Scientific Bulletin. Series F. Biotechnologies, Vol. XXII, 2018
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

REVIEW ON SOME CURRENT SKIN ANTISEPTICS

Getuța DOPCEA[1, 2], Florentina MATEI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]SC EUROFINS EVIC PRODUCT TESTING ROMANIA SRL,
64-66 Mărăşeşti Blvd, District 4, Bucharest, Romania

Corresponding author email: getuta.dopcea@gmail.com

Abstract

This article will review some current antiseptics used for skin disinfection: povidone iodine (PVP-I), chlorhexidine digluconate (CHG) and octenidine dihydrochloride (OCTD). These antiseptics are used in both healthcare and household products, for different types of applications preoperative skin preparation, surgical hand scrub, skin wound cleanser, skin wound protectant, hand hygiene, oral care and body wash. In the past years more and more concerns have been raised due to increased resistance to antiseptics and antibiotics of pathogens over the years. Some studies support the cross-resistance to antibiotics triggered by antiseptics. Increased resistance and cross-resistance may result from inadequate skin disinfection, over dilution of the antiseptics and environmental residues of antiseptics. More studies are required to assess the mechanisms of resistance and cross-resistance to antiseptics and antibiotics and also the impact of sub-lethal antiseptic concentration on clinical isolates. Usage guidelines of chlorhexidine were proposed recently, but usage guidelines should address all valuable skin antiseptics in order to be used only in cases where there is a clear benefit.

Key words*: resistance, cross-resistance, povidone iodine, chlorhexidine digluconate, octenidine dihydrochloride.*

INTRODUCTION

Antiseptics are antimicrobial substances that are applied to living tissues or human skin. Both antibiotics and antiseptics use had led to the emergence of new mechanisms of microbial resistance. Over the past years, the problem of nosocomial infections and increased antiseptics and antibiotics resistance and cross-resistance is worrisome in many countries and solutions are see ked. This article will review three of the most used antiseptics: povidone iodine, octenidine dihydrochloride and chlorhexidine digluconate.

1. POVIDONE IODINE

Povidone iodine (PVP-I) was discovered in 1952 by Shelanski . PVP-I is a complex of iodine and polyvinylpyrrolidone (povidone). Povidone is a polymer without biocidal activity, but having affinity to cell membranes it delivers the elemental iodine to the target. PVP-I has a better chemical stability and it is less reactive compared to previous elemental iodine formulations, showing increased safety and tolerability on the skin and mucous membranes. PVP-I also brings better solubility of iodine and a sustained way of releasing free inorganic iodine in solution. The antimicrobial activity is given by the quantity of free iodine released in the solution (Gottardi, 2001; Ripa et al., 2003). PVP-I has broad-spectrum antimicrobial properties and efficacy against biofilms (Bigliardi et al., 2017).

Different concentrations of PVP-I are used. Formulations of 10% PVP-I contain 1% available iodine and yield free iodine concentrations of 1 ppm (Anderson, 1989). 10% PVP-I is used as preoperative skin preparation, catheter site disinfection, wound antisepsis, anogenital antisepsis. 7.5% PVP-I is used as preoperative skin preparation, surgical hand scrub and antiseptic handwash. 5% PVP-I is used for preoperative skin preparation, preoperative ocular antisepsis, wound antisepsis, catheter site disinfection. 0.5% PVP-I is used for mouth/throat antisepsis. 0.5% PVP-I is used for vaginal antisepsis (Ripa et al., 2003; Kıvanç et al., 2016; Firanek et al., 2016; Kieran et al., 2017; Silas et al., 2017). A recent *in vitro* study showed that a single application of PVP-I at concentrations equal and higher

than 2.5% and three applications of 30 seconds of PVP-I at 0.7% concentrations produced a 3-$\log_{10}$ reduction of *Staphylococcus epidermidis* colonies (Silas et al., 2017).

Iodine rapidly penetrates the cell wall of microorganisms and inactivates cells by forming complexes with amino acids and unsaturated fatty acids, resulting in impaired protein synthesis and alteration of cell membranes (WHO, 2009). The antimicrobial effect of PVP-I can be strongly affected by temperature, exposure time, concentration of total available iodine, the amount and type of organic and inorganic compounds (e.g. alcohols and detergents) and rising pH (WHO, 2009; Wiegand et al., 2015).

An exploratory clinical study showed excellent antibacterial efficacy with no detectable microorganisms by the tenth day and rapid reepithelialization of 10% PVP-I ointment (Vogt et al., 2017).

In a recent randomized controlled trial (RCT), 5% PVP-I was significantly more effective in decolonizing *Staphylococcus aureus* at 4 hours post-application compared to saline, PVP-I being associated with only 21% *S. aureus* positive patients compared with 59% for saline (Rezapoor et al., 2017). Another study showed that 5% PVP-I significantly reduced the resident *S. aureus* from the anterior nares of human subjects compared to saline at 1, 6 and 12 h after swabbing application (Anderson et al., 2015). Also 5% PVP-I showed >2.0 Log_{10} CFU reduction in methicillin-resistant *S. aureus* (MRSA) regardless of mupirocin sensitivity in an *in vitro* test (Anderson et al., 2015).

During an *in vitro* evaluation, PVP-I was effective against all 81 *Acinetobacter baumannii* isolates tested, and their logarithmic reduction ≥ 5 were observed in 100% of the isolates in their undiluted form (Lanjri et al., 2017).

In two recent *in vitro* studies, PVP-I was effective against *Candida auris*, when tested according to EN 13624:2013 (Moore et al., 2017) and inhibited *C. auris* isolates at concentrations 0.07%-1.25% (Abdolrasouli et al., 2017).

Studies have shown that PVP-I exhibits antibacterial activity, particularly against the *Pseudomonas* species and *S. aureus* that are prevalent in biofilms (Kunisada et al., 1997; Drosou et al., 2003).

A PVP-I ointment tested against biofilms of *Pseudomonas aeruginosa*, multi-species biofilms of *Candida albicans* and MRSA, resulted in no viable *P. aeruginosa*/ *C. albicans*/ MRSA biofilm material recovered after 4 and 24 hours from the treatment (Hoekstra et al., 2016).

Yamasaki et al. (2017) reported at least a 7 Log_{10} reduction in viability of bacteria produced by PVP-I in biofilm studies. A few recent RCTs suggest that CHG based antiseptics are superior to PVP-I. In a large RCT performed on 2349 randomly assigned patients undergoing catheter insertions, compared 2% CHG - 70% IPA (isopropyl alcohol) (1181 patients) with 5% PVP-I - 69% ethanol (EtOH) (1168 patients), demonstrated that CHG-IPA was associated with lower incidence of catheter-related infections compared with PVP-I - EtOH, 0.28 and 1.77 per 1000 catheter-days, respectively; hazard ratio 0·15, 95% CI 0·05-0·41; p=0·0002 (Mimoz et al., 2015). In another large RCT involving 1132 randomized (796 included in the analysis set) patients undergoing catheter insertions, Yasuda et al. (2017) compared 0.5% and 1.0% alcohol based CHG with 10% aqueous PVP-I and found that the incidence of catheter colonization was 3.7, 3.9 and 10.5 events per 1000 catheter-days in 0.5% CHG, 1% CHG and PVI groups, respectively ($p = 0.03$), confirming that 0.5% and 1.0% alcohol based CHG are superior to 10% aqueous PVP-I for the prevention of intravascular catheter colonization.

