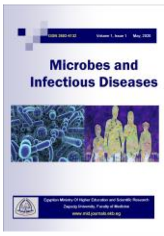
Microbes and Infectious Diseases 2023; Article-In-Press, **DOI:** 10.21608/mid.2023.206322.1514



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Evaluating the antibacterial activity of potassium aluminium sulphate (alum) combined with other antibiotics

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ARTICLE INFO

Article history:
Received 16 April 2023
Received in revised form 22 April 2023
Accepted 23 April 2023

Keywords:
Potassium aluminium sulphate (alum)
Ceftriaxone
Amoxicillin
E. coli
Synergism
gentamicin

ABSTRACT

Background: Antibiotic resistance is one of the most serious biomedical problems that require new agents to combat bacterial pathogens. Potassium aluminium sulphate (alum) has recently drawn the attention of the scientific community as an efficient, safe and eco-friendly inorganic compound with antimicrobial activity. **Methods:** Ten samples of bacteria, five types of *Eschericha coli* (*E. coli)* and five types of *S. aureus* were isolated from patients. *Eschericha coli* isolated from urine sample by sterile container. *Staphylococcus aureus (S. aureus)* isolated from wound samples by a cotton swab. The samples were cultured on the following media (Mannitol salt agar, eosin methylene blue agar, and blood agar). Well diffusion method used to evaluate the antimicrobial activity. Alum aqueous solution with two concentrations (1% and 2%) was determined as well as amoxicillin, gentamicin, and ceftriaxone. **Results*:*** *E. coli* is sensitive by 50%for both concentrations of alum (1%, 2%), *S. aureus* is 100% resistant to a concentration of 1% of alum and 50% resistant to a concentration of 2% of alum. Combination of amoxicillin with alum for each concentration (1% and 2%) has no significant effect on the activity of amoxicillin for both *E. coli and S. aureus* (*p*= 97 and *p*= 0.62) respectively. Combination of ceftriaxone with alum for each concentration (1% and 2%) has no significant effect. The combination of gentamicin with alum for each concentration in *E. coli* has no significant effect. But for *S. aureus* the mean of gentamicin sensitivity was 39.50± 3.87, the mean is decreased after combination with 1% alum 41.50± 4.35 and 2% alum 40± 5.83 with significant statically differences (*p*= 0.02). **Conclusions:** The white alum effect is dose response, and greater concentration will lead to enhanced decrease on bacterial growth. This study suggests more than 1% and 2% concentrations of white alum could be used as antimicrobial agent. The effect of 1% and 2% alum combined with ceftriaxone and amoxicillin shoed no significant differences (*p*> 0.05).

Introduction

Bacterial antibiotic resistance is one of the most serious modern biomedical problems that prioritize the search for new agents to combat bacterial pathogens [1-3].

DOI: 10.21608/MID.2023.206322.1514
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Potassium aluminium sulphate (PAS) commonly referred to as potash alum (alum) has recently drawn the attention of the scientific community as an efficient, safe and eco-friendly inorganic compound, commercially available and cost effective. Alum, however, has found applications in a wide spectrum of human activities such as pharmaceutical, cosmetic, food, textile and synthetic industries. It has an antimicrobial agent and numerous applications [4].

Alum is a mineral found in nature in both pure and impure forms. It is a mineral salt. It is a colourless, clear, odourless, crystalline mass or granular powder with a sweetish astringent flavor [5]. A study suggests that alum have antibacterial actions against some pathogens and may be used for the treatment and prophylaxis against diseases [6].

Escherichia coli (*E. coli)* are the gram-negative organism most frequently isolated in adult patients with bacteremiaand in severe cases it may lead to death [7-8]. In general, *E. coli* are a part of the normal commensal gut microbiota of healthy human populations. However, some strains can cause intestinal or extraintestinal infections due to specific virulence factors (VFs).Isolates that are capable of gaining access to and surviving in the bloodstream are known as extraintestinal pathogenic *E. coli* (ExPEC) and cause a variety of infections, including urinary tract infections (UTI), sepsis, and neonatal meningitis. The most common extra-intestinal site colonized by these bacteria is the urinary tract [9-10].

Staphylococcus aureus (S. aureus) cells are Gram-positive and appear in spherical shape. They are often in clusters resembling bunch of grapes when observed under light microscope after Gram staining. The name ‘Staphylococcus’ was derived from Greek, meaning bunch of grapes (staphyle) and berry (kokkos). The scanning electron microscopic observation reveals roughly spherical shaped cells with smooth surface [11-12]. The diameter of the cells ranges from 0.5 to 1.0 μm [13].

