# Antiseptic Efficacy of Povidone Iodine and Chlorhexidine Gluconate Skin Preparation Solutions Used in Burns Surgery

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**The authors devised a comparative prospective study to determine the in vitro microbicidal efficacy of skin preparation solutions in the concentrations and temperatures used in the burns theatre against common bacterial and fungal microorganisms. A panel of 10 microorganisms Staphylococcus aureus, streptococcus pyogenes, enterococcus faecalis, escherichia coli, pseudomonas aeruginosa, candida albicans, bacillus cereus were assembled comprising 8 common strains of *S. aureus* (including methicillin resistant staphylococcus aureus (MRSA)), *S. pyogenes*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans*, *B. cereus*, and multi-drug resistant *Klebsiella* and *Acinetobacter*. These were cultured in the following formulations: 1) povidone iodine (PVP-iodine) 10% stored at room temperature (25$^0$C), 2) PVP-iodine stored at 40 to 42$^0$C, 3) chlorhexidine digluconate stored at room temperature diluted with warmed saline to concentrations of 4%, 2%, 1%, 0.8%, and 0.5%. All 3 formulations met DIN EN (Deutsches Institut für Normung) (European Standards) requirements for antiseptics. Both antiseptics showed the same high bactericidal and fungicidal efficacy ($P$ = < 0.05). For chlorhexidine, all minimum inhibitory concentrations at both 24 and 48 hours were very low (< 0.5mg/L), but for PVP-iodine the minimum inhibitory concentrations were much higher and ranged from 64 to 512 mg/L. All concentrations of chlorhexidine tested were superior to PVP-iodine with no bacterial growth. There was a small amount of growth in some of the PVP-iodine treated groups, but this was not clinically significant. (J Burn Care Res 2018;39:440–444)**

Povidone iodine (PVP-iodine) and chlorhexidine digluconate are commonly used antimicrobial agents in surgical practice. A number of studies have compared their efficacy clinically and in vitro. Koburger et al[1] have shown that when a prolonged contact time is required, chlorhexidine digluconate is superior to PVP-iodine, conversely for an immediate antimicrobial effect, PVP-iodine is superior to chlorhexidine. Chlorhexidine has also been shown to be superior to PVP-iodine in preventing surgical-site infections.[2,3]

In our burns practice, 10% PVP-iodine is stored in a warming cupboard at 40$^0$C. This is to prevent excessive cooling of the patient during skin preparation and is contrary to the manufacturers' instructions which state that PVP-iodine should be stored at room temperature only. We designed an in vitro study to investigate whether warming PVP-iodine affects its efficacy.

Chlorhexidine is purchased from the manufacturer as a 4% solution and diluted with sterile water to 1% for use as a skin preparation agent in theatre. We also investigated the efficacy of chlorhexidine over a range of concentrations (4%, 2%, 1%, 0.8%, and 0.5%).

In order to compare the efficacy of these 2 agents under our defined conditions, each test solution was incubated for a predetermined time period to establish the rate of antiseptic activity against a panel of 10 microorganisms.

## MATERIALS AND METHODS

The microbiocidal efficacy of the test solutions was determined following DIN EN 1040, 1275, 58940–7, and 58940–8 European standard microbiological tests.[4–7] These are known as the quantitative suspension test and the microdilution tests

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respectively—both of these tests are detailed below. We exposed test organisms to the following 3 formulations for predetermined periods of time: 1) PVP-iodine 10% stored at room temperature, 2) PVP-iodine 10% stored at 40 to 42$^0$ C, 3) chlorhexidine digluconate (stored at room temperature) diluted with warmed sterile saline (40–42$^0$C) to concentrations of 4%, 2%, 1%, 0.8%, and 0.5%. Test microorganisms were also incubated with 0.9% saline solution as a growth control.

The PVP-iodine was not diluted. The iodine and chlorhexidine agents were prepared in an identical manner to that used in preparing theatre cases. Four percentage chlorhexidine was diluted to the appropriate test strength with warmed 0.9% saline.

The following microorganisms were used in the study: *Staphylococcus aureus* ATCC 25923, *S. aureus* NCTC 12493 (methicillin-resistant strain), *Streptococccus pyogenes* ATCC 19615, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* (wild type, Klebsiella pneumonia carbapenemase klebsiella pneumonia carbapenemase-positive clinical isolate), *Bacillus cereus* NCTC 10320, *Candida albicans* ATCC 90028, and *Acinetobacter baummannii* (wild-type clinical isolate).

### Quantitative Suspension Test

The quantitative suspension test is based on DIN EN 1040 and 1275 European standard tests.[4,5] Test organisms were cultured on blood agar (Oxoid, Basingstoke, United Kingdom) and after 18 to 24-hour incubation at 37°C transferred into 5 ml saline. Concentrations were adjusted to between $10^6$ and $10^7$ (colony forming units (cfu)/ml (0.5 McFarland). One milliliter of the test organism suspension and 1 ml of saline were mixed and incubated for 2 minutes. One milliliter of the bacterial test suspension was added to 9 ml of each product and mixed. After a contact time of 1, 5, 10, and 60 minutes, 1 ml of the mixture of bacteria and product was transferred to a tube containing 8 ml neutralizer (D3435 Dey-Engley Neutralising Broth; Sigma (Sigma-Aldrich Company Ltd., Dorset, England)) and 1 ml sterile water. All experiments included mixture of organism and saline instead of product as a control.

