

Effectiveness of ethanol and methanol alcohols on different isolates of *staphylococcus* species

Abstract

Introduction: This is a perspective analytical study. The objectives of which were to identify the different *Staphylococcus* species that infects Human and to study their sensitivity to Alcohol. The study identified the best type and concentration of Alcohol to be used for cleaning the infected wounds using different isolates of *Staphylococci* known for its higher resistance to both antibiotics and alcohol.

Materials and methods: Isolates were sub cultured into Nutrient broth and incubated for 3-5hours. Then, 200µl of sub cultured Nutrient broth was added to 2ml Alcohol each time with different concentrations starting from 100% to 50% and a loop full of the mix was streaked at 10seconds, 30seconds, 1minute, and 5minutes.

Results: This study showed that *S. aureus* was the predominant as the most pathogenic species accounting for 50% of all isolates. This followed by *S. epidermidis* which accounted for 26.7% of isolates. Other species that were isolated included: *S. intermedius* (6.7 %), *S. schleiferi* (6.7 %), *S. saprophyticus* (3.3 %), *S. sciuri* (3.3 %), and *S. lugdunensis* (3.3 %). Ethanol effectiveness on *Staphylococcus* species showed higher effectiveness at higher concentrations (100%, 90% and 80%) and the effectiveness become as lower with lower concentrations (70%, 60%, and 50%) especially with shorter exposure times. Methanol effectiveness on *Staphylococcus* species showed also higher bactericidal effects of the absolute (100%) and the 90% concentrations while lower concentrations (80%, 70%, 60%, and 50%) gave higher resistant rates of *Staphylococci* especially with shorter exposure times.

Conclusion: The best concentrations found to be bactericidal for both Ethanol and Methanol were the Absolute and 90% with 100% and 90% effectiveness rate respectively. The results also showed that Ethanol is much better a disinfectant than Methanol in all concentrations and especially at the lower concentrations.

Keywords: *staphylococci*, Alcohol, Ethanol, methanol, bactericidal

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Introduction

Staphylococcus species are Gram-positive, non-motile, non-sporing cocci of varying size occurring singly, in pairs and in irregular clusters. They are facultative anaerobes with fermentative metabolism. Their optimum growth temperature is 30°C-37°C.¹ *Staphylococci* are classified mainly by their production of coagulase enzyme that clots plasma into Coagulase positive *Staphylococci* and Coagulase negative *Staphylococci*.² Alcohol refers to two water-soluble chemical compounds, ethyl alcohol and isopropyl alcohol, which have germicidal characteristics. These Alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60%-90% solutions in water. Alcohols are not recommended for sterilizing medical and surgical materials principally because they lack sporicidal action and they cannot penetrate protein-rich materials.³

Materials and methods

Study design

This is perspective analytical case control study that used to identify the different *Staphylococcus* species that infects Human and to study their sensitivity to Alcohol. The study was carried out in the period between February 2015 and September 2015. The study was carried out at Tropical Medicine Research Institute, Khartoum, Sudan.

Collections of specimens

Samples were obtained according to site of infection. Samples from wounds were collected using sterile swabs and also biopsy was taken. Urine samples had been collected in sterile urine containers. Stool samples had been collected in stool containers.

Cultivation of specimens

Wound swabs were cultivated on Blood agar and MacConkey agar, and then sub cultured on Mannitol salt agar. Urine specimens were cultivated on CLED medium and then sub cultured on Mannitol salt agar. Stool specimens were firstly cultivated on Selenite-F-Broth and then sub cultured on XLD media Mannitol salt agar. *Staphylococcus* isolates were then tested for their Coagulase production that categorized them into two groups. Then *Staphylococcus* species were identified according to biochemical tests and sugars assimilation tests. Sugars used for the identification of *Staphylococcus* species included: Lactose, Maltose, Sucrose, Mannitol, Raffinose, and Fructose.

Methodology for alcohol sensitivity

Isolates were sub cultured into Nutrient broth and incubated for 3-5hours. Then, 200µl of sub cultured Nutrient broth was added to 2ml Alcohol each time with different concentrations starting from 100% to 50% and a loop full of the mix was streaked at 10seconds, 30seconds, 1minute, and 5minutes. The media were incubated at 37°C for 24 hours aerobically and then results were recorded.

Statistical analysis

Results were analyzed by computer using Microsoft office excel program for the tables and the charts presentation.

