The Forgotten Role of Alcohol: A Systematic Review and Meta-Analysis of the Clinical Efficacy and Perceived Role of Chlorhexidine in Skin Antisepsis

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Abstract

Background: Skin antisepsis is a simple and effective measure to prevent infections. The efficacy of chlorhexidine is actively discussed in the literature on skin antisepsis. However, study outcomes due to chlorhexidine-alcohol combinations are often attributed to chlorhexidine alone. Thus, we sought to review the efficacy of chlorhexidine for skin antisepsis and the extent of a possible misinterpretation of evidence.

Methods: We performed a systematic literature review of clinical trials and systematic reviews investigating chlorhexidine compounds for blood culture collection, vascular catheter insertion and surgical skin preparation. We searched PubMed, CINAHL, the Cochrane Library, the Agency for Healthcare Research and Quality website, several clinical trials registries and a manufacturer website. We extracted data on study design, antiseptic composition, and the following outcomes: blood culture contamination, catheter colonisation, catheter-related bloodstream infection and surgical site infection. We conducted meta-analyses of the clinical efficacy of chlorhexidine compounds and reviewed the appropriateness of the authors’ attribution.

Results: In all three application areas and for all outcomes, we found good evidence favouring chlorhexidine-alcohol over aqueous competitors, but not over competitors combined with alcohols. For blood cultures and surgery, we found no evidence supporting chlorhexidine alone. For catheters, we found evidence in support of chlorhexidine alone for preventing catheter colonisation, but not for preventing bloodstream infection. A range of 29 to 43% of articles attributed outcomes solely to chlorhexidine when the combination with alcohol was in fact used. Articles with ambiguous attribution were common (8–35%). Unsubstantiated recommendations for chlorhexidine alone instead of chlorhexidine-alcohol were identified in several practice recommendations and evidence-based guidelines.

Conclusions: Perceived efficacy of chlorhexidine is often in fact based on evidence for the efficacy of the chlorhexidine-alcohol combination. The role of alcohol has frequently been overlooked in evidence assessments. This has broader implications for knowledge translation as well as potential implications for patient safety.

Introduction

Skin antisepsis has been an indispensable part of medical practice for more than a century. After a period of increased attention in the 1970s and 1980s that temporarily waned, there is now renewed interest in its role as a simple and effective measure for preventing healthcare-acquired infections.

The most commonly used substances for skin antisepsis are (1) alcohols (ethanol, isopropanol and n-propanol), (2) chlorhexidine, commonly available as chlorhexidine gluconate (CHG), and (3) povidone-iodine (PVI), an organic iodine complex. Among these antiseptics, alcohols are microbiologically most active but have no appreciable residual activity [1–3]. CHG and PVI are less effective, but have residual activity on skin, which is pronounced for CHG but small for PVI. The usual active concentrations are about 70–90% (v/v) for alcohols, 0.5–4% (w/v) for CHG, and 5–10% (v/v) for PVI (or, instead of total PVI, 0.5–1% “available” iodine). Both CHG and PVI are available as aqueous solutions where they are the sole active ingredients, and they can be combined with alcohols, thereby creating enhanced antiseptics with two active components. There is also iodine tincture, which is an alcoholic solution of elemental iodine and potassium iodide.

Among the antiseptics, CHG has attracted considerable attention through several prominent clinical studies concerning vascular catheters and surgery [4–6]. CHG became a topic of discussion and a subject of keynote presentations at conferences. Preference for CHG, in particular over its main competitor, PVI,
was expressed in several practice recommendations and evidence-based guidelines for skin antisepsis [7–10]. We noticed an inconsistent interpretation of findings in some primary studies and subsequent reviews. Several articles that evaluated the efficacy of the combination of alcohols plus CHG attributed the study outcomes solely to the CHG component [11–13]. These articles effectively concluded that CHG was the only agent responsible for positive outcomes and that CHG per se was superior to PVI per se when in fact CHG-alcohol versus PVI alone had been tested.

This apparent misinterpretation of evidence and an increasing number of recommendations that were focussing prominently or exclusively on the efficacy of the CHG component prompted us to reassess the evidence by way of systematic review. We posed the following questions: (1) What is the evidence for the efficacy of CHG alone or combination antiseptics containing it for blood culture collection, vascular catheter insertion, and surgical skin preparation? (2) How common is the attribution of efficacy from a combination of antiseptics to CHG alone in the primary literature and in systematic reviews? (3) Has this misattribution had an effect on practice recommendations and evidence-based guidelines?

**Methods**

**Literature Search Strategy**

Exhaustive searches for primary and secondary literature were performed in three areas of skin antisepsis: (1) blood culture collection, (2) vascular catheter insertion, and (3) surgical skin preparation. For the purpose of this review, primary literature was defined as randomised clinical trials (RCTs) and non-randomised clinical studies, and secondary literature was defined as systematic reviews. Searches were performed using PubMed, CINAHL, the Cochrane Library, the Agency for Healthcare Research and Quality website, several clinical trials registries, and a CHG product manufacturer’s website (CareFusion, San Diego, CA, USA). Apart from the selection of databases, no specific limits on publication dates and language were applied. The full literature search strategy is provided in Text S1, and a PRISMA flow diagram in Figure 1. A PRISMA Checklist is provided in Checklist S1.

**Selection Criteria**

All included primary and secondary articles had to have evaluated any CHG-containing antiseptic against any other antiseptic in one of the three areas of interest. The following outcomes had to be reported: (1) for blood culture studies, the rate of blood culture contamination, (2) for vascular catheter studies, the rates of microbial catheter colonisation and/or catheter-related bloodstream infection (CR-BSI), and (3) for surgery articles, the rate of surgical site infections. The following interventions were excluded: antiseptic cloth wiping or bathing in the preoperative phase, antisepsis only during catheter maintenance but not at insertion, non-superficial skin antisepsis, and where skin antisepsis was only part of a multifactorial intervention. Further information on eligibility criteria is provided in Text S1.

