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IN VITRO ACTIVITY OF CHLORHEXIDINE GLUCONATE AGAINST METHICILLIN-RESISTANT AND -SENSITIVE *STAPHYLOCOCCUS AUREUS* STRAINS

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Abstract - A wide variety of antimicrobial cationic agents, such as chlorhexidine gluconate, are commonly used in antiseptic preparations in the prevention of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). In this study was investigated the activity of chlorhexidine gluconate against MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA). The quantitative suspension test was carried out with 1% and 4% chlorhexidine gluconate and contact time of 30 s, 60 s and 120 s. Since the plasmid-borne gene *qacA/B* confers resistance to cationic antiseptic agents in *S. aureus*, activity was also examined with regard to the presence of *qacA/B*. The results indicate that neither 1% nor 4% chlorhexidine gluconate achieved a $\log_{10}$ reduction factors (RF) >5 against MRSA and MSSA strains at 30 s, 60 s and 120 s. At all concentrations, the RF for MRSA *qacA/B* negative strains were significantly higher when compared to MRSA *qacA/B* positive strains at 60 s and 120 s.

Key words: Antiseptics; chlorhexidine gluconate; methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, resistance, *qacA/B* gene; quantitative suspension tests

INTRODUCTION

The methicillin-resistant *Staphylococcus aureus* (MRSA) strains have become one of the most important nosocomial pathogens causing a wide range of diseases, from localized skin infections to life-threatening conditions such as pneumonia and endocarditis (Hiramatsu et al., 2001; Shamsudin et al., 2012). Since the hands of healthcare workers are the major source of transmission of nosocomial pathogens, treatment of hands with appropriate antiseptics is the most important measure to be taken in breaking the chain of transmission (Goroncy-Bermes et al., 2001). In both the United Kingdom and United States, antiseptic soaps and scrubs containing 2% and 4% chlorhexidine are mainly available for this purpose (Goroncy-Bermes et al., 2001).

Excessive use of antiseptic agents may result in the emergence of MRSA with reduced antiseptic susceptibility or even antiseptic resistance. At least 12 antiseptic resistance genes (*qacA* to *qacJ*, *smr* and *norA*) have been identified in *Staphylococcus* species, of which four (*qacA*, *qacB*, *qacC* and *norA*) are found mainly in clinical isolates of *S. aureus* (Noguchi et al., 2006). The *qacA* gene, located on chromosomes and on plasmids (pSK1 family), confers resistance to monovalent cations (ethidium, benzalkonium, cetrimide) and divalent cations (chlorhexidine, penta-

midine). The *qacB* gene, located on several plasmids such as β-lactamase and heavy metal-resistance plasmids (pSK23), confers resistance to monovalent cations and at low levels to some divalent compounds. Both determinants have been identified to confer resistance by means of proton motive force-dependent multidrug efflux (Paulsen et al., 1997).

The aim of the study was to determine the antimicrobial activity of chlorhexidine gluconate (1% and 4%) against MRSA and MSSA strains.

MATERIALS AND METHODS

A total of 100 randomly collected clinical strains of *S. aureus*, isolated from blood cultures and swabs, were used in this study. By polymerase chain reaction (PCR), the *mecA* gene was detected in 50 (50%) strains, which confirms that they were MRSA. The presence of *qacA/B* genes was also determined by PCR as shown previously (Opačić et al., 2010).

To determine the antimicrobial (bactericidal) activity of chlorhexidine gluconate, the quantitative suspension test was carried out according to Kampf et al. (1998). Briefly, per 0.1 mL of bacterial suspension was transferred to a control tube containing 9.9 mL sterile water (diluent) and to a reaction tube containing 9.9 mL antiseptic. The contact times were 30 s, 60 s and 120 s, as the biocides chosen for this study are used mainly as skin antiseptics and therefore have to be effective within a short time. After the contact times, a 10-fold dilution was made in the tube containing the neutralizer (combination of Tween 80 (3%), L-histidine (0.1%) and sodium thiosulfate (0.5%)). The combination of Tween 80 (3%), L-histidine (0.1%) and sodium thiosulfate (0.5%) was used to neutralize the activity of chlorhexidine gluconate.

