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THE EFFECTS OF ALCOHOL-BASED ANTISEPTIC SOLUTIONS AGAINST STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PYOGENES

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ABSTRACT

Introduction: Staphylococcus aureus and Streptococcus pyogenes are gram positive bacteria which can cause various diseases. The use of antiseptics is an effort that can be done in order to break the chain of transmission and reduce morbidity due to infection by microorganism. Alcohol as the main ingredients of antiseptics is probably the oldest and most widely used in various antiseptic products on the market. Despite their easy and practical use, reports of incidents of resistance to alcohol-based antiseptic agents to bacteria have been reported from several studies.

Aim: Determine the inhibition of alcohol-based antiseptic solutions against Staphylococcus aureus and Streptococcus pyogenes bacteria.

Methods : Kirby-Bauer disc-diffusion assays as susceptibility test. Zone of inhibition of growth diameter was performed in mm, with criteria resistance if ≤ 6 mm and sensitive if > 6 mm. We used 4 solutions namely A) ethanol 70%, B) ethanol 70% + H2O2 0,15%, C) ethanol 80%, D) ethanol 80% + H2O2 0,15%.

Result: The assay results on Staphylococcus aureus showed the zone of inhibition of growth for solution ethanol 70%, ethanol 70% + H2O2 0,15%, ethanol 80%, and ethanol 80% + H2O2 0,15% are less than 6 mm which indicated that the antiseptic solution do not inhibit bacterial growth, as well as for all solutions against Streptococcus pyogenes. Different results were obtained from solution D (ethanol 80% + H2O2 0.15%) on S. aureus which indicate the zone diameter is 21 mm and classified having the ability to inhibit bacterial growth (sensitive).

Conclusion: Exposure of gram positive bacteria, Staphylococcus aureus and Streptococcus pyogenes to clinically relevant concentrations of ethanol based antiseptic with or without hydrogen peroxide addition is not effective in inhibiting bacteria. Only an 80% alcohol-based antiseptic solution with the addition of H2O2 is still effective in inhibiting S. aureus . Further research needs to be done to review the recommended antiseptic formula and the need to add other ingredients to make an effective antiseptic in order to prevent infection.

Keywords: *Alcohol, Staphylococcus aureus, Streptococcus pyogenes*

ABSTRAK

Pendahuluan: Staphylococcus aureus dan Streptococcus pyogenes merupakan bakteri gram positif yang dapat menyebabkan berbagai penyakit. Penggunaan antiseptik merupakan upaya yang dapat dilakukan dalam rangka memutus rantai penularan dan mengurangi angka kesakitan akibat infeksi mikroorganisme. Alkohol sebagai bahan utama antiseptik mungkin merupakan yang tertua dan paling banyak digunakan dalam berbagai produk antiseptik yang beredar di pasaran. Meskipun penggunaannya mudah dan praktis, laporan kejadian resistensi agen antiseptik berbahan dasar alkohol terhadap bakteri telah dilaporkan dari beberapa penelitian.

Tujuan: Mengetahui daya hambat larutan antiseptik berbahan dasar alkohol terhadap bakteri Staphylococcus aureus dan Streptococcus pyogenes.

Metode : Uji difusi cakram Kirby-Bauer sebagai uji kerentanan. Zona hambat diameter pertumbuhan diukur dalam satuan mm, dengan kriteria resistensi jika ≤ 6 mm dan sensitif jika > 6 mm. Kami menggunakan 4 larutan yaitu A) etanol 70%, B) etanol 70% + H2O2 0,15%, C) etanol 80%, D) etanol 80% + H2O2 0,15%.

Hasil: Hasil pengujian terhadap Staphylococcus aureus menunjukkan zona hambat pertumbuhan untuk larutan etanol 70%, etanol 70% + H2O2 0,15%, etanol 80%, dan etanol 80% + H2O2 0,15% kurang dari 6 mm. yang menunjukkan bahwa larutan antiseptik tidak menghambat pertumbuhan bakteri, demikian juga untuk semua larutan melawan Streptococcus pyogenes. Hasil berbeda diperoleh dari larutan D (etanol 80% + H2O2 0,15%) pada S. aureus yang menunjukkan diameter zona 21 mm dan tergolong mempunyai kemampuan menghambat pertumbuhan bakteri (sensitif).

Kesimpulan: Paparan bakteri gram positif, Staphylococcus aureus dan Streptococcus pyogenes pada konsentrasi antiseptik berbasis etanol yang relevan secara klinis dengan atau tanpa penambahan hidrogen peroksida tidak efektif dalam menghambat bakteri. Hanya larutan antiseptik berbahan dasar alkohol 80% dengan penambahan H2O2 yang masih efektif menghambat S. aureus. Perlu dilakukan penelitian lebih lanjut untuk mengkaji formula antiseptik yang dianjurkan dan perlunya penambahan bahan lain untuk membuat antiseptik yang efektif guna mencegah infeksi.

