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Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant

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Summary The efficacy of a new skin disinfectant, 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (IPA) (ChloraPrep®), was compared with five commonly used skin disinfectants against *Staphylococcus epidermidis* RP62A in the presence or absence of protein, utilizing quantitative time-kill suspension and carrier tests. All six disinfectants [70% (v/v) IPA, 0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG, 0.5% (w/v) CHG in 70% (v/v) IPA and 10% (w/v) aqueous povidone iodine (PI)] achieved a $\log_{10}$ reduction factor of 5, in colony-forming units/mL, in a suspension test (exposure time 30 s) in the presence and absence of 10% human serum. Subsequent challenges of *S. epidermidis* RP62A in a biofilm (with and without human serum) demonstrated reduced bactericidal activity. Overall, the most effective skin disinfectants tested against *S. epidermidis* RP62A were 2% (w/v) CHG in 70% IPA and 10% (w/v) PI. These results suggest that enhanced skin antisepsis may be achieved with 2% (w/v) CHG in 70% (v/v) IPA compared with the three commonly used CHG preparations [0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG and 0.5% (w/v) CHG in 70% (v/v) IPA].

Introduction

Coagulase-negative staphylococci are frequently associated with catheter-related bloodstream infections.[1,2] A characteristic feature of these micro-organisms is their ability to adhere and form biofilms on prosthetic devices, resulting in resistance to antimicrobial agents. In order to reduce the risk of microbial colonization and subsequent sepsis of peripheral vascular catheters, it is recommended that the skin insertion site should be disinfected for 30 s with an antimicrobial solution.[3] A chlorhexidine preparation is preferred;

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however, povidone iodine (PI) or 70% isopropyl alcohol (IPA) may be used.[4-6] These agents use different modes of action to achieve antisepsis, which may be reduced in the presence of organic matter.[7,8] Two percent chlorhexidine gluconate (CHG) preparations have not been universally available in the UK. Recently, a 2% (w/v) CHG in 70% (v/v) IPA solution (ChloraPrep®: Medi-Flex® Incorporated; Kansas, USA) for skin decontamination has been developed and is currently under review for approval by the Medicines and Healthcare Products Regulatory Agency (UK) for marketing authorization. Clinical studies have demonstrated that this skin disinfectant provided a significantly better and more persistent antimicrobial activity than 70% (v/v) IPA or 2% (w/v) aqueous CHG at 24 h in patients receiving pre-operative skin antisepsis on abdominal and inguinal sites ($N=106$).[9] This enhanced residual antimicrobial activity may also potentially reduce the risk of subsequent phlebitis for patients requiring a peripheral vascular catheter.

The criterion for determining the antimicrobial activity of a disinfectant is usually the rate of reduction of the number of viable micro-organisms when exposed to the antiseptic agent. The most widely recognized definition with regards to bactericidal activity is a $\log_{10}$ reduction factor of 5.[10] Assessing the efficacy of a disinfectant may be undertaken by various quantitative in vitro methods including suspension tests and carrier tests.[11]

The aim of the present study was to determine the antimicrobial efficacy of 2% CHG in 70% (v/v) IPA, which has recently become available in the UK, and to compare it with 70% (v/v) IPA, 10% (w/v) aqueous PI, 0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG and 0.5% (w/v) CHG in 70% (v/v) IPA utilizing quantitative in vitro time-kill tests against *S. epidermidis* RP62A at 30 s. Suspension tests were used to determine the effectiveness of the disinfectant in reducing the potential risk from impaction on insertion of vascular catheters. Although biofilm formation develops following medical device insertion, some disinfectants have residual activity. Therefore, in addition to the suspension tests, carrier tests were undertaken to evaluate the potential inhibition of biofilms on disinfectant activity.

Methods

Six skin disinfectants were evaluated: 70% (v/v) IPA (BDH; Poole, UK) was prepared by diluting 100% (v/v) IPA in sterile distilled water; 0.5% (w/v) and 2% (w/v) aqueous CHG (Sigma; St Louis, USA) were prepared by diluting 20% (w/v) CHG in sterile distilled water; 0.5% (w/v) CHG in 70% (v/v) IPA (Adams Healthcare; Leeds, UK); 2% (w/v) CHG in 70% (v/v) IPA (Medi-Flex® International; Kansas, USA) and 10% (w/v) aqueous PI (Seton Healthcare; Oldham, UK).