**Data Extraction and Quality Assessment**

Data were extracted on study design, antiseptics compared and their composition, main outcomes, and the authors’ interpretation of the study results. All primary (RCTs and non-RCTs) and secondary articles were rated to assess the authors’ attribution of study outcomes from CHG-containing antiseptics (qualitative synthesis), while only RCTs were selected for subsequent meta-analyses (quantitative synthesis). All RCTs were appraised for risk of bias using a domain-based approach recommended by the Cochrane Collaboration [14]. Further details and the results of risk of bias assessment are provided in Text S1 and Tables S1, S2 and S3.

**Assessment of Authors’ Attribution (Qualitative Synthesis)**

Attribution was rated as “correct” if study authors recognised that the combination of both CHG and alcohol was used and therefore responsible for the outcomes. It was rated as “incorrect” if authors clearly attributed study outcomes derived from the combination of CHG and alcohol to CHG alone. It was rated as “intermediate” if there were ambiguous statements, such as when authors recognised the antiseptic properties of alcohols but also made statements suggesting that CHG alone might be responsible. It was rated as “not applicable” if CHG alone without alcohol had been used.

**Meta-analyses of Clinical Efficacy (Quantitative Synthesis)**

Meta-analyses to quantify the clinical efficacy of CHG compounds were performed using the RevMan software [15] by computing relative risks (RR) and 95% confidence intervals (CI). Only RCTs that were clinically homogenous and had tested the same basic antiseptic components were pooled together. In the absence of statistical heterogeneity, a fixed-effects model was used for analysis, and in the presence of statistical heterogeneity ($I^2\geq50\%$, $p<0.1$), both fixed-effects and random-effects models were used in a sensitivity analysis.

**Survey of Tertiary Literature**

The impact of the conclusions in the primary and secondary literature on perceptions in the medical community and on practice recommendations was gleaned from a non-exhaustive survey of the tertiary literature. Tertiary literature was defined as any other articles commenting on the role of CHG in skin antisepsis, including narrative reviews, professional websites and e-mail discussion forums, clinical practice recommendations, and evidence-based guidelines.

**Results**

**Skin Antisepsis for Blood Culture Collection**

A total of 12 articles met the inclusion criteria for blood culture collection; this included 10 primary studies [11,16–24] and two systematic reviews [25,26] (Table 1). Among the primary studies, four were RCTs. All of the articles evaluated CHG-alcohol combinations, none evaluated aqueous CHG.

Correct attribution was found in seven articles (58%), ambiguous statements (intermediate ranking) in one (8%), and incorrect attribution in four (33%). Among the ones with incorrect attribution, three noted the presence of alcohol in the CHG-containing preparation but did not associate it with the efficacy of the antiseptic, while one published abstract listed and discussed CHG alone, and the presence of alcohol was found out through correspondence. Both systematic reviews recognised the importance of alcohols.

Two parallel-group RCTs [16,22], one within-subject trial with each subject experiencing both interventions [17] and a cluster-randomised cross-over trial [24] were subjected to meta-analyses (Figure 2). The results showed that the combination of CHG plus alcohol was significantly better than aqueous PVI alone (RR: 0.45; 95% CI: 0.32–0.63) and that there was no significant difference between CHG-alcohol versus sequential isopropanol and iodine.
tincture (RR: 1.17; 95% CI: 0.75–1.82). A single comparison of CHG-alcohol versus sequential isopropanol and PVI [24] also showed no significant difference (RR: 1.61; 95% CI: 0.98–2.64). The results of the non-RCTs are listed in Table 1 but were not included in meta-analyses.

The Malani et al systematic review [25] included four trials, two examining CHG-containing antiseptics. The authors found no clear evidence favouring any particular type of antiseptic, however, they identified possible benefits from prepackaged kits and alcohol-containing antiseptics. The Caldeira et al review [26] included six trials, three examining CHG-containing antiseptics. Several conclusions were made: (1) alcoholic iodine tincture was better than aqueous PVI, (2) alcoholic CHG was better than aqueous PVI, (3) alcoholic products were better than non-alcoholic ones, and (4) alcohol alone was not inferior to any iodine products. The authors commented that alcohol alone may be sufficient.

We identified several tertiary sources that contained unsubstantiated statements concerning the efficacy of CHG. A Clinical and Laboratory Standards Institute (CLSI) guideline for blood culture collection [7] stated: “chlorhexidine gluconate [without reference to the presence of alcohol]... is the recommended skin disinfectant for older infants, children, and adults”. A standard textbook on phlebotomy [27] contained similar statements. Several contributions to the discussion forum ClinMicroNet (American Society for Microbiology) discussed “chlorhexidine” (without reference to alcohols) and its benefits for blood culture collection.

In contrast, we did not find any relevant evidence supporting the use of CHG alone prior to blood culture collection.

**Skin Antisepsis for Vascular Catheter Insertion**

A total of 20 articles met the inclusion criteria for vascular catheter insertion; this included 18 primary studies [28–45] and two systematic reviews [46,47] (Table 2). Among the primary studies, 15 were RCTs. Four studies evaluated aqueous CHG, 13 evaluated CHG-alcohol combinations, and two evaluated a triple combination of CHG, benzalkonium chloride and benzyl alcohol. There were four studies with three study arms.

Judgement of attribution was not applicable to three studies, as they used aqueous CHG only. Among the remaining 17 articles, correct attribution was found in six articles (35%), ambiguous statements (intermediate ranking) in another six articles (35%), and incorrect attribution in five articles (29%). Three original articles correctly listed the presence of alcohol but did not associate it with antiseptic efficacy, while for one abstract, the presence of alcohol was found out through correspondence.

Four meta-analyses were performed (Figure 3); this included two analyses (catheter colonisation and CR-BSI) on aqueous CHG versus aqueous PVI (3 trials each) and two analyses on CHG-alcohol versus aqueous PVI (7 and 8 trials, respectively). The comparison of aqueous CHG with aqueous PVI indicated a significantly lower risk of catheter colonisation in the CHG group (RR 0.41; 95% CI: 0.18–0.95), but lacked significance for CR-BSI (RR 0.66; 95% CI: 0.31–1.41). The comparison of CHG-alcohol with PVI alone indicated significant benefits for CHG plus alcohol for both catheter colonisation (RR 0.62; 95% CI: 0.39–0.98) and CR-BSI (RR 0.44; 95% CI: 0.26–0.73). Statistical heterogeneity was detected in both groupings for the outcome of catheter colonisation. For aqueous CHG versus aqueous PVI (Figure 3A), the source of heterogeneity appeared to be the trial of Vallés et al [43], for CHG-alcohol versus aqueous PVI (Figure 3C), potential sources were the trials of Humar et al [35] and Maki et al [36].

Additional single-trial comparisons included (1) the combination of CHG, benzalkonium chloride and benzyl alcohol versus aqueous PVI [32], showing a benefit for the CHG preparation for catheter colonisation (RR 0.43; 95% CI: 0.22–0.82) but not for CR-BSI (RR 0.85; 95% CI: 0.17–4.16), (2) the same combination...
versus PVI plus alcohol [41], showing a benefit for the CHG preparation for colonisation (RR 0.52; 95% CI: 0.34–0.80) but not CR-BSI (RR 0.40; 95% CI: 0.13–1.24), and (3) a trial of CHG-alcohol versus sequential alcohol and aqueous PVI [34] being insignificant for colonisation (RR 0.51; 95% CI: 0.21–1.19). Again, the results of the non-RCTs are listed individually in Table 2 but were not included in meta-analyses. Again, the results of the non-RCTs are listed individually in Table 2 but were not included in meta-analyses.

The first systematic review [46] included eight trials, two examining 2% aqueous CHG, one a triple combination with CHG, and five examining CHG-alcohol combinations. The comparator for all was 10% aqueous PVI. The authors pooled all studies and found a significant risk reduction for colonisation and CR-BSI in the CHG-containing group. However, all study outcomes were solely attributed to CHG. Only a brief passage in the Discussion mentioned that only the subset of studies testing alcoholic CHG had produced a significant reduction in CR-BSI, but not the ones testing aqueous CHG. It was concluded that this may have been due to inadequate statistical power from the smaller number of studies with aqueous CHG. The second systematic review [47] included seven trials, five examining CHG-containing antiseptics against different competitors. It compared both aqueous CHG and CHG-alcohol combinations versus different antiseptics in a non-CHG group. It found a benefit of CHG-containing solutions for preventing device colonisation.

Again, we found several examples in the tertiary literature that referred to CHG alone where the CHG-alcohol combination would have been relevant. A follow-up article [48] on the 2002 systematic review of Chaiyakumapruk et al [48] examined the clinical and economic benefits of CHG for vascular catheter site care. It commented on the benefits of CHG in preventing CR-BSI, even though that had only been demonstrated for the CHG-alcohol combination. A seminal article on the Keystone Project [4]...

Table 1. Primary studies and systematic reviews evaluating chlorhexidine-containing antiseptics for the prevention of blood culture contamination.

| Referencea Study design Antiseptics comparedb Main outcomesc Comments Attributiond | A: CHG 0.5% + ALC (7%); B: PVI aq 10% | A: 14/1019; B: 34/1022; p<0.05 | Advantage of CHG + ALC over PVI aq | Incorrect |
| --- | --- | --- | --- | --- |
| Mimoz et al. 1999 [16] (M, C) RCT | A: CHG + IPA 70%; B: IPA 70% seq IT (I2 2%, ETH 47%) | A: 1/215; B: 3/215; NS | Study design equivalent to RCT | Correct |
| Trautner et al. 2002 [17] (M, C) RCT† | A: CHG 2% + IPA 70%; B: IT (composition?) | A: 158/5802; B: 186/5936; NS | Composition of IT could not be clarified | Incorrect |
| Barenfanger et al. 2004 [18] Non-RCT† | A: CHG 2% + IPA 70%; B: Unknown | A: 40/1870; B: 304/4072; p<0.05 | Weak study design, comparator unknown | Correct |
| Madeo et al. 2008 [19] Non-RCT† | A: CHG 2% + IPA 70%; B: IPA 70% | Complex outcomesg | Weak study design, thoughtful analysis | Correct |
| McLellan et al. 2008 [20] Non-RCT† | A: CHG 2% + IPA 70%; B: IPA 70% | A: 23/687; B: 37/612; p<0.05 | Alcohol in arm A only revealed by correspondence | Incorrect |
| Stonyepher 2008 [21] Non-RCT† | A: CHG 2% + IPA 70%; B: IPA aq 10% | A: 34/1068; B: 74/1078; p<0.05 | Advantage of CHG + ALC over PVI aq | Correct |
| Suwanpimolkul et al. 2008 [22] (C) RCT | A: CHG 0.5% + ETH 70%; B: PVI aq 10% | A: 169/7606; B: 251/7158; p<0.05 | Confounder: staff training before CHG + IPA study arm | Intermediate |
| Tepus et al. 2008 [23] Non-RCT† | A: CHG 2% + IPA 70%; B: IPA 70% seq IT (I2 2%, ETH 47%) | A: 14/3482; B: 122/4942; p<0.05 | Attribution criticised in letter to the editor | Incorrect |
| Marlowe et al. 2010 [24] Non-RCT† Cluster-randomised cross-over trial | A: CHG 3.15% + IPA 70%; B: PVI aq 10% | A: 41/4534; B: 25/4261; p<0.05 | Use of IPA before PVI and IT in arms B and C, clarified by author | Correct |
| Malani et al. 2007 [25] Systematic review 4 eligible trials, 2 with CHG-containing antiseptics | 4 eligible trials, 2 with CHG-containing antiseptics | No clear evidence; possible benefits from packaged kits and alcohol-based antiseptics | Results overall inconclusive | Correct |
| Caldeira et al. 2011 [26] Systematic review 6 eligible trials, 3 with CHG-containing antiseptics | Alcoholic products > non-alcoholic ones; ALC + CHG > PVI aq; CHG compounds vs iodine compounds inconclusive; ALC alone not inferior to iodine products | Article appropriately analyses different ingredients and compositions of antiseptics | Correct |

ALC, alcohol (when alcohol type not known); aq, aqueous; CHG, chlorhexidine gluconate; ETH, ethanol; IPA, isopropanol; IT, iodine tincture; PVI, povidone iodine; RCT, randomised clinical trial; seq, sequential application; vs, versus; %, percentage not specified; >, (in systematic reviews), performing better than.

aAnnotation with (M) or (C) denotes whether original studies were included in the systematic reviews of Malani et al [25] (M) or Caldeira et al [26] (C).

bA, B, and C denote different study arms.

cOutcome: number of contaminated blood cultures per cultures obtained in each study arm. Significance is indicated either by NS (not significant) or p<0.05 (when significant).

dAttribution: assesses whether study outcomes derived from alcohol plus CHG were attributed to CHG alone by authors.

eIn this trial, all subjects received both antiseptics at the same time, outcomes were assessed blindly.

fThese studies were classified as non-randomised cluster cross-over trials. Some had been conducted by prospective sequential implementation of different antiseptic regimens in clinical units [18,20,21], some by retrospective comparison of antiseptic regimens [11,19,23].

gThis study had complex outcomes from several pre- and post-intervention intervals showing that rigorous training and application may be more important than the choice of antiseptic.

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that described evidence-based procedures to decrease CR-BSIs in
108 intensive care units mentioned skin preparation with
“chlorhexidine” without referring to alcohol. In fact, almost all
units had used a CHG-alcohol combination from one company
(CareFusion, correspondence). Both the 2002 Centers for Disease
Control (CDC) guidelines for intravascular catheters [8] and the
draft of the 2011 guidelines [9] recommended preparing the skin
with a 2% chlorhexidine-based preparation for central venous
catheters. This was classified as Category IA evidence. The draft
was followed by a public comment phase, and the final 2011
guideline [49] recommended a 0.5% chlorhexidine skin
preparation with alcohol. The final guideline also stated that the
relative efficacy of CHG-alcohol versus PVI-alcohol was unre-
solved.

Overall, we found strong evidence supporting the efficacy of
CHG-alcohol antisepsis for catheter insertion and maintenance,
particularly when compared with aqueous PVI. We also found
evidence favouring aqueous CHG over aqueous PVI, but this was
limited to the outcome catheter colonisation. In single trials, a
CHG-containing triple combination was better than PVI-alcohol
[41], and CHG-alcohol was better than alcohol alone [42], both in
terms of catheter colonisation.

**Skin Antisepsis before Surgery**

A total of 14 articles met the inclusion criteria for surgical skin
antisepsis; this included 11 primary studies [6,50–59] and three
systematic reviews [12,13,60] (Table 3). Among the primary
studies, 9 were RCTs. All primary articles evaluated CHG-alcohol
combinations, none aqueous CHG.

Among all primary and secondary articles, correct attribution to
both CHG and alcohol was found in five articles (36%),
ambiguous statements (intermediate ranking) in three articles
(21%), and incorrect attribution in six articles (43%).

Five RCTs evaluated CHG-alcohol versus aqueous PVI and
were included in a meta-analysis (Figure 4). This showed a
significant advantage of CHG-alcohol in reducing surgical site
infections (RR 0.65; 95% CI: 0.50–0.85). The remaining RCTs
evaluated various CHG-alcohol against various iodine-alcohol
combinations, but these studies were very heterogeneous. For two
larger trials [50,53], the types and concentrations of alcohol could
not be clarified. Two smaller trials [52,56] had satisfactory alcohol
concentrations but had few outcomes only, and one trial [54] used
an alcohol concentration (23%) far below the antimicrobially
active range in the PVI-containing arm. Given that different
alcohol types and concentrations can easily tip the efficacy in
favour of one or another preparation [61], we elected not to
perform additional meta-analyses. Again, the non-RCT studies are
listed in Table 3 but were not included in meta-analyses.

The first systematic review [60] included 7 trials, of which only
one had a CHG-containing arm. It concluded that there was
insufficient evidence to support a particular antiseptic over
another. Another systematic review [12] included 9 trials
comparing CHG-containing versus iodine-containing antiseptics.
The authors examined two outcomes, surgical site infections and
microbial skin cultures. The majority of studies (5 trials) compared
CHG-alcohol with aqueous PVI. The authors pooled all CHG-
containing versus all iodine-containing trials – without accounting
for other ingredients – and found a significant risk reduction for
both outcomes in favour of the CHG-containing preparations.

The conclusion was that skin antisepsis with CHG is more effective
than with iodine. We further examined the included articles that
assessed microbial skin cultures and found that none reported
whether neutralisers were used in the testing. However, suitable
neutralisers are essential for antimicrobial efficacy assessment
[62,63]. The third systematic review [13] examined six trials; the
authors pooled any CHG-containing versus any PVI-containing
antiseptics without accounting for other ingredients and concluded
that CHG per se was superior to povidone-iodine per se.

Again, we found examples of tertiary publications that
contained unsubstantiated statements about the role of CHG. A
narrative Current Concepts review on the prevention of periop-
erative infection [64] concluded: “chlorhexidine gluconate is
superior to povidone-iodine for preoperative antisepsis”. A
surgical care initiative by Washington State hospitals [65]
| Reference | Study design | Antiseptics compared | Outcomes catheter colonisation | Outcomes CR-BSI | Comments | Attribution |
|-----------|--------------|----------------------|-------------------------------|-----------------|-----------|-------------|
| Maki et al. 1991 (28) (C) | RCT; CVCs, ACs; insertion and maintenance | A: CHG aq 2%; B: PVI aq 10% | A: 5/214; B: 21/227; C: 11/227; only A&B p<0.05 | A: 1/214; B: 6/227; C: 3/227; all NS | Seminal study; only arms A vs B in colonisation significant | Not applicable |
| Sheehan et al. 1993 [29] (C) | RCT; CVCs, ACs; insertion and maintenance | A: CHG aq 2%; B: PVI aq 10% | A: 3/169; B: 12/177; p<0.05 | A: 1/169; B: 1/177; NS | Conference abstract; colonisation significant | Not applicable |
| Garland et al. 1995 [30] | Non-RCT; PVCs; only insertion, not maintenance | A: CHG 2% / IPA 70%; B: PVI aq 10% | A: 20/418; B: 38/408; p<0.05 | A: 2/418; B: 0/408; NS | Only colonisation significant | Incorrect |
| Meffre et al. 1999 [31] (C) | RCT; PVCs; insertion and maintenance | A: CHG 0.5% + ALC (7%); B: PVI aq 10% | A: 9/568; B: 22/549; p<0.05 | A: 3/568; B: 3/549; NS | Conference abstract; colonisation significant | Correct |
| Mimoz et al. 1996 [32] (C) | RCT; CVCs, ACs; insertion and maintenance | A: CHG 0.25% + BAK 0.025% + BALC 4%; B: PVI aq 10% | A: 12/170; B: 24/145; p<0.05 | A: 3/170; B: 3/145; NS | Synergistic combination of three antiseptics in arm A | Correct |
| Legras et al. 1997 [33] (C) | RCT; CVCs, ACs; insertion and maintenance | A: CHG 0.5% + ALC (7%); B: PVI aq 10% | A: 19/179; B: 31/224; NS | A: 0/208; B: 4/249; NS | Differences non-significant | Intermediate |
| Cobbett and Letblanc 2000 [34] (C) | RCT; PVCs; insertion yes, maintenance not specified | A: CHG 0.5% + IPA 70%; B: ALC (7%) seq PVI aq 10%; C: PVI aq 10% seq ALC (7%) | A: 6/83; B: 12/80; C: 11/81; All NS | ND | Differences non-significant, also when B and C pooled vs A | Correct |
| Humar et al. 2000 [35] (C) | RCT; CVCs; insertion and maintenance | A: CHG 0.5% + ALC (7%); B: PVI aq 10% | A: 36/116; B: 27/116; NS | A: 4/193; B: 5/181; NS | Differences non-significant; sole study with slight disadvantage of CHG + ALC vs PVI aq | Intermediate |
| Maki et al. 2001 [36] (C) | RCT; CVCs, PICCs, ACs; insertion and maintenance | A: CHG 1% + ALC 75%; B: PVI aq 10% | A: 43/422; B: 192/617; p<0.05 | A: 4/422; B: 23/617; p<0.05 | Largest study; biggest difference between study arms | Intermediate |
| Langgartner et al. 2004 [37] (R) | RCT; CVCs; insertion was studied; maintenance all with CHG + ALC | A: CHG 0.5% + IPA 70%; B: PVI aq 10%; C: CHG 0.5% + IPA 70% seq PVI aq 10%; D: CHG 0.5% + IPA 70% seq ALC (7%) | A: 11/45; B: 16/52; C: 2/43; A:C, B:C p<0.05 | ND | Arm C (sequential protocol) significantly better than A or B | Correct |
| Astle and Jensen 2005 [38] (R) | RCT; CVCs (hemodialysis); insertion and maintenance | A: CHG 0.5% + IPA 70%; B: ExSept | ND | A: 1/64; B: 1/57; NS | Study did not report catheter colonisation | Incorrect |
| Kelly et al. 2005 [39] | RCT; CVCs, ACs; insertion and maintenance | A: CHG 2% + IPA 70%; B: PVI aq 10% | A: 4/82; B: 15/82; p<0.05 | A: 1/82; B: 8/82; p<0.05 | Conference abstract; alcohol in arm A only revealed by correspondence | Incorrect |
| Balamongkhon et al. 2007 [40] | Non-RCT; insertion and maintenance | A: CHG 2% + ETH 70%; B: PVI aq 10% | ND | A: 3/120; B: 7/192; NS | Weak study design, difference non-significant | Intermediate |
| Mimoz et al. 2007 [41] (R) | RCT; CVCs; insertion and maintenance | A: CHG 0.25% + BAK 0.025% + BALC 4%; B: PVI aq 10% | A: 2/25; B: 31/243; p<0.05 | A: 4/24; B: 10/239; NS | Rare study with PVI-alcohol; difference for colonisation significant | Intermediate |
| Small et al. 2008 [42] (R) | RCT; PVCs; only insertion, not maintenance | A: CHG 2% + IPA 70%; B: PVI aq 10% | A: 18/91; B: 39/79; p<0.05 | ND | Significant difference; but mean colony counts lower in IPA alone group | Correct |
| Vallès et al. 2008 [43] (R) | RCT; CVCs, ACs; insertion and maintenance | A: CHG 2% + ALC (7%); B: CHG 2% aq; C: PVI aq 10% | A: 34/226; B: 38/211; C: 48/194; only A:C p<0.05 | A: 9/226; B: 9/211; C: 9/194; all NS | Only difference in arms A vs C in colonisation significant | Correct |
| Garland et al. 2009 [44] | RCT; PICCs; insertion and maintenance | A: CHG 0.5% + ALC (7%); B: PVI aq 10% | A: 3/24; B: 1/24; NS | A: 0/24; B: 0/24; NS | Small study; focus on skin tolerability in neonates | Incorrect |
| Ishizuka et al. 2009 [45] | Non-RCT; CVCs; insertion studied; maintenance all PVI aq | A: CHG aq 0.05%; B: PVI aq 10% | ND | A: 14/286; B: 6/298; NS | CHG concentration very unusually low | Not applicable |
announced that it would mandate that skin preparation should be done with “chlorhexidine”, citing the study of Darouiche et al [6]. The 2010 national Australian infection control guidelines [10] state that “chlorhexidine” (without reference to alcohol) should preferably be used for skin preparation in surgery. The UK National Institute for Health and Clinical Excellence (NICE) issued a public review proposal for its surgical guidelines [66], citing new evidence of benefits of CHG over PVI for surgical skin preparation.

As for blood cultures, we did not find any relevant evidence supporting CHG alone for pre-incisional preparation of superficial skin in surgery. In fact, aqueous CHG commonly fails US regulatory requirements for patient preoperative skin preparation [67,68].

**Discussion**

We found a high proportion of primary and secondary literature and some prominent tertiary sources that attributed the efficacy of the combination of CHG and alcohol to CHG alone. The rates of incorrect attribution among the articles that we assessed ranged from 29% for catheters to 43% for surgery. The rates of incorrect and ambiguous attribution combined ranged from 42% for blood cultures to 65% for catheters. For surgery, there were more articles with incorrect (43%) than with correct attribution (36%). These conclusions were found at all levels of evidence gathering and knowledge translation, including primary clinical trials, systematic reviews, clinical practice recommendations and evidence-based guidelines.

The omission of alcohols in the process of evidence assessment can be seen, for example, in the draft CDC catheter guidelines [9] which initially recommended CHG alone for central venous catheter insertion and maintenance. This was subsequently changed to CHG-alcohol in the final guidelines [49]. We are unaware of the sequence of events, but assume that the change may have come through external submissions during the public comment phase. This change effectively rectified the section on skin antisepsis in the final guidelines. Another area of impact is a potentially mistaken rejection of alternatives or competitor products on the basis that they do not contain CHG, even if they have not been sufficiently tested in clinical trials. This appears to be affecting PVI plus alcohol in surgery, by way of negative implication [10,12,13,65,66].

In our analyses, we found good evidence favouring CHG-alcohol combinations over aqueous PVI, the most commonly tested alternative, in all three areas of skin antisepsis. This is a comparison of two active agents against one. However, this superiority does not hold against PVI plus alcohol or other competitors combined with alcohol, either due to equivalent performance in meta-analyses (for blood cultures) or a lack of relevant studies (for catheters and surgery). For blood cultures, alcohols alone may be effective, according to another analysis [26]. For surgery, the question of CHG-alcohol versus iodine-alcohol is unresolved. For both blood cultures and surgery, we found no evidence that CHG alone is effective. For vascular catheters, the situation is more complex. There is evidence that CHG alone is superior to PVI alone for preventing colonisation, but its effect did not reach significance for CR-BSI. In contrast, CHG-alcohol was superior to PVI alone for both outcomes, colonisation and CR-BSI.

Each of the three applications has different biological and functional requirements. Blood culture collection requires immediate activity at the venipuncture site, but no prolonged action. Alcohols, with their strong immediate activity that typically exceeds those of CHG and PVI by about a factor of 10 [1,2,61], fulfill this requirement well. Surgery requires significant immediate activity before incision and some persistent activity during the operation for several hours. Thus, surgical skin preparation is expected to benefit from the immediate action of alcohols plus persistent or enhanced activity from added CHG or PVI [67,69]. Vascular catheter sites also require good immediate activity before insertion, but since catheters often stay in place for

### Table 2. Cont.

| Reference* | Study design* | Antiseptics compared* | Outcomes catheter colonisation* | Outcomes CR-BSI* | Comments | Attribution* |
|------------|---------------|------------------------|-------------------------------|----------------|----------|--------------|
| Chaiyakunapruk et al. 2002 [46] | Systematic review | 8 eligible trials, 2 with CHG aq, 1 with CHG plus other compounds, 5 with CHG + ALC; comparator for all PVI aq 10% | Relative risk for CHG-containing vs PVI aq was about 0.5 (50%) for colonisation and CR-BSI | See comments under colonisation | Seminal review; basis for multiple recommendations; only CHG + ALC but not CHG aq significant in CR-BSI | Incorrect |
| Rickard and Ray-Barruel 2010 [47] | Systematic review | 7 examined any CHG-containing antiseptic prior to catheter insertion | Any CHG vs any others performed significantly better in colonisation but not in CR-BSI; same for any CHG vs any PVI | See comments under colonisation | Article available on internet; part of Australian national infection control guidelines | Intermediate |

ACs, arterial catheters; ALC, alcohol (when alcohol type not known); aq, aqueous; BAK, benzalkonium chloride; BALC, benzyl alcohol; CHG, chlorhexidine gluconate; CR-BSI, catheter-related bloodstream infection; CVCs, central venous catheters; ETH, ethanol; IPA, isopropanol; ND, not determined; PICCs, peripherally inserted central venous catheters; PVCs, peripheral venous catheters; PVI, povidone iodine; RCT, randomised clinical trial; seq, sequential application; vs, versus; ?%, percentage not specified.

*Annotation with (C) or (R) denotes whether original studies were included in the systematic reviews of Chaiyakunapruk et al [46] (C) or Rickard and Ray-Barruel [47] (R).

*Mention of insertion and maintenance refers to whether the assigned study antiseptic was used prior to catheter insertion only, or both, for insertion and maintenance.

*A, B, and C denote different study arms.

-Outcome: number of catheters colonised or CR-BSIs per catheters inserted in each study arm. Significance is indicated either by NS (not significant) or p<0.05 (when significant).

-Attribution: assesses whether study outcomes derived from alcohol plus CHG were attributed to CHG alone by authors.

*These studies were classified as non-randomised cluster cross-over trials. They had been conducted by prospective sequential implementation of different antiseptic regimens in clinical units.

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Figure 3. Meta-analyses of skin antiseptics for the prevention of vascular catheter-related infection. (A) Aqueous CHG versus aqueous PVI, outcome catheter colonisation. (B) Aqueous CHG versus aqueous PVI, outcome catheter-related bloodstream infection. (C) CHG plus alcohol versus aqueous PVI, outcome catheter colonisation. (D) CHG plus alcohol versus aqueous PVI, outcome catheter-related bloodstream infection. References and abbreviations are as provided in Table 2.

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Table 3. Primary studies and systematic reviews evaluating chlorhexidine-containing antiseptics for the prevention of surgical site infections.

| Reference* | Study design | Antiseptics comparedb | Main outcomesc | Comments | Attributiond |
|------------|-------------|------------------------|----------------|----------|--------------|
| Berry et al. 1982 | RCT; mixed surgery, including abdominal | A: CHG 0.5% + ALC (7%); B: PVI 10% + ALC (7%) | A: 44/453; B: 61/413; p<0.05 | ALC type and content in both study arms unknown; difference significant | Incorrect |
| Brown et al. 1984 | RCT; mixed surgery, including obstetric, abdominal | A: CHG 0.5% + IPA 70%; B: PVI aq (0.7% av I2) seq PVI aq (7%) | A: 23/378; B: 29/359; NS | Different non-significant | Incorrect |
| Ostrander et al. 2005 | RCT; clean foot and ankle surgery | A: CHG 2% + IPA 70%; B: IPOV (0.7% av I2) + IPA 74%; C: Chloroxylenol 3% | A: 1/40; B: 0/40; C: 2/40; all NS | Also skin microbial counts studied, but methodology not adequately described | Intermediate |
| Veiga et al. 2008 | RCT; elective clean plastic surgery | A: CHG 0.5% + ALC (7%); B: PVI 10% + ALC (7%) | A: 0/125; B: 4/125; NS | Difference non-significant; ALC type and content unknown | Incorrect |
| Cheng et al. 2009 | RCT; clean foot surgery | A: CHG 2% + IPA 70%; B: PVI 10% + IPA 23% | A: 0/25; B: 0/25; NS | Small study; focus on skin counts; IPA content in arm B far below active range | Intermediate |
| Paocharoen et al. 2009 | RCT; general surgery, including clean, clean-contaminated and contaminated cases | A: CHG 4% + IPA 70%; B: PVI aq (7%) | A: 5/250; B: 8/250; NS | Difference non-significant | Incorrect |
| Saltzman et al. 2009 | RCT; clean shoulder surgery, including arthroscopic | A: CHG 2% + IPA 70%; B: IPOV (0.7% av I2) + IPA 74%; C: PVI aq scrub & paint (0.75% & 1.0% av I2) | A: 0/50; B: 0/50; C: 0/50; NS | Small study; focus on skin counts; microbiological methods potentially inadequate | Correct |
| Swenson et al. 2009 | Non-RCT; mixed general surgery* | A: CHG 2% + IPA 70%; B: PVI aq seq PVI aq 10%; C: IPOV (0.7% av I2) + IPA 74% | A: 68/827; B: 72/1514; C: 38/794; A:B, A:C p<0.05 | Significantly more infections in CHG + ALC arm, but only superficial ones | Correct |
| Darouiche et al. 2010 | RCT; mixed clean-contaminated surgery, including abdominal | A: CHG 2% + IPA 70%; B: PVI aq 10% scrub & paint | A: 39/409; B: 71/440; p<0.05 | Seminal study; significant difference in favour of CHG + ALC over PVI aq | Correct |
| Sistla et al. 2010 | RCT; elective clean inguinal hernia surgery | A: CHG 2.5% + ETH 70%; B: PVI aq 10% | A: 14/200; B: 19/200; NS | Difference non-significant | Correct |
| Levin et al. 2011 | Non-RCT; elective gynaecological laparotomy surgery* | A: CHG aq 2% seq IPA 70%; B: PVI aq 10% seq PVI 10% + ETH 65% | A: 5/111; B: 21/145; p<0.05 | Weak study design; significant difference | Correct |
| Edwards et al. 2004 | Systematic review | 7 eligible trials, only 1 with a CHG-containing arm [50] | Overall inconclusive due to lack of well-designed studies | Review from 2004, updated 2009; lack of studies at the time | Intermediate |
| Lee et al. 2010 | Systematic review | 9 eligible trials, 5 studied CHG + ALC vs PVI, 4 studied CHG + ALC vs PVI + ALC (including 1 both), 1 studied CHG aq vs PVI aq for mucous membranes | “Chlorhexidine” superior to iodine, based on majority CHG + ALC vs PVI aq outcomes | Analyzed both infection rates and microbial skin counts; criticised in letters to the editor | Incorrect |
| Noorani et al. 2010 | Systematic review | 6 eligible trials, 3 studied CHG + ALC vs PVI, 2 CHG + ALC vs PVI + ALC, 1 CHG aq vs PVI aq for mucous membranes | “Chlorhexidine” superior to iodine, based on majority CHG + ALC vs PVI aq outcomes | Attribution criticised in letters to the editor | Incorrect |

ALC, alcohol (when alcohol type not known); aq, aqueous; av, available (referring to available iodine as opposed to total iodine complex); CHG, chlorhexidine gluconate; ETH, ethanol; IPA, isopropanol; IPOV, iodine povacrylex; PVI, povidone iodine; RCT, randomised clinical trial; seq, sequential application; vs, versus; %, percentage not specified.

*Annotation with (E), (L), or (C) denotes whether original studies were included in the systematic reviews of Edwards et al [60] (E), Lee et al [12] (L), or Noorani et al [13] (C).