Gentamicin is an aminoglycoside antibiotic used in the treatment of several Gram-negative infections. It should be indicated based on patient age, symptoms, signs at presentation, and local antimicrobial resistance patterns to enhance the probability of successful treatment in bacterial septicemia, meningitis, urinary tract infections, gastrointestinal tract infections, and soft tissue infections [14-15].

Ceftriaxone is an extended- spectrum third-generation cephalosporin. It is a greatly effective antibacterial with high potency covering wide variety of gram- negative and gram- positive specious and has been extensively prescribed in healthcare facilities including for empirical treatment [16].

Amoxicillin is one of the most commonly used antibiotics in the primary care setting. It is an amino-penicillin, created by adding an extra amino group to penicillin to battle antimicrobial resistance. Amoxicillin covers a wide variety of gram-positive bacteria, with some added gram-negative coverage compared to penicillin. Like penicillin, it covers most *Streptococcus* species and is also effective against other gram positive bacteria. Because amoxicillin is well absorbed after oral administration, it is extensively used in out-patient settings [17].

The aims of the current study were evaluation the antibacterial action of 1% and 2% alum against both *E. coli* and *S. aureus* and to evaluate combination of alum with other antibiotic to determine the possible synergism effect.

Material and Methods

Specimen collection and cultivation

Ten samples of bacteria, five types of *E. coli* and five types of *Staphylococcus aureus* were isolated from patientsduring the period 1/12/2022 to 8/2/2023.A urine sample was collected from a number of patients in a clean container from the midstream of urine The samples were cultivated on culture media (blood agar, MacConkey agar and eosin methylene blue agar (EMB agar)), then the cultured dishes were kept at a temperature of 37C° for 24 hours in incubator, and in the positive results that showed bacterial growth, they were transplanted again on new dishes of EMB as a selective media to growth *E. coli* as a metallic green sheen colonies. Wound samples were collected from a number of patients by a cotton swab, the samples were cultured on the following media (Mannitol salt agar, and blood agar) after which they were kept at a temperature of 37 °C for 24 hours in incubator, and then the pure staphylococcal colonies were isolated on the medium.

Preparation of alum concentrations

Alum was purchased from the local market of Hila city. 1gm of potassium sulfate (alum) dissolved in 100ml of distilled water to prepare 1% concentration of alum. 2gm of alum was dissolved in 100ml of distilled water to prepare 2% concentration of alum.

Antimicrobial activity testing

Loop full growth from bacterial isolate was inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×108 CFU / ml. Muller- Hinton agar was inoculated with 50 µg of bacterial inoculum .Using corked borer, wells were made on the cultured media (6mm).where in each dish there are 5 holes, and these holes are filled with 50 µgof 1% of alum and the other hole with 50 microns of 2% of alum and other agents (amoxicillin, gentamicin, and ceftriaxone); in the other dish for the same type of bacteria, the wells are filled with 50 µgof amoxicillin + 50 µg(1% of alum) and the second hole is filled with 50 µgof amoxicillin + 50 µg(2% of alum) and other antibiotics as amoxicillin were add with different concentrations of alum.

The dishes are kept in the incubator at a temperature of 37C° for 24 hours, after which we notice the formation of inhibition zones the diameter of which was measured in millimeter.

Data analysis

All the statistical analysis was done by using SPSS 26 software and Excel app. For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. For correlation analysis, Spearman's correlation for non-parametric analysis was used. One way ANOVA used to compare the mean of more than two groups, *p* value < 0.05 considered significant.

Results

Figure 1 showed the sensitivity range and the resistance of bacteria (Escherichia Coli and*Staphylococcus aureus*) against alum concentrations.*E. coli*was sensitive by 50%for both concentrations of alum (1%, 2%), for *Staphylococcus aureus*, it showed 100% resistant to a concentration 1% of alum and 50% resistance to a concentration 2% of alum.

Table 1 showed mean of only Amoxicillin and amoxicillin combined with alum concentrations. Combination of amoxicillin with alum for each concentration (1% and 2%) have no significant effect for acting as synergism or antagonism effect on the activity of amoxicillin for both *E. coli* and *S. aureus* (*p*= 97 and *p*= 0.62) respectively.