Kata kunci: Alkohol, Staphylococcus aureus, Streptococcus pyogenes

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INTRODUCTION

Controlling the transmission of infectious diseases is one of the main challenges in today's medical world. Bacterial infection plays a significant role as a cause of illness and death in the world. Among them are gram-positive Staphylococcus bacteria which grow in clusters and *Streptococcus* which form in chains. Both are classified as gram-positive bacteria in the shape of coccus (1).

Staphylococcus aureus is a microorganism that can be both commensal and pathogenic and is suspected to be the agent of various diseases ranging from skin infections, septic arthritis, meningitis, sepsis, pneumonia, endocarditis, food poisoning to toxic shock syndrome. S. aureus as a commensal bacteria can be found in the ears, nose and in various other body locations (2). The optimal temperature for them to grow is 30°C to 37°C and is facultative anaerobic. Compared to other *Staphylococcus* species, S. *aureus* belongs to the most pathogenic species and was found most frequently from various samples taken from patients in hospital. Almost all patients with chronic renal failure undergoing hemodialysis, diabetic and chronic dermatitis have skin areas with S. *aureus* colonies. S. *aureus* pathogenicity is associated with its ability to produce enzyme catalase and coagulase (1,3,4).

Streptococcus pyogenes is a gram-positive group A cocci and another gram-positive bacteria which is clinically pathogen. These bacteria cause respiratory tract infections ranging from mild to moderate degrees such as pharyngitis and tonsillitis. They are also colonizes the skin causing pyoderma, such as impetigo, cellulitis, erysipelas and if it penetrates deeper into the tissue, it can cause fatal infections such as sepsis and streptococcal toxic-like syndrome. Recurrent infections can trigger autoimmune disorders, including acute glomerulonephritis (AGN), rheumatic fever, and rheumatic heart diseases(1,4).

The use of antiseptics is an effort that can be done in order to break the chain of transmission and reduce morbidity due to infection with microorganisms, including bacteria. Antiseptics are defined as biocides or antimicrobial processes apply at the skin or mucous membranes. Biocides defined as chemical or physical agent agent that typically inactivates broad spectrum of microorganisms and are divided into antiseptics and disinfectants (5). Antiseptics are used on skin or living tissue, while disinfectants on non-living objects. Antiseptic active ingredients that are widely used and have been approved by WHO include alcohol, iodine,

chlorhexidine gluconate (CHG), chlorine derivatives, triclosan, chloroxylenol (PCMX), and quaternary ammonium compounds (3).

Alcohols as the main ingredients of antiseptics is probably the oldest and most widely used in various antiseptic products on the market. They are commonly used in general public and health care provider such as hospitals and other health facilities. Alcohols are consider bactericidal rather than bacteriostatic, they also are fungicidal, and virucidal but do not destroy bacterial spores. The alcohol level recommended by WHO is 60 to 90%, while the Ministry of Health of the Republic of Indonesia recommends an alcohol concentration of 70 to 80% (3,6).

The types of alcohol that are often used are ethanol, isopropanol or isopropyl alcohol, and n-propanol (3,6,7). Relatively affordable and rapid antimicrobials with little or no residues little to no residues or environmental concerns following application are the advantages of alcohol. It is also relatively stable, odorless, and nontoxic. The ethanol-based antiseptic was found to have a broad spectrum of bactericidal activity to the common species causing nosocomial infections and the relevant emerging pathogens (5,8). Various studies prove alcohol-based antiseptic remove organisms more effectively and require less time, and also irritate skin less often than handwashing with soap or other antiseptic agents and water (9).

The bactericidal mechanism of alcohol, especially ethanol, is known through the process of dehydration and denaturation of bacterial protein components, resulting in impaired bacterial growth and metabolism. The reactive hydroxyl (-OH) group will forms hydrogen bonds with proteins, which leads to a loss of structure and function, end up to precipitation. Ethanol also causes plasma membrane leakage which leads to bacterial death (2,5,10).

Despite their easy and practical use, reports of incidents of resistance to alcohol-based antiseptic agents to bacteria have been reported from several studies (3,5). Its massive use allows bacteria to mutate to develop self-defense mechanism against the bactericidal effects of alcohol. This study aims to test the efficacy of an alcohol-based antiseptic against gram-positive bacteria, Staphylococcus aureus and Streptococcus pyogenes.