In a retrospective cohort study including a total of 4,259 patients undergoing abdominal hysterectomy, 70.5% (n=3,005) of the patients were assigned to CHG and 29.5% (n=1,254) were assigned to PVP-I. The unadjusted rate of SSI (surgical site infection) was 2.6% (95% CI 2.1-3.3; n=79) for CHG and 3.6% (95% CI 2.7-4.8; n=45; P=.09) for the PVP-I group, but for the matched groups the rate of SSI was 1.5% (95% CI 0.8-2.6; n=12) for the CHG-alcohol group and 4.7% (95% CI 3.5-6.4; n=40) for the PVP-I group (P<.001). This study suggested that CHG based skin antisepsis was associated with overall lower odds of SSI compared with PVP-I (Uppal et al., 2017).

The results of Privitera et al. (2017) meta-analysis confirmed that CHG is superior to PVP-I for both SSI incidence (risk ratio, 0.70; 95% confidence interval, 0.52-0.92) and bacterial skin colonization (risk ratio, 0.45; 95% confidence interval, 0.36-0.55).

WHO (WHO, 2016) strongly recommended the use of an alcohol-based antiseptic solution preferably based on CHG for surgical site preparation on intact skin, based on a meta-analysis of available studies showing alcohol-based CHG is beneficial in reducing SSI rates compared to alcohol-based PVP-I.

In a RCT involving 388 patients undergoing clean or clean contaminated surgeries, 220 patients were treated with 10% PVP-I and 186 patients were treated with 2% CHG – 70% IPA, Bibi et al. (2015) showed lower infection rates in CHG group (7.1% infection rate) compared with PVP-I group (10% infection rate), but it was not statistically significant (p=0.324). In this study *P. aeruginosa* (23.5%) was the predominant pathogen associated with SSI followed by *S. aureus* (17.6%) (Bibi et al., 2015).

Kieran et al. (2017), in a RCT involving 304 neonates, found no statistically significant differences (p=0.631) in preventing catheter-related blood stream infection between 2% CHG – 70% IPA group and 10% aqueous PVP-I group, but more infants treated with PVP-I had thyroid dysfunction. Ghobrial et al. (2017), in prospective study performed on 6959 spinal surgery patients (0.992% SSI), showed that there is no significant difference (p = 0.728) in the incidence of SSI between 7.5% PVP-I group (33 [1.036%] of 3185) and 2% CHG – 70% IPA group (36 [0.954%] of 3774).

Concurrent use of CHG and PVP-I could be an option to concerns regarding development of acquired bacterial resistance. Following a prospective SSI database analysis, Davies and Patel (2016) suggested that the use of both CHG and PVP-I significantly reduced SSI rates compared to CHG or PVP-I alone. In a two arm RCT enrolling 407 patients, the detection of viable bacteria in the samples taken after disinfection was significantly lower (p = 0.009) in the group assigned to a sequential application of PVP-I and CHG (59 [29.1%]) compared to the group treated only with PVP-I (85 [41.7%]) (Patrick, 2017).

2. OCTENIDINE DIHYDROCHLORIDE

Octenidine dihydrochloride (OCTD) (N,N'-(1,10-Decandiyldi-1(4-H)-pyridynyl-4yliden) bis(1-octanamine)-dihydrochloride) is a bispyridine antimicrobial compound exhibiting antiseptic activity against a wide range of bacteria (Gram-positive and negative), some fungus and dental plaque agents (Patters et al., 1983; Harke, 1989). OCTD was introduced as a topical antiseptic agent more than 25 years ago and now it is used as an antiseptic in many applications and represents an alternative to older substances such as CHG, PVP-I or triclosan (Hübner et al., 2010). In a pH range 5-9 the bactericidal activity of OCTD is not affected (Wiegand et al., 2015). By present no clinically significant local and systemic side effects were associated with OCTD (Willy et al., 2017).

OCTD at concentrations of 0.1% and lower has bactericidal and fungicidal effect (Harke, 1989). 0.05% OCTD is preferred as a chronic wound preparation and for decolonization of multi drug resistant organisms from wounds a 0.1% OCTD/phenoxyethanol solution is preferred (Kramer et al., 2017). As an antimicrobial wash and coating OCTD at concentrations of 0.01, 0.05 and 0.1 produced >5 Log_{10} CFU/cm^2 reductions of *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7, within 2 minutes (Upadhyay et al., 2016).

OCTD demonstrated a significant >6 Log_{10} bacterial reduction factor within 30 seconds contact time in the presence of organic load (0.6% albumin), against six MRSA isolates and two methicillin-sensitive *S. aureus* (MSSA) isolates (Conceição et al., 2016). Another recent in vitro study, performed according to standard method EN 13727, demonstrated the effectiveness (>5 Log_{10} bacterial reduction factor within 1 minute contact time) of OCTD at both concentrations, 0.01% and 0.05%, against all five different tested species: *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii* and *P. aeruginosa* (for each species using five clonally unrelated isolates, including a susceptible wild type strain, and four multidrug-resistant Gram-negative isolates) (Alvarez-Marian, 2017).

Kung et al. (2016) showed in a *in vitro* study that OCTD is effective against *Trichomonas.*

vaginalis, the 50% effective concentration (EC50) values ranged from 5.7 to 21.37µg/mL after 5 min, from 6.48 to 10.82µg/mL after 15 min and from 0.68 to 2.11µg/mL after 30 min of treatment, suggesting that OCTD could be a promising treatment of bacterial and fungal vaginal infections.

In a study which included 36 MRSA-positive patients, octenidine achieved complete decontamination of 24 patients (67%) (Danilevicius et al., 2015).

OCTD demonstrated a significant reduction of skin flora from the arm and showed a long-term effect after 24 h, when tested according to Deutschen Gesellschaft für Mikrobiologie und Hygiene standard method 13 (Brill et al., 2015).

Similar results in reduction of MRSA acquisition, after five-day cycles of routine bathing of intensive care patients, between CHG and OCTD were reported (Spencer et al., 2013). A large prospective study performed on MRSA-colonized patients showed that daily OCTD body washes was not associated with either a significant reduction in MRSA transmission, MRSA infections, or MRSA bacteremia (Harris et al., 2015).

OCTD based mouth rinses showed comparable results with CHG based mouth rinses, regarding antibacterial and antiplaque efficacy in the human oral cavity (Welk et al., 2016; Decker et al., 2017).

In clinical study involving patients undergoing elective isolated coronary artery bypass graft, Reiser et al. (2017) showed that there was no significant difference ($p=0.39$) in SSI between the control group (15.4% SSI rate) and the group treated with OCTD (13.3%), which consisted in OCTD ointment three times daily and showering the night before and on the day of the surgery with OCTD soap.

OCTD proved *in vitro* antibiofilm activity against *S. aures* and *E. coli*, but not on *Candida tropicalis* (Slobodnikova et al., 2014).