After neutralization, 1 ml aliquots were cultured on blood agar plates and incubated at 37°C for 48 hours. Colonies were counted at 24 and 48 hours and the viable count of the mixture calculated. The reduction of bacteria was calculated as the difference in viable counts before and after the application time. Two replicates of each experiment were performed.

In DIN EN, the concentration of an antiseptic agent is considered adequately bactericidal or fungicidal if a reduction in the quantitative suspension test of at least 5 (bactericidal) or 4 (fungicidal) log steps is achieved.[4–7]

### Microdilution Test

To determine the minimum inhibitory concentrations (MICs) of the antiseptics, DIN 58940-727 and 58940-828 were followed. The MIC is defined as the lowest concentration of an antimicrobial substance required to inhibit the growth of a microorganism following an overnight incubation. This is a strong indicator of antimicrobial potency. The test microorganisms were cultivated on blood agar at 37°C for 18 to 24 hours; thereafter, 1 colony was transferred into 1 mL of Mueller–Hinton bouillon and diluted to reach $10^5$cfu/ml. Tests were performed using 96-well micro-titre plates. Each well was filled with 100 ml of test solution and 100 ml of test microorganism suspension. The presence of turbidity was evaluated with the naked eye as the indicator for bacterial growth after 24 and 48 hours. This allowed the calculation of the MIC.

## RESULTS

### Quantitative Suspension Test

All formulations tested fulfilled DIN EN requirements for antiseptics (i.e., a reduction of 5 log steps was archived). The control plates of all tested microorganisms treated with saline grew > 100,000 (CFU/ml). There was significantly impaired growth of the organisms tested in both of the PVP-iodine groups. Small numbers of *S. aureus* (17.5 cfu/ml), *E. faecalis* (23 cfu/ml), and *B. cereus* (1 cfu/ml)

**Table 1.** Quantitative suspension tests for organisms 1 to 10 vs room temperature PVP-iodine (mean cfu/ml)

| No. | Organism | 1 min | 5 min | 10 min | 60 min |
|---|---|---|---|---|---|
| 1 | *S. aureus* | 17.5 | 0 | 0 | 0 |
| 2 | MRSA | 0 | 0 | 0 | 0 |
| 3 | *S. pyogenes* | 0 | 0 | 0 | 0 |
| 4 | *E. faecalis* | 23 | 0 | 0 | 0 |
| 5 | *E. coli* | 0 | 0 | 0 | 0 |
| 6 | *P. aeruginosa* | 0 | 0 | 0 | 0 |
| 7 | *K. pneumoniae* | 0 | 0 | 0 | 0 |
| 8 | *B. cereus* | 1 | 13.5 | 1 | 0 |
| 9 | *C. albicans* | 0 | 0 | 0 | 0 |
| 10 | *A. baummannii* | 0 | 0 | 0 | 0 |

PVP-iodine, povidone iodine; MRSA, methicillin resistant staphylococcus aureus.

**Table 2.** Quantitative suspension tests for organisms 1 to 10 vs warmed PVP-iodine (mean cfu/ml)

| No. | Organism | 1 min | 5 min | 10 min | 60 min |
|---|---|---|---|---|---|
| 1 | *S. aureus* | 0 | 0 | 0 | 0 |
| 2 | MRSA | 0 | 0 | 0 | 0 |
| 3 | *S. pyogenes* | 0 | 0 | 0 | 0 |
| 4 | *E. faecalis* | 0 | 0 | 0 | 0 |
| 5 | *E. coli* | 0 | 0 | 0 | 0 |
| 6 | *P. aeruginosa* | 0 | 0 | 0 | 0 |
| 7 | *K. pneumoniae* | 0 | 0 | 0 | 0 |
| 8 | *B. cereus* | 1 | 0 | 0 | 0 |
| 9 | *C. albicans* | 0 | 0 | 0 | 0 |
| 10 | *A. baummannii* | 0 | 0 | 0 | 0 |

PVP-iodine, povidone iodine; MRSA, methicillin resistant staphylococcus aureus.

were grown in the room temperature PVP-iodine group (Table 1) and a 1 cfu/ml of *B. cereus* was grown in the warmed PVP-iodine group (Table 2). In both these cases, the scanty numbers of bacteria grown showed acceptable antimicrobial activity. In the room temperature group, *B. cereus* still showed activity after 5 and 10 minutes of exposure but not after 60 minutes. In contrast, there was no bacterial growth in any of the concentrations tested in the chlorhexidine group.

### Microdilution Test

For chlorhexidine, the MICs were all very low (< 0.5 mg/l), but for PVP-iodine, they were much higher and ranged from 64 to 512 mg/l depending on the test organism (Table 4).