METHODS AND MATERIAL

The research design used in this study is in vitro experimental post test only control group. The antiseptic solution tested consisted of four types, namely 1) solution A consisting of ethanol 70% v / v,

glycerin 8% v / v, and water (add 100% v / v), 2) solution B consisting of ethanol 70 % v / v, H2O2 0.15% v / v, glycerin 8% v / v, and water (add 100% v / v), 3) C solution consisting of ethanol 80% v / v, glycerin 8% v / v, water (add 100% v / v), 4) solution D consisting of ethanol 80% v / v, H2O2 0.15% v / v, glycerin 8% v / v, water (add 100% v / v) . Concentrations are given as either percentage of volume (= ml / 100 ml, abbreviated % v / v). All the solutions were prepared as previously described in WHO guidelines (3).

The Gram positive bacteria used were Staphylococcus aureus ATCC 25923 and Streptococcus pyogenes ATCC 19615 that were obtained from the Central Health Laboratory Ministry of Health of the Republic of Indonesia, Surabaya, East Java, Indonesia.

Susceptibility test

The susceptibility test is used to determine the inhibitory effect of a substance against microorganisms. Staphylococcus aureus ATCC 25923 and Streptococcus pyogenes ATCC 19615 were cultured in blood agar for 24 hours. McFarland standards are used to prepare bacterial suspensions to be equivalent to the 0.5 McFarland. The bacterial suspension was then inoculated on Muller Hinton agar. The inhibitory test method used in this study is the Kirby-Bauer disc-diffusion method by immersing the blank disc in A-D solution then placing the disc on Mueller Hinton Agar (MHA) media that has been inoculated with a suspension of bacteria to be tested and incubated for 24 hours (11,12). The antiseptic solution A-D was tested on the culture of each bacteria. The interpretation of resistance and susceptibility is determined through the size of the zone of inhibition of growth. The zone of inhibition is interpreted resistance if the diameter is ≤ 6 mm and is interpreted sensitive if it is > 6 mm. Antibiotics used for controls were Gentamycin 10 mcg for Staphylococcus aureus ATCC 25923 and Clindamycin 2 mcg for Streptococcus pyogenes ATCC 19615. Controls were treated like previously described.

Study was carried out at the Center for Health Laboratory of the Ministry of Health of the Republic of Indonesia, 18th Karang Menjangan Street, Surabaya, East Java, Indonesia. Data were analyzed descriptively.

RESULT

After being incubated for 24 hours, the test material for A-D solution was observed and measured the zone of inhibition of growth. The zone margin is the area showing no obvious, visible growth that can be detected with the unaided eye. The zone diameter is shown in Table 1.

Table 1. Zone of inhibition of growth from disc diffusion assay

Antiseptic solutions	*Staphylococcus aureus* ATCC 25923	*Streptococcus pyogenes* ATCC 19615
A (Ethanol 70%)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)
B (Ethanol 70% + H_2O_2 0,15%)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)
C (Ethanol 80%)	Resistance (≤ 6 mm)	Resistance (≤ 6 mm)
D (Ethanol 80% + H_2O_2 0,15%)	**Sensitive** (21 mm)	Resistance (≤ 6 mm)
Controls (Gentamycin 10 mcg/ Clindamycin 2 mcg)	Sensitive (22 mm)	Sensitive (30 mm)

The assay results on Staphylococcus aureus showed the inhibition zone for solution A, B, and C were less than 6 mm which indicated that the antiseptic solution did not inhibit bacterial growth, as well as for all solutions against Streptococcus pyogenes as shown in figure 1-2. Different results were obtained from solution D (ethanol 80% + H2O2 0.15%) on S. aureus which indicates the zone diameter is 21 mm so it is classified as having the ability to inhibit bacterial growth (sensitive).

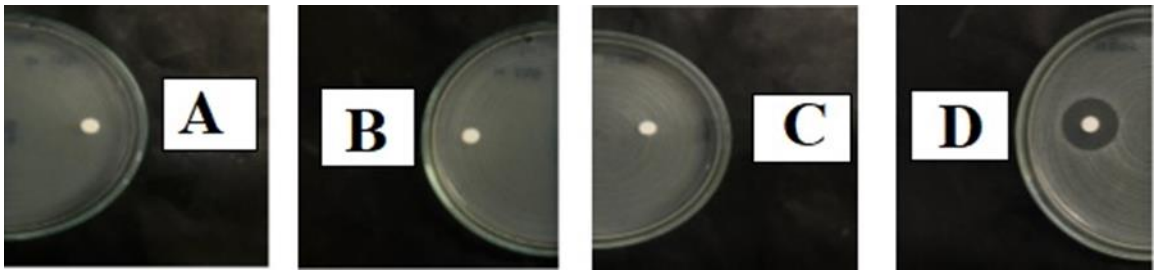

Figure 1. Disc diffusion assay of Stapylococcus aureus ATCC 25923

A.Ethanol 70% B. Ethanol 70% + H2O2 0,15% C. Ethanol 80% D. Ethanol 80% + H2O2 0,15%