Evaluation of the efficacy of the antimicrobial agents was undertaken at 30 s; the recommended time for disinfecting the intended skin site of a peripheral vascular catheter prior to insertion.[3]

A neutralizing agent was prepared containing 2% (v/v) Tween 80 (BDH; Poole, UK), 1.17% (w/v) lecithin (Fisher Scientific; Loughborough, UK), 0.1% (v/v) Triton X-100 (Sigma; St Louis, USA) and 0.5% (w/v) sodium thiosulphate (BDH; Poole, UK) in sterile distilled water. This was sterilized at 121 °C for 15 min and then stored at 4 °C until required. Prior to commencing the antimicrobial time-kill studies, verification of the effectiveness and non-toxicity of the chosen neutralizing agent against the range of antimicrobial agents and the efficacy of the antimicrobial agents against the challenge micro-organisms were determined.

S. epidermidis RP62A stored on microbank beads (Pro-Lab Diagnostics; Ontario, Canada) was revived by placing one bead in 3 mL brain heart infusion (BHI) broth (Oxoid; Basingstoke, UK) and incubating at 37 °C in air for 24 h. *S. epidermidis* RP62A is a reference biofilm-positive strain and 'slime' producer, which was confirmed under current test conditions by Freeman *et al.*'s technique.[12]

In the suspension test, 10 μL broth containing 3×10^6 colony-forming units (cfu) *S. epidermidis* RP62A was added to 990 μL disinfectant and mixed. After 30 s contact time at room temperature, 100 μL suspension was removed and added to 900 μL neutralizing agent, mixed and left to dwell for 5 min. Serial dilutions were inoculated on to BHI agar plates which were incubated at 37 °C in air for up to 48 h. Further suspension tests were undertaken by adding 10% (v/v) human serum (Sigma; St Louis, USA) to the suspension prior to adding the disinfectant. The evaluations were carried out in triplicate.

To evaluate the efficacy of the disinfectants against a biofilm, a carrier test was undertaken with a 96-well polystyrene flat-bottomed microtitre tray (Immulon® 2HB Thermo Labsystems; Franklyn, MA, USA). A suspension of *S. epidermidis* RP62A was diluted in BHI to approximately 1×10^4. Two-hundred-microlitre aliquots of the suspension were inoculated into 16 wells of a sterile microtitre tray. This was then covered with a microplate sealer (Greiner-Bio-One; Gloucester, UK) and incubated at 37 °C in air for 24 h. Confirmation of biofilm production was undertaken by O'Toole and Kolter's[13] technique. To determine the efficacy of the disinfectants against a biofilm in the presence of protein, the carrier test was repeated; a suspension of *S. epidermidis* RP62A was diluted in BHI to approximately 1×10^4 cfu/mL and 10% human (v/v) serum was added.

The cells in suspension in each well were removed by inversion of the plate; the wells were then washed with 250 μL phosphate-buffered saline (PBS). Two-hundred microlitres of the selected disinfectant was added to each well and allowed to dwell for 30 s. The disinfectant was aspirated and 250 μL neutralizing agent was added to each well and left for 5 min. The neutralizing agent was removed by inversion of the tray, and the microtitre wells were washed with PBS. Removal of the biofilm from the microtitre well was undertaken by adding a 200-μL aliquot of BHI to each inoculated well. With a sterile pipette tip, the walls of the microtitre wells and base were scraped 10 times and the BHI was removed from each well and collected. This procedure was repeated a further three times and the inoculum was mixed thoroughly. Previous studies had demonstrated that four consecutive scrapes were required to remove >99% of the micro-organisms in a biofilm attached to a micro-titre well; successive scrapes failed to statistically reduce this number further. The numbers of viable *S. epidermidis* RP62A in suspension were enumerated by serial dilutions, and 100 μL of each dilution was inoculated on to BHI agar plates. The plates were then incubated at 37 °C in air for up to 48 h. Tests and controls were carried out 16 times.

Statistical analysis

Data were compared using the Mann-Whitney *U*-test. *P* values of equal to or less than 0.05 were regarded as significant.

Results

In all tests, the controls containing no disinfectant resulted in a complete recovery of the initial inocula.

