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Efficacy of aluminum potassium sulfate against *Staphylococcus* species in wound infections compared to meropenem and amoxyclav

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ABSTRACT

Background: The worldwide increase in multidrug resistance of pathogenic bacteria has led to an urgent need for identifying an alternative strategy. Natural products have been used for centuries in treating human diseases and they contain components of therapeutic value. Alum has become a favorite antimicrobial against bacteria. **Material and Methods:** *Staphylococcus* species isolated from wounds in sixty Iraqi patients, isolates were cultured for overnight on blood agar, then were sub-cultured on Mannitol Salt Agar to distinguish between different species. Biochemical tests were done for these bacteria. Well diffusion method for antimicrobial sensitivity used to evaluate 20% aluminum potassium sulfate (alum) activity. Antibacterial activity of meropenem and amoxyclav was assessed by disk diffusion method. **Results:** The total number of isolated *staphylococcus* species were 60 including 30 *Staphylococcus aureus* and 30 coagulase negative *staphylococci*. The antibacterial activity of 20% alum was 100 % active against all *staphylococcus* species, while 83.3% and 78.3% of isolates were sensitive to meropenem and amoxyclav respectively. The comparison of the mean of inhibition zone diameter between *S. aureus* (29.23± 5.39) mm with Coagulase negative *staphylococcus* (30.2± 3.88) mm showed no significant differences ($p= 0.42$). Twenty percent of alum (29.5± 5.3) mm is more effective than meropenem (26.1± 5.8) mm significantly ($p= 0.02$). Alum (29.5± 5.3) mm was significantly more effective than amoxyclav (20.2± 3.9) mm ($p < 0.001$). **Conclusion:** 20% alum is more effective (superior) than meropenem and amoxyclav against *staphylococcus* species with promising natural antibacterial agent to be used in clinical medicine.

Introduction

Despite the great progress in human medicine, infectious disease caused by bacteria are still a serious problem in public health. It is estimated that *staphylococci* species have high rate of resistance to antibiotics [1]. The worldwide increase in multidrug resistance of pathogenic bacteria has led to an urgent need for identifying an alternative strategy to counter bacterial infection [2]. The latest research has been focused on identifying the potential antimicrobial agent from the natural

resources [3]. Natural products have been used for centuries in treating human diseases and they contain components of therapeutic value. They are environmentally safer and easily available [4].

Aluminum potassium sulfate (Alum) has become a favorite antimicrobial and can therefore be used as a natural inhibiting the growth of the bacteria. The addition of alum to water results in the production of chemical precipitates which remove pollutants. Removal of suspended solids, algae phosphorus, heavy metals and bacteria occurs

primarily by enmeshment and adsorption onto aluminum hydroxide precipitate [5].

Alum occurs naturally in rocks that are located in areas where sulfide materials and potassium-bearing minerals. It is crystallized double sulfate, generally odorless, colorless and crystalline solids that turn white in air, which is used as an astringent and antiseptic in various food preparation processes such as pickling and fermentation and as a flocculant for water purification. Food and Drug Administration over the counter advisory panel has recommended alum as category I active ingredient in mouthwashes. Alum is used medicinally in many subunit vaccines as an adjuvant to enhance the body's response to immunogens. Alum is also widely used in some rural areas for the treatment of pediatric cough [6-7].

A value of 0.2 mg/l alum in drinking water is recommended by WHO based on aesthetic consideration for decontamination of water. Careful operation of filtration or any other process is mandatory for successful treatment of water to obtain both an aesthetically acceptable product and effective disinfection [8].

Alum has recently drawn the attention of the scientific community as an efficient, safe and eco-friendly inorganic compound, commercially available and cost effective. It demonstrated a high propensity of antimicrobial activity in a variety of systems traditionally and scientifically. Several In vitro and In vivo studies report that alum individually or in synergism have antimicrobial properties against a broad spectrum of bacterial and fungal species and harness other activities beneficial to humans. Alum, however, has found applications in a wide spectrum of human activities such as pharmaceutical, cosmetic, food, textile and synthetic industries [9].

The aim of current study was to evaluate the antibacterial activity of alum against *staphylococcus* species and comparing results with antibiotic sensitivity of both amoxycylav and meropenem.

Material and methods

Staphylococcus species were isolated from sixty Iraqi patients in Al- Shomali General Hospital, Babylon city, Iraq. Isolates were obtained from wounds and were overnight cultured on blood agar, and then were sub-cultured on Mannitol Salt Agar to distinguish between different species, then were biochemically tested [10].

Alum solution preparation was 20%, by dissolving 20 grams of white alum in 100 ml distilled water then putting the solution in autoclave 120 °C fore 20 min.

The antibiotic susceptibility testing was done by the standard well diffusion method on Mueller-Hinton agar. Turbidity standard protocol was followed in order to have homogenized bacterial inoculum suspension, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. Agar well diffusion method is widely used to evaluate the antimicrobial activity of alum; the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm is punched aseptically with a sterile cork borer or a tip, and a volume (50 µL) of the alum was introduced into the well. Then, agar plates were incubated under suitable conditions. The disc diffusion method used to evaluate the activity of meropenem 10 µg and amoxycylav 20 µg.

For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. T-test was applied to examine measurement data, *p* value < 0.05 considered significant.

Results

The antibacterial activity of 20% alum was 100 % active against *staphylococcus* species, while 83.3% of isolates were sensitive to meropenem. The activity of amoxycylav was 78.3% (**Figure 1**).