## DISCUSSION

In our study, both antiseptics demonstrated effective antimicrobial activity in all formulations tested. Chlorhexidine was the most effective antimicrobial agent at all concentrations and all contact times. This was correlated in both the quantitative suspension test and the microdilution test. Chlorhexidine also demonstrated a lower MIC than PVP-iodine (with a 1,000-fold difference in concentration). The MIC for PVP-iodine was particularly high (> 512 mg/l) against *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *C. albicans*, which is a potential concern as *Klebsiella*, *Pseudomonas*, and *Candida* species are 3 of the 4 most common pathogens in burn wound infections.[8]

The mechanisms of chlorhexidine and PVP-iodine activity have been studied extensively previously.[9–11] Microbial uptake of chlorhexidine by *S. aureus* and *E. coli* has been shown to be very rapid and dependent on the antiseptic concentration and pH[9]. More recent studies with [14C] chlorhexidine diacetate showed that the maximal antimicrobial effect occurs within 20 seconds.[11] Chlorhexidine damages the outermost layers of the bacterial cell wall but this is not bactericidal alone. The molecule crosses the cell wall, most likely by diffusion and damages the inner cell membrane, which results in leakage of intracellular components. Higher concentrations of chlorhexidine have been shown to cause coagulation of the intracellular constituents in situ.[11]

Aqueous or alcoholic iodine containing antiseptics have been used for more than 150 years; however, they may cause irritation. Aqueous iodine solutions are particularly unstable, forming a complex equilibrium with typically at least 7 iodine species with molecular iodine (I2) as the primary bactericidal moiety.[12] Problems with stability have been overcome by the development of iodophores, or iodine "carrying" or "releasing" compounds, where the carrier acts as a reservoir for the bactericidal-free iodine. The most widely known of these iodophores is PVP-iodine. Iodophores are considered less effective than alcoholic iodine against some spores and fungi.[13]

In our study, *B. cereus* grew 2 cfu/ml in 10 minutes in the room temperature PVP-iodine group. This was the only spore-forming organism tested in

**Table 3.** Control plates—organisms 1 to 10 vs normal saline

| No. | Organism | 1 min | 5 min | 10 min | 60 min |
|---|---|---|---|---|---|
| 1 | *S. aureus* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 2 | MRSA | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 3 | *S. pyogenes* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 4 | *E. faecalis* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 5 | *E. coli* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 6 | *P. aerugenes* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 7 | *K. pneumoniae* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 8 | *B. cereus* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 9 | *C. albicans* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |
| 10 | *A. baummannii* | > 100,000 | > 100,000 | > 100,000 | > 100,000 |

MRSA, methicillin resistant staphylococcus aureus.

**Table 4.** Minimum inhibitory concentrations of chlorhexidine and room temperature PVP-iodine (mg/l)

| | | Chlorhexidine (mg/l) | | PVP-Iodine (mg/l) | |
|---|---|---|---|---|---|
| **No.** | **Organism** | **MIC 24 hr** | **MIC 48 hr** | **MIC 24 hr** | **MIC 48 hr** |
| 1 | *S. aureus* | < 0.5 | < 0.5 | 512 | 512 |
| 2 | MRSA | < 0.5 | < 0.5 | 256 | 256 |
| 3 | *S. pyogenes* | < 0.5 | < 0.5 | 64 | 64 |
| 4 | *E. faecalis* | < 0.5 | < 0.5 | 512 | 512 |
| 5 | *E. coli* | < 0.5 | < 0.5 | > 512 | > 512 |
| 6 | *P. aerugenes* | < 0.5 | < 0.5 | > 512 | > 512 |
| 7 | *K. pneumoniae* | < 0.5 | 0.5 | > 512 | > 512 |
| 8 | *B. cereus* | < 0.5 | < 0.5 | 512 | 512 |
| 9 | *C. albicans* | < 0.5 | < 0.5 | > 512 | > 512 |
| 10 | *A. baummannii* | < 0.5 | < 0.5 | 512 | 512 |

PVP-iodine, povidone iodine; MIC, minimum inhibitory concentration; MRSA, methicillin resistant staphylococcus aureus.

this study. Importantly, *B. cereus* does not produce any spores in 24-hour cultures and therefore our results cannot be used/interpreted for sporicidal or sporostatic activity. Bacterial spores possess an intrinsic resistance to bactericides and high concentrations may be required to achieve a sporicidal effect. The most important spore-producing bacteria are those of *Bacillus* and *Clostridium* species.[14] Glutaraldehyde, iodine, and chlorine-releasing agents are effective sporicides.[15] In contrast, high-concentration chlorhexidine, alcoholics, phenols, and quaternary ammonium compounds are normally sporicidal[16] but may be sporostatic. Iodine-containing antiseptics possess superior sporocidal activity when compared with chlorhexidine but are not the agent of choice when a sporicidal effect is required.

From a surgical point of view, the quick action of skin preparation agents is important because in burn cases once the patients' skin is prepped the procedure typically starts within 1 to 5 minutes. Consequently, the ideal skin preparation agent should have a quick action. Chlorhexidine was superior to PVP-iodine in this respect, with the most bacterial growth in the PVP-iodine groups occurring 1 to 5 minutes after exposure.

The antimicrobial activity of both PVP-iodine and chlorhexidine is impaired in the presence of organic matter.[17,18] First, it provides a physical barrier that reduces contact with bacteria. Second, organic matter has also been shown to neutralize disinfectants—in particular chlorhexidine and iodine-containing compounds.[19] Chlorhexidine is a cationic agent and its antimicrobial activity is reduced in the presence of nonionic surface-active agents.[20] Anionic compounds may have similar effects—these include phospholipids, serum, and solid culture mediums such as agar.[21,22] The skin is a complex matrix of organic matter and it is reasonable to assume that this may affect antimicrobial activity. In practice, in an in vivo setting, chlorhexidine is a very effective antimicrobial agent. Darouiche et al[23] showed a significant difference in infection rates in patients undergoing clean-contaminated surgery (9.5% chlorhexidine-alcohol vs 16.1% povidone-iodine; $P = .004$).