Four further 10-fold dilutions were then made in the tubes containing the diluent. Aliquots were transferred from each tube to sheep blood agar plates and spread over the agar. The plates were incubated at 37° C for 24 h. Water (six 10-fold dilutions) and neutralizer served as a control. Tests were run in duplicate. Colony forming units (cfu) were counted from all plates and the means were calculated for each experiment. Reduction factors (RF) were calculated using the following formula: $F = \log_{10}\text{cfu (control)} - \log_{10}\text{cfu (antiseptic)}$. Log_{10} reductions of >5 were taken as indicative of satisfactory bactericidal activity.

Statistics

Data were compared using the *t* test; $p \leq 0.05$ were significant.

RESULTS

Chlorhexidine gluconate (1%) was not bactericidal (RF<5) for MRSA and MSSA at 30 s, 60 s and 120 s. At a concentration of 4%, RF<5 were also observed against MRSA and MSSA at all contact times. However, at both concentrations, the RF for MSSA were higher when compared to MRSA ($p>0.05$) at 120 s (Table 1). In a previous study (Opačić et al., 2010), the *qacA/B* gene, responsible for the resistance to monovalent and divalent cations, was detected in 17 of the total 100 strains (17%): in 16 out of the 50 MRSA (32%) and in 1 out of the 50 MSSA (2%) strains, $p<0.05$. At all concentrations, the reduction factors for MRSA *qacA/B* negative strains were significantly higher when compared to MRSA *qacA/B* positive strains at 60 s and 120 s, $p<0.05$ (Table 2). Irrespective of the contact time, the RF for MSSA *qacA/B* negative strains were not significantly higher when compared to MSSA *qacA/B* positive strains, $p>0.05$ (Table 3).

DISCUSSION

In a hospital setting, *S. aureus* including MRSA may produce bloodstream and surgical-site infections causing human infections of the skin and soft tissues, bones and joints, normal heart valves and abscesses. In addition to MSSA, MRSA is now a huge burden for most healthcare institutions and is by far the most significant antibiotic-resistant acquired pathogen the world has ever encountered (European Antimicrobial Resistance Surveillance System, 2005). The transfer of pathogenic bacteria via the hands of

Table 1. Comparative activity of chlorhexidine gluconate (1% and 4%) against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) at various contact times (30 s, 60 s and 120 s).

Concentration of chlorhexidine gluconate	Contact time	Mean RF*		*p* value
		MRSA	MSSA	
1%	30 s	0.19	0.16	0.261
	60 s	0.38	0.38	0.948
	120 s	0.69	0.72	0.644
4%	30 s	0.30	0.25	0.267
	60 s	0.51	0.51	0.974
	120 s	0.88	0.94	0.501

* Mean of reduction factors (N=50)

Table 2. Comparative activity of chlorhexidine gluconate (1% and 4%) against methicillin-resistant *Staphylococcus aureus* (MRSA) with regard to the *qacA/B* gene at various contact times (30 s, 60 s and 120 s).

Concentration of chlorhexidine gluconate	Contact time	Mean RF*		*p* value
		MRSA	MRSA	
		qacA/B$^-$	*qacA/B*$^+$	
1%	30 s	0.22	0.14	0.066
	60 s	0.43	0.29	0.026
	120 s	0.77	0.52	0.007
4%	30 s	0.34	0.22	0.091
	60 s	0.57	0.37	0.016
	120 s	0.96	0.72	0.028

* Mean of reduction factors (N=50)

Table 3. Comparative activity of chlorhexidine gluconate (1% and 4%) against methicillin-sensitive *Staphylococcus aureus* (MSSA) with regard to the *qacA/B* gene at various contact times (30 s, 60 s and 120 s).

