from the fingers after use of any hand sanitiser.

Microbe	Microbial number (colony forming unit per hand; mean \pm SD)			
	Baseline	10 mins	20 mins	4 hours
Bacteria	71 \pm 88	45 \pm 54	36 \pm 47	68 \pm 71
Fungi	1 \pm 1	1 \pm 2	1 \pm 2	3 \pm 9

fingers after the use of the capric acid-based sanitiser being 4.3 times less than the quaternary ammonium-based and 1.4 times less than the alcohol-based sanitisers (Figure 1). The numbers of microbes isolated from fingers after the use of the alcohol-based sanitiser were also less than after use of the quaternary ammonium-based ($p < 0.0001$) sanitiser, with the numbers isolated from the alcohol-based sanitiser being 3.2 times less than the quaternary ammonium-based sanitiser.

Four hours after hand washing, the numbers of microbes recovered from fingers after the use of the capric acid-based sanitiser ($66.8 \text{ cfu} \pm 5.9 \text{ SEM}$) remained significantly less than the quaternary ammonium-based sanitiser ($84.4 \text{ cfu} \pm 7.9 \text{ SEM}$; $p = 0.006$) but was not significantly different to the alcohol-based sanitiser ($69.9 \text{ cfu} \pm 8.7 \text{ SEM}$; $p = 0.648$), with the numbers recovered from fingers after the use of the capric acid-based sanitiser being 1.2 times less than the quaternary ammonium-based sanitiser (Figure 1). In addition, the numbers of microbes from fingers 4 hours after the use of the alcohol-based sanitiser were significantly less than the quaternary ammonium-based sanitiser ($p = 0.001$), with the numbers of microbes being also 1.2 times less.

When each individual hand wash was analysed over time, there were some significant differences (Figure 1). The number of microbes was reduced significantly after baseline at 10 mins with the quaternary ammonium-based ($p = 0.003$),

the capric acid-based ($p = 0.001$) and the alcohol-based ($p = 0.0001$) sanitisers. After 20 mins, the numbers of microbes were only reduced compared to baseline for the capric acid-based and the alcohol-based ($p < 0.0001$ for both) sanitisers. The numbers of microbes returned to near baseline levels with all handwashes after 4 hours ($p > 0.05$).

There was a significant increase in the numbers of bacteria isolated from fingers at baseline when chocolate agar with Tween 80 and lecithin was used ($89 \text{ cfu} \pm 26.4 \text{ SEM}$ vs. $164 \text{ cfu} \pm 27.0 \text{ SEM}$, $p = 0.011$) compared to chocolate agar alone (Table 5). There was no difference between the numbers of bacteria isolated from fingers at baseline using chocolate agar alone ($89 \text{ cfu} \pm 26.4 \text{ SEM}$ vs. $71 \text{ cfu} \pm 15.9 \text{ SEM}$, $p = 0.283$; Table 5). There was a significant reduction in the numbers of microbes from fingers over time when either of the two chocolate agars were used (Table 5). For chocolate agar alone, the average reduction in numbers from baseline to 10 minutes was $18\% \pm 26$ ($p = 0.002$) and to 20 minutes was $47\% \pm 17$ ($p = 0.0001$).

Similarly, when chocolate agar containing Tween 80 and lecithin was used, the average reduction in numbers from baseline to 10 minutes was $17\% \pm 23$ ($p = 0.001$) and to 20 minutes was $48\% \pm 9$ ($p = 0.0001$). The average reductions in the numbers of bacteria isolated from fingers after the use of the capric acid-based sanitiser were not different whether chocolate agar alone or with Tween 80 and lecithin was used

Table 5. The effect of using Tween 80 and lecithin on microbial numbers from fingers after using Doxall.

Agar	Numbers of bacteria (colony forming unit per hand mean \pm standard error)			Comparison between times: P-value
	Baseline	10 mins	20 mins	
Chocolate agar alone	89 \pm 26.4	75 \pm 36.8	37 \pm 14.0	0.002
Chocolate agar plus Tween 80 and lecithin	164 \pm 27.0	118 \pm 9.0	81 \pm 6.0	0.0001
Comparison between agar types: P-value	0.011	0.012		

($p = 0.876$). Interestingly, the difference in bacterial numbers between chocolate agar with and without Tween 80 and lecithin was maintained, and the difference increased but not significantly ($p > 0.12$), from baseline (2.4 ± 2.7 times) 10 minutes (5.1 ± 7.2 times) and 20 minutes (5.6 ± 9.2 times) after use of the capric acid hand sanitiser.

Discussion

The current study demonstrated the efficacy of hand sanitisers to reduce the microbial load on fingers after use, and the length of time the effect lasted. The data found significant differences between the hand sanitisers and also could identify when a hand sanitiser was most effective (i.e. resulted in the greatest reduction in numbers of culturable microbes on fingers). The study attempted to use real-world conditions as far as possible by allowing participants to return to their normal day-to-day activities immediately after the application of the hand washes. Most people used computers during this time in the study. Two hand washes, alcohol-based or capric acid-based, significantly reduced the numbers of microbes that could be cultured from fingers 10 and 20 minutes after washing. The study also showed that laboratory assays of hand sanitisers (MIC, MBC) did not correlate with activity on hands.

Similarly to another report,¹⁵ there was minimal activity of the alcohol-based the hand sanitiser even at 100% on the agar plate assay. As was speculated previously,¹⁵ this may be due to rapid evaporation of the alcohol, and this is partly confirmed in the broth-based assay where the alcohol-based hand sanitiser was very active, giving MIC at 10% dilution. The lack of correlation between the laboratory-based assays and the clinical trial was intriguing. It is possible that the low activity of Doxall in laboratory-based assays, but high activity in the clinical trial may be due to complexes forming with the antimicrobial ingredient capric acid in laboratory media which reduces activity. Alternatively, there is the possibility of synergy between capric acid and endogenous antimicrobials on hands, which is discussed in more detail below.

Computer keyboards and mice have been reported to be contaminated with a variety of microbes. In university settings, *staphylococci* are the most commonly isolated bacteria from computer keyboards,^{22,23} and these were the most common isolates from fingers of participants in the current study. *Staphylococci* are also commonly isolated from computer keyboards in hospital settings.²⁴ The fingers of clinical trial participants at the University of New South Wales have been previously shown to be colonised commonly by *staphylococci*.¹⁷

Producing a hand sanitiser with prolonged antimicrobial activity on the skin has been suggested as one way of obtaining the most benefit from hand hygiene and overcoming some of the issues with compliance.^{2,4} Two hand sanitisers produced a significant reduction in the numbers of bacteria on fingers ten and twenty minutes after use. Alcohol has well-known antimicrobial activity, affecting microbial membranes and proteins. Capric acid is antimicrobial with activity against several bacteria and fungi.²⁵⁻²⁷ The mode of action of capric acid, which is positively charged, is most likely by acting on the negatively charged lipids in microbial membranes.^{25,26} This is a similar mode of action to quaternary ammonium compounds, but the hand sanitiser containing this as the antimicrobial ingredient was not as effective as the other two used in the current study.

Initially no potential disinfectant neutralisers were incorporated into the culture media as the phase of growth of cells, especially stationary vs. logarithmic phase, may affect the activity of disinfectants.²⁸ Therefore, it was hypothesised that allowing the disinfectants to continue to have activity whilst the microbes were growing on the agar plates would allow the disinfectants the most chance of affecting the microbes. However, in the absence of neutralisers it is not possible to determine whether the disinfectants were bactericidal or bacteriostatic as they may have been present throughout the incubation of the agar plates. Quaternary ammonium and alcohol are both well-known bactericidal agents.^{18,19}

As there is less information regarding the activity of capric acid-based hand sanitisers, the present investigation studied whether the effect of this on bacteria on hands was bactericidal or bacteriostatic by incorporating disinfectant neutralisers Tween 80 and lecithin²⁹ into the chocolate agar. At baseline (prior to use of any of the hand sanitiser), there was a significant increase in bacteria cultured on the agar containing Tween 80 and lecithin. This suggests that there are bacteriostatic substances naturally occurring on fingers that are neutralised by Tween 80 and/or lecithin. Indeed, lauric, capric, stearic, oleic, sapienic and hexadecenoic acid that are present on human skin are antibacterial and are active against *staphylococci*.^{30,31} The data further suggest that the average amount of capric acid loaded onto participant's fingers was bactericidal as there was the same amount of bacterial reduction using chocolate agar in the presence or absence of neutralisers.

There was variation between the difference in the numbers of bacteria isolated from fingers of participants at baseline on the two types of chocolate agar; ranging from 0.7 to 9 times. This suggests that participants may have different amounts of antibacterial substances on their fingers, possibly as the result of producing different amounts of endogenous fatty acids or having used different types of hand sanitisers or soaps prior to the study. Future studies should consider using a wash-in approach, i.e. having participants use the same types of hand washing procedures prior to examining effects of different hand sanitisers.

It may also be interesting to sample the fingers of participants and determine whether the concentrations of antibacterial substances such as fatty acids correlate with an innate ability to reduce microbial growth in bacterial media. The similar amount of reduction in bacterial numbers when using the capric acid-containing sanitiser at baseline and 10 and 20 minutes after use suggests that this hand sanitiser may not alter the endogenous antimicrobial substances on fingers, but rather provide an additional amount of bacterial killing.

The concentration of each antimicrobial ingredient in the hand sanitisers was not available in the literature or from the manufacturers. However, it is known that quaternary ammonium compounds can be toxic by disrupting mitochondrial function, altering oestrogen signalling and inhibiting cholesterol synthesis in mammalian cells in laboratory culture.^{32,33} Certain quaternary ammonium compounds can also be toxic or induce an inflammatory response from cells of the ocular surface.³⁴ Therefore, it is likely that manufacturers need to balance antimicrobial activity with toxicity when formulating hand sanitisers containing quaternary ammonium compounds. Capric acid is a naturally occurring

fatty acid and is on the Federal Drug Administration's list of substances that are 'generally regarded as safe' (<https://www.fda.gov/food/food-additives-petitions/food-additive-status-list#ftnD>). This may mean that higher concentrations can be used to give maximum antimicrobial effect whilst maintaining low toxicity.

This study has a number of potential limitations. The fingers of subjects were not cultured between 20 minutes and four hours after use of each hand sanitiser and so the data do not show exactly how long the sanitisers that were active at 20 minutes remain active. Future studies should examine more time points. Also, it would be useful to examine other hand sanitisers that contain similar disinfectants to determine whether the results are consistent across the disinfectant types. The current study did not culture fingers for viruses, and this should be attempted in future studies.

Conclusions

The current study found that hand sanitisers containing capric acid or alcohol as their disinfecting agents produced a greater reduction in microbial numbers of fingers than a hand sanitiser containing a quaternary ammonium compound. The microbial numbers on fingers after use of the alcohol or capric acid containing hand sanitisers were significantly reduced up to 20 minutes after used, but the hand sanitiser containing a quaternary ammonium compound did not significantly reduce the numbers of microbes on fingers.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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