Chlorhexidine activity is also pH dependent.[19] None of these are desirable features of a skin preparation agent. The effective pH range of chlorhexidine is between 5 and 7. Above a pH of 8, the base may precipitate from aqueous solutions.[24] Skin pH has been shown to be between 5 and 8, which falls within the working pH of chlorhexidine.[25,26]

The MIC values for chlorhexidine were lower than those for PVP-iodine, and after 24 hours, chlorhexidine was bactericidal for all tested organisms. One drawback of our study was that it was in vitro and the conditions did not reflect the in vivo environment. Experiments were performed without defined interfering substances, for example, protein load and therefore do not completely represent clinical conditions. Further investigations under physiological conditions would provide further insight into the influence of protein load on the antimicrobial activity of the antiseptic agents.

## CONCLUSION

Both antiseptics performed well in our study with all formulations tested fulfilling DIN EN requirements for antiseptics. Warmed PVP-iodine demonstrated good antibacterial activity, despite the manufacturers advice that it should only be stored at room temperature. All concentrations of chlorhexidine tested were superior to PVP-iodine with no bacterial growth. There was a small amount of growth in both of the PVP-iodine–treated groups (Tables 1, 2), but this was not clinically significant as only small numbers of colonies were formed.

Both antiseptics showed effective long-term activity, whereas chlorhexidine demonstrated superior short-term activity vs PVP-iodine in the short term.

Finally, although the bactericidal component of skin preparation agents is important, an often overlooked feature of skin preparation in the diluting effect of the agent used, which alone will reduce bacterial load.

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# Implications of chlorhexidine use in burn units for wound healing☆

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ABSTRACT

Chlorhexidine is known to be a potent antiseptic with evidence of a beneficial role in burn care. Nevertheless, several *in vitro* studies have reported cytotoxicity on cultured cells, while *in vivo* and *clinical data* seem to show more controversial results. In the frame of this work, we aimed to evaluate the use of chlorhexidine in burn units worldwide be sending a survey to professionals of the field. We associated survey results to those perspectives reported in the literature to update recommendations for the use of chlorhexidine in specific protocols for burn management. The survey results showed that there is no clear consensus on the use of chlorhexidine regarding the concentrations, the type of excipient and the cleansing after application. Literature searches showed evidence that the skin of premature infants appears to be more sensitive to chlorhexidine that adult skin, with more reported cases of adverse effects. It was also determined that aqueous formulations of chlorhexidine do not appear to be necessarily less efficient than with alcohol as an excipient, and that lower concentrations are as efficient as higher concentrations. In view of this study, we have adjusted our protocols for the use of aqueous formulations at low concentrations and investigated further the role of washing after application in order to standardize the indication of chlorhexidine and minimize the probability of adverse effects.



## 1. Introduction

Skin is the main physical and chemical barrier against pathogens, notably by producing antimicrobial peptides [1]. This barrier is destroyed by burns rendering the body more prone to infections due to a lack of vascularity, immune deficiency and repeated surgery [2]. Infections can inhibit wound healing by invasion and dissemination of micro-organisms, which can extend the inflammatory phase with an increase of pro-inflammatory cytokines and proteases, inducing disproportionate granulation tissue degradation associated with impaired healing [3]. With a prevalence of infection in burn units of approximatively 66% and more than 11 million people affected annually by burn injuries worldwide [4], wound infection has become the major cause of mortality,

☆ Part of this manuscript content can be found on the SERVAL Institutional Repository of our University (http://serval.unil.ch), as this work was done in the context of a Masters project at our University Hospital.

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morbidity and wound healing delay [5,6]. Furthermore, rate of mortality in burn patients is three times higher in patients who are infected by pathogens [7]. Therefore, infection still remains a major challenge of wound care in burn units.

Clinically, infection can be seen by a discoloration of the wound, sloughing of burned tissue, increased edema or particular odor, and is usually characterized by a load of more than $10^5$ microorganisms per gram of tissue [8]. Microorganisms that infect burn wounds are heterogeneous depending on the anatomical site and time after injury and they can include bacteria, virus and fungi [9,10]. Most of the time, Staphylococcus and Pseudomonas are responsible for infections and sepsis in burn cases [11]. Commonly, antiseptics are used in order to significantly reduce bacterial load of the skin or mucous membranes [12].

Reference to antiseptic substances can be traced back to the time of ancient Egyptians, who used coniferous resin for antimicrobial effect in the process of embalming mummies [13]. Also, in early medical practices, the anti-bacterial effects of honey, vinegar and wine were well known [14,15]. However, major developments for antiseptics took place from the 19th century to the 20th century. Briefly, Bernard Courtois, discovered iodine in 1811 [16], which is presently a commonly used antiseptic agent. Bromine, carbolic acid and sodium hypochlorite were established to be effective in treating gangrene during the Civil War (1861–1865) [17]. In 1897, Joseph Lister, inspired by the work of Louis Pasteur on bacteria, discovered the antiseptic properties of phenol (carbolic acid) in surgery [18]. During the First World War, irrigation of wounds with Dakin solution (composed of sodium hypochlorite) was initiated. Later during the Vietnam war, several studies against infections permitted the development of Mafenide acetate and silver sulfadiazine, two topical antimicrobials still used routinely today [17].

The mechanisms of action and effects of the contemporary antiseptic agents are currently not totally determined and understood. In particular, Chlorhexidine has been used since 1954 and studied for wound care only in the last 20–30 years. While it is a potent antiseptic, chlorhexidine has been reported in relation to serious burn injuries under certain circumstances [19]. Nowadays, specific guidelines for the use of chlorhexidine in burn units is of benefit. The state of the literature for Chlorhexidine reports numerous concentrations and in different excipients for the same indication. We therefore evaluated the worldwide use of chlorhexidine in burn units and associated this information into perspective with the effects reported in the literature. Overall, this allows an update for recommendations of specific protocols for burn patient care.

## 2. Methods

In order to evaluate the use of Chlorhexidine in burn units, a survey was sent worldwide to 213 professionals of burn care. The survey encompassed questions about the performed practice in burn wound management and associated concentrations of antiseptics containing Chlorhexidine. The survey was sent twice in the same year at an interval of 5 months in order to obtain a maximum number of responses. We compared the responses of the external medical units with the internal protocols of our hospital, as the Burn Center of the Lausanne University Hospital (CHUV) was among the first burn units to receive certification from the European Burns Association. Data were also compared to the existing literature on patient care involving Chlorhexidine. For more details on the survey questions and criteria for the literature review, please refer to the *Supporting Information*.

## 3. Results

### 3.1. Survey

In order to have a worldwide overview of the use of Chlorhexidine in burn units, a survey was sent out to 213 professionals of burn care, among which 36 were filled, thus representing a participation rate of 17%.

The health care category, medical practice and geographical region of the participants who completed the survey are presented in Fig. 1. In summary, the majority of the responses came from physicians (77%) and nurses (23%), working in Burn Care (53%) and Plastic Surgery (38%), mainly in Europe (67%) followed by the Middle East (24%).

Participants acknowledged the use of Chlorhexidine on burn wounds by 54%, with Chlorhexidine concentrations varying from 0.02% to 4% (Fig. 2a-b). The most common Chlorhexidine concentrations used in burn care were found to be 0.05% (31% of the participants) and 4% (23% of the participants), which represents a concentration difference of 100-fold. Also, for approximately 31% of the responses, the practitioner did not know the Chlorhexidine concentration used (no specific response). As an alternative substance instead of Chlorhexidine, Betadine® was the most used disinfectant (40%), followed by saline solution (11%), soap and water (6%) and hydrogen peroxide (3%). Overall, 40% of survey participants did not answer this specific question (Fig. 2c).

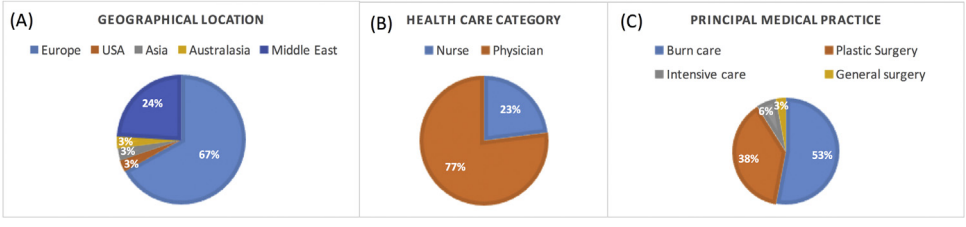


**Fig. 1 – Survey results: (A) geographical location of the survey participants, (B) health care category as physician or nurse, and (C) field of practice of the participants.**

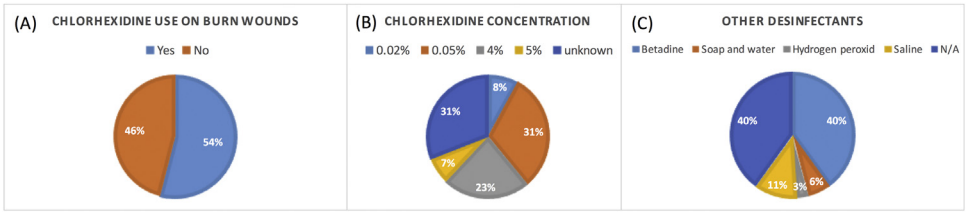


**Fig. 2 – Survey results: (A) The fraction of survey participants that use Chlorhexidine, (B) the used concentrations of Chlorhexidine in burn care, and (C) other disinfectants used in burn care.**

The survey participants were also asked if they washed the burn wounds after disinfection, with 60% of the participants responding positively (Fig. 3a); this question was asked as we postulated that rinsing the patient after disinfection may reduce side effects. Nevertheless, 63% of the participants reported the observations of side effects (Fig. 3b), such as skin irritation, dryness, inhibition of wound healing and pseudomonas contamination. Moreover, the results have revealed different shower frequencies depending on the hospitals; only three hospitals reported to not use shower systems. Shower frequency shows two trends of either a daily shower (35%) or only when indicated (22%). In addition, other practice was found, such as a shower twice a week of every other day (Fig. 3c).

Reported protocols for burn wound cleaning varied between the different hospitals. Among the responses received, some even reported that they have no Standard Operating Procedures (SOPs) in place and they transfer the patient directly to specialized centers. However, for a majority, one or the other of the following SOP's is implemented in routine: (i) Disinfection with Betadine® only or Betadine® wiped afterward with saline solution. In case of allergy of Betadine®, washed only with saline water. (ii) Disinfection with Chlorhexidine at concentration 0.0.5% and 4%. Some of them used only Chlorhexidine or after a first wash with Betadine® and normal saline solution. Others used Chlorhexidine at first and then washed with soapy water, normal saline water or betadine®. (iii) Application of MEBO® ointment on the burn. Thereafter, old ointment removed and apply the new layer three times per day. (iv) Application of Flamazine® directly on the wound. Also used to remove the eschar and then clean with normal saline solution. (v) Bath or wash with soapy water and wiped with saline water.

Results regarding the type of cover or dressing used and if cellular therapy was routinely used showed that no specific type of dressing is more frequently used than others (Fig. 4a). Among the 33% of other employed wound covers, mentioned included Mebo ointment®, Flamazine®, Flaminal®, Silver sulfadiazine, hypochlorite solution for some infected wounds, dressings Mepitel®, Mepiplex®, Acticoat® or Urogotul SSD®, Polyfax® and Biobrane®. Likewise, the majority of the survey participants (60%) did not answer if they used specific cellular therapy techniques (Fig. 4b).

### 3.2. *Literature review on the use of Chlorhexidine for burns and in wound care*

As previously mentioned, burn wounds are subject to infections because the pathophysiology of burns implies loss of physical barrier, blood vessel damage and a subsequent immunosuppressed state, hence the use of antiseptics such as Chlorhexidine in burn management. Following a burn, the wound is sterile except for the presence of some microorganisms deep in the sebaceous glands and hair follicles [20]. Therefore, recommendations to use Chlorhexidine have been reported in the literature to keep burn wounds sterile and to prevent colonization from microorganisms [21,22]. Nevertheless, there are inconsistent results regarding the indication. One article advises use only on large burns to prevent sepsis [23] while two other publications suggest use only for superficial burns as a disinfectant [21,24]. Other indications were reported included use within dressings or embedded in surgical scrub-brushes during strong debridement procedures [9,20].

Contradictory results can be found in the literature regarding Chlorhexidine effect on wound healing, as Wasiak et al. reported in their review article that Chlorhexidine dressings do not reduce the time of burn wound healing compared to hydrocolloid dressings [25]. On the other hand, avoidance of disinfectants such as Chlorhexidine has been advised due to inhibitory effects on wound healing [26–28]. Notably, the majority of adverse effects reported due to Chlorhexidine concerned premature newborns [29–34]. Extremely low birth weight populations seemed to be very susceptible to Chlorhexidine and adverse skin reactions appeared mostly by burns [29]. Also, in comparison to silver sulfadiazine, Chlorhexidine dressings used in clinics did show less interference with wound reepithelialization [25,35].

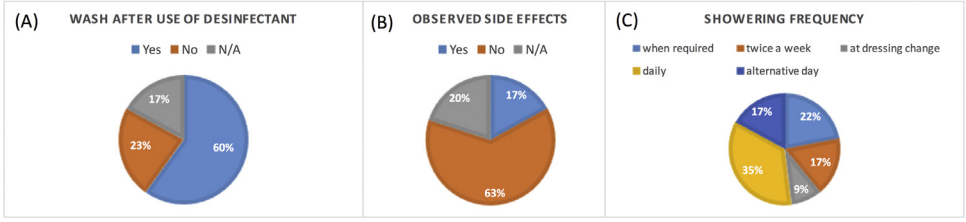


**Fig. 3 – Survey results: (A) fraction of the survey participants that shower their burn patients after Chlorhexidine use, (B) the fraction of observed side effects after Chlorhexidine use, and (C) and showering frequency of the burn patients.**

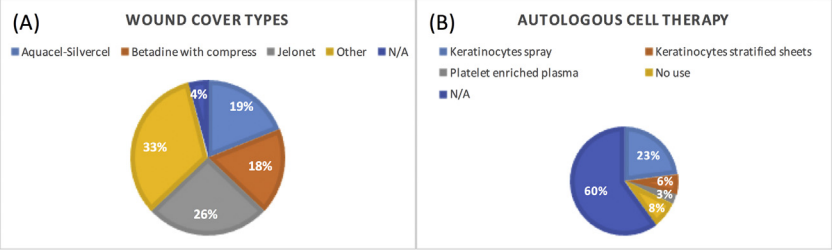


**Fig. 4 – Survey results: (A) different types of wound covers used by the survey participants for the burn care, (B) different types of cell therapies used by the survey participants for the burn care.**

In animals, results are similar to clinical data, as Chlorhexidine was also found to inhibit the healing process compared to saline water [36]. At a concentration of 4% Chlorhexidine, the effect was more pronounced than at 0.05%, as expressed in a delay of the formation of the granulation tissue and tissue thickness decrease. On the other hand, authors of another report studied the effect of 0.05% Chlorhexidine on wounds and concluded that Chlorhexidine was more beneficial than normal saline solution [37], and it was reported that the concentration of Chlorhexidine which is cytotoxic *in vitro* is not cytotoxic *in vivo*. Finally, neutral effects on wound healing have also been stated, as some authors have reported that either Chlorhexidine does not interfere with the reepithelialization of the wound [38] and that no difference in toxicity of Chlorhexidine on wound healing [39] nor on the delay in the wound healing process were noted [40,41].

### 3.3. *Lausanne University Hospital (CHUV) internal data*

Chlorhexidine is an antiseptic largely used in the CHUV for cleansing or for disinfection of hands, skin, wounds or materials. Concentrations vary from 0.02% to 4% and Chlorhexidine can be found to be used in alcoholic or aqueous solution. Table 1 summarizes the use of Chlorhexidine in the different protocols of the CHUV. From the different protocols in use at the CHUV, what appeared relevant is that Chlorhexidine is mainly present for disinfection of skin before surgery and for hand wash procedures. In burn care at CHUV, disinfection with 0.05% Chlorhexidine or Betadine for a duration of 10 min is a required step prior to any skin grafting procedure. Indeed, prior to a skin autograft procedure Betadine 10% is employed for 10 min. on both, the wound and the donor site. However, if a topical or a dressing containing silver is also applied on the wound, such as Ialugen plus or Aquacel Ag+Extra; in that case Chlorhexidine 0.05% is preferred. If the autograft procedure involves a cell therapy, such as CEA, Chlorhexidine 0.05% for 10 min. is also preferred. Interestingly, from 2009 to 2015, about 11.5 million units of chlorhexidine in alcoholic solution were used at CHUV, corresponding to nearly 4 million liters. In comparison, only 450,000 liters were in aqueous solution, which represents 10-fold less (Table 2). The yearly overall use of Chlorhexidine corresponds to 750,000 liters (Fig. 5).

## 4. Discussion

In the frame of this study, the first aim was to provide an overview of the use of Chlorhexidine in burn care by sending out a survey to professionals in the field concerning their practice regarding Chlorhexidine use. Overall, the majority of the responses came from European practitioners, and most of them use Chlorhexidine in concentrations varying from 0.02%

**Table 1 – Different indication of Chlorhexidine use at CHUV.**

| Chlorhexidine concentration | Clinical use |
|---|---|
| 0.02% | Vesical wash.<br>Disinfection of wounds on ear-nose-throat area. |
| 0.05% | Disinfection of the external urethral orifice before placing a urinary catheter or vulval cleansing if prescribed.<br>Cleansing and dressing of burned patient. |
| 0.5% | Disinfection of surgery field or intact skin before an invasive surgery, blood sample or injection.<br>Disinfection of central venous catheter and venous catheter site by oncologic patients.<br>Disinfection of the skin before introduction of a pacemaker.<br>Disinfection of intravenous connections, gloves, taps. |
| 2% | Replacement of one-way valve of veinous catheter.<br>Desinfection of parenteral feeding field.<br>Impregnated gloves for cleansing.<br>Disinfection of skin before connection on hemodialysis fistula.<br>Hand wash.<br>Impregnated dressings for intensive care of burn patients. |
| 4% | Hand wash.<br>Preoperative patient wash whether or not colonized by multidrug-resistant germs.<br>Entire cleansing of neutropenic patient. |

**Table 2 – Quantities of Chlorhexidine-containing solutions produced at CHUV (namely its in-house pharmacy) between 2009-2015 for disinfection purposes. The overall quantity corresponds to nearly 4.5 million liters.**

| Product | Quantity (units) |
|---|---|
| Chlorhexidine Alcoolique Colorée Braun sol 2% 1 flac 100 mL | 632, 200.00 |
| Chlorhexidine Alcoolique Colorée Braun sol 2% 1 flac 500 mL | 3, 589, 000.00 |
| Chlorhexidine Alcoolique Incolore sol 2% 1 flac 250 mL | 7, 519, 750.00 |
| Chlorhexidine Aqueuse Bichsel sol 0.100% 1 flac 100 mL | 465, 700.00 |
| Chlorhexidine Aqueuse Braun sol 0.500% 1 flac 100 mL | 133, 200.00 |
| Chlorhexidine Aqueuse CHUV sol 0.050% 1 flac 500 mL | 787, 500.00 |
| Chlorhexidine Aqueuse CHUV sol 2% 1 flac 100 mL | 69, 900.00 |
| Chlorhexidine Incolore Braun teinture 0.500% 1 flac 500 mL | 10, 000.00 |
| Hibidil Stérile sol 0.050% 5 flac 15 mL | 1, 157, 475.00 |
| Hibiscrub sol 4% 1 flac 250 mL | 1, 250.00 |
| Lifo-Scrub sol 4% 1 flac 100 mL | 1, 168, 400.00 |

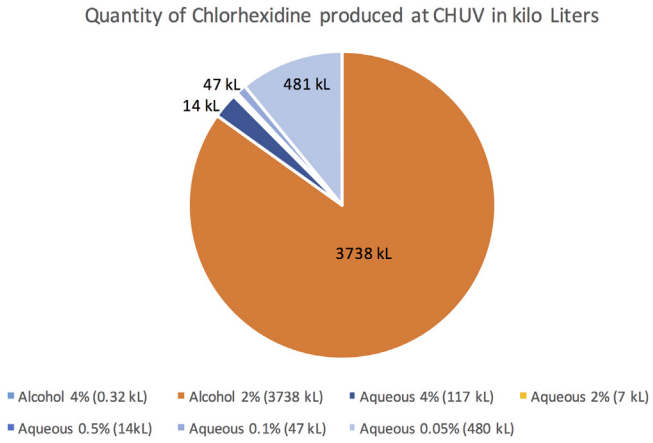


**Fig. 5 – Overall quantities in liters of Chlorhexidine-containing solutions produced at CHUV between. 2009-2015.**

to 5%. Approximately 20% of side effects were reported when using Chlorhexidine such as skin irritation, dryness, inhibition of wound healing and contamination with Pseudomonas. Nevertheless, we were not able, from the received answers, to determine a dose-dependent correlation with observed side effects and if the side-effects were due to the association of alcohol with Chlorhexidine. Strikingly, most of the participants did not know the concentration of Chlorhexidine used, indicating a lack of consensus in the concentration to use. Interestingly, 60% of the professionals who responded to the survey wash the patient after the use of a disinfectant, while this practice is typically not recommended in the literature. This could illustrate that healthcare professionals have the assumption that washing may reduce potential adverse effects. Nevertheless, efficacy of this procedure of post-disinfection wash has to be investigated to be proven. Importantly, it should be investigated if a post-disinfection wash would cancel the antiseptic effect of the Chlorhexidine, especially it has been shown that disinfection efficacy can be improved to some extent by omitting the preceding washing step and by awaiting the evaporation of the alcohol [42].