Concentration of chlorhexidine gluconate	Contact time	Mean RF*		*p* value
		MSSA	MSSA	
		qacA/B$^-$	*qacA/B*$^+$	
1%	30 s	0.16	0.02	0.273
	60 s	0.39	0.08	0.278
	120 s	0.72	0.52	0.619
4%	30 s	0.25	0.11	0.469
	60 s	0.51	0.14	0.268
	120 s	0.94	0.75	0.682

* Mean of reduction factors (N=50)

staff in healthcare centers is known as a source of outbreaks of nosocomial infections and might be significantly reduced by the use of antiseptics (Messager et al., 2001).

Chlorhexidine gluconate has been incorporated into a number of hand-hygiene preparations. Aqueous or detergent formulations containing 0.5% or 0.75% chlorhexidine are more effective than plain

soap, but they are less effective than antiseptic detergent preparations containing 4% chlorhexidine gluconate. Preparations with 2% chlorhexidine gluconate are slightly less effective than those containing 4% chlorhexidine (Lowbury and Lilly, 1973; Paulson, 1994).

The effect of chlorhexidine-containing scrubs on microorganisms is controversial. Although the antimicrobial efficacy of this active agent was demonstrated by some investigators, others recorded resistance to chlorhexidine, especially in the case of gram-negative species. However, a limited effectiveness of chlorhexidine has also been shown with MRSA (Goroncy-Bermes et al., 2001). Suller and Russell (1999) showed MRSA strains to be less susceptible than MSSA to chlorhexidine. In contrast, Baddour (2008) described a remarkably pronounced effect of chlorhexidine against both MRSA and MSSA.

Against MRSA, the chlorhexidine-containing scrub and the nonmedicated handwash products were significantly less active ($\log_{10}$ RF values of 1.00 and 2.60, respectively), whereas the alcoholic hand disinfectant demonstrated the highest activity ($\log_{10}$ RF=5.22). In fact, 2% chlorhexidine was not sufficiently effective against MRSA at the recommended contact time of 1 minute as was the case with vancomycin-resistant enterococci and high-level gentamicin-resistant enterococci (Goroncy-Bermes et al., 2001). Neither chlorhexidine detergent scrub (4%) nor liquid soap (20% palm oil and potassium fatty acid) was completely effective in removing MRSA from contaminated fingertips (Huang et al., 1994). On the other hand, at a concentration of 2%, $\log_{10}$ RF>5 was observed against MSSA at 30 s, 60 s and 5 min and against MRSA only after 60 s and 5 min. At all mentioned reaction times, chlorhexidine gluconate (4%) was highly bactericidal ($\log_{10}$ RF>5) for MRSA and MSSA (Kamf et al., 1998), which is in contrast to the results of this study.

The varying effect of chlorhexidine upon clinical strains, as observed in different tests, is of importance as it may mean that certain strains will have an ability to survive chlorhexidine treatment and that the use of biocides could act as a selective pressure to allow these strains to predominate (Vali et al., 2008). Many investigators call attention to the fact that the *qacA/B* genes are present in 47.9%, 63%, 80% and 83.3% of Japanese, European, Brazilian and Malaysian clinical MRSA strains, respectively (Noguchi et al., 2005; Mayer et al., 2001; Miyazaki et al., 2007; Shamsudin et al., 2012). In addition, *qacA/B* genes are detected in 2 (4%) of 50 MSSA strains (Nakipoğlu et al., 2012). Since nucleotide sequencing analysis revealed the presence of two genetic variants in *qacA* (V1 and V2) and four variants in *qacB* (V1-V4), some findings revealed that *qacA* and *qacB* are prevalent in MRSA and that variable genetic variants of these genes may be responsible for the different resistance levels against antiseptic substances (Alam, 2003).

In conclusion, this study gives insight into the activity of chlorhexidine gluconate (1% and 4%) with insignificant differences between their effects against methicillin-resistant and -sensitive *S. aureus* strains.

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