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Review

Addressing the challenges in antisepsis: focus on povidone iodine

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ABSTRACT

Objectives: Using antiseptics in wound care can promote healing by preventing and treating infection. However, using antiseptics can present many challenges, including issues with tolerability, inactivation by organic matter and the emergence of antimicrobial resistance/cross-resistance. This review discussed the key challenges in antisepsis, focusing on povidone-iodine (PVP-I) antiseptic.

Methods: Literature searches were conducted in PubMed, in January 2019, with a filter for the previous 5 years. Searches were based on the antimicrobial efficacy, antiseptic resistance, wound healing properties, and skin tolerability for the commonly used antiseptics PVP-I, chlorhexidine gluconate (CHG), polyhexanide (PHMB), and octenidine (OCT). Additional papers were identified based on author expertise.

Results: When compared with CHG, PHMB and OCT, PVP-I had a broader spectrum of antimicrobial activity against Gram-negative bacteria, actinobacteria, bacterial spores, fungi and viruses, and a similar and broad spectrum of activity against Gram-positive bacteria. PVP-I was also highly effective at eradicating bacterial biofilms, which is a vitally important consideration for wound care and infection control. Despite a long history of extensive use, no resistance or cross-resistance to PVP-I has been recorded, which is in contrast with other antiseptics. Despite previous misconceptions, it has been shown that PVP-I has low allergenic properties, low cytotoxicity and can promote wound healing through increased expression of transforming growth factor beta.

Conclusion: With increased understanding of the importance of tackling antimicrobial resistance and bacterial biofilms in acute and chronic wound care, alongside improved understanding of the challenges of antiseptic use, PVP-I remains a promising agent for the management of antisepsis.

1. Introduction

The use of topical antimicrobial agents in wound care can drastically aid the healing process by preventing and treating infections in wounds [1]. Antiseptics have a broad spectrum of activity against bacteria, actinobacteria, fungi and viruses; they are therefore well suited for the treatment of wounds [2]. Their high efficacy against both planktonic and sessile bacterial communities is particularly desirable, as wound healing is often delayed by the formation of biofilms, which are bacterial communities that are often tolerant to antibiotic treatments [3]. However, the use of antiseptics can present many challenges, including issues with tolerability, inactivation by organic matter and the emergence of antiseptic resistance, where microbes are resistant to antiseptics [1].

Abbreviations: CFU, colony forming unit; CHG, chlorhexidine gluconate; ESKAPE, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.; FBS, foetal bovine serum; MDR, multidrug-resistant; MFS, major facilitator superfamily; MBC, minimum bactericidal concentration; MEM, Eagle's Minimal Essential Medium; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-sensitive Staphylococcus aureus; OCT, octenidine; PHMB, polyhexanide; PHMG, polyhexamethylene guanidine hydrochloride; PVP-I, povidone-iodine; Qac, quaternary ammonium compounds; RF, reduction factor; TGF-beta, transforming growth factor beta; VRE, VRSA, vancomycin-resistant; VSE, vancomycin-susceptible; WHO, World Health Organization; XDR, extensively drug-resistant.

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According to the World Health Organization (WHO), antimicrobial resistance is a priority for global health action and is one of the biggest threats to health, food and security [4]. It is well established that the prevalence of resistance to topical antibiotics is increasing due to the misuse and overuse of these agents, in particular mupirocin and fusidic acid [5–8]. However, it is becoming increasingly clear that resistance to some antiseptics is also on the rise; this is therefore a key challenge that needs to be addressed [9].

A major concern with the rising prevalence of antibiotic resistance, defined as the resistance of microbes specifically to antibiotics, is the emergence of multidrug-resistant (MDR) nosocomial infections. The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.) are the leading cause of nosocomial infections across the world [10], with many ESKAPE bacteria becoming MDR [10]. In an age of increasing resistance, antiseptics might provide an alternative and viable option to effectively target these organisms.

This article discusses key challenges in antisepsis, namely antimicrobial efficacy, antiseptic resistance, antibiotic and antiseptic cross-resistance, wound healing and tolerability, focusing on povidone-iodine (PVP-I) in comparison with other commonly used antiseptics.

2. Methods

This article resulted from a focus meeting on 'antiseptics in clinical practice' held in December 2018, which was attended by all but one of the authors. In order to expand the microbiology expertise of the authors, Professor Surbhi Malhotra-Kumar (University of Antwerp) was invited to co-author the article after this event.

Six literature searches were conducted in PubMed in January 2019. A filter for the previous 5 years was used to identify recent advances and challenges in topical antisepsis. Search terms were chosen based on discussions during the focus meeting. Searches were based on the commonly used antiseptics – PVP-I, chlorhexidine gluconate (CHG), polyhexanide (PHMB) or octenidine (OCT) – with the following additional search terms (synonyms were included in all searches): Search 1: «'Antimicrobial spectrum' OR named microorganisms OR 'biofilm'» AND «terms relating to activity/efficacy»; Search 2: «'Cross-resistance' OR 'drug-resistance'»; Search 3: «'ESKAPE' OR individual ESKAPE organism names [*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.] OR 'nosocomial isolate' OR 'nosocomial infection' OR 'hospital-acquired infection'» AND «terms relating to activity/efficacy»; Search 4: «'Organic material' OR 'organic soil' OR 'Blood' OR 'in vivo' OR 'ex vivo' OR 'real life'» AND «'efficacy' OR 'activity' OR 'inactivation'»; Search 5: «'Wound' OR 'burn' OR 'ulcer' OR 'wound healing'» AND «'effect' OR 'efficacy' OR 'activity' OR 'promote' OR 'prevent'»; and Search 6: «'Tolerability' OR 'cytotoxicity' OR 'irritation' OR 'pain' OR 'colour' OR 'apoptosis' OR 'allergy' OR 'adverse effects'».

Based on their abstracts, only papers identified by the searches that were considered directly relevant were included in this review article. The manuscript included additional papers that were identified by authors outside of the literature search. Papers were considered relevant based on authors' expertise in microbiology and antisepsis in surgical and dermatological practice.

3. Results and Discussion

The results from the literature search are summarised in Table 1.