Results

Staphylococcal isolates

In this Study 30 strains were isolated. As shown in (Figure 1), *S. aureus* was the predominant as the most pathogenic species accounting for 50% of isolates, followed by *S. epidermidis* which accounted for 26.7% of isolates. Other species that were isolated include: *S. intermedius* (6.7%), *S. schleiferi* (6.7%), *S. saprophyticus* (3.3%), *S. sciuri* (3.3%), and *S. lugdunensis* (3.3%).

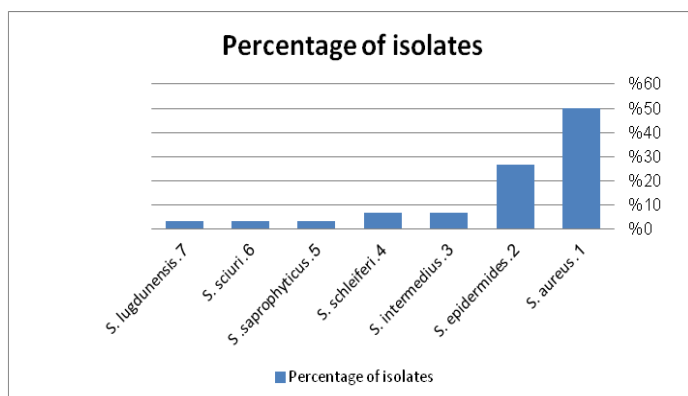


Figure 1 Shows the percentage of isolates from the 30 samples in a columnar chart.

Bactericidal effect of ethanol on staphylococcus species

Result shows that higher concentrations of ethanol are more effective than lower concentrations in the disinfection process. It also shows that more time of exposure results in more effective disinfection by Ethanol. As shown in Table 1 & Figure 2 Bactericidal affect of different concentrations of ethanol (100%, 90%, 80%, 70%, 60%, and 50%) on the different *Staphylococcus* species at different exposure times (10seconds, 30seconds, 1minute, and 5minutes) by percentage.

Table 1 The Bactericidal effect of Ethanol on *Staphylococcus* species

Time seconds/ conc. of ethanol	10	30	60	300
100%	100%	100%	100%	100%
90%	90%	100%	100%	100%
80%	90%	90%	100%	100%
70%	50%	60%	80%	90%
60%	20%	40%	90%	90%
50%	0%	0%	10%	70%

Bactericidal effect of methanol on staphylococcus species

Result shows that higher concentrations of Methanol are more effective than lower concentrations in the disinfection process. It also shows that more time of exposure results in more effective disinfection by Methanol. As shown in Table 2 & Figure 3 Bactericidal effect of different concentrations of Methanol (100%, 90%, 80%, 70%, 60%,

and 50%) on the different *Staphylococcus* species at different exposure times (10seconds, 30seconds, 1minute, and 5minutes) by percentage.

Table 2 The Bactericidal effect of Methanol on *Staphylococcus* species

Time seconds/ conc. of ethanol	10	30	60	300
100%	100%	100%	100%	100%
90%	80%	100%	100%	100%
80%	50%	50%	100%	100%
70%	30%	50%	80%	100%
60%	0%	30%	50%	90%
50%	0%	0%	0%	40%