In an *in vitro* study, Narayanan et al. (2016) showed that 0.3%, 0.6% and 0.9% concentrations of OCTD were effective in significantly ($p<0.05$) inactivating *A. baumannii* isolates, either multidrug resistant or drug susceptible isolate, when tested on different surfaces (polystyrene, stainless steel and catheters).

Amalaradjou et al. (2009) showed that OCTD can be effective in preventing the establishment of Listeria monocytogenes biofilms and also to eliminate the preformed biofilms.

OCTD (94 ± 1% reduction rate) and CHG (91 ± 1% reduction rate) demonstrated good efficacy in disinfection of MRSA-biofilms (Gunther et al., 2017). Another *in vitro* study showed that 0.1% OCTD is effective against the following biofilm forming microrganisms: *Enterococcus faecalis, S. aureus, Candida albicans*, within 30 seconds exposure (Ghivari et al., 2017).

OCTD was the most effective when compared, at minimal effective concentration, with CHG and PVP-I, according to standard test method EN 1040 and 1275 against *S. aureus, P. aeruginosa* and *C. albicans* (Koburger et al., 2010).

According to Brill et al. (2015) CHG and OCTD showed comparable efficacy in reducing the resident skin from the arm.

Cherian et al. (2016) showed the 0.1% OCTD was more effective than 2% CHG against *E. faecalis*.

3. CHLORHEXIDINE DIGLUCONATE

Chlorhexidine digluconate (CHG) was first synthesized in the 1950s in United Kingdom and introduced into the USA in the 1970s (Davies et al., 1954; Denton, 2001). CHG is probably one of the most widely used skin antiseptic, due to its broad-spectrum efficacy and low skin irritation. CHG is a cationic bisguanide, water-soluble is generally compatible with other cationic molecules, such as quaternary ammonium compounds. CHG is used as skin antiseptic prior to surgical, catheter insertion or other clinical procedures, in dressings, in healthcare personnel hand wash, in patients bathing (Davies et al., 1954, Paulson, 2003; Denton, 2001; McDonnell and Russell, 1999; Vali et al., 2017).

The antimicrobial activity of CHG is reduced in the presence of anionic detergents (sodium lauryl sulfate) found in natural soaps and mouth rinsing products, various inorganic anions, non-ionic surfactants, and hand creams containing anionic emulsifying agents; CHG forms salts of low solubility with anions (Paulson, 2003; Denton, 2001; Barkvoll et al., 1989; Larson, 1995; Walsh et al., 1987).

Aqueous and alcohol based CHG preparations are available in different concentrations ranging between 0.4% to 4% (WHO, 2009; Vali et al., 2017).

The CHG mechanism of action is known to be related to the binding to negatively charged bacterial and attachment to cytoplasmic membranes and subsequent disruption of this membranes resulting in precipitation of cellular contents, thus affecting the osmotic equilibrium of the cell (Rotter, 1999; Larson, 1995; Vali et al., 2017).

Antimicrobial activity of CHG is influenced by pH, but in a pH range 5-9 the bactericidal activity of CHG is optimal, however pH influence varies with each microorganism (Paulson, 2003; Wiegand et al., 2015).

Double application of CHG (first application: standard hand rub and rinse of the 4% CHG; second application: aqueous solution of 5% CHG applied with no further rinsing of the hands) under the conditions of EN 12791 test method, was superior to single CHG application ($p<0.01$) and n-propanol ($p<0.05$) and surpassed EN12791 (Herruzo and Vizcaino, 2017).

In recent *in vitro* studies, CHG was effective against MRSA obtained from Canary black pigs (Espigares et al., 2017), and also against *Acinetobacter* spp. including international clone II at a concentration of 1000mg/L and exposure time for at least 30s (Hayashi et al., 2017).

Hennig et al. (2017) demonstrated in a study performed according to EN 12791 (2016) that alcohol based CHG formulation (mean log reduction factors of 1.42 ± 0.79 and 1.24 ± 0.90 immediately and after 6 h) achieved significantly lower ($p \leq 0.025$ immediately after application and $p \leq 0.01$ after 6 h) mean log reduction factors compared with alcohol-only formulation (mean log reduction factors of 1.96 ± 1.06 immediately after application and 1.67 ± 0.71 after 6 h) in either immediately or after 6 h.

Previous studies reported no impact of CHG bathing on healthcare associated infections (HAI) (Climo et al., 2013; Bass et al., 2013), while others claim to reduce the HAIs (Climo et al., 2009; Vernon et al., 2006).

Recent studies conducted over long period of time claim that CHG bath reduces most of the HAIs. In quasi-experimental interventional, 9years study, Mendez et al. (2016) showed that CHG bathing significantly reduced the vancomycin-resistant *Enterococcus* (VRE) colonization and infection rates ($p=0.001$), but not for multidrug-resistant (MDR) Gram-negative pathogens. Another recent prospective study conducted in 4 medical units from Canada for over 7 months, confirmed that daily bathing with CHG significantly decreased the hospital-associated infections with VRE by 36% (23.2 vs. 36.0 cases *per* 10,000 patients, $p=0.03$) and also with MRSA by 55% (5.1 vs. 11.4 cases per 10,000 patients, $p=0.04$) compared with control cohorts which were assigned to non-medicated soap and water bathing (Lowe et al., 2017), although skin CHG concentrations were lower when rinsing with water after CHG solution bath compared with CHG solution without rinsing (Alserehi et al., 2017). During an over 18 months study performed in a hospital from Mexico, which included 158 isolates, 2% CHG bath was associated with a reduction in antibiotic resistance and favored the reduction of MRSA isolates and a temporary reduction of ST5-MRSA-II (New York/Japan) clone (Velázquez-Meza et al., 2017). Also, in a prospective, quasi-experimental, single-center study, performed on 237 patients, 4% CHG bath significantly reduced the percentage of contaminated blood cultures in treated group (6.3%) compared with daily cleaning with common soap (15.0%) (Garrido-Benedicto, 2017).

In an *in vitro* study, Wang and Ren (2017) reported significant log reductions of biofilm cells of *S. mutans* and *S. aureus* achived by CHG combined with low-level direct current.

4. RESISTANCE AND CROSS-RESISTANCE

Resistance has been defined as the temporary or permanent ability of an organism and its progeny to remain viable and/or multiply under conditions that would destroy or inhibit other members of the strain (Cloete, 2003; Oancea and Stoia, 2010). Antiseptic-resistance gene expression can be induced by exposure to subinhibitory concentrations of antiseptics (Smith et al., 2008).

Among mechanisms of resistance that have been reported we can mention: efflux pumps (Paulsen et al., 1996; Levy, 2002; DeMarco et al., 2007; Jaglic et al., 2012), inactivation of the active ingredient (Kaulfers, 1995), changes in the cell wall structure (Kaulfers, 1995), changes in membrane fluidity (Gadea et al., 2017).

The most frequent antiseptic antiseptic-resistance genes are listed in Table 1 along with the corresponding primers.

The qacA/B is found in the chromosome and on plasmids of *S. aureus* (Liu et al., 2009; Reich et al., 2016). qacA and qacB are part of the largest known protein family of secondary transporter systems called "Major Facilitator Superfamily" (Wassenaar et al., 2015). The qacA gene confers resistance to ethidium bromide and CHG (Mayer et al., 2001; Shamsudin et al., 2012; Reich et al., 2016). The qacB gene confers resistance to monovalent organic cations and some bivalent compounds (Mayer et al., 2001; Shamsudin et al., 2012; Opacic, 2010).

The smr gene is part of the "Small Multidrug Resistance" protein family and it is identical with qacC gene. The smr gene is regarded as an antiseptic resistance gene (Grinius et al., 1992; Mayer et al., 2001; Shamsudin et al., 2012; Vali et al., 2017; Liu et al., 2015; Shi et al., 2015).

The qacE and qacΔE1 (functional deletion variant of qacE) genes can be found in Gram-negative species and they are corelated with reduced susceptibility to CHG and other biocides (Wassenaar et al., 2015; Guo et al., 2015).

Efflux genes and specific antibiotic resistance genes are located together on mobile genetic elements and antiseptic-resistance and cross-resistance to antibiotics may be aquired together (Yamamoto et al., 1988; Wales and Davies, 2015). Resistance to several antibiotics in association with qac genes has been reported in different clinical isolates (Zhang et al., 2011; Babaei et al., 2015).

Gomaa et al. (2017) reported a significant correlation between the presence of qac genes and MBL-encoding bla_{VIM} (Verona integron-encoded metallo-β-lactamases) (p=0.0064; coexistence detected in 68.1% of the isolates) and bla_{NDM-1} (New-Delhi-metallo- β - lactamase) (p=0.0467; coexistence detected in 68.1% of the isolates) genes and a significant correlation between qac genes carriage and increased MICs of CHG (p = 0.0096).

Until now, no development of resistance to PVP-I or OCTD has been reported (Al-Doori et al., 2007; Willy et al., 2017; Bigliardi et al., 2017).

On the other hand, many studies reported resistance and cross-resistance to CHG. Gomaa et al. (2017) reported higher MIC of CHG than the concentrations recommended for disinfection in 54.5% of all metallo-β-lactamase producing *Acinetobacter baumannii* clinical isolates. Wu et al. (2016) reported cross-resistance of 14 clinical isolates of *S. aureus* to at least one antibiotic following exposure to CHG at. sublethal doses for up to 14 days, sugesting that antibiotics and antiseptics could have similar antimicrobial mechanisms.

Table 1: Antiseptic-resistance genes

Gene name	Primer	Sequence	Reference
qacA/B	qacAB-Forward qacAB-Reverse	GCAGAAAGTGCAGAGTTCG CCAGTCCAATCATGCCTG	Noguchi et al., 2005
qacE	qacE-Forward qacE-Reverse	ATGAAAGGCTGGCTT TCACCATGGCGTCGG	Kucken et al., 2000; Mahzounieh et al., 2014
qacΔE1	qacΔE1-Forward qacΔE1-Reverse	TAGCGAGGGCTTTACTAAGC ATTCGAAATGCCGAACACCG	Wang et al., 2007 Mahzounieh et al., 2014
smr	smr-Forward smr-Reverse	GCCATAAGTACTGAAGTTATTGGA GACTACGGTTGTTAAGACTAAACCT	Noguchi et al., 2005
cepA	cepA- Forward cepA-Reverse	CAACTCCTTCGCCTATCCCG TCAGGTCAGACCAAACGGCG	Fang et al., 2002

The main mechanisms of reduced susceptibility to CHG are CHG efflux and changes in outer membrane content (Wand et al., 2016; Hassan et al., 2013; Costa et al., 2013; Rajamohan et al., 2010; Russell, 2002; Tattawasart et al., 2000). The use of efflux pump inhibitors together with CHG may be an option for preventing the efflux of the CHG from *P. aeruginosa* resulting in a increased efficacy of CHG (Mombeshora et al., 2017). Bhardwaj et al. (2017) identified adaptive changes in genes with predicted or experimentally confirmed roles in CHG susceptibility (efrE), global nutritional stress response (relA), nucleotide metabolism (cmk), phosphate acquisition (phoU), and glycolipid biosynthesis (bgsB). Also Bhardwaj identified a link between serial sub-inhibitory CHG exposure and reduced daptomycin susceptibility.

Guzmán Prieto et al. (2017) concluded that increased CHG resistance may have been selected by microorganisms exposure to antiseptics.

CONCLUSIONS

Povidone iodine remains an effective antiseptic due to its antimicrobial properties and lack of resistance.

Octenidine dihydrochloride is a relatively recent antiseptic, compared with povidone iodine and chlorhexidine digluconate, but with promising antimicrobial results and with no resistance reported until now.

Chlorhexidine digluconate is a valuable antiseptic, but it should be used as a targeted antiseptic in applications where there are proven benefits in order to avoid new bacterial resistance cases.

There is clinical evidence that chlorhexidine digluconate based antiseptics are superior to povidone iodine based antiseptics.

New antiseptic treatments such as successive multiple-antiseptics application (chlorhexidine digluconate and povidone iodine) or antiseptic application along with low level direct current could help in fighting against resistant organisms and biofilm-producing organisms.

There is an increased reduced susceptibility to both antibiotics and antiseptics. Reduced susceptibility to some antiseptics and the potential for cross resistance to some antibiotics highlights the need to restrict the use of antiseptics. Hand hygiene could have a good impact in reduction and transmission of resistant organisms.

Antimicrobial effectiveness of antiseptics should be assessed periodically to overcome dissemination of resistant organisms.

The effect of prolonged exposures at low level concentrations of antiseptics on clinical pathogens should be assessed. Standardized methods could be developed to test and to oversee if antiseptics can trigger resistance and cross-resistance, in order to comply with the UE and USA regulations regarding the biocides marketing.

REFERENCES

Abdolrasouli A., Armstrong-James D., Ryan L., Schelenz S., 2017. *In vitro* efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with *Candida auris*. Mycoses, 60(11):758-763.

Al-Doori Z., Goroncy-Bermes P., Gemmell C.G., Morrison D., 2007. Low-level exposure of MRSA to octenidine dihydrochloride does not select for resistance. Journal of Antimicrobial Chemotherapy, 59(6):1280-1.

Alserehi H., Filippell M., Emerick M., Cabunoc M.K., Preas M.A., Sparkes C., Johnson J.K., Leekha S., 2017. Chlorhexidine gluconate bathing practices and skin concentrations in intensive care unit patients. American Journal of Infection Control, pii: S0196-6553(17)31002-7.

Alvarez-Marin R., Aires-de-Sousa M., Nordmann P., Kieffer N., Poirel L., 2017. Antimicrobial activity of octenidine against multidrug-resistant Gram-negative pathogens. European Journal of Clinical Microbiology & Infectious Diseases, 36(12):2379-2383.

Amalaradjou M.A., Norris C.E., Venkitanarayanan K., 2009. Effect of octenidine hydrochloride on planktonic cells and biofilms of *Listeria monocytogenes*. Applied and environmental microbiology, 75(12):4089-92.

Anderson M.J., David M.L., Scholz M., Bull S.J., Morse D., Hulse-Stevens M., Peterson M.L., 2015. Efficacy of skin and nasal povidone-iodine preparation against mupirocin-resistant methicillin-resistant *Staphylococcus aureus* and *S. aureus* within the anterior nares. Antimicrobial Agents Chemotherapy, 59(5):2765-73.

Anderson R.L., 1989. Iodophor antiseptics: intrinsic microbial contamination with resistant bacteria. Infection Control and Hospital Epidemiology, 10:443–446.

Babaei M.R.; Sulong A.; Hamat R.A.; Nordin S.A.; Neela V.K., 2015. Extremely high prevalence of antiseptic resistant Quaternary Ammonium

Compound E gene among clinical isolates of multiple drug resistant Acinetobacter baumannii in Malaysia. Annals of Clinical Microbiology and Antimicrobials, 14, 11.

Barkvoll P. , Rølla G., Svendsen K., 1989. Interaction between chlorhexidine digluconate and sodium lauryl sulfate *in vivo*. Journal of Clinical Periodontology, 16(9):593-5.

Bass P., Karki S., Rhodes D., Gonelli S., Land G., Watson K., Spelman D., Harrington G., Kennon J., Cheng A.C., 2013. Impact of chlorhexidine-impregnated washcloths on reducing incidence of vancomycin-resistant enterococci colonization in hematology-oncology patients. American Journal of Infection Control, 41(4):345-8.

Bhardwaj P., Hans A., Ruikar K., Guan Z., Palmer K.L., 2017. Reduced chlorhexidine and daptomycin susceptibility in vancomycin-resistant *Enterococcus faecium* after serial chlorhexidine exposure. Antimicrobial Agents and Chemotherapy, 62(1).

Bibi S., Shah S.A., Qureshi S., Siddiqui T.R., Soomro I.A., Ahmed W., Alam S.E., 2015. Is chlorhexidine-gluconate superior than Povidone-Iodine in preventing surgical site infections? A multicenter study. Journal of Pakistan Medical Association, 65(11):1197-201.

Bigliardi P.L., Alsagoff S.A.L., El-Kafrawi H.Y., Pyon J.K., Wa C.T.C., Villa M.A., 2017. Povidone iodine in wound healing: A review of current concepts and practices. International Journal of Surgery, 44: 260-268.

Brill F.H.H., Radischat N., Goroncy-Bermes P., Siebert J., 2015. Residual antiseptic efficacy of octenidine dihydrochloride versus chlorhexidine gluconate in alcoholic solutions. Antimicrobial Resistance and Infection Control, 4:P33.

Cherian B., Gehlot P.M., Manjunath M.K.., 2016. Comparison of the Antimicrobial Efficacy of Octenidine Dihydrochloride and Chlorhexidine with and Without Passive Ultrasonic Irrigation - An Invitro Study. Journal of Clinical and Diagnostic Research, 10(6):ZC71-7.

Climo M.W., Sepkowitz K.A., Zuccotti G., Fraser V.J., Warren D.K., Perl T.M., Speck K., Jernigan J.A., Robles J.R., Wong E.S., 2009. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. Critical Care Medicine, 37(6):1858-65.

Climo M.W., Yokoe D.S., MD, Warren D.K., Perl T.M., Bolon M., Herwaldt L.A., Weinstein R.A., MD8, Sepkowitz K.A., Jernigan J.A., Sanogo K., Wong S.E., 2013. Daily Chlorhexidine Bathing-Effect on Healthcare-associated BSI and MDRO Acquisition. The New England Journal of Medicine, 368(6): 533–542.

Cloete TE., 2003. Resistance mechanisms of bacteria to antimicrobial compounds. International Biodeterioration & Biodegradation. Volume 51, Issue 4, 277-282.

Conceição T., de Lencastre H., Aires-de-Sousa M., 2016. Efficacy of octenidine against antibiotic-resistant *Staphylococcus aureus* epidemic clones. Journal of Antimicrobial Chemotherapy, 71(10):2991-4.

Costa S.S., Viveiros M.M., Amaral L., Couto I., 2013. Multidrug Efflux Pumps in *Staphylococcus aureus*: an Update. The open microbiology journal, 7:59–71.

Danilevicius M., Juzénienė A., Juzénaitė-Karneckienė I., Veršinina A., 2015. MRSA decontamination using octenidine-based products. British Journal of Nursing, 24(15):S36.

Davies B.M., Patel H.C., 2016. Does chlorhexidine and povidone-iodine preoperative antisepsis reduce surgical site infection in cranial neurosurgery?. Annals of the Royal College of Surgeons of England, 98: 405–408.

Davies G.E., Francis J., Martin A.R., Rose F.L., Swain G., 1954. 1:6-Di-4'-chlorophenyldiguanidohexane (hibitane); laboratory investigation of a new antibacterial agent of high potency. British Journal of Pharmacology and Chemotherapy, 9(2):192-6.

Decker E.M., Bartha V., Kopunic A., von Ohle C.., 2017. Antimicrobial efficiency of mouthrinses versus and in combination with different photodynamic therapies on periodontal pathogens in an experimental study. Journal of Periodontal Research, 52(2):162-175.

DeMarco C.E., Cushing L.A., Frempong-Manso E., Seo S.M., Jaravaza T.A., Kaatz G.W., 2007. Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy,51:3235e3239.

Denton G.W., 2001. Chlorhexidine. In: Block S.S., Disinfection, Sterilization, and Preservation. Philadelphia, Lippincott Williams and Wilkins, Fifth Edition, 321-336.

Drosou A., Falabella A., Kirsner R.S., 2003. Antiseptics on wounds: an area of controversy. Wounds, 15:149–66.

Espigares E., Roldan E.M., Espigares M., Abreu R., Castro B., Dib A.L., Arias Á., 2017. Phenotypic Resistance to Disinfectants and Antibiotics in Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from Pigs. Zoonoses and Public Health, 64(4):272-280.

Fang C.T., Chen H.C., Chuang Y.P., Chang S.C., Wang J.T., 2002. Antimicrobial Agents and Chemotherapy, 46(6):2024-8.

Firanek C., Szpara E., Polanco P., Davis I., Sloand J., 2016. Comparison of Disinfection Procedures on the Catheter Adapter-Transfer Set Junction. Peritoneal Dialysis International, 36(2):225-7.

Gadea R., Glibota N., Pérez Pulido R., Gálvez A., Ortega E., 2017. Adaptation to Biocides Cetrimide and Chlorhexidine in Bacteria from Organic Foods: Association with Tolerance to Other Antimicrobials and Physical Stresses. Journal of Agricultural and Food Chemistry, 65(8):1758-1770.

Garrido-Benedicto P., Cueto-Quintana P., Farré-Termens E., Mariné-Cabré M., Riba-Reig J., Molina-Chueca R., 2017. Effect of daily cleaning with chlorhexidine on the incidence of contamination of blood cultures

in the critical patient. Enfermeria Intensiva, 28(3):97-104.

Ghivari S.B., Bhattacharya H., Bhat K.G., Pujar M.A., 2017. Antimicrobial activity of root canal irrigants against biofilm forming pathogens- An *in vitro* study. Journal of Conservative Dentistry, 20(3):147-151.

Ghobrial G.M., Wang M.Y., Green B.A., Levene H.B., Manzano G., Vanni S., Starke R.M., Jimsheleishvili G., Crandall K.M., Dididze M., Levi A.D., 2017. Preoperative skin antisepsis with chlorhexidine gluconate versus povidone-iodine: a prospective analysis of 6959 consecutive spinal surgery patients. Journal of Neurosurgery. Spine, 24:1-6.