3.1. Antimicrobial efficacy of antiseptics

3.1.1. Antimicrobial spectrum

As mentioned, the antimicrobial activity of antiseptics against ESKAPE pathogens is particularly desirable, given their leading role in nosocomial infections [10]. PVP-I was found to be effective against *Acinetobacter baumannii* (*A. baumannii*), *Klebsiella pneumoniae* (*K. pneumoniae*), methicillin-sensitive *Staphylococcus aureus* (*S. aureus*) (MSSA), methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecium* (*E. faecium*), *Enterobacter* spp., and *Pseudomonas aeruginosa* (*P. aeruginosa*) (Table 2A) [11–15]. Similarly, CHG was found to be effective against some MDR Gram-negative organisms (*A. baumannii, K. pneumoniae, Escherichia coli [E. coli]*), vancomycin-resistant *Enterococci, Enterobacte*r spp., and nosocomial MSSA, MRSA and *E. faecium* isolates, although the efficacy of CHG against MDR *K. pneumoniae and P. aeruginosa* was variable (Table 2B) [11,13,16–19]. CHG was found to have antimicrobial activity against MDR *A. baumannii* international clone II (IC II), although less than for non-IC II isolates [20]. PHMB was found to be effective against all ESKAPE pathogens and had higher bactericidal activities against MRSA, carbapenem-resistant *K. pneumoniae*, and ceftazidime-resistant *Enterobacter* spp. than CHG (Table 2C) [21–23]. A slight bactericidal advantage over CHG was observed against vancomycin-resistant *E. faecium* (VRE), ciprofloxacin-resistant and levofloxacin-resistant *Acinetobacter* spp., and MDR *P. aeruginosa* [21]. OCT was found to be effective against all ESKAPE pathogens, including MDR Gram-negative and Gram-positive pathogens associated with nosocomial infections such as *K. pneumoniae, Enterobacter cloacae, A. baumannii, P. aeruginosa*, and antibiotic-resistant *S. aureus* epidemic clones (Table 2D) [24–29].

Overall, PVP-I had a broader spectrum of antimicrobial activity compared with other commonly-used antiseptics (PHMB, CHG and OCT), targeting a wider range of Gram-negative bacteria, fungi and viruses, and a similar and broad range of Gram-positive bacteria (Table 3) [2,30]. Additionally (unlike PHMB, CHG and OCT), PVP-I exhibited antimicrobial activity against actinobacteria and bacterial spores. The differences in the spectrum of activity between antiseptics may be due to their varying mechanisms of action. While PHMB, CHG and OCT primarily act via cell wall and plasma membrane disruption, PVP-I has been found to have multiple mechanisms of action (Figure 1) [31–35]. For example, PVP-I interacts with several enzymes, including viral enzymes such as haemagglutinin, neuraminidase and sialidase [36]. Enzyme inhibition may therefore be one example of why PVP-I is effective against a wide range of viruses as well as bacteria. In contrast, CHG and PHMB have been found to primarily disrupt the viral envelope and have limited efficacy against non-enveloped viruses [37,38].

3.1.2. Effect of organic material on antiseptic efficacy

The efficacy of antiseptics can be diminished by organic material, such as blood, which is typically present in wounds [2]. Historically, the antimicrobial activities of wound antiseptics have been tested using in vitro suspension tests, which do not accurately represent wound conditions [39]. Recently, a new phase 2/step 2 in vitro test method was proposed, in which wound antiseptics are tested against microbial test suspensions pre-dried on a metal carrier; this setting is more representative of antiseptic treatment of a wound than suspension tests [39]. Using this new phase 2/step 2 method, the potentially inhibiting influence of organic material was tested on PVP-I, CHG, PHMB, and OCT. PVP-I had the shortest time to efficacy against *S. aureus, E. faecium* and *P. aeruginosa* even in the presence of blood (Figure 2) [39]. Despite being widely used as an antiseptic, CHG was a far less effective antiseptic than PVP-I in the absence or presence of organic material (Figure 2) [39].

Table 1
Ability of commonly used antiseptics to address key challenges in antisepsis.

Challenge	Povidone-iodine	Chlorhexidine gluconate	Polyhexanide	Octenidine
Broad antimicrobial spectrum	Broad spectrum of activity against Gram-positive bacteria, Gram-negative bacteria, fungi and viruses [2]	Broad spectrum of activity against Gram-positive bacteria. Narrow spectrum of activity against Gram-negative bacteria, fungi and viruses [2]	Broad spectrum of activity against Gram-positive bacteria and Gram-negative bacteria. Narrow spectrum of activity against fungi and viruses [2]	Broad spectrum of activity against Gram-positive bacteria. Narrow spectrum of activity against Gram-negative bacteria, fungi and viruses [2]
Effective against ESKAPE pathogens	Effective against all ESKAPE pathogens [11,14,15,43]	Effective against most ESKAPE pathogens, excluding *Klebsiella pneumonia* (variable activity) and *Pseudomonas aeruginosa* (limited activity) [11,16,18-20]	Effective against all ESKAPE pathogens [22-24]	Effective against all ESKAPE pathogens [25-30]
Effective in organic material	Shortest time to efficacy against *Staphylococcus aureus, Enterococcus faecium* and *Pseudomonas aeruginosa* in the presence of blood [40]	Less effective than PVP-I in the presence of organic material [40]	Less effective than PVP-I in the presence of organic material [40]	Less effective than PVP-I in the presence of organic material [40]
Effective against biofilms	Highly effective at eradicating biofilms, including MRSA, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Candida albicans* [14]	Less effective at eradicating *Acinetobacter baumannii, Escherichia coli*, MRSA, and *Pseudomonas aeruginosa* in biofilms than free-form bacteria but higher efficacy in young vs. mature biofilms [44,45]	Effective against MRSA biofilm [23]	Effective against *Staphylococcus aureus, Acinetobacter baumannii*, MRSA, and VRSA biofilms [27,28]
Lack of antimicrobial resistance/cross-resistance	No observed antimicrobial resistance/cross-resistance [35,36]	CHG resistance observed in *Staphylococcus epidermidis, Acinetobacter baumannii* and *Mycobacterium abscessus*. Cross-resistance to colistin has been observed [34,54-57]	Reduced susceptibility of MRSA and associated cross-resistance to daptomycin has been observed [66]	Repeated exposure to *Staphylococcus aureus* leads to bacterial resistance [59]
Provides beneficial effects on wound healing	Promotes wound healing through increased expression of TGF-β, neovascularisation and re-epithelialisation [73]	Promotes healing of full-thickness skin wounds but can cause skin irritation [76]	Induces inflammation in vitro, which may be detrimental for healing wounds [80]	Has a greater efficacy for wound healing than silver dressings [82]
Provides acceptable tolerability	Allergy is largely overestimated and allergic reaction to PVP-I is rare [89]	Allergic reaction is well recognised [89]	Allergic reaction is rare [89]	Allergic reaction is rare [89]