Another lack of consensus was observed for the frequency of showering of the burn patients, which varied from daily to three times a week. This observation was already reported in the literature, as wound cleansing frequency is based rather on ritualized patterns and preferences of the healthcare staff rather than on evidence-based practice [43,44].

The frequency of wound cleansing should be dictated by several factors, including the amount of exudation, the presence of debris/necrotic tissues, and the half-life of the wound dressing active component when present [43]. A too frequent cleansing may be destructive to the newly formed tissue and alter the wound healing process, namely by removing the exudate which could be essential to keep the wound moist and also which may contain growth factors supporting wound healing [43,45].

Regarding the use of Chlorhexidine related in the literature, we investigated the reports of potential adverse effects at two levels, *in vivo* studies and *clinical case reports*, since the *in vitro* cytotoxicity of Chlorhexidine is well documented [45–48]. The *in vivo* studies revealed controversial effects of Chlorhexidine related to the healing process of wounds, which is similar to results documented for povidone-iodine [49]. These contradictory results raise several questions that still remain unexplored, such as: (i) why there is a significant difference in the toxicity results of Chlorhexidine between *in vitro* and *in vivo* studies and what are the mechanisms underlying these differences? (ii) why products containing Chlorhexidine for clinical disinfection are at a concentration well above effective concentrations against microorganisms and proven to be cytotoxic *in vitro?* (iii) Does the concentration have an effect on germs selection such as Pseudomonas? (iv) is there a synergistic effect of alcohol with Chlorhexidine on disinfection and on side-effects?

Regarding the clinical cases reported in the literature, adverse effects where observed for adult patents but most of the adverse effects concerned neonatal cases. There could be multiple reasons why most of the burns by Chlorhexidine were on neonate infants, but the most probable reason is the different and more sensitive skin type, as neonates have a thinner stratum corneum and reduced dermo-epidermal cohesion implying a higher skin permeability [50]. In several case reports [30–33,51,52], authors mentioned the use of alcohol in the preparation on premature infant skin as the cause for irritation. Alcohol is well known for its antibacterial properties and is probably enhancing the disinfection when associated with Chlorhexidine. However, alcohol solutions and providone-iodine with alcohol are also known to induce burns [31,31,32,33,53–56], which might be another

explanation that the alcoholic excipient can be a factor of adverse effect. These types of formulations should therefore be avoided in the neonatal population.

High concentrations of Chlorhexidine often used are not proven to be more effective than lower concentrations [57], while its toxicity is dose dependent. Therefore, there is no reason to use Chlorhexidine at higher concentrations especially on fragile skin such as on burn wounds. An interesting element worth to note is that povidone-iodine and Chlorhexidine are rarely used simultaneously notwithstanding that the incompatibility of these two agents is not evident. Some studies suggest a synergistic antiseptic effect of these two compound when used together [58,59], without necessarily implying further adverse effect of the combination. Nevertheless, this needs to be confirmed by a randomized trial.

In summary, case reports found in the literature mention skin reactions, burns and allergic reactions mainly on extremely low birth weight infants but these adverse effects also can occur on adults.

Dose dependent toxicity of Chlorhexidine is well demonstrated *in vitro,* but *in vivo* results are rare and seem contradictory. Due to the toxicity of Chlorhexidine, its effects on wound healing and specifically on burn wound healing should appear to be harmful but are not really known. Opinions of authors are heterogeneous in the literature [60], though the role of Chlorhexidine in the prevention and treatment of infections have been clearly demonstrated. Finally, further studies are required on the effects of Chlorhexidine on burns and adult population.

Based on the literature review and the results of the survey, minimal changes may diminish adverse reactions and we therefore recommend: (i) inform the medical workers that Chlorhexidine can be harmful if not used properly; (ii) use Chlorhexidine in an aqueous solution at a low concentration (0.05%), especially on fragile skin; (iii) different packaging design and color between aqueous and alcoholic solutions could be implemented in order to avoid confusions.

## 5. Conclusions

In conclusion, the role of Chlorhexidine in burn care is still a controversy. Effects on wound healing and reepithelization are still contradictory. On the other hand, its large spectrum of action and its role in the treatment of bacterial-resistant infections is well established. As a topical solution, within dressings or brushes and with different solutions could prevent or treat infections of burn wounds. We report herein that there is no clear consensus on the use of Chlorhexidine regarding the concentrations, the type of excipient and the cleansing after application. Literature searches showed evidence that the skin of premature infants appears to be more sensitive to Chlorhexidine then that of adult skin, with more reported adverse effects seen for neonatal skin. Aqueous formulations of Chlorhexidine as well as lower concentrations do not appear to be necessarily less efficient than with alcohol as the excipient. Therefore, we recommend to use lower concentrations, within an aqueous solution and investigate the role of washing after application in order to standardize the indication of Chlorhexidine and minimize the probability of adverse effects for overall burn care.

## Conflict of interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.burns.2019.12.008.

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