Gomaa F.A.M., Helal Z.H., Khan M.I., 2017. High Prevalence of blaNDM-1, blaVIM, qacE, and qacED1 Genes and Their Association with Decreased Susceptibility to Antibiotics and Common Hospital Biocides in Clinical Isolates of *Acinetobacter baumannii.* Microorganisms, 5(2).

Gottardi W., 2001. Iodine and Iodine Compounds. In: Block S.S., Disinfection, Sterilization, and Preservation. Philadelphia, Lippincott Williams and Wilkins, Fifth Edition, 159-184.

Grinius L., Dreguniene G., Goldberg E.B., Liao C.H., Projan S.J., 1992. A staphylococcal multidrug resistance gene product is a member of a new protein family. Plasmid 27:119 –129.

Günther F., Blessing B., Tacconelli E., Mutters N.T., 2017. MRSA decolonization failure-are biofilms the missing link? Antimicrobial Resistance and Infection Control, 6:32.

Guo W., Shan K., Xu B., Li J., 2015. Determining the resistance of carbapenem-resistant *Klebsiella pneumoniae* to common disinfectants and elucidating the underlying resistance mechanisms. Pathogens and Global Health, 109:184e192.

Guzmán Prieto A.M., Wijngaarden J., Braat J.C., Rogers M.R.C., Majoor E., Brouwer E.C., Zhang X., Bayjanov J.R., Bonten M.J.M., Willems R.J.L., van Schaik W., 2017. The Two-Component System ChtRS Contributes to Chlorhexidine Tolerance in *Enterococcus faecium.* Antimicrobial Agents and Chemotherapy, 61(5).

Harke H.P., 1989. Octenidine dihydrochloride, properties of a new antimicrobial agent. Zentralblatt für Hygiene und Umweltmedizin, 188(1-2):188-93.

Harris P.N.A., Le B.D., Tambyah P., Hsu L.Y., Pada S., Archuleta S., Salmon S., Mukhopadhyay A., Dillon J., Ware R., Fisher D.A., 2015. Antiseptic Body Washes for Reducing the Transmission of Methicillin-Resistant *Staphylococcus aureus*: A Cluster Crossover Study. Open Forum Infectious Diseases, 2(2):ofv051.

Hassan K.A., Jackson S.M., Penesyan A., Patching S.G., Tetu S.G., Eijkelkamp B.A., Brown M.H., Henderson P.J., Paulsen I.T., 2013. Transcriptomic and biochemical analyses identify a family of chlorhexidine efflux proteins. Proceedings of the National Academy of Sciences of the United States of America, 110(50):20254-9.

Hayashi M., Kawamura K., Matsui M., Suzuki M., Suzuki S., Shibayama K., Arakawa Y., 2017. Reduction in chlorhexidine efficacy against multi-drug-resistant *Acinetobacter baumannii* international clone II. Journal of Hospital Infection, 95(3):318-323.

Hennig T.J., Werner S., Naujox K., Arndt A., 2017. Chlorhexidine is not an essential component in alcohol-based surgical hand preparation: a comparative study of two handrubs based on a modified EN 12791 test protocol. Antimicrobial Resistance & Infection Control, 6:96.

Herruzo R., Vizcaino M.J., 2017. Surgical antisepsis of hands: a two-step procedure with chlorhexidine to surpass the EN 12791. Journal of Hospital Infection, pii: S0195-6701(17)30650-3.

Hoekstra M.J., Westgate S.J., Mueller S., 2016. Povidone-iodine ointment demonstrates *in vitro* efficacy against biofilm formation. International Wound Journal, 14: 172-179.

Hübner N.O., Siebert J., Kramer A., 2010. Octenidine dihydrochloride, a modern antiseptic for skin, mucous membranes and wounds. Skin Pharmacology and Physiology, 23(5):244-58.

Jaglic Z.; Cervinkova D., 2012. Genetic basis of resistance to quaternary ammonium compounds—The qac genes and their role: A review. Veterinary Medicine, 57, 275–281.

Kaulfers P.M.,1995. Epidemiologie und Ursachen mikrobieller Biozidresistenzen. Zentralbl Hygiene Umweltmed, 197:252e259.

Kieran E.A., O'Sullivan A., Miletin J., Twomey A.R., Knowles S.J., O'Donnell C.P.F., 2017. 2% chlorhexidine-70% isopropyl alcohol versus 10% povidone-iodine for insertion site cleaning before central line insertion in preterm infants: a randomised trial. Archives of Disease in Childhood. Fetal and Neonatal, Edition 2017 Oct 26. pii: fetalneonatal-2016-312193.

Kıvanç S.A., Kıvanç M., Bayramlar H., 2016. Microbiology of corneal wounds after cataract surgery: biofilm formation and antibiotic resistance patterns. Journal of Wound Care, 25(1):12, 14-9.

Koburger T., Hübner N.O., Braun M., Siebert J., Kramer A., 2010. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. Journal of Antimicrobial Chemotherapy, 65(8):1712-9.

Kramer A., Dissemond J., Kim S., Willy C., Mayer D., Papke R., Tuchmann F., Assadian O., 2017. Consensus on Wound Antisepsis: Update 2018. Skin Pharmacology and Physiology, 31(1):28-58.

Kücken D., Feucht H., Kaulfers P., 2000. Association of qacE and qacEDelta1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. FEMS Microbiology Letters, 183(1):95-8.

Küng E., Pietrzak J., Klaus C., Walochnik J., 2016. *In vitro* effect of octenidine dihydrochloride against *Trichomonas vaginalis*. International Journal of Antimicrobial Agents, 47(3):232-4.

Kunisada T., Yamada K., Oda S., Hara O., 1997. Investigation on the efficacy of povidone-iodine against antiseptic-resistant species. Dermatology, 195 Suppl. 2:14-8.

Lanjri S., Uwingabiye J., Frikh M., Abdellatifi L., Kasouati J., Maleb A., Bait A., Lemnouer A., Elouennass M., 2017. *In vitro* evaluation of the susceptibility of *Acinetobacter baumannii* isolates to antiseptics and disinfectants: comparison between clinical and environmental isolates. Antimicrobial Resistance & Infection Control, 6:36.

Larson E.L, 1995. APIC guideline for handwashing and hand antisepsis in health care settings. American Journal of Infection Control, 23(4):251–269.

Levy S.B., 2002. Active efflux, a common mechanism for biocide and antibiotic resistance. Journal of Applied Microbiology, 92(Suppl):65se71s.

Liu Q., Liu M., Wu Q., Li C., Zhou T., Ni Y., 2009. Sensitivities to biocides and distribution of biocide resistance genes in quaternary ammonium compound tolerant *Staphylococcus aureus* isolated in a teaching hospital. Scandinavian Journal of Infectious Diseases, 41:403e409.

Liu Q., Zhao H., Han L., Shu W., Wu Q., Ni Y., 2015. Frequency of biocideresistant genes and susceptibility to chlorhexidine in high-level mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MuH MRSA). Diagnostic Microbiology and Infectious Disease, 82:278e283.

