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Inhibition of swarming in *Proteus mirabilis* by Alum (Hydrated Aluminum Potassium Sulfate)

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Abstract : The antibacterial activity of different concentrations of Alum (Hydrated Aluminum Potassium Sulfate) were examined against Proteus mirabilis that causes upper urinary tract infections .During our experiments we found that the following concentrations (0.0, 0.05, 0.1, 0.3, 0.5, 0.6, 0.7 , 0.8, 0.9 mg/ ml) were given gradual effect on swarm, a phenotype that was associated with motility. These concentrations were used to inhibit swarming and motility the two important virulence factors coupled closely with the ability to invade urothelial cells. The present study revealed that Alum began significantly inhibited swarming at 0.3 mg/ml for all seven isolates of P. mirabilis that tested in the study . It was considered that the concentration 0.6 mg/ml completely inhibition the phenomenon of swarming because the bacteria appear as separated small colony on nutrient agar . The diameter of swarming was decreased by increasing the concentration of Alum. Although it was found that this bacteria losses the motility on semi-solid media in 0.6 mg/ ml of alum(Hydrated Aluminum Potassium Sulfate) after 24 hrs incubation but the Minimum Inhibition Concentration (MIC) of Alum was 0.8 mg/ml .The study revealed that Alum has the potential effect against P. mirabilis .

Key words : *Proteus mirabilis* ,Alum ,inhibition swarming

Introduction

Proteus mirabilis, is a motile Gram-negative enteric bacterium, it is an important pathogen of the urinary tract, and is the primary infectious agent in patients with indwelling urinary catheters **(1)**. *Proteus mirabilis* is a common cause of complicated urinary tract infections . These infections are predominant in individuals with urinary tract abnormalities or patients with long-term catheters, such as those in nursing homes or hospitals. Bacteriuria with *P. mirabilis* may lead to fever, pyelonephritis**(2),** bacteraemia and even death **(3)**. During *P. mirabilis* infection, the urinary tract often becomes obstructed by urinary stones resulting from urease production **(4) .**In some cases the organisms can enter the blood stream and cause **(5)** .

These organisms colonize the catheter **(6)** forming surface biofilm communities . As the biofilm develops it obstructs the flow of urine through the catheter **(7).**The gram-negative bacterium *Proteus mirabilis* is well known for its ability to motile, and elongated swarmed cells that rapidly spread over a surface. When cultured on a nutrient agar plate, a strain of *P. mirabilis* typically is able to colonize the whole plate within 24 hrs **(8)** . Many materials was used for inhibiting swarming like *p*-nitrophenylglycerol **(9)**and Resveratrol **(10)** .

Aluminum Potassium Sulfate or, potash alum($KAl(SO4)_2 \cdot 12H_2O$) is used as an astringent and antisepsis in various food preparation, react acid to litmus , and as a flocculent for water purification among other things in crystal form **(11)** .It is successful in controlling profuse bleeding by ensuring effective blood clotting **(12).**

The aim of this study was to test the inhibition of *P. mirabilis* swarming by Alum(Aluminum Potassium Sulfate)

Material and Methods

Bacterial isolates

Seven bacterial isolates of *P. mirabilis* were isolated from clinical samples using MacConkey and blood agar **(13).** Seven clinical isolates were identified as *P. mirabilis* using API 20E system of classification **(14)** were used throughout this study. All these strains were motile swarming and produce

urease obtained from infections in Ramadi General Hospital **.**

Biological activity of Alum on bacteria Seven bacterial isolates of *P. mirabilis* were tested against Aluminum Potassium Sulfate in concentrations (0.9 , 0.8, 0.7 , 0.6 , 0.5 , 0.3 , 0.1 ,0.05, and 0.0 mg/ ml) to determine the Minimum Inhibition Concentration MIC in the agar **(10)** .

The Alum stock solution prepared in concentration 5mg/ml was sterilized by filtration through amilli pore filter with a pore diameter of 0.45mm . All concentrations were prepared from stock solution in a sterile conditions

Inhibition of *P. mirabilis* swarming by Alum. to test the effect of Alum on *P. mirabilis* swarming, 5 µl of a standardized test inoculums containing 10^8 cells/ml was freshly prepared using the direct colony suspension method, with the aid of the 0.5 McFarland turbidity standard **(15)** the Bacteria were inoculated onto the centre of nutrient agar, swarming agar plates. containing various concentrations of Alum, and the migration distance of the bacteria was measured after 18 hrs incubation at 37°C. **(10) (16) .**

Assay of motility test to test the effect of Alum on *P. mirabilis* motility, the bacteria were inoculated onto semi-solid media(agar concentration 0.4%) **(13)** containing (0.0 ,0.05, 0.1, 0.3, 0.5 ,0.6, 0.7, 0.8 , 0.9, