Clinical Trial/Experimental Study

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Effectiveness of surgical hand antisepsis using chlorhexidine digluconate and parachlorometaxylenol hand scrub

Cross-over trial

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Abstract

Background: Chlorhexidine and parachlorometaxylenol (PCMX) are antiseptics recommended for surgical hand antisepsis. To our knowledge, PCMX has not been evaluated for bactericidal efficacy "in vivo.

Methods: We conducted a randomized, double-blind, controlled crossover trial to compare the bacterial loads on fingertips and fingernails under laboratory conditions after use of antiseptic test products, including chlorhexidine digluconate 4%, PCMX 3%, and a reference solution of propan-1-ol 60% (P-1). We assessed bacterial load after a prewash with soft soap, immediately after application of an antiseptic, and 3 hours after application and wearing of sterile, powder-free gloves. Our procedures followed those specified by European Norm (EN) 12791 for evaluating surgical hand antiseptics and using cotton swab for fingertips and fingernails.

Results: Chlorhexidine digluconate 4% and PCMX 3% did not decrease bacterial load on the hands. The bactericidal performances of chlorhexidine digluconate 4% and PCMX 3% did not differ significantly. Chlorhexidine digluconate 4% and PCMX 3% increased bacterial load on the fingertips after participants had worn gloves for 3 hours. Fingernails had greater bacterial loads than skin on the fingertips.

Conclusions: Chlorhexidine digluconate 4% and PCMX 3% had similar bactericidal efficacy, but they failed to meet the EN 12791 efficacy standard. Fingernails should be a particular focus of antisepsis in preparation for surgery.

The trial was registered at ClinicalTrials.gov (ID: NCT02500758).

Abbreviations: CHG = chlorhexidine gluconate, EN = European Norm, P-1 = propan-1-ol 60%, PCMX = parachlorometaxylenol, PVI = povidone iodine.

Keywords: antisepsis, randomized controlled trial, surgical site infection

1. Introduction

Surgical site infections (SSI) are among the most common hospital-acquired infections worldwide despite significant developments in surgical technique.[1] Surgical hand antisepsis has long been used to prevent SSI. Intact surgical gloves are the most important barrier to the bi-directional migration of microorganisms between the hands of surgical team members and the patient.[2] However, undetected perforations of surgical gloves are common and increase in frequency with duration of glove wear.[3–6] The risk of glove defects is related to the type of surgery, ranging from 7% in urologic surgery to 65% in cardiothoracic surgery.[7,8] Surgical hand antisepsis is also essential for preventing chronic infections associated with contaminated implants.[9]

The 5 main products currently marketed for preoperative antisepsis are alcohol, chlorhexidine, iodine/iodophors, parachlorometaxylenol (PCMX), and triclosan,[10,11] with povidone iodine (PVI) and chlorhexidine gluconate (CHG) the most commonly used for skin preparations. They are available in aqueous and alcoholic preparations and in different concentrations.[12–14] PVI and CHG are effective against a wide range of gram-positive and Gram-negative bacteria, viruses, and fungi, though CHG has greater residual antiseptic activity on the skin after application[15] and superior efficacy in bacterial reduction.[16–18] Although PCMX is a recommended antiseptic to prevent SSI,[10,11] we are aware of no formal in vivo evaluations of its bactericidal power.

We compared the efficacy of a PCMX 3% formulation with CHG 4%[16–18] in surgical hand antisepsis. We used an standard approach for assessing anti-bacterial efficacy: European Norm (EN) 12791 to determine bactericidal efficacy in vivo during

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surgical hand scrub preparations[19,20] and cotton swab to determinate bacterial load from fingertips and fingernails independently.

2. Methods

We conducted a randomized, double-blind, controlled crossover trial to compare the number of colony forming units per mL (CFU/mL) on the fingertips, fingernails, and web spaces between fingers under laboratory conditions after use of antiseptic test products, including CHG, PCMX, and a reference solution. The study was performed from January to June, 2016, at Universidad Complutense de Madrid (Spain).

2.1. Participants

Twenty-five healthy participants of last courses in the health sciences were assessed for eligibility. Twenty Participants completed the study, aged 25 to 55 years old, mean 29, 95 ± 9, 02, median 28. They participated in the study after giving written informed consent.

These participants met the inclusion criteria of having short and clean fingernails, no cuts/abrasions on their fingers, no history of skin disorders, including allergies to any ingredient in tested solutions, no recent antibiotic/antimicrobial use, no recent use of medicated soap or cream on hands, and no painted nails[17] at least 1 week before the study. All participants received formal training on standard surgical hand scrubbing (with non-antimicrobial soap) and sterile gloving.[22] The ethics committee of the Hospital Clínico San Carlos, Madrid, Spain, approved this study (ID: 14/186-TFM) and the trial was registered at ClinicalTrials.gov (ID: NCT02500758).

The sample size was calculated with the software from Unidad de Epidemiología Clínica y Bioestadística. Complexo Hospitalario Universitario de A Coruña. Universidade A Coruña (www.fisterra.com) to detect a difference in bacterial load reduction equal to that observed previously between CHG and PVI were 0.94 ± 1.11 log10 and 0.15 ± 1.11 log10, respectively[18] with 80% statistical power ($\alpha = .05$, 2-tailed test), at least 18 participants are required. Our inclusion of 20 participants surpasses this limit and meets the EN 12791 sample size requirement.[20] Furthermore, assuming a loss to follow up rate of 25%, at least 25 participants were included in the study.

2.2. Experimental design

Experimental procedures followed those specified by EN 12791 for assessing surgical hand scrubs, with propan-1-ol 60% (P-1) as a reference control.[21] Participants were randomly assigned to 1 of 2 groups (n = 10 each), a "Latin-square design" is used. In 1 group, participants first used P-1. In the second and third test sessions, participants in this group used CHG and PCMX, respectively. In the other group, participants used PCMX, P-1, and CHG in the first, second, and third test sessions, respectively (Fig. 1). Between test sessions, there was a washout period of at least 1 week to allow normal skin flora to reconstitute.[18,21] Random assignments were based on computer generated randomization routine using EpiData software version 3.02 (EpiData Association, Odense, Denmark) and allocations were concealed with sealed, numbered, tamperproof, opaque envelopes that were opened only after participants consented to participate. Participants were blinded to CHG and PCMX administration, but not P-1 administration because it was applied with a sterile syringe according to EN 12791. The laboratory assessor of bacterial load outcomes was blinded to participants' allocations.

2.3. Materials

The soft soap used in pre-antisepsis scrubbing was composed of 50 parts linseed oil, 0.5 parts potassium hydroxide, 7 parts 96% ethanol (volume concentration), and up to 1000 grams distilled water that had been sterilized in a autoclave at 121°C for 15 minutes. The CHG formulation included CHG 4%, propan-2-ol 1% to 5%, lauryldimethylamine oxide 1% to 5%, and glycerol 1% to 5% (Dispomedic Scrub C, CV Medica, Sarral, Tarragona, España). The PCMX formulation included PCMX 3%, water, sodium laureth sulfate, triethyleneglycol, cocamide propylbetaine, chloroxylenol, aloe barbadensis, lanolin, parfum, methylchloroisothiazolinone, and methylisothyazolinone (Dispomedic Aloe PCMX, CV Medica, Sarral, Tarragona, España). The P-1 formulation included propan-ol-1 60% (volume concentration). Participants wore powder free sterile surgical gloves (Peha-Taft Classic , Bastos Medical, S.L., Barcelona, Spain) after application of test antiseptics.

2.4. Procedures

2.4.1. Antiseptic application and bacterial sampling. Our procedures strictly followed norm EN12791.[20] For each test session, samples for bacterial counts were taken immediately after a prewash (before application of the test antiseptic), immediately after application of the test antiseptic, and 3 hours after application.

For the prewash, soap was poured into cupped dry hands and rubbed vigorously on to the skin up to the wrists. The hands were then rinsed with running tap water and dried thoroughly with clean disposable paper towels. Immediately after drying, the 5 fingertips were rubbed for 1 minute on the base of a 90 mm diameter sterile petri dish containing 10 mL of trypticase soy broth (TSB) without neutralizer. A separate petri dish was used for each hand. To isolate the source of any bacteria found, we also scrubbed cotton-tipped swabs (Copan Italia S.p.A., Brescia, Italy) for 10 seconds in the medial nailfold at the subungual aspect of the free border of the nail of the thumb, and from the fingertip of the thumb, respectively. Cotton-tipped swabs have been used for bacterial sampling on skin of the foot and ankle previously.[23–26]

Next, the test antiseptics were applied. For P-1, 3 mL of P-1 was poured into participants' cupped dried hands. Participants then rubbed the liquid vigorously into the skin up to the wrists according with the standard handrub procedure.[22] When hands were nearly dry, additional 3 mL aliquots of P-1 were applied until the hands had been wet for 3 continuous minutes. 10 cc of P-1 was used by each participant. CHG and PCMX were applied according to the manufacturer's instructions. Fingernails were brushed with a sterile brush, and hands and forearms were washed over a period of 3 minutes. After washing, hands were rinsed with running tap water for 15 seconds and dried with a sterile disposable towel. As soon as participants' hands were dry after antiseptic application, we used the same bacterial sampling procedure as after the prewash described above, but for 1 randomly selected hand only, taking care to avoid contamination of the other hand.

Participants then donned gloves. After participants had worn the gloves for 3 hours, according to the reference surgical hand-disinfection procedure described in norm EN 12791.[20] The

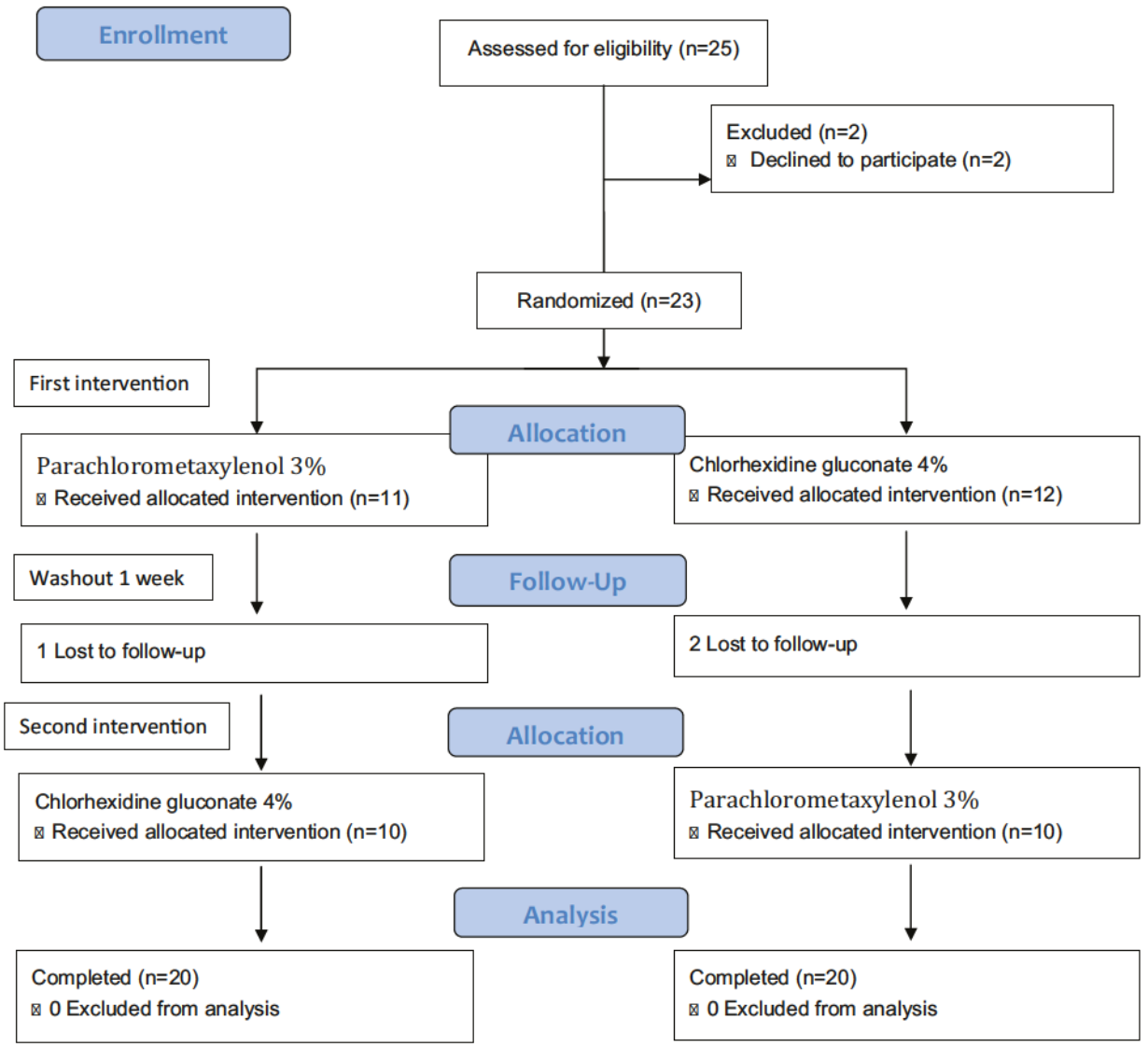

Figure 1. shows the flow chart of study population starting from those eligible to those included in the analysis.

gloves were removed and the bacterial sampling were repeated again, this time for the hand not sampled immediately after the antiseptic application.

2.4.2. Microbiologic processing. For prewash samples, 1:10 and 1:100 dilutions were prepared in TSB. For each dilution, 0.1 mL was spread over trypticase soy agar (TSA) in 2 Petri dishes with a sterile glass spatula. No more than 5 minutes elapsed between sampling and seeding. Dishes were incubated for 24 hours at 37±2°C. After an initial count of the CFU, Petri dishes were incubated for another 24 hours to detect slow-growing colonies.[20] For both rounds of samples after application of antiseptics, 1.0 mL and 0.1 mL of undiluted solution and a 1:10 dilution of it were plated, incubated, and assessed as for the prewash samples. The mean number of CFU per mL (log10 values) in duplicate dishes was calculated after correction for the dilution factor.

The nail and skin swabs were resuspended in 2 mL of 0.9% sodium chloride and diluted 10-fold. At least 3 dilutions of each sample were spread onto TSA (20 μL in each plate). Plates were incubated at 35°C, and colonies were counted (log10 values per cm^2 of skin) after 24 to 48 hours. The limits of detection in the nailfold and skin tests were 1.33×10^2 and 1×10^2 CFU/cm^2, respectively.[24–26] The laboratory investigator was blinded to the antiseptic test products individual participants had received.

2.5. Outcome measures

The primary outcome measures were the log10 CFU values for the different sample types at the 3 assessment points (prewash, immediate post-application, and 3 hours post-application) and the immediate and 3-hour reduction factors (prewash—immediate and prewash—3 hours, respectively).

Secondary outcome measures were adverse effects participants reported about the different antiseptics.

2.6. Statistical analysis

We computed means and standard deviations for the primary outcomes. These variables were not normally distributed

Table 1

Immediate and 3-hour bactericidal effects of antiseptic products using European Norm 12791.

	Immediate effect (log 10 CFU/ml) means ± SD					3-hour effect (log 10 CFU/ml) means ± SD				
	Prewash	Immediate post-application	P value	RFI	*P* value RFI	Prewash	3-hours post-application	*P* value*	RF3	P Value RF3
CHG 4%	3.71 ± 0.58	3.87 ± 0.58	0.076	-0.16 ± 0.62	CHG/P-1 0,0002	3.9 ± 0.48	4.65 ± 0.8	.001	-0.75 ± -0.32	CHG/P-1 0,0002
PCMX 3%	4.07 ± 0.63	3.96 ± 0.39	0.254	0.11 ± 0.60	PCMX/P-1 0,0016	3.89 ± 0.75	4.54 ± 0.62	.001	-0.65 ± 0.67	PCMX/P-1 0,0001
P-1 60%	3.47 ± 1.13	2.08 ± 1.00	0.001	1.38 ± 1.20	CHG/PCMX .162	3.68 ± 1.20	2.37 ± 1.00	.001	1.32 ± 0.84	CHG/PCMX 0.614

CFU = colony-forming units, CHG = chlorhexidine gluconate, P-1 = propan-1-ol 60%, PCMX = parachlorometaxylenol, RF3 = reduction factor 3 hours effect expressed by decimal logarithms of log prevalue 3 hours" minus log "postvalue 3 hours", RFI = reduction factor Immediate effect expressed by decimal logarithms of log "prevalue immediate" minus log "postvalue inmediate".

according to the Shapiro–Wilk test ($P < .05$). Therefore, to compare prewash and post-application values, we calculated Wilcoxon signed-rank test. We computed Kruskal Wallis tests to compare antiseptics' reduction factors. Statistical significance was set at $P < .05$. We performed the analyses with SPSS 19.0 (Chicago, IL).

3. Results

Assessed according UN12791, P-1 reduced bacterial load substantially, at both immediate and 3-hour post-application assessments (Table 1). CHG and PCMX, however, did not change bacterial load appreciably at the immediate assessment ($P > .05$), and they both actually increased bacterial load by 3-hours post-application ($P < .001$). P-1 had significantly greater bactericidal efficacy reduction factors than CHG at immediate and 3-hours ($P = .0002$) and PCMX at immediate and 3-hours ($P < .001$). The reduction factors for CHG and PCMX were not significantly different from each other at either immediate and 3-hours ($P = .162$ and .614 respectively).

Bacterial loads collected with swab were higher from fingernail samples (Table 2) than for fingertips and P-1 reduced bacterial load considerably from fingernail ($P < .0001$), but the reductions for CHG and PCMX were very small ($P > .05$). P-1 had significantly greater bactericidal efficacy than CHG and PCMX at immediate and 3-hours ($P = .0001$ and .0004 respectively), and the reduction factors for CHG and PCMX were not significantly different at either immediate and 3-hours ($P = .56280$ and .6951 respectively).

Collected with swab the fingertips (Table 3) had lower bacterial loads than fingernails. P-1 still decreased bacterial load at this location at both immediate and 3-hours ($P = .0007$ and .0011 respectively), but CHG and PCMX produced only negligible changes in bacterial load at both immediate and 3-hours ($P > .05$). P-1 was a significantly better antisepsis power than PCMX ($P < .01$) and showed similar bactericidal power with CHG ($P = .177$). The bactericidal performances of CHG and PCMX were not significantly different at fingertips at immediate and 3-hours effect ($P > .05$)

There were no adverse effects.

4. Discussion

We evaluated the bactericidal efficacy of CHG and PCMX in surgical hand antisepsis, following the procedures specified in EN 12791.[20] To our knowledge, PCMX has not previously been evaluated in this context, although the US Centers for Disease Control and Prevention have recommended it to prevent SSI.[10,11]

In our randomized, double-blind, controlled, crossover trial, CHG, and PCMX did not decrease bacterial load on the fingerstips and fingernails after surgical hand antiseptis. The bactericidal performances of CHG and PCMX did not differ significantly. However, the reference antiseptic, P-1, reduced bacterial loads substantially at immediate effect and maintained these reductions even after participants had worn sterile, powder-free surgical gloves for 3 hours. CHG and PCMX actually produced a modest increase in bacterial load after participants had worn gloves for 3 hours. Consequently, neither CHG nor PCMX met the EN12791 criteria for non-inferiority relative to P-1 in our study.

The increase in bacterial load 3 hours after the application of CHG and PCMX is a paradoxical effect similar to that previously described after surgical hand antisepsis and use of powdered gloves.[27]

Our participants, however, wore sterile, powder-free gloves. Unlike P-1, the CHG and PCMX antiseptic products both have excipients, which might counteract their antibacterial effects. Edmonds and colleagues[28,29,31] showed that excipients can influence bactericidal efficacy in antiseptics. If the paradoxical effect we observed is replicated, this and other possible explanations merit investigation.

The lack of bactericidal efficacy we observed for CHG is consistent with research showing CHG's poor antiseptic

Table 2

Immediate and 3-hour bactericidal effects of antiseptic products on fingernails using cotton swab.

	Immediate effect (log 10 CFU/mL) means ± SD					3-hour effect (log 10 CFU/mL) means ± SD				
	Prewash	Immediate post-application	*P* value	RFI	P Value RFI	Prewash	3-hours post-application	P value	RF3	*P* value RF3
CHG 4%	4.78 ± 0.88	4.60 ± 0.79	.1730	0.18 ± 0.71	CHG/P-1 0.0001	4.55 ± 1.04	3.99 ± 1.20	0.0349	0.56 ± 1.03	CHG/P-1 0,0004
PCMX 3%	4.95 ± 0.92	4.82 ± 0.75	.6143	0.12 ± 0.77	PCMX/P-1 0.0001	4.45 ± 1.28	4.15 ± 1.24	0.2197	0.30 ± 1.20	PCMX/P-1 0,0004
P-1 60%	4.96 ± 0.84	2.28 ± 0.64	.0001	2.68 ± 1.03	CHG/PCMX 0.5628	4.98 ± 1.29	2.34 ± 0.83	0.0001	2.64 ± 1.34	CHG/PCMX 0,6951

CFU = colony-forming units, CHG = chlorhexidine gluconate, P-1 = propan-1-ol 60%, PCMX = parachlorometaxylenol, RF3 = reduction factor 3 hours effect expressed by decimal logarithms of log prevalue 3 hours" minus log "postvalue 3 hours", RFI = reduction factor Immediate effect expressed by decimal logarithms of log "prevalue immediate" minus log "postvalue inmediate".

Table 3

Immediate and 3-hour bactericidal effects of antiseptic products on fingertips using cotton swab.

	Immediate effect (log 10 CFU/mL) means ± SD					3-hour effect (log 10 CFU/mL) means ± SD				
	Prewash	Immediate post-application	*P* value	RFI	*P* value RFI	Prewash	3-hours post-application	*P* value	RF3	*P* value RF3
CHG 4%	2.55 ± 0.85	2.29 ± 0.72	.2762	0.26 ± 0.81	CHG/P-1 0,177	2.33 ± 0.81	2.23 ± 0.76	.5995	0.11 ± 0.05	CHG/P-1 0,116
PCMX 3%	2.77 ± 1.21	2.78 ± 1.11	.6008	0.00 ± 0.79	PCMX/P-1 0,010	2.53 ± 0.82	2.41 ± 0.73	.2397	0.12 ± 0.72	PCMX/P-1 0,025
P-1 60%	2.35 ± 0.69	1.71 ± 0.24	.0007	0.64 ± 0.71	CHG/PCMX 0,270	2.28 ± 0.64	1.65 ± 0.15	.0011	0.63 ± 0.64	CHG/PCMX 0,7938

CFU = colony-forming units, CHG = chlorhexidine gluconate, P-1 = propan-1-ol 60%, PCMX = parachlorometaxylenol, RF3 = reduction factor 3 hours effect expressed by decimal logarithms of log prevalue 3 hours" minus log "postvalue 3 hours", RFI = reduction factor Immediate effect expressed by decimal logarithms of log "prevalue immediate" minus log "postvalue inmediate".

performance in surgical preparation hand[18] and toenail scrubs.[24,25,30] However, Marchetti and colleagues found that hand-rubbed CHG (Hisbiscrub) was an effective antiseptic and met the EN 12791 standards.

Pre-operative surgical hand antisepsis using CHG or PCMX do not can reduce Significantly, but not eradicate, the resident flora on the fingertips and fingernails, and thus it does not eliminate the risk of contamination of microorganisms into the surgical site if the integrity of the glove is breached. Based on our findings and those of others.[3,11]

We recommend further research adding before or after a hand wash with alcohol solution when using these antiseptics to increase its efficacy for surgical hand antisepsis

We found that the fingernails had greater bacterial loads than skin on the fingertips and the first web spaces of the hand. This suggests that fingernails should be a particular focus of antisepsis in preparation for surgery.

Moreover, CHG and PCMX is not the only recommended antiseptic products that did not met the EN 12791requirements because some alcohol-based hand rubs recommend by the World Health Organization for both hygienic and pre-surgical hand treatment with formulations based on ethanol 80% v/v and 2-propanol 75% v/v[22] also failed to meet the EN 12791 criteria.[30] These and other results, in combination with our own, underline the need for further rigorous evaluation of surgical hand antiseptic products.

A limitation of the study is that the European Standard Norm EN 12791 specifies a test method simulating practical conditions for establishing whether a product for surgical hand antisepsis reduces the release of hand flora according to its requirements when used for the antisepsis of the clean hands of volunteers, so further research is needed in an operating theatre environment or clinical setting

In conclusion, as surgical hand antiseptics, CHG 4% and PCMX 3% had similar bactericidal efficacy, but failed to meet the EN 12791 efficacy standard. PCMX did not show paradoxical overcolonization at immediate effect as showed by CHG and fingernails had high bacterial load and thus should be a particular focus of pre-surgical antisepsis.

Author contributions

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