+A, B, and C denote different study arms.

Outcome: surgical site infections per number of surgical procedures in each study arm. Significance is indicated either by NS (not significant) or p<0.05 (when significant).

*Attribution: assesses whether study outcomes derived from alcohol plus CHG were attributed to CHG alone by authors.

*These studies were classified as non-randomised cluster cross-over trials. One had been conducted by prospective sequential implementation of different antiseptic regimens in clinical units [57] and one by retrospective comparison of antiseptic regimens after sequential implementation [59].

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and abbreviations are as provided in Table 3. 

requirement is fulfilled well by CHG [1,2,67]. 

good persistent action is also required. This 

Figure 4. Meta-analysis of skin antiseptics for the prevention of surgical site infection. 

is reflected by the commonly-used term ''chlorhexidine in alcohol'' 

alcohol may not be universally regarded as an effective antiseptic. 

is suggested by some text passages in the draft CDC catheter guidelines [9]. In any 

Second, authors may regard alcohol simply as a solvent for CHG. This 

misattribution. However – even though this is speculative – some 

Our findings have broader implications. An important scientific 

a week or more, good persistent action is also required. This 

Another fact deserves consideration. Most catheter studies used 

It is unusual for systematic reviews, but nevertheless we 

Our review has several limitations. First, it is partially 

This has broader implications for knowledge translation as well as 

CHG in skin antisepsis is often in fact based on evidence for the 

and references to alcohol as a ''base solution'' for CHG. Second, 

Our findings also have potential implications for patient safety. 

When following recommendations to use “chlorhexidine”, care-

In summary, there is good evidence that CHG-alcohol is 

superior to aqueous PVI – an important competitor – in all three 

areas of skin antisepsis. However, this does not apply to 

competitors combined with alcohols. The perceived efficacy of 

CHG plus alcohol versus aqueous PVI. References 

Role of Chlorhexidine in Skin Antisepsis 

| Study or Subgroup | CHG + ALC Events | Total | PVI aq Events | Total | Weight | Risk Ratio M-H, Fixed, 95% CI | Risk Ratio M-H, Fixed, 95% CI |
|-------------------|------------------|-------|---------------|-------|--------|-----------------------------|-----------------------------|
| Brown 1984        | 23               | 378   | 29            | 359   | 23.8%  | 0.75 [0.44, 1.28]            |                            |
| Paocaroen 2009    | 5                | 250   | 8             | 250   | 6.4%   | 0.63 [0.21, 1.88]            |                            |
| Saltzman 2009     | 0                | 50    | 0             | 50    |        | Not estimable                |                            |
| Darouiche 2010    | 39               | 409   | 71            | 440   | 54.7%  | 0.59 [0.41, 0.85]            |                            |
| Sistla 2010       | 14               | 200   | 19            | 200   | 15.2%  | 0.74 [0.38, 1.43]            |                            |
| Total (95% CI)    | 1287             | 1299  | 100.0%        |       |        | 0.65 [0.50, 0.85]            |                            |

0.1 0.2 0.5 1 2 5 10 

Favours CHG + ALC  Favours PVI aq 

[caption]

Our findings have broader implications. An important scientific principle – the fact that it is generally not possible to attribute effects to only one factor when several factors have been tested together – has frequently been overlooked. The individual published analyses may have been done correctly at a technical level of evidence assessment [14], but the conclusions appear incorrect. What are possible causes, and what are further implications?

First, it may be a matter of subjective views held by authors. If, for example, alcohol is regarded as a mere solvent for CHG, then authors are unable to draw appropriate conclusions. This means that the assessment of evidence remains susceptible to subjective influences, and this will continue to require attention in this area as well as in other subject areas. Second, this highlights the principle of biological plausibility. In the CHG example, plausibility could have been checked by what is known from microbiological studies of antiseptics [1,2,61,67,69]; this would have indicated that alcohol is a key component. While biological plausibility is part of the Bradford-Hill Criteria in epidemiological studies [71], there is currently no explicit requirement to address this in clinical trials and systematic reviews [14,72]. However, we think this should become a requirement.

Our findings also have potential implications for patient safety. When following recommendations to use “chlorhexidine”, caregivers may inappropriately use CHG on its own, in aqueous solution, as this is readily available. The clinical impact from blood cultures and vascular catheterisation may be small, because contaminated blood cultures do not directly harm patients and CHG alone appears to exert some protective effect in vascular catheterisation. However, tangible negative consequences may arise in surgery, because marked differences in surgical infection rates have been observed between different antiseptic regimens [6,57]. Conversely, if caregivers are unaware of the presence and significance of alcohols, they might accidentally use alcohol compounds for antisepsis on mucous membranes, where they are contraindicated.

In summary, there is good evidence that CHG-alcohol is superior to aqueous PVI – an important competitor – in all three areas of skin antisepsis. However, this does not apply to competitors combined with alcohols. The perceived efficacy of CHG in skin antisepsis is often in fact based on evidence for the efficacy of the CHG-alcohol combination. In conjunction, the role of alcohol has frequently been overlooked in evidence assessments. This has broader implications for knowledge translation as well as potential implications for patient safety.
Table S1 Results of risk of bias assessment for studies evaluating antiseptics for surgical skin preparation.

Table S2 Results of risk of bias assessment for studies evaluating antiseptics for vascular catheter insertion.

Table S3 Results of risk of bias assessment for studies evaluating antiseptics for surgical skin preparation.

Text S1 Eligibility criteria, literature search strategy and risk of bias assessment.

Checklist S1 PRISMA Checklist.

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Author Contributions

Conceived and designed the experiments: MM ESYC. Performed the experiments: MM. Analyzed the data: MM ESYC. Contributed reagents/materials/analysis tools: MM ESYC. Wrote the paper: MM ESYC. Read and approved the final paper: MM ESYC.

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