Table 2 revealed the combination of gentamicin with alum for each concentration (1% and 2%), in *E. coli* has no significant effect for acting as synergism or antagonism effect on the activity of gentamicin. But for *S. aureus* the mean of gentamicin sensitivity was 39.50± 3.87, the mean is decreased after combination with 1% alum 41.50± 4.35 and 2% alum 40± 5.83 with significant statically analysis (*p*= 0.02)

Combination of ceftriaxone with alum for each concentration (1% and 2%) had no significant effect for acting as synergism or antagonism effect on the activity of ceftriaxone for both *E. coli* and S, aureus (*p*= 90 and *p*= 0.62) respectively**(Table 3)**.

Table 4 revealed there is no correlation between gentamicin, ceftriaxone, and amoxicillin with each other (*p*> 0.05)

Figure 1. Resistance of bacteria to alum concentrations

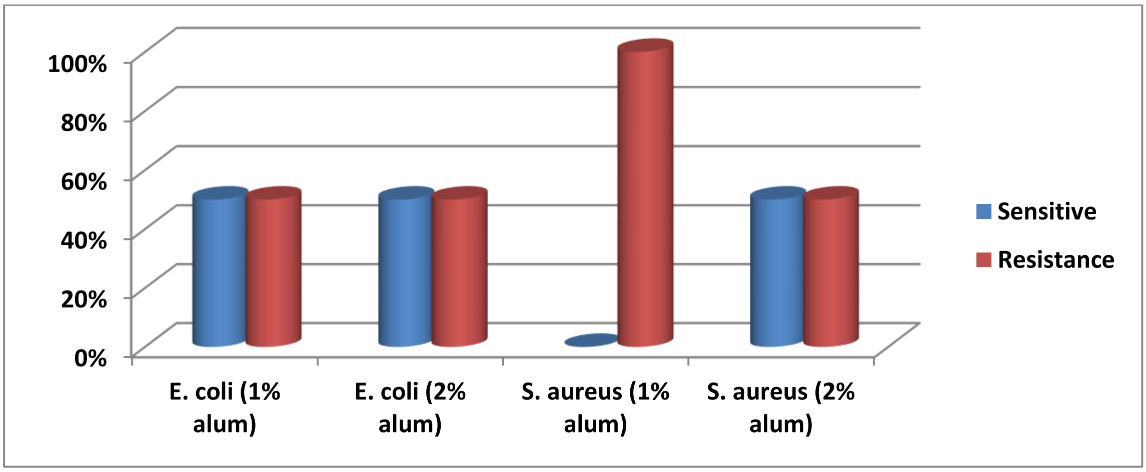

Table 1. The effect of amoxicillin and alum on bacterial sensitivity.

Variables	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	***p* value**
Amoxicillin (AM) and *E. coli*						
AM only	10	40.00	12.936	21	50	0.97
AM plus 1% alum	10	38.50	9.950	24	45	
AM plus 2% alum	10	39.75	9.845	25	45	
Amoxicillin (AM) and *S. aureus*						
AM only	10	29.25	1.500	27	30	0.62
AM plus 1% alum	10	33.75	11.087	25	50	
AM plus 2% alum	10	32.75	3.202	30	36	

Table 2. The effect of gentamicin and alum on bacterial sensitivity**.**

Variables	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	***p* value**
Gentamicin (GEN) and *E. coli*						
GEN only	10	39.50	3.87	36	45	0.82
GEN plus 1% alum	10	41.50	4.35	36	45	
GEN plus 2% alum	10	40.00	5.83	34	45	
Gentamicin (GEN) and *S. aureus*						
GEN only	10	45.50	4.43	40	50	0.02
GEN plus 1% alum	10	37.75	4.92	34	45	
GEN plus 2% alum	10	36.75	2.50	34	40	
Variables	**N**	**Mean**	**Std. deviation**	**Minimum**	**Maximum**	***P* value**
Gentamicin (GEN) and *E. coli*						
GEN only	10	39.50	3.87	36	45	0.82
GEN plus 1% alum	10	41.50	4.35	36	45	
GEN plus 2% alum	10	40.00	5.83	34	45	
Gentamicin (GEN) and *S. aureus*						
GEN only	10	45.50	4.43	40	50	0.02
GEN plus 1% alum	10	37.75	4.92	34	45	
GEN plus 2% alum	10	36.75	2.50	34	40	

Table 3. The effect of ceftriaxone and alum on bacterial sensitivity.

Variables	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	***p* value**
Ceftriaxone (CEF) and *E. coli*						
CEF only	10	43.50	5.066	39	50	0.90
CEF plus 1% alum	10	42.50	2.887	40	45	
CEF plus 2% alum	10	43.50	2.380	40	45	
Ceftriaxone (CEF) and *S. aureus*						
CEF only	10	44.50	5.260	40	50	0.62
CEF plus 1% alum	10	37.75	14.500	16	45	
CEF plus 2% alum	10	40.25	6.946	30	45	

Table 4. Correlation between different antibiotics.