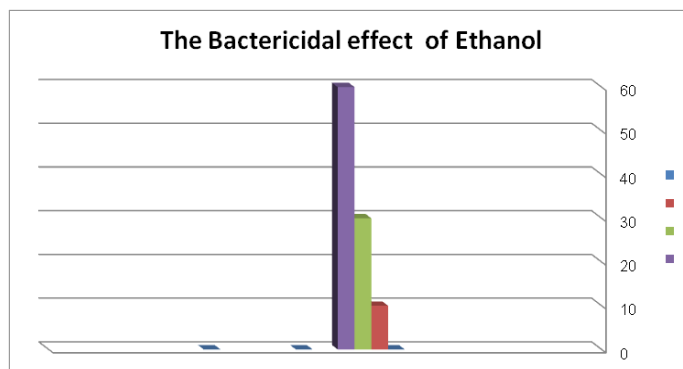


Figure 2 The Bactericidal effect of Methanol on *Staphylococcus* species

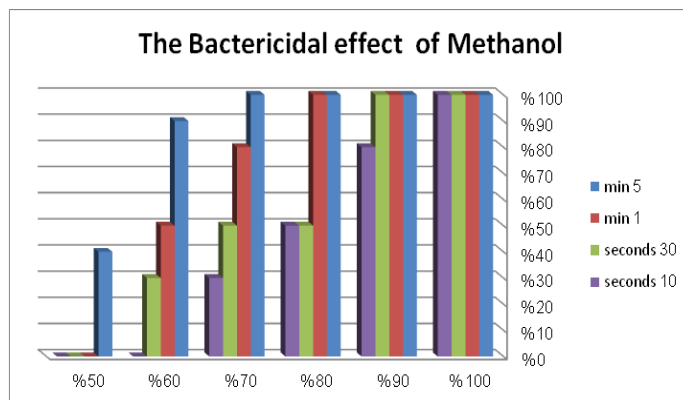


Figure 3 The Bactericidal effect of Methanol on *Staphylococcus* species

Discussion

Our study showed that *S. aureus* was the most predominant pathogenic species accounting for 50% of isolates, followed by *S. epidermidis* which accounted 26.7% of isolates. Other species that were isolated include: *S. intermedius* (6.7%), *S. schleiferi* (6.7%), *S. saprophyticus* (3.3%), *S. sciuri* (3.3%), and *S. lugdunensis* (3.3%). These results are similar to the finding of Seif Eldin et al., (2000) who found in 92% of all pus cultures from patient with abscesses, microbial agents was identified, the large majority being *S. aureus* (69%). These results also agree partially with Tanusri et al.,⁴ who found among the *Staphylococci* isolates 116(86.57%) were coagulase positive *Staphylococcus aureus*. And a total of 18(13.43%) Coagulase-

negative *Staphylococci* were isolated. Sixteen isolates (11.94%) out of 134 were identified as *Staphylococcus epidermidis* and two isolates (1.49%) were *Staphylococcus haemolyticus*. In Sudan, there was no relevant published data on the bactericidal effects of Ethanol and Methanol on different species of *Staphylococci*. The study proved that higher concentrations of both Ethanol and Methanol are more effective than the lower concentrations, and this finding does not agree with the CDC that proved that 70% (commercial spirit) Ethanol is the most effective bactericidal agent. Their explanation for this is that the mechanism of alcohol killing is by denaturation of proteins and absolute ethyl alcohol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water because proteins are denatured more quickly in the presence of water (CDC, 2008). The study showed that 70% Ethanol is only 50% effective in 10seconds exposure time and 70% Methanol is 30% effective in 10seconds of exposure. The results of this study showed resistance of *Staphylococci* to lower concentrations of Ethanol and Methanol including the commercial spirit (70%). In a study done by the CDC,³ the bactericidal activity of various concentrations of ethyl alcohol (ethanol) was examined against a variety of microorganisms in exposure periods ranging from 10seconds to 1hour. *Pseudomonas aeruginosa* was killed in 10seconds by all concentrations of ethanol from 30% to 100% (v/v), and *Serratia marcescens*, *E. coli* and *Salmonella typhosa* were killed in 10seconds by concentrations of ethanol from 40% to 100%. The Gram-positive organisms *S. aureus* and *Streptococcus pyogenes* were slightly more resistant, being killed in 10seconds by ethyl alcohol concentrations of 60%–95%.

Results

The Results of this study showed that Ethanol is more effective than Methanol as a Bactericidal agent, this finding also agrees with the study done by the CDC that proved Methyl alcohol (methanol) has the weakest bactericidal action of the alcohols and thus is seldom used in healthcare.³

Conclusion

The results showed that the most frequent isolates among the *staphylococcus* species was *S. aureus* as the major human cause of disease followed by *S. epidermidis* and then other *Staphylococci*. The results of ethanol effectiveness on *Staphylococcus* species showed higher effectiveness of higher concentrations (absolute, 90% and 80%) and the effectiveness becomes lower with lower concentrations (70%, 60%, and 50%) especially with shorter exposure times. The results of Methanol effectiveness on *Staphylococcus* species showed also higher bactericidal effects of the absolute and the 90% concentrations while lower concentrations (80%, 70%, 60%, and 50%) showed higher resistant rates of *Staphylococci* especially

with shorter exposure times. The best concentrations found to be bactericidal for both ethanol and Methanol are the higher Absolute and 90% with 100% and 90% effectiveness respectively. The results also showed that Ethanol is much better disinfectant than Methanol in all concentrations.

Recommendations

From results of this study it's recommended that:

- I. Ethanol 80% and above (90% and absolute) are to be used for disinfection of surfaces and for wound cleaning instead of 70% (commercial spirit).
- II. Methanol must not be used as a disinfectant except in its very high concentrations (Absolute and 90%).
- III. Future studies are recommended with bacteria other than *staphylococci* especially that infect the wounds.
- IV. Future studies on the surfaces disinfected with different concentrations of ethanol and other alcohols.
- V. Future research is needed to study other different disinfectants and their effectiveness on *Staphylococci* and also on other bacteria.

Acknowledgements

None.

Conflict of interest

The authors declare no conflict of interest.

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