Lowe C.F., Lloyd-Smith E., Sidhu B., Ritchie G., Sharma A., Jang W., Wong A., Bilawka J., Richards D., Kind T., Puddicombe D., Champagne S., Leung V., Romney M.G., 2017. Reduction in hospital-associated methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* with daily chlorhexidine gluconate bathing for medical inpatients. American Journal of Infection Control, 45(3):255-259.

Mahzounieh M., Khoshnood S., Ebrahimi A., Habibian S., Yaghoubian M.., 2014. Detection of Antiseptic-Resistance Genes in *Pseudomonas* and *Acinetobacter* spp. Isolated From Burn Patients. Jundishapur Journal of Natural Pharmaceutical Products, 9(2):e15402.

Mayer S., Boos M., Beyer A., Fluit A. C., Schmitz F. J., 2001. Distribution of the antiseptic resistance genes qacA, qacB, and qacC in 497 methicillin-resistant and –susceptible European isolates of *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 47, 896–897.

McDonnell G., Russell A.D., 1999. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clinical microbiology reviews, p. 147-179.

Mendes E.T., Ranzani O.T., Marchi A.P., da Silva M.T., Filho J.U.A., Alves T., Guimarães T., Levin A.S., Costa S.F., 2016. Chlorhexidine bathing for the prevention of colonization and infection with multidrugresistant microorganisms in a hematopoietic stem cell transplantation unit over a 9-year period Impact on chlorhexidine susceptibility. Medicine, 95:46.

Mimoz O., Lucet J.C., Kerforne T., Pascal J., Souweine B., Goudet V., Mercat A., Bouadma L., Lasocki S., Alfandari S., Friggeri A., Wallet F., Allou N., Ruckly S., Balayn D., Lepape A., Timsit J.F., 2015. Skin antisepsis with chlorhexidine-alcohol versus povidone iodine-alcohol, with and without skin scrubbing, for prevention of intravascular-catheter-related infection (CLEAN): an open-label, multicentre, randomised, controlled, two-by-two factorial trial. 386(10008):2069-2077.

Mombeshora M., Mukanganyama S., 2017. Development of an accumulation assay and evaluation of the effects of efflux pump inhibitors on the retention of chlorhexidine digluconate in *Pseudomonas aeruginosa* and *Staphylococcus aureus.* BMC Research Notes, 10:382.

Moore G., Schelenz S., Borman A.M., Johnson E.M., Brown C.S., Yeasticidal activity of chemical disinfectants and antiseptics against *Candida auris.* Journal of Hospital Infection, 97(4):371-375.

Narayanan A., Nair M.S., Karumathil D.P., Baskaran S.A., Venkitanarayanan K., Amalaradjou M.A., 2016. Inactivation of *Acinetobacter baumannii* Biofilms on Polystyrene, Stainless Steel, and Urinary Catheters by Octenidine Dihydrochloride. Frontiers in Microbiology, 7:847.

Noguchi N., Suwa J., Narui K., Sasatsu M., Ito T., Hiramatsu K., Song J.H., 2005. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes qacA/B and smr of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. Journal of Medical Microbiology, 54(Pt 6):557-65.

Oancea S., Stoia M., 2010. Antibiotic resistance of bacterial pathogens: the magnitude of the problem from two perspectives - Romanian and worldwide. Rom. Biotech. Lett., 15 (5): 5519-5529.

Patrick S., McDowell A., Lee A., Frau A., Martin U., Gardner E., McLorinan G., Eames N., 2017. Antisepsis of the skin before spinal surgery with povidone iodinealcohol followed by chlorhexidine gluconate-alcohol versus povidone iodine-alcohol applied twice for the prevention of contamination of the wound by bacteria: a randomised controlled trial. The Bone & Joint Journal, 99-B(10):1354-1365.

Patters M.R., Anerud K., Trummel C.L., Kornman K.S., Nalbandian J., Robertson P.B., 1983. Inhibition of plaque formation in humans by octenidine mouthrinse. Journal of Periodontal Research, 18(2):212-9.

Paulsen I.T., Brown M.H., Skurray R.A., 1996. Proton-dependent multidrug efflux systems. Microbiology Rev., 60:575– 608.

Paulson D.S., 2003. Chlorhexidine gluconate. In: Paulson DS, Handbook of Topical Antimicrobials Industrial Applications, Industrial applications in consumer products and Pharmaceuticals: New York, Marcel Dekker, 117-122.

Privitera G.P., Costa A.L., Brusaferro S., Chirletti P., Crosasso P., Massimetti G., Nespoli A., Petrosillo N., Pittiruti M., Scoppettuolo G., Tumietto F., Viale P., 2017. Skin antisepsis with chlorhexidine versus iodine for the prevention of surgical site infection: A systematic review and meta-analysis. American Journal of Infection Control, 45(2):180-189.

Rajamohan G., Srinivasan V.B., Gebreyes W.A., 2010. Novel role of *Acinetobacter baumannii* RND efflux

transporters in mediating decreased susceptibility to biocides. Journal of Antimicrobial Chemotherapy, 65(2):228-32.

Reich P.J., Boyle M.G., Hogan P.G., Johnson A.J., Wallace M.A., Elward A.M., Warner B.B., Burnham C.A., Fritz S.A., 2016. Emergence of communityassociated methicillin-resistant *Staphylococcus aureus* strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clinical Microbiology and Infection, 645:e1e8.

Reiser M., Scherag A., Forstner C., Brunkhorst F.M., Harbarth S., Doenst T., Pletz M.W., Hagel S., 2017. Effect of pre-operative octenidine nasal ointment and showering on surgical site infections in patients undergoing cardiac surgery. Journal of Hospital Infection, 95(2):137-143.

Rezapoor M., Nicholson T., Tabatabaee R.M., Chen A.F., Maltenfort M.G., Parvizi J., 2017. Povidone-Iodine-Based Solutions for Decolonization of Nasal *Staphylococcus aureus*: A Randomized, Prospective, Placebo-Controlled Study. The Journal of Arthroplasty. 32(9):2815-2819.

Ripa S., Bruno N., Reder R.F., Casillis R., 2003. Clinical applications of Povidone-Iodine as a topical antimicrobial. In: Paulson DS, Handbook of Topical Antimicrobials Industrial Applications, Industrial applications in consumer products and Pharmaceuticals: New York, Marcel Dekker, 77-98.

Rotter M., 1999. Hand washing and hand disinfection. In: Mayhall CG, ed. Hospital epidemiology and infection control, 2nd ed. Philadelphia, PA, Lippincott Williams & Wilkins, 1339–1355.

Russell A.D., 2002. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. Symposium series (Society for Applied Microbiology), 121-135S.

Shamsudin M. N., Alreshidi M. A., Hamat R. A., Alshrari A. S., Atshan S. S., Neela V., 2012. High prevalence of qacA/B carriage among clinical isolates of meticillin-resistant *Staphylococcus aureus* in Malaysia. Journal of Hospital Infection, 81, 206–208.

Shelanski H.A., 1952. Mixtures of Polymeric N-Vinyl Pyrrolidone and Halogens. United States Patent Office 2, 739, 922.

