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Comparison of efficacy of some hand antiseptics against meticilline-resistant *Staphylococcus aureus*

Bazı el antiseptiklerinin metisiline-dirençli Staphylococcus aureus'a karşı etkilerinin karşılaştırılması

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SUMMARY

The effectiveness of four different hand antiseptics (chlorohexidine gluconate 4 %, benzalconium chloride 10 %, 2 - propanol 70 % and mixture of ethanole 40 % + isopropanol 30 % + chlorohexidine gluconate 0.1 %) were assesed by the quantitative suspension test against meticilline-resistant Staphylococcus aureus (S.aureus ATCC 43300). Antiseptics which contain benzalconium chloride 10 %, 2 - propanol 70 %, chlorohexidine gluconate 4 % were found effective at in-use concentrations and a contact time of 5 min., however the antiseptic which contains mixture of ethanole 40 % + isopropanole 30 % + chlorohexidine gluconate 0.1 % was not found effective.

Key words: *antiseptics, MRSA, quantitative suspension test*

ÖZET

Dört farklı el antiseptiğinin (% 10'luk benzalkonyum klorür, % 70'lik 2 - propanol, % 4'lük klorheksidin glukonat ve % 40'lık etanol + % 30'luk isopropanol + % 0.1'lik klorheksidin glukonat karışımının), metisiline-dirençli Staphylococcus aureus (S.aureus ATCC 43300)'a karşı etkileri, kantitatif süspansiyon testi kullanılarak değerlendirilmiştir. % 10'luk benzalkonyum klorür, % 70'lik 2 - propanol ve % 4'lük klorheksidin glukonat içeren antiseptikler, kullanılması önerilen konsantrasyonlarda ve 5 dakikalık temas süresinde etkili bulunmuştur. Bununla birlikte % 40'lık etanol + % 30'luk isopropanol + % 0.1'lik klorheksidin glukonat karışımı içeren antiseptik aynı etkiyi göstermemiştir.

Anahtar kelimeler: *antiseptikler, MRSA, kantitatif süspansiyon testi*

INTRODUCTION

Antiseptics are used to clean and disinfect wounds, mucous membranes, hands and operation sites. They are also occasionally used to treat carriers and dispersers of multi-resistant strains of bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) (1).

MRSA infections increase mortality, prolong hospitalization and more costly than infections caused by methicillin susceptible *Staphylococcus aureus* (MSSA) (2).

The purpose of this study was to determine the germicidal activity of four different hand antiseptics against MRSA.

MATERIAL AND METHOD

Bacteria

MRSA *(S.aureus ATCC 43300)* was obtained from University of Ankara, Faculty of Pharmacy, Department of Pharmaceutical Microbiology.

Antiseptics

The following hand antiseptics were tested: mixture of ethanole 40 % + isopropanole 30 % + chlorohexidine gluconate 0.1 %, chlorohexidine gluconate 4 %, 2 - propanol 70 %, benzalconium chloride 10 %.

Sterile distilled water was used as a diluent and disinfectant control. The disinfectants were stored

in the dark at room temperature.

Neutralization/recovery system

Neutralizer efficacy is important for accurate determination of the efficacy of an antiseptic or disinfectant. Neutralizer (0.5 % Tween 80 in tryptase soy broth) was previously tested to determine whether it was appropriate to inactivate each of the chemicals.

To mimic test conditions, 100 μl of sterile distilled water was added to 900 μl of the disinfectant at the highest use concentration, mixed and left for 1 min. then 10 μl of this mixture was added to 990 μl of the neutralization/recovery medium. 10 μl of the undiluted test suspension of MRSA was added to this mixture (neat), vortex mixed for 20 s. and serially diluted to 10^{-5} in Ringer's solution only. 100 μl of the neat and subsequent dilutions were spread onto Tryptase Soy Agar (TSA) (Merck, Darmstadt, Germany) in duplicate, using sterile dispencers. The plates were incubated at 37 °C for 24 h. and colony-forming units (cfu) were enumerated. The undiluted test suspension was used as the initial count.

The test was repeated using water instead of the disinfectant as the control. The neutralizer was deemed suitable as there was no difference in colony size, growth rate or the number of cfu retrieved from tests and controls. This shows the neutralization/recovery system was effective and not inhibitory (3).

Assessment of Antiseptic Activity

Susceptibility testing was performed using the quantitative suspension test. A single

isolated colony of bacteria was removed from TSA plates and grown separately in 10 ml of Tryptase Soy Broth (TSB) (Merck, Darmstadt, Germany) for 24 h. at 37°C. After incubation, the tubes were centrifuged for 20 min. at 2000 rpm with a rotor centrifuge. The cell pellets were washed with 10 ml of TSB. Then bacterial suspensions in TSB were adjusted to the Mc Farland 0.5 standard. In brief, 100 μl of bacterial suspension was added to 900 μl of the antiseptic or disinfectant solutions at room temperature for a contact time of 5 minutes, and then 10 μl was removed to 990 μl of the neutralization system and serially diluted to 10^{-1} to 10^{-3}. 100 μl of each dilution was placed onto TSA plates in duplicate by the spread-plate technique and incubated at 37°C for 18 to 24 h. Then surviving colonies were enumerated and expressed as colony-forming units per milliliter. The reduction rate was calculated as the expression of the disinfectant efficacy, according to the following formula:

log10 reduction = log10 pre-disinfection count - log10 disinfection count.

Log10 reductions of 5 or more were taken as an indication of satisfactory microbicidal activity (4).

RESULTS

The results of the suspension tests are presented as log10 reductions of test bacteria after 5 minutes of contact. Chlorohexidine gluconate 4 %, 2 - propanol 70 %, benzalconium chloride 10 %, when tested at a contact time of 5 min., achieved the pass criteria of at least an microbicidal effect (ME) (log reduction) of 5. Mixture of ethanole 40 % + isopropanol 30 % + chlorohexidine gluconate 0.1 % showed minimal activity, mean ME of 3.19. (Table 1).

Table 1. Efficacy of some hand antiseptics against MRSA (*S.aureus* ATCC 43300) by the Quantitative Suspension Test after 5 minutes of contact.

	Chlorohexidine gluconate 4 %	Benzalconium chloride 10 %	Propanol 70 %	Mixture of ethanole 40 % + isopropanole 30 % + chlorohexidine gluconate 0.1 %
Log10 reductions of microbial count (inoculum size: 1 to 2 x 10^8)	7.15	7.15	7.15	3.19

DISCUSSION

MRSA is a type of *Staphylococus* that is resistant to antibiotics called beta-lactams. Beta-lactam antibiotics include methicillin and other more common antibiotics such as oxacillin, penicilin and amoxicillin. In the outbreaks of MRSA, the environment has not played a significant role in the transmission of MRSA. MRSA is transmitted most frequently by direct skin-to-skin contact. The majority of MRSA infections occur among patients in hospitals or other healthcare settings; however, it is becoming more common in the community setting. You can protect yourself from infections by practicing good hygiene applications (5).

The importance of efficacy in choosing the right hand hygiene product is reflectedin the new Centers for Disease Control and Prevention guideline on hand hygiene. The best antimicrobial efficacy can be achieved with ethanol (60 to 85 %), isopropanol (60 to 80 %), and n-propanol (60 to 80 %). The activity is broad and immediate. The combination of alcohols may have a synergistic effect.

The antimicrobial efficacy of chlorhexidine (2 to 4 %) is lower and slower. Additionally, this agent has a risk of bacterial resistance. It is often supported by the mechanical removal of pathogens during hand washing. Taking the antimicrobial efficacy and the mechanical removal together, it is still less effective than the alcohols. Plain soap and water has the lowest efficacy of all (6).

The quantitative suspension test has been used to measure the antimicrobial effectiveness of such agents. This test clarifies a linkage between the time and the concentration used in the procedure. It does not require much equipment, and is easy and inexpensive to perform (4).

All of the hand antiseptics that we used in our study was effective on MRSA, except the mixture of ethanole 40 % + isopropanole 30 % + chlorohexidine gluconate 0.1 %. In this sample percentage of the alcohol is lower. This study confirms that the best antimicrobial efficacy can be achieved with alcohol (60 to 80 %). Also the percentage of chlorohexidine gluconate is lower in this sample.

According to these results, chlorohexidine gluconate 4 %, propanol 70 %, benzalconium chloride 10 % can be used against MRSA. However mixture of ethanole 40 % + isopropanole 30 % + chlorohexidine gluconate 0.1 % can not showed adequate efficacy against MRSA.

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