Variables		**Ceftriaxone**	**Amoxicillin**
Gentamicin	Pearson Correlation	0.55	-0.11-
	Sig. (2-tailed)	0.15	0.78
Ceftriaxone	Pearson Correlation		0.22
	Sig. (2-tailed)		0.60

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 [18-19].

Today, demands for the production of antimicrobial substances in health care have increased. Therefore, discovering a suitable material to introduce as a proper anti-bacterial agent has been considered as a challenge. Different materials have been reported as antimicrobial agents Excessive use of antibiotics can lead to emergence of antibiotic resistant bacterial strains [20].

The present study demonstrates that 2% and 1% concentrations of white alum decrease the growth of *E. coli* in vitro.

These findings agree with a study mentioned that the 0.5% and 0.25% concentrations of white alum have no significant effect on the growth . *E coli*, although these concentrations have decreased the growth of *E. coli*. A value of 0.2 mg/l in drinking water is recommended [6].

Another study tested different alum concentrations against *Proteus mirabilis*, which causes urinary tract infections. Study's findings revealed that potential antimicrobial activity was demonstrated by the possible loss of bacteria motility in culture media [21].

Among varieties of commercially available alums, potash alum, is very much common both for household, pharmaceutical and medicinal applications. Alum concentrations at (4.4 – 35.0 mg/mL) showed zones of inhibition which ranged from 0.005 – 0.438cm against *E. faecalis*, 0.019 – 0.421cm against *E. faecium*, 0.011 – 0.455cm against *S. aureus*, 0.013 – 0.430cm against *E. coli*, And 0.025 – 0.485cm against *K. pneumoniae* respectively at 24hr incubation [22].

Bactericidal effect of alum had been observed but the mechanism was not prominent. It was postulated that it effect was due to reduction in acidity or deleterious effects on bacterial cell wall. Another study revealed a more pronounced antibacterial activities on Gram negative bacteria ; *Pseudomonas aeruginosa* (21mm) and *E.coli* (20mm) rather than Gram positive, *S. aureus* and *Bacillus subtilis* (19mm) respectively [23].

The current study showed that combination of amoxicillin with alum for each concentration (1% and 2%) have no significant effect for acting as synergism or antagonism effect on the activity of amoxicillin for both E. coli and S, aureus (*p*= 97 and *p*= 0.62) respectively. Combination of ceftriaxone with alum for each concentration (1% and 2%) have no significant effect for acting as synergism or antagonism effect on the activity of ceftriaxone for both E. coli and S, aureus *(p*= 90 and *p*= 0.62) respectively.

Undoubtedly, combination strategy may improve drug performance by synergism which May proffer a better alternative to microbial resistance single Drug/substance as this protocol is usually more effective at low Dosage. Some major advantages of combination therapy are cost Effectiveness, less side Effects associated with higher drug dosage can be minimized without compromising performance. However, encouraging the use of drugs in combination would improve the prevention and management of microbial infections therapy [24-26].

Alum crystals are highly soluble in water and when used under arm, they are dissolved by the body's sweat leaving a dry thin layer on the skin's surface which prevents sweat to come in contact with odor-causing bacteria. Further studies are required to investigate the safety, allergy and efficacy of alum on human skin when used as antiperspirant. According to this study a concentration of 10 or 20 mg/mL could be considered appropriate for formulation of deodorant lotion, cream and gel. Lower concentration can be used when mixed with cefotaxime or tetracycline. Alum has excellent antimicrobial inhibitory effects microorganisms especially commixed with antibiotics [27].

Alum is composed of potassium, aluminium, hydrogen, sulphur and oxygen [21]. Aluminium sulphate is the commonest and most widely used coagulant for water treatment worldwide. Aluminium is a well-known adsorbent for phosphorous (P), a nutrient highly associated with eutrophication in most water bodies. Thus, this paper provides a review on P adsorption from aqueous media using alum sludge waste material generated by water treatment plants that use aluminium sulphate as sole coagulant [28].

Conclusions

The white alum effect is dose response, and greater concentration will lead to enhanced decrease on bacterial growth.This study suggests more than 1% and 2% concentrations of white alum could be used as antimicrobial agent.

Acknowledgements: None.

Conflict of interest: There is no conflict of interest

Financial disclosure: None

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