Shi G.S., Boost M., Cho P., 2015. Prevalence of antiseptic-resistance genes in staphylococci isolated from orthokeratology lens and spectacle wearers in Hong Kong. Investigative Ophthalmology & Visual Science; 56:3069e3074.

Silas M.R., Schroeder R.M., Thomson R.B., Myers W.G., 2017. Optimizing the antisepsis protocol: Effectiveness of 3 povidoneiodine1.0% applications versus a single application of povidoneiodine 5.0. Journal of Cataract & Refractive Surgery, 43(3):400-404.

Slobodníková L., Hupková H., Koreň J., Záborská M., 2014. Antibiofilm activity of agents for disinfection of skin, mucosa, and wound on microorganisms isolated from patients with catheter-related infections. Epidemiol Mikrobiol Imunol, 63(3):168-72.

Smith, K.; Gemmell, C.G., Hunter, I.S., 2008. The association between biocide tolerance and the presence or absence of qac genes among hospital-acquired and community-acquired MRSA isolates. Journal of Antimicrobial Chemotherapy, 61, 78–84.

Spencer C., Orr D., Hallam S., Tillmanns E., 2013. Daily bathing with octenidine on an intensive care unit is associated with a lower carriage rate of meticillin-resistant *Staphylococcus aureus*. Journal of Hospital Infection. 83(2):156-9.

Tattawasart U., Maillard J.Y., Furr J.R., Russell A.D., 2000. Outer membrane changes in *Pseudomonas stutzeri* resistant to chlorhexidine diacetate and cetylpyridinium chloride. International Journal of Antimicrobial Agents, 16(3):233-8.

Upadhyay A., Chen C.H., Yin H., Upadhyaya I., Fancher S., Liu Y., Nair M.S., Jankelunas L., Patel J.R., Venkitanarayanan K., 2016. Inactivation of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 on cantaloupes by octenidine dihydrochloride. Food Microbiololy, 58:121-7.

Uppal S., Bazzi A., Reynolds R.K., Harris J., Pearlman M.D., Campbell D.A., Morgan D.M., 2017. Chlorhexidine-Alcohol Compared With Povidone-Iodine for Preoperative Topical Antisepsis for Abdominal Hysterectomy. Obstetrics and Gynecology, 130(2):319-327.

Vali L., Dashti A.A., Mathew F., Udo E.E., 2017. Characterization of Heterogeneous MRSA and MSSA with Reduced Susceptibility to Chlorhexidine in Kuwaiti Hospitals. Frontiers in Microbiology, 8:1359.

Velázquez-Meza M.E., Mendoza-Olazarán S., Echániz-Aviles G., Camacho-Ortiz A., Martínez-Reséndez M.F., Valero-Moreno V., Garza-González E., 2017. Chlorhexidine whole-body washing of patients reduces methicillinresistant *Staphylococcus aureus* and has a direct effect on the distribution of the ST5-MRSA-II (New York/Japan) clone. Journal of Medical Microbiology, 66(6):721-728.

Vernon M.O., Hayden M.K., Trick W.E., Hayes R.A., Blom D.W., Weinstein R.A., 2006. Chlorhexidine gluconate to cleanse patients in a medical intensive care unit: the effectiveness of source control to reduce the bioburden of vancomycin-resistant enterococci. Archives of Internal Medicine, 166(3):306-12.

Vogt P.M., Hauser J., Mueller S., Bosse B., Hopp M., 2017. Efficacy of Conventional and Liposomal Povidone-Iodine in Infected Mesh Skin Grafts: An Exploratory Study. Infectious Diseases and Therapy, 6(4):545-555.

Wales A.D., Davies R.H., 2015. Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens. Antibiotics, 4, 567-604.

Walsh B., Blakemore P.H., Drabu Y.J., 1987. The effect of handcream on the antibacterial activity of chlorhexidine gluconate. Journal of Hospital Infection, 9(1):30–33.

Wand M.E., Bock L.J., Bonney L.C., Sutton J.M., 2016. Mechanisms of Increased Resistance to Chlorhexidine and Cross-Resistance to Colistin following Exposure of *Klebsiella pneumoniae*

Clinical Isolates to Chlorhexidine. Antimicrobial Agents and Chemotherapy, 61(1).

Wang C., Cai P., Guo Y., Mi Z., 2007. Distribution of the antiseptic-resistance genes qacEDelta1 in 331 clinical isolates of *Pseudomonas aeruginosa* in China. Journal of Hospital Infection, 66(1):93-5.

Wang H., Ren D., 2017. Controlling *Streptococcus mutans* and *Staphylococcus aureus* biofilms with direct current and chlorhexidine. AMB Express, 7(1):204.

Wassenaar T.M., Ussery D., Nielsen L.N., Ingmer H., 2015. Review and phylogenetic analysis of qac genes that reduce susceptibility to quaternary ammonium compounds in *Staphylococcus* species. European Journal of Microbiology and Immunology, 5:44e61.

Welk A., Zahedani M., Beyer C., Kramer A., Müller G.., 2016. Antibacterial and antiplaque efficacy of a commercially available octenidine-containing mouthrinse. Clinical Oral Investigations, 20(7):1469-76.

WHO (World Health Organization), 2009. Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva.

WHO (World Health Organization), 2016. Global Guidelines for the prevention of surgical site infection. Geneva.

Wiegand C., Abel M., Ruth P., Elsner P., Hipler U.C., 2015. pH Influence on Antibacterial Efficacy of Common Antiseptic Substances. Skin Pharmacology and Physiology, 28:147–158

Willy C., Scheuermann-Poley C., Stichling M., von Stein T., Kramer A., 2017. Importance of wound irrigation solutions and fluids with antiseptic effects in therapy and prophylaxis : Update 2017. Der Unfallchirurg, 120(7):549-560.

Wu D., Lu R., Chen Y., Qiu J., Deng C., Tan Q., 2016. Study of cross-resistance mediated by antibiotics, chlorhexidine and *Rhizoma coptidis* in *Staphylococcus aureus.* Journal of Global Antimicrobial Resistance, 7:61-66.

Yamamoto T.; Tamura Y.; Yokota T., 1988. Antiseptic and antibiotic resistance plasmid in *Staphylococcus aureus* that possesses ability to confer chlorhexidine and acrinol resistance. Antimicrobial Agents and Chemotherapy, 32, 932–935.

Yamasaki K., Saito F., Ota R., Kilvington S., 2017. Antimicrobial efficacy of a novel povidone iodine contact lens disinfection system. Contact Lens & Anterior Eye, pii: S1367-0484(16)30195-3.

Yasuda H., Sanui M., Abe T., Shime N., Komuro T., Hatakeyama J., Matsukubo S., Kawano S., Yamamoto H., Andoh K., Seo R., Inoue K., Noda E., Saito N., Nogami S., Okamoto K., Fuke R., Gushima Y., Kobayashi A., Takebayashi T., Lefor A.K., 2017. Comparison of the efficacy of three topical antiseptic solutions for the prevention of catheter colonization: a multicenter randomized controlled study. Critical Care, 21(1):320.

Zhang M.; O'Donoghue M.M.; Ito T.; Hiramatsu K.; Boost M.V., 2011. Prevalence of antiseptic-resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonising nurses and the general population in Hong Kong. Journal of Hospital Infection, 78, 113–117.