Table I outlines the results of the suspension and carrier tests in both the presence and absence of protein. Efficacy of the disinfectant activity is represented as the $\log_{10}$ reduction factor of the initial cfu/mL. None of the skin disinfectants tested achieved a $\log_{10}$ reduction factor >5 in all four tests. Four disinfectants [70% (v/v) IPA, 0.5% (w/v) CHG in 70% (v/v) IPA, 2% (w/v) CHG in 70% (v/v) IPA and 10% (w/v) aqueous PI] achieved a $\log_{10}$ reduction factor >5 at 30 s in the suspension tests, both in the presence and absence of human serum, and in the carrier test when challenged with *S. epidermidis* RP62A in a biofilm.

When evaluating the effectiveness of the six disinfectants against *S. epidermidis* RP62A in a biofilm enriched with 10% (v/v) human serum, 70% (v/v) IPA, 0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG and 0.5% (w/v) CHG in 70% (v/v) IPA achieved a $\log_{10}$ reduction factor between 2 and 4 at 30 s. In comparison, 2% (w/v) CHG in 70% (v/v) IPA and 10% (w/v) aqueous PI achieved a $\log_{10}$ reduction factor of between 4 and 5. There was no statistical difference between the two disinfectants on analysis ($P=0.28$).

Table I Comparing the efficacy of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (IPA) against five standard skin disinfectants on *Staphylococcus epidermidis* RP62A after 30 s of contact time utilizing suspension and carrier tests

Antiseptic	$\log_{10}$ reduction factor in cfu/mL of *S. epidermidis* RP62A			
	Suspension test	Suspension test with 10% human serum	Carrier test: biofilm	Carrier test: biofilm enriched with 10% human serum
2% (w/v) CHG in 70% (v/v) IPA	6.5	6.3	5.3	**4.7**
70% (v/v) IPA	6.5	6.3	5.4	**2.8**
0.5% (w/v) aqueous CHG	6.5	6.3	**4.1**	**2.3**
2% (w/v) aqueous CHG	6.5	6.3	**4.8**	**2.8**
0.5% (w/v) CHG in 70% (v/v) IPA	6.5	6.3	5.8	**3.6**
10% (w/v) aqueous povidone iodine	6.5	6.3	5.9	**4.4**

cfu, colony-forming units. Bold type indicates a failure to achieve a $\log_{10}$ reduction factor of 5.

Discussion

This study compared the antimicrobial effectiveness of 2% (w/v) CHG in 70% (v/v) IPA with five standard skin disinfectants. The findings demonstrated that the range of disinfectants tested were capable of achieving a $\log_{10}$ reduction factor of 5, in cfu/mL, when in suspension both in the presence and absence of protein. However, when challenged with *S. epidermidis* RP62A in a biofilm (with or without protein), the antimicrobial effectiveness was reduced, thus reflecting previous reports that disinfectants may be inhibited in the presence of organic matter.[7,8]

The application of effective skin antisepsis is essential in the strategy to reduce catheter-related sepsis. The Centers for Disease Control and Prevention[4] recommend the use of a 2% chlorhexidine-based preparation for skin decontamination prior to line insertion, but do not specify the use of either an aqueous solution or one in 70% IPA. Pratt *et al*.[5] and the National Institute for Clinical Excellence guidelines[6] recommend an alcoholic chlorhexidine solution but do not specify a concentration. This study supports the recommendation of a chlorhexidine in alcohol product. Indeed, the in vitro results suggest that 2% (w/v) CHG in 70% (v/v) IPA offers an improved antimicrobial effect compared with all three standard preparations of CHG currently available in the UK [0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG and 0.5% (w/v) CHG in 70% (v/v) IPA] when challenged with *S. epidermidis* RP62A in a biofilm in the presence of 10% human serum ($P=0.0001$).

Further in vitro studies are required to assess the potential clinical effectiveness of 2% (w/v) CHG in 70% (v/v) IPA against a wider range of pathogens. In addition, assessment of the residual antiseptic activity on the skin compared with other commercially available chlorhexidine preparations needs to be studied. This study, however, suggests that 2% (w/v) CHG in 70% (v/v) IPA may offer advantages over the other chlorhexidine products available. In vivo studies are required to assess the effectiveness of this product in the clinical situation.

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