Abbreviations: CHG, chlorhexidine gluconate; ESKAPE, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.; MRSA, methicillin-resistant *Staphylococcus aureus*; PVP-I, povidone-iodine; VRSA, vancomycin-resistant *Staphylococcus aureus*; TGF-beta, transforming growth factor beta

3.1.3. *Efficacy against biofilms*

In the real-world setting, bacteria predominantly exist as communities of cells in biofilms. Biofilms are heterogeneous structures containing a variety of microorganisms surrounded by a protective matrix, which can attach to inert and organic surfaces [40]. A recent systematic review and meta-analysis found the prevalence of biofilms in chronic wounds to be 78.2%, suggesting that biofilms are present in the majority of chronic non-healing wounds [41]. As some ESKAPE pathogens have been observed to begin the formation of biofilms within 24 hours, acute wounds are also often affected by biofilm formation [3]. The presence of biofilms delays wound healing and biofilm microorganisms are particularly resistant to host defences and antimicrobial treatment [3,40]. As such, there is a vital need for antiseptics that are effective against biofilms in the treatment of both acute and chronic wounds.

Several studies have been conducted to assess the efficacy of commonly used antiseptics against biofilms, including PVP-I, CHG, PHMB, and OCT. Low-dose PVP-I (0.25% w/w) eradicated robust biofilms of MDR *S. aureus, K. pneumoniae, P aeruginosa*, and *Candida albicans* in vitro [14]. Following dilution, PVP-I was more effective than other topical antimicrobials at removing biofilms of *P. aeruginosa* and multi-species biofilms of MRSA and *C. albicans* [42]. In addition, PVP-I completely eradicated both *S. aureus* and *P. aeruginosa* biofilms within 15 minutes of application, while CHG completely eradicated *S. aureus* biofilms only [18].

CHG has been found to have antimicrobial activity against *Streptococcus mutans* biofilm, but has been found to be less effective than alexidine and cetrimide [43]. In a further study, CHG had limited efficacy when acting on a multi-species biofilm (*K. pneumoniae, P. aeruginosa, S. aureus*, and *E. faecalis*), particularly in a simulated wound-bed setting [19]. CHG was also less effective at eradicating *A. baumannii, E. coli*, MRSA, and *P. aeruginosa* in biofilms than in their free form, but had higher efficacy in young versus mature biofilms in another study [44,45].

Studies investigating the efficacy of PHMB against biofilms have shown PHMB to be effective against MRSA biofilm in a porcine wound model [22]. Wound cleansing with PHMB or saline solution also reduced the bacterial load in venous leg ulcers, with no difference between treatment groups; however, biofilm was still present after cleansing with PHMB or saline [23].

OCT rapidly inactivated *S. aureus*, MRSA and vancomycin-resistant *S. aureus* biofilms on polystyrene plates, stainless steel coupons and urinary catheters in the presence or absence of serum proteins [26]. OCT was also effective against *A. baumannii* biofilms on polystyrene, stainless steel and urinary catheters, and

Table 2
(A) Antimicrobial activity of povidone-iodine against ESKAPE pathogens. (B) Antimicrobial activity of chlorhexidine against ESKAPE pathogens. (C) Antimicrobial activity of polyhexanide against ESKAPE pathogens. (D) Antimicrobial activity of octenidine against ESKAPE pathogens.

(A)

Antiseptic	Pathogen	Efficacy
Povidone-iodine	Nosocomial MRSA, MSSA, *Enterococcus faecium, Enterococcus faecalis*	7.5% solution effective (vs. before exposure) on all pathogens after 3 minutes of contact time in quantitative suspension test [11]
	Acinetobacter baumannii	10% solution effective (removal rate = 98.48%) after 60 seconds of contact time vs. skin culture taken prior, in topical application of artificially contaminated hands [12]
	Nosocomial *Enterobacter* spp., *Klebsiella pneumoniae, Acinetobacter* spp.	10% solution 'immediately' effective on all pathogens vs. skin culture taken before topical application ($P < 0.001$) [13]
	Biofilms of MSSA, *Klebsiella pneumoniae, Pseudomonas aeruginosa*	All pathogens completely eradicated after 24 hours of incubation with 0.25% solution or 0.25% gel, in biofilm eradication assay [14]
	Escherichia coli ATCC 25922 reference strain, nosocomial *Acinetobacter baumannii*	4% PVP-I was effective (> 5 $\log_{10}$ reduction) against all pathogens in microplate assay [15]

(B)

Antiseptic	Pathogen	Efficacy
Chlorhexidine	Nosocomial MRSA, MSSA, *Enterococcus faecium, Enterococcus faecalis*	4% solution was effective (vs. before exposure) on all pathogens after 3 minutes of contact time in quantitative suspension test [11]
	Nosocomial *Enterobacter* spp., *Klebsiella pneumoniae, Acinetobacter* spp.	10% solution 'immediately' effective on all pathogens vs. skin culture taken 'immediately' before topical application ($P < 0.001$) [13]
	Klebsiella pneumoniae	0.005% chlorhexidine diacetate solution (urinary catheter maintenance solution) effective against all strains within recommended contact time (15 minutes) 0.05% chlorhexidine gluconate solution (antiseptic mouthwash with ethanol and 4% isopropyl alcohol) effective against all strains within 15 minutes and all strains (except for NCTC13368) within 5 minutes 0.5% CHG solution (pre-operative skin preparation) effective against all strains within 5 minutes 0.004–0.012% CHG solution (hand disinfectant with patented combination of antimicrobial agents) effective against all strains within 5 minutes 0.06% CHG (antimicrobial skin cleanser/hand wash) effective against all strains within 5 minutes [16]
	Extensively drug-resistant *Pseudomonas aeruginosa*, XDR *Acinetobacter baumannii*, XDR *Klebsiella pneumoniae*, MRSA, VRE	0.5%, 1% and 2% solutions effective (99–100% reduction vs. pre-exposure) against XDR *Pseudomonas aeruginosa*, XDR *Acinetobacter baumannii* and XDR *Klebsiella pneumoniae* after 15 seconds of contact time, and against MRSA and VRE after 1 minute of contact time in quantitative suspension test [17]
	3-day mature biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*	0.015% solution completely eradicated (no viable cells/6 $\log_{10}$ CFU reduction vs. before exposure, $P < 0.001$) *Staphylococcus aureus* biofilm and was effective (3.96 $\log_{10}$ CFU reduction vs. before exposure, $P < 0.01$) against *Pseudomonas aeruginosa* biofilm after 15 minutes exposure in ex vivo porcine skin explant model 0.015% solution eradicated (no viable cells vs. before exposure, $P < 0.001$) both *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms after 24 hours of exposure in ex vivo porcine skin explant model [18]
	Multi-species *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis* (1:1:1:1) biofilm	After 24 hours of incubation with CHG solution: • 0.1% solution eradicated ($P < 0.01$ vs. control) *Staphylococcus aureus* and *Klebsiella pneumoniae*, and significantly reduced *Pseudomonas aeruginosa* and *Enterococcus faecalis* ($P < 0.01$ vs. control) • 1% solution eradicated ($P < 0.01$ vs. control) *Staphylococcus aureus* and *Enterococcus faecalis* and significantly reduced ($P < 0.01$ vs. control) *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* • 2% solution eradicated ($P < 0.01$ vs. control) *Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis* and significantly reduced ($P < 0.01$ vs. control) *Klebsiella pneumoniae* • 4% solution eradicated all pathogens ($P < 0.01$ vs. control) Standard wiping technique with 2% CHG wipes: • 2% wipes significantly more effective ($P < 0.01$ vs. isopropyl alcohol) than 70% IPA against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Enterococcus faecalis* Biofilm embedded in artificial agar plate wound bed: • Sterile gauze soaked in 0.5% CHG significantly reduced($P < 0.01$ vs. sterile gauze) *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus faecalis*, and eradicated ($P < 0.01$ vs. sterile gauze) *Staphylococcus aureus* Commercially available Tulle Gras dressing impregnated with 0.5% CHG in soft white paraffin eradicated ($P < 0.01$ vs. sterile gauze) *Staphylococcus aureus* but no significant reduction (vs. sterile gauze) in *Pseudomonas aeruginosa, Klebsiella pneumoniae* or *Enterococcus faecalis* [19]
	Acinetobacter baumannii international clone II (IC II) and non-IC II	0.1% CHG solution effective against *Acinetobacter baumannii* IC II and *Acinetobacter baumannii* non-IC II (reduced below detection vs. before exposure, $P < 0.05$) after 30 seconds of exposure [20]

(*continued on next page*)

Table 2 (*continued*)

(C) Antiseptic	Pathogen	Efficacy
Polyhexanide	MRSA, MSSA, *Klebsiella pneumoniae*, carbapenem-resistant *Klebsiella pneumoniae*, and *Enterobacter* spp., ceftazidime-resistant *Enterobacter* spp., *Enterococcus faecium, Acinetobacter* spp., MDR and wide-type *Pseudomonas aeruginosa*, and VSE and VRE	0.1–3.2% PHMG significantly more effective (based on MIC, $P < 0.05$) than chlorhexidine against MRSA, MSSA, *Klebsiella pneumoniae, Enterobacter* spp., *Enterococcus faecium, Acinetobacter* spp., and *Pseudomonas aeruginosa* in a modified Mueller-Hinton broth microdilution PHMG eradicated MRSA, carbapenem-resistant *Klebsiella pneumoniae*, ceftazidime-resistant *Enterobacter* spp., VRE, ciprofloxacin-resistant and levofloxacin-resistant *Acinetobacter* spp. and MDR *Pseudomonas aeruginosa* faster than CHG at 2 x MIC in time-kill suspension test Similar efficacy (based on MIC, $P > 0.05$) to CHG against VSE and VRE, wide-type ciprofloxacin-resistant and levofloxacin-resistant *Acinetobacter* spp. and wide-type and MDR *Pseudomonas aeruginosa* [21]
	MRSA biofilm	0.1 % PHMB + propyl-betaine solution was effective (97.85% reduction vs. baseline [$P < 0.05$], 99.64% reduction vs. sterile water [$P < 0.05$] and $P < 0.05$ vs. octenidine) against MRSA biofilm after 3 days in an in vivo porcine wound model [22]
	Staphylococcus aureus and *Pseudomonas aeruginosa* dominated leg ulcers	0.1% PHMB and 0.1% undecylenamidopropyl betaine reduced bacterial load (vs. baseline) of *Staphylococcus aureus* and *Pseudomonas aeruginosa* but was not significantly more effective ($P > 0.05$) than saline on venous leg ulcers of human patients [23]
(D) Antiseptic	**Pathogen**	**Efficacy**
Octenidine	Antibiotic-resistant *Staphylococcus aureus* epidemic clones (MRSA, MSSA, high-level mupirocin-resistant MRSA, low-level mupirocin-resistant MRSA, mupirocin-susceptible MRSA, mupirocin-susceptible MSSA	0.001% solution was effective (bacterial reduction of > 6 $\log_{10}$ vs. before exposure) against all isolates after 30 sec exposure in quantitative suspension tests [24]
	MDR Gram-negative pathogens (*Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter baumannii* and *Pseudomonas aeruginosa*)	0.01% and 0.05% were fully effective (bacterial reductions of > 5 $\log_{10}$ vs. before exposure) against all isolates after 1 min contact time [25]
	MSSA, MRSA and vancomycin-resistant *Staphylococcus aureus* planktonic cells and biofilms	2 mM effective ($P < 0.05$ vs. 0 mM) at inactivating planktonic cells and preventing biofilm formation of all pathogens 10 mM and 5 mM effective ($P < 0.05$ vs. 0 mM negative control) against fully formed biofilms of all pathogens [26]
	Acinetobacter baumannii biofilms	0.3%, 0.6%, and 0.9% were effective ($P < 0.05$ vs. 0% negative control) in significantly inactivating *Acinetobacter baumannii* biofilms on all tested surfaces in a microliter plate assay [27]
	Enterococcus faecalis biofilm	0.1% solution significantly more effective (greater proportion dead cells) against *Enterococcus faecalis* biofilm than 1% alexidine and 2% chlorhexidine in a root dentin disc biofilm model [28]
	Staphylococcus aureus, Pseudomonas aeruginosa and *Klebsiella pneumoniae*	Effective against *Staphylococcus aureus* (3.8 $\log_{10}$ reduction), *Klebsiella pneumoniae* (4.8 $\log_{10}$ reduction) and *Pseudomonas aeruginosa* (3.4 $\log_{10}$ reduction) after 15 min exposure [29]

Abbreviations: CFU, colony forming unit; CHG, chlorhexidine gluconate; ESKAPE, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-sensitive *Staphylococcus aureus;* PHMB, polyhexanide; PHMG, polyhexamethylene guanidine hydrochloride; PVP-I, povidone-iodine; VRE, vancomycin-resistant *Enterococcus faecium*; VSE, vancomycin-susceptible *Enterococcus faecium*; XDR, extensively drug-resistant

Table 3
Summary of the antimicrobial activity for povidone-iodine, polyhexanide, chlorhexidine, and octenidine. Adapted from Lachapelle et al.[2]

Antiseptic	Vegetative bacteria			Bacterial spores	Fungi	Viruses
	Gram-positive	Gram-negative	Actinobacteria			
Povidone-iodine, 10%	BC +++, BS	BC +++, BS	BC ++	SC ++	FC +++, BS	VC ++, BS
Polyhexanide	BC +++, BS	BC +++, BS	NA	NA	FC ++, NS	VC +, NS
Chlorhexidine gluconate	BC +++, BS	BC +++, NS	NA	NA	FC ++, NS	VC +, NS
Octenidine	BC ++, BS	BC ++, NS	NA	NA	FC ++, NS	VC +, NS

+, weak; ++, medium; +++, high
Abbreviations: BC, bactericidal; BS, broad-spectrum; FC, fungicidal; NA, no activity; NS, narrow-spectrum; SC, sporicidal; VC, virucidal

was equally effective against biofilms of MDR and drug-susceptible isolates [27]. Interestingly, OCT had greater antimicrobial activity against *E. faecalis* biofilm on root dentin discs than CHG and alexidine [28].

3.2. Antiseptic resistance

Resistance to antiseptics can be intrinsic to the microorganism (e.g. impermeability or chromosomally-mediated inactivation) or acquired through mutation or acquisition of plasmids and transposons (e.g. efflux pumps, mutation of the target site) [37,46]. Despite extensive clinical use of PVP-I over several decades, and rigorous testing of isolates, there have been no reports of resistance or increased bacterial tolerance to antiseptic treatment [34,35]. This favourable resistance profile is likely to be due to the fact that iodine has multiple modes of action (Figure 1) [34,35]. Unlike PVP-I, CHG has been found to act on one specific bacterial target: the bacterial cell wall [47]. Therefore, adaptations in this target can result in resistance to CHG, as demonstrated by the upregulation of major facilitator superfamily efflux pump genes and Qac (quaternary ammonium compounds) efflux proteins in *K. pneumoniae* and *Staphylococci*, respectively [33,48]. Despite the fact that recent reports have suggested that the use of CHG does not promote CHG resistance among pathogens [49–51], there is overwhelming evidence to the contrary in seven distinct studies [52–58].

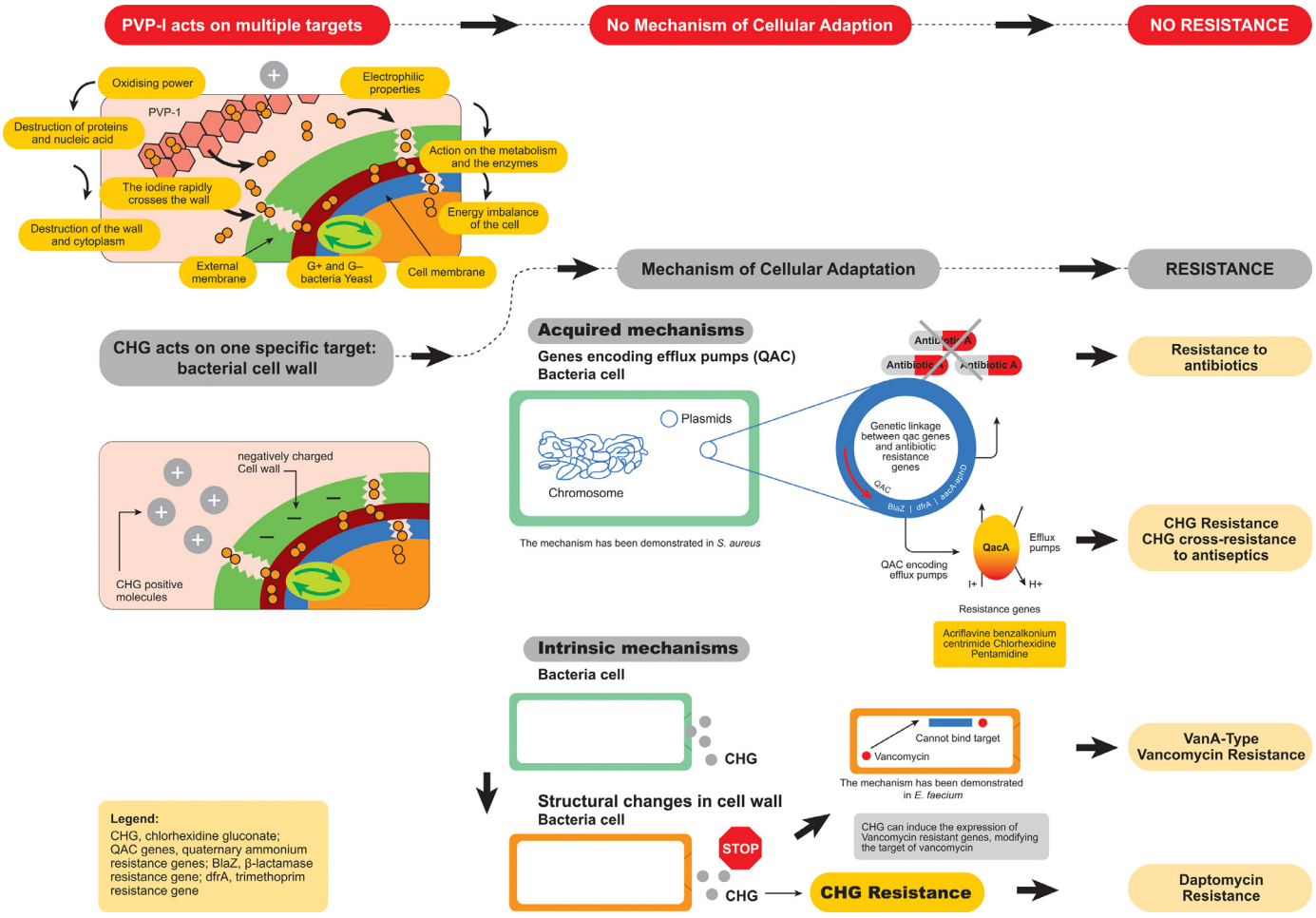

Figure 1. Povidone iodine (PVP-I) acts on multiple bacterial targets and therefore no resistance or antibiotic cross-resistance has been reported [34,35]. CHG acts on one specific target: the bacterial cell wall [47]. Expression of acquired resistance genes leads to CHG resistance, and may also lead to cross-resistance to antibiotics [48]. Adaptations to the bacterial cell wall lead to greater resistance to CHG alongside vancomycin and daptomycin [33,58,70]. Abbreviations: CHG, chlorhexidine gluconate; QAC genes, quaternary ammonium resistance genes; BlaZ, β-lactamase resistance gene; dfrA, trimethoprim resistance gene; aacA-aphD, aminoglycosides resistance genes

MSSA and MRSA strains with reduced susceptibility to CHG have been reported [52], with a cross-sectional study showing that CHG exposure was associated with reduced CHG susceptibility in MRSA isolates [53]. The results of a European study suggest that resistance to CHG is more common in MRSA than in MSSA [59]. This may result from greater expression of QacA/B efflux pump genes, which is the most common determinant of CHG resistance in MRSA [60]. In recent reports, decreased susceptibility to CHG has also been found among clinical isolates of *Staphylococcus epidermidis* associated with surgical site infections [54], and among MDR clinical isolates of *S. epidermidis* from bloodstream infections [55]. Clinical isolates of *A. baumannii* with reduced susceptibility to CHG have been observed [56], and clinically prevalent strains of *Mycobacterium abscessus* that are resistant to CHG (but not to PVP-I) have also been identified [57]. VRE appears to have developed resistance to CHG in Danish hospitals, where use of CHG is widespread, highlighting a need for continued surveillance of the emergence of resistance (Figure 1) [58]. Interestingly, recent data indicate that exposure to sub-lethal concentrations of CHG and triclosan may contribute to the emerging problem of antibiotic resistance by stimulating horizontal transfer of antimicrobial resistance genes between bacteria [48,61,62]. In one study, exposure of *E. coli* to sub-lethal triclosan concentrations upregulated the sex pilus-encoding *TraA* gene and promoted horizontal gene transfer to a greater degree than lethal concentrations [61]. The induction of horizontal gene transfer and antiseptic resistance may involve an array of processes more complex than those following lethal doses of CHG and triclosan. This includes disturbances in osmoregulation and respiratory activity, the dissipation of proton motive force, oxidative stress, the triggering of SOS responses, and the induction of error-prone DNA replication [48,61,63,64]. These reports of resistance are of particular concern because exposure to sub-minimum inhibitory concentrations (MICs) of CHG and triclosan is a common occurrence following the use of personal hygiene products [62]. The true consequences of this exposure, within the human body and the environment, are currently unknown and more research is needed [62].

In a study investigating the antimicrobial activity of PHMB against Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa, Moraxella catarrhalis, Haemophilus influenza*) in the presence or absence of antibiotics, no resistance and no antagonism with antibiotics was observed [65]. Furthermore, reduced susceptibility of MRSA to PHMB was not observed following PHMB decolonisation treatment in vivo. However, in the same study, prolonged stepwise exposure to low concentrations of PHMB in vitro promoted reduced susceptibility of MRSA to PHMB [66].

In a cross-sectional study, no direct association was found between OCT exposure and reduced susceptibility in MRSA isolates [53]. Although another study found a demonstrable reduction in the susceptibility of MRSA isolates following OCT exposure, the re-

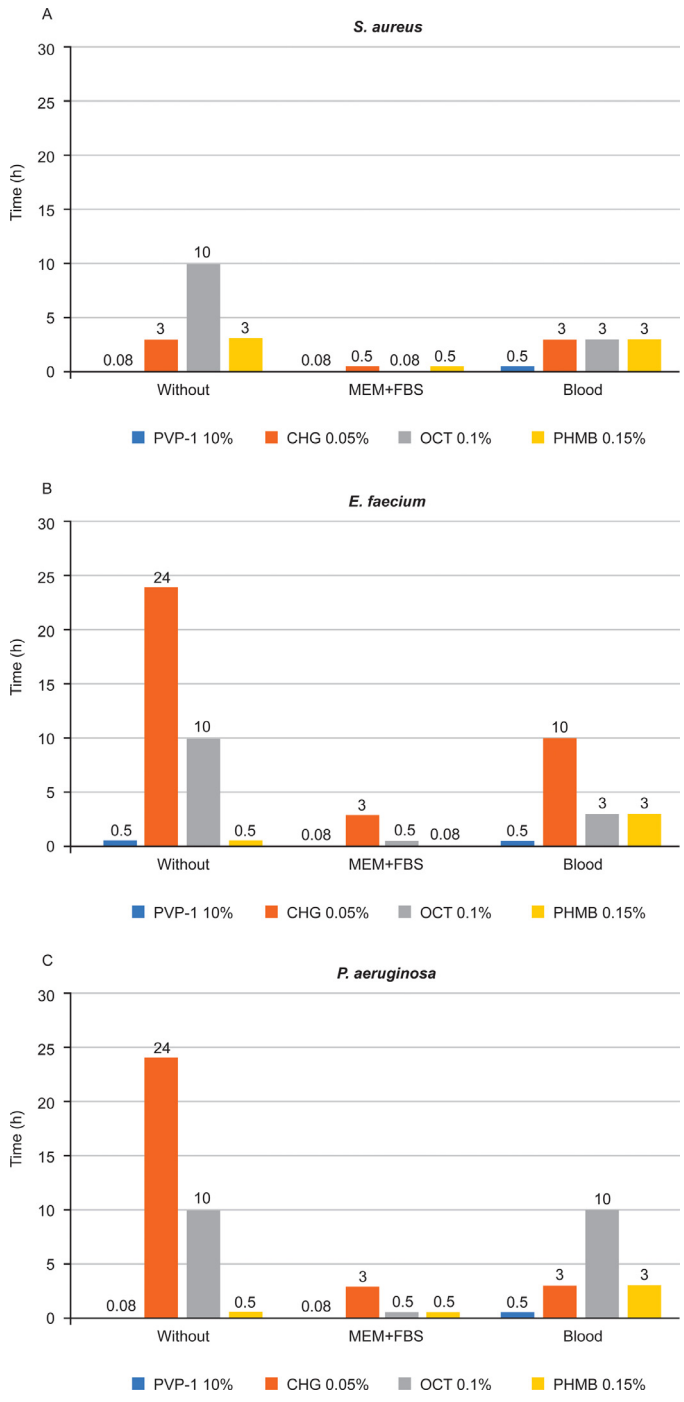

Figure 2. Required exposure time to achieve efficacy in the presence (log_{10} RF $\geq$ 3) and absence (log_{10} RF $\geq$ 5) of organic material for (A) *Staphylococcus aureus*, (B) *Enterococcus faecium* and (C) *Pseudomonas aeruginosa*. Bacteria were dispersed in either aqueous solution, organic material solution (MEM [with Earl's salts and L-glutamine] and 10% FBS) or 30% organic blood material solution (MEM [with Earl's salts and L-glutamine] and 10% FBS with human erythrocytes). Stainless steel test carriers were placed in Petri dishes coated with the resulting test solutions, which were dried for 60 minutes. Antiseptic compounds or negative controls were then applied to the test carrier surface. Antiseptic action was terminated after 0.08 h, 0.5 h, 3 h, 10 h, and 24 h. Bacteria were recovered and re-incubated, and the resulting CFU and log_{10} RF determined. Adapted from Schedler et al. [39]. Abbreviations: CFU, colony forming unit; CHG, chlorhexidine gluconate; FBS, foetal bovine serum; MEM, Eagle's Minimal Essential Medium; OCT, octenidine; PHMB, polyhexanide; PVP-I, povidone iodine; RF, reduction factor.

sulting MIC and minimum bactericidal concentration (MBC) were still below clinical concentrations [59].

3.2.1. Development of cross-resistance to last-line antibiotics

Although there is concern about the development of resistance to antiseptics themselves, a potentially greater concern is cross-resistance between antiseptics and antibiotics [67]. Cross-resistance can be defined as resistance to a particular antiseptic that results in concomitant resistance to antibiotics [33]. Exposure of clinical *K. pneumoniae* isolates to CHG can lead to resistance to CHG, and also cross-resistance to the antibiotic colistin. As very few antibiotics, in particular colistin, are effective against the majority of carbapenem-resistant *K. pneumoniae* isolates, such loss of colistin efficacy has serious implications for successful treatment of MDR *K. pneumoniae* infections (Figure 1) [33]. Whole genome sequencing analysis of CHG-adapted strains of *K. pneumoniae* has shown genetic changes to the PhoPQ two-component signalling system and/or a putative Tet repressor gene *smvR*. These genetic changes increase the activity of efflux pumps and explain the increased CHG resistance. Cross-resistance to colistin following CHG-adaptation is likely due to upregulation of the operon containing *pmrK*. This alters lipopolysaccharide modification and reduces the lipid A net negative charge, causing a reduced binding affinity of colistin. Alongside this, *phoPQ* mutations that arise through CHG-adaptation are linked to lipid A modifications and further colistin resistance [33]. In Gram-positive bacteria, the *qac* family of genes encode for the Qac efflux proteins, which play a role in the efflux of CHG and antiseptic resistance. The *qac* genes are located on a bacterial plasmid that also encode multiple resistance genes, with the use of CHG adding a selective pressure for bacteria that are also resistant to multiple antibiotics (Figure 1) [48].

Wound infections due to *Enterococcus* spp. are increasing worldwide and strains of *E. faecium* with tolerance to antiseptics have begun to emerge [68,69]. Serial exposure to sub-inhibitory concentrations of CHG selects for VRE with reduced susceptibility to CHG, and also isolates with reduced susceptibility to daptomycin (Figure 1) [70]. Furthermore, a clinical MDR *E. faecium* isolate with tolerance to CHG appears also to be tolerant to the antibiotic bacitracin via the same genetic determinant [68]. Incubation of several *S. aureus* strains with sub-lethal doses of CHG for up to 14 days did not decrease susceptibility to CHG in the majority of strains, but did result in cross-resistance to tetracycline in all isolates [71].

Alongside the resistance issues observed with the use of CHG, prolonged in vitro exposure to low concentrations of PHMB selects for MRSA, with reduced susceptibility to PHMB and concomitant resistance to daptomycin [66]. Exposing *P. aeruginosa* to increasing concentrations of OCT over several days lead to increased tolerance to OCT and also CHG [72].

This research into cross-resistance between antiseptics and antibiotics is promising. However, further investigation is needed to understand the concentrations of antiseptics that are required for the promotion of horizontal gene transfer, as this area is currently poorly understood [62]. Research in this area is imperative, as increasing antimicrobial resistance may have grave consequences as pathogens become more resistant to antibiotics and antiseptics.

3.3. Wound healing and skin tolerability

When considering antiseptics for wound management, attention should be paid to their efficacy in reducing the microbial burden and also their effect on the healing wound [73]. An ideal antiseptic for wound care should promote healing and exhibit good local tolerability [34].

3.3.1. Wound healing

In a rodent model of acute skin wounds, PVP-I treatment enhanced wound healing through increased expression of transforming growth factor beta, neovascularisation and re-epithelialisation [74]. PVP-I has also been found to have haemostyptic (an astringent that stops bleeding) and anti-inflammatory effects in periapical surgery [75] and to reduce production of reactive oxygen species by human polymorphonuclear neutrophils [76]. Compared with controls, PVP-I significantly increased the healing rate of chronic leg ulcers with no apparent cytotoxicity towards dendrocytes, with the densities in microvessels and dendrocytes higher in PVP-I-assigned lesions than in those receiving silver sulfadiazine or CHG [77]. Similarly, treating leg ulcers with hydrocolloid dressings in combination with daily applications of PVP-I increased the healing rate and reduced deleterious bacteria-related inflammation, alongside the reduction in inflammation caused by the inhibitory effect of PVP-I on leukotriene B4 and leukocyte extravasation, compared with hydrocolloid alone [75,78].

A recent systematic review concluded that topical PHMB may promote healing of chronic stalled wounds and alleviate wound-related pain [79]. A preclinical study in mice indicated that PHMB has favourable effects on microcirculation, angiogenesis and epithelialisation in wound healing [80]. Furthermore, topical application of PHMB, but not OCT, increased microcirculation of the human skin in vivo, which is important for wound healing [73]. However, PHMB induced inflammation in vitro via activation of NF-kB and subsequent cytokine secretion, which may be detrimental for healing wounds [81]. OCT gel significantly reduced the size of chronic venous leg ulcers compared with modern dressings [82], and in a clinical study comparing the efficacy of OCT and silver dressings, the rate of healing was faster and reduction in pain level greater in the OCT group versus the silver group [83].

Cytotoxicity tests have shown that PVP-I is better tolerated by murine fibroblasts than CHG, PHMB and OCT. Interestingly, treatment with PVP-I led to a revitalisation of murine fibroblasts, which was not observed with CHG, PHMB or OCT [84]. When tested on human fibroblasts, PHMB, hydrogen peroxide, CHG and OCT were 100% cytotoxic at their MBC; in contrast, some cell viability remained for PVP-I at the MBC [85]. Additional in vitro studies have indicated cytotoxic effects of PVP-I, PHMB and CHG [86–89]. However, it should be noted that these experiments were not carried out in vivo and many used considerably lower concentrations than those used in clinical practice. In vitro experiments must therefore be interpreted with caution, as they may not necessarily be reflective of clinical settings [34]. Further investigation of antiseptics in wound management in vivo is required, using concentrations of antiseptics as applied in clinical practice.

3.3.2. Tolerability

Allergy to PVP-I has historically been overestimated, mainly due to confusion between allergy and irritation [90]. In a study that retested all patients who reacted positively to a PVP-I patch test to ensure that any false positives were not recorded, the prevalence of allergic contact dermatitis caused by PVP-I was estimated to be 0.4% [90]. When comparing the allergenic properties of commonly-used antiseptics, allergic contact dermatitis was rare for PVP-I, OCT and PHMB, but more common for CHG [90]. There have also been reports of urticarial and anaphylactic reactions for CHG, anaphylactic reactions for PHMB, and aseptic tissue necrosis for OCT [90–93]. In recent years there has been a surge in confirmed cases of anaphylaxis caused by CHG, yet awareness of CHG as an allergen has been found to be low [94]. Diagnosis is easy to miss but presentation can be severe and occur at any time [95], and increased awareness of CHG allergy is needed in healthcare settings [95].

In terms of irritation, PVP-I 10% was significantly less irritating on the skin than CHG 5% [2]. Worryingly, cases of CHG-induced chemical burns in very low birth weight infants have been reported [96]. Recently, the irritative potency of selected antiseptics was assessed using a semi-in vivo testing method (hen's egg test on chorio-allantoicmembrane); PHMB caused no irritation, while CHG solution and OCT gel caused severe irritation [97].

Unlike the other antiseptics discussed in this review, PVP-I is sometimes associated with the induction of thyroid dysfunction. Although PVP-I has been shown to have a transient effect on thyroid function in some susceptible patients, there were no major consequences of this effect on the health of these patients [98]. Additionally, further studies have shown that thyroid dysfunction in those individuals exposed to iodine antiseptics does not differ from that found in the general population [98–103].

In addition to allergic reaction and irritation, the use of PVP-I is often associated with pain. A prospective study investigating the prevalence of adverse reactions to commonly used antiseptics showed a transient burning sensation to be experienced by 4–7% of patients, with no significant difference between antiseptics [104]. A further study using mouse models showed PVP-I to cause pain by stimulating a subset of sensory neurons that express TRPA1 and TRPV1 channels. The mechanisms by which antiseptics induce pain are not fully understood and this finding improves the understanding of the adverse effects of antiseptic use, while providing an insight into potential methods of reducing pain for patients [105].

4. Conclusions

When treating wounds with antiseptics, a number of potential challenges need to be taken into consideration, including: antimicrobial spectrum and efficacy in the real-world setting; antiseptic resistance and antimicrobial cross-resistance; effect on wound healing; and tolerability. Although CHG is the most widely used antiseptic, it has a number of undesirable properties such as its association with resistance/cross-resistance, reduced efficacy in the presence of organic material and allergic reactions.

When compared with other commonly used antiseptics – including CHG, PHMB and OCT – PVP-I had several advantages. PVP-I had the broadest spectrum of activity, was highly effective at eliminating ESKAPE pathogens and biofilms, and maintained efficacy in the presence of blood, making PVP-I a highly desirable antiseptic in the management of wounds and nosocomial infections. Furthermore, in an era where resistance to antiseptics and antibiotics is on the rise, a key feature of PVP-I that sets it apart from other antiseptics is the lack of resistance/cross-resistance attributed to this antiseptic. This is even more noteworthy given that PVP-I has been widely used for decades.

As discussed, an ideal antiseptic for wound care should not only reduce the microbial burden of a wound, but also promote wound healing. PVP-I has been found to promote wound healing while exhibiting low levels of cytotoxicity. Furthermore, PVP-I has been highly tolerated, with the prevalence of allergic reaction being 0.4%. Although pain can be experienced when using antiseptics, this is not unique to PVP-I and increased understanding could lead to improved techniques to manage any adverse side effects.

Moving forwards, further research is needed to understand the future implications of cross-resistance between antiseptics and antibiotics. Large and well-controlled trials of topical antiseptics in wound care and skin infections are needed, as the rationale for their selection and use in clinical practice has largely been based on empirical evidence and small clinical and pre-clinical studies. Finally, healthcare facilities need to be mindful of the issues associated with antiseptics, in particular resistance/cross-resistance, to

ensure that wounds are effectively treated without causing detrimental effects.

Declaration of Competing Interest

Authors are responsible for disclosing all financial and personal relationships between themselves and others that might be perceived by others as biasing their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist.

RB: No conflict of interest

BB: No conflict of interest

JL: Received honoraria as speaker and was member of advisory boards for Mylan

SMK: No conflict of interest

VMSF: No conflict of interest

SM: Received honoraria as speaker and was member of advisory boards for Mylan

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