The comparison of the mean of inhibition zone diameter between *S. aureus* 29.23± 5.39 mm with Coagulase negative *staphylococcus* 30.2± 3.88 mm showed no significant differences (*p*= 0.42) (**Table 1**).

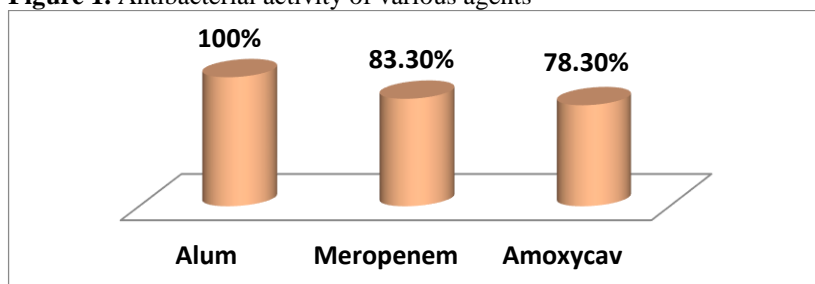
Table 1. Comparison of inhibition mean between *S. aureus* and coagulase negative staph species

	Groups	No.	Mean (mm)	Std. Deviation	<i>p</i> value
Alum	<i>S. aureus</i>	30	29.23	5.39	0.42
	Coagulase negative	30	30.20	3.88	

20% alum 29.5± 5.3 mm is more effective than meropenem 26.1± 5.8 mm significantly (*P*= 0.02). Alum 29.5± 5.3 mm was significantly more effective than amoxycylav 20.2± 3.9 mm (*p*<0.001) (**Table 2**).

Table 2. Comparison inhibition zone of different agents

Bacteria	Groups	Mean (millimeter)	Std. Deviation	p value
<i>Staphylococcus</i> species	Alum (20%)	29.5	5.3	0.02
	Meropenem	26.1	5.8	
	Alum (20%)	29.5	5.3	< 0.001
	Amoxyclav	20.2	3.9	

Figure 1. Antibacterial activity of various agents

Discussion

Antibiotics are essential in modern medicine and are used to treat various infectious diseases caused by bacteria. However, the emergence of antibiotic-resistant bacteria has become a global public health threat, leading to increased morbidity, mortality, and healthcare costs. Therefore, it is crucial to determine the antibiotic sensitivity of bacterial strains to choose appropriate antibiotic therapy [12-14].

The current study showed antibacterial activity of 20% alum was 100 % active against *staphylococcus* species. Another study revealed that the alum has bacteriostatic activity against axillary normal bacterial flora. The diameter of inhibition zone has increased with increasing concentrations to 9% of potash alum stock solutions [15].

Alum in concentration 5 gm/100 ml showed high inhibition effect against bacteria including *Staph. aureus* compared with Ceftriaxone and Tobramycin antibiotics. As well as the alum in concentration (10 and 5) gm/100 ml sterilized by gauze showed high inhibition effect against all bacterial isolates under study compared with the antibiotics (ampicillin, tobramycin and ceftriaxone) [16].

Another study mentioned that antibacterial activity by inhibition zone (mm) estimation for alum on tested isolates, the inhibitory effect was very strong, highest on *E. coli* and *C. albicans* (resistant to most antibiotics) observed sensitive to alum in concentration 16 % and 20% in *E. coli* [17]. Antibacterial activity of Alum against isolates at different concentrations (w/v%), produced zone

diameters of inhibition for the isolates, ranging from 9.3 - 21.2 mm [18].

A study found that 60% alum concentration gives inhibition zone of 29 mm diameter on *Pseudomonas aeruginosa*, while 50 and 40% alum concentrations were 25 and 22 mm diameter, respectively. Consequently, alum is utilized in wound and burns disinfection and in treatment of ulcers in the oral cavity [19]. Another study also mentioned that alum susceptibility trend was as *K. pneumoniae* > *S. aureus* > *E. faecalis* > *E. coli* > *E. faecium* at highest tested concentration (35mg/ml) and 24hr of incubation period [20].

Alum solution showed excellent inhibitory effects on all bacterial strains at concentrations (7.5–18.75 mg/mL). All tested bacteria showed no growth with alum at concentrations of 7.5, 10, 15 and 20 mg/mL [21].

The current study found that 20% alum with a inhibition zone diameter of 29.5 ± 5.3 mm was more effective than meropenem, 26.1 ± 5.8 mm, significantly ($p = 0.02$). Alum with a inhibition zone diameter of 29.5 ± 5.3 mm was significantly more effective than augmentin 20.2 ± 3.9 mm ($p < 0.001$).

Antibacterial susceptibility profiles of alum were determined against bacteria isolated from shellfish bivalve oysters. In-vitro bioassay using disc and agar well diffusion techniques with different concentrations; 1.5, 2.0, 2.5 and 3.0% of Alum were used to determine the susceptibility profiles of these isolates and compared with standard antibiotic, Ofloxacin as control. Alum exhibited high levels of sensitivity on *Escherichia*

coli (16.0mm) and *Pseudomonas aeruginosa* and *Vibrio* species (13.0mm) respectively [22].

Conclusion

20% alum is more effective than meropenem and amoxyclav against *staphylococcus* species with promising branch as a natural antibacterial agent. More studies on Alum side effects and toxic effects on human body are recommended.

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