Figure 2. Disc diffusion assay of Streptococcus pyogenes ATCC 19615

A.Ethanol 70% B. Ethanol 70% + H2O2 0,15% C. Ethanol 80% D. Ethanol 80% + H2O2 0,15%

DISCUSSION

In this study, the main ingredients of antiseptic solution used was ethanol 70% and 80% as the WHO recommended range. The use of alcohol more than 80% can coagulate the proteins in the bacterial cell wall, thus inhibiting further penetration into the cell and inhibits antibacterial activity furthermore. Glycerin addition aimed as skin conditioning agents because frequent use of alcohol-based antiseptics tends to cause skin irritation, dryness, and even surface tissue damage (3,5).

Disc diffusion assay showed that increasing ethanol dose up to 80% still did not show inhibition activity against S. aureus and S. pyogenes. The publication by Elzain, et al. reported that the optimal levels of ethanol and methanol for eradicating gram-positive bacteria are at levels of 90-100%, while lower concentrations (80%, 70%, 60%, and 50%) gave higher resistant rates (4). Another study suggests the use of an antiseptic that has at least 80% ethanol as the active ingredient because the antiseptic containing a total amount of up to 70% alcohol is significantly less effective (13) . Apart from that fact, the safety factor should still be considered for the use of antiseptics with higher ethanol levels because of the inflammable risk and skin irritation.

The addition of hydrogen peroxide (H2O2) in solutions B and D showed sensitivity to S. aureus at 80% ethanol levels, while S. pyogenes showed no inhibition against bacterial growth. The low concentration of H2O2 is incorporated in the formulations to help eliminate contaminating bacterial spores that cannot be destroy by ethanol. H2O2 is a strong oxidant that works as a biocide through the oxidation process and in combination with the presence of short-lived breakdown products such as the superoxide and hydroxyl radicals will amplify the process of microorganism damage. Hydrogen peroxide is classified as safe for the environment because it will quickly degraded into water and oxygen. Although H2O2 is considered more effective against gram-positive than gram-negative bacteria, the resistance results obtained in this study are probably due to the presence of enzyme catalase and other peroxidases. They reduces sensitivity to H2O2 due to the enzyme action which degrades peroxide (3,5).

Bacterial resistance to antiseptic agents can occur through several mechanisms. One of them is bacterial efflux systems that have been shown to efflux biocides. Staphylococcus aureus is known to have cytoplasmic proteins NorA which can reduce the accumulation of the

biocide and other substances in the cytoplasm (14).

The ability of gram-positive bacteria including S. aureus and S. pyogenes to form biofilms increases the incidence of resistance to antimicrobials and toxic chemicals, also resist the clearance mechanisms of the immune system (10,15). Biofilm are complex communities of organisms containing layers of bacteria within a glycocalyx, or other sources define it as communities of microorganisms developed on or associated with surfaces (5,16). Biofilm will inhibit the penetration of the biocide material and the components it produces can neutralize the activity of antimicrobial substances such as enzyme catalase and peroxide that have been previously mentioned. Unfortunately, exposure ethanol to biofilms has been shown to increase the number of biofilms formed and also enhances the biofilm-promoting genes, intercellular adhesin (ica) gene, beside other several antibiotics resistance genes. The formation of biofilm formation will increase as the higher concentration of ethanol (2,10).

The intercellular adhesin (ica) gene which consists of icaA and icaD expression regulate the production of polysaccharide intercellular adhesin, the major exopolysaccharide produced in some positive bacteria. Ethanol exposure showed increased ica expression by modulating the repressive gene icaR (15).

Several species of gram positive bacteria including Staphylococcus sp. and Streptococcus sp. have external capsules and slime layers on their cell walls. Its structure is a glycocalyx and consists of polysaccharide chains covers the entire cell surface. Although the contribution of external capsules and slime layers to resistance to antiseptic agents is not widely known, it is thought to function as a barrier of biocides penetration (5).

CONCLUSION

Exposure of gram positive bacteria, Staphylococcus aureus and Streptococcus pyogenes to clinically relevant concentrations of ethanol based antiseptic with or without hydrogen peroxide addition is not effective in inhibiting bacteria. Only an 80% alcohol-based antiseptic solution with the addition of hydrogen peroxide (H2O2) is still effective in inhibiting Staphylococcus aureus bacteria. Further research needs to be done to review the recommended antiseptic formula and the need to add other ingredients to make an effective antiseptic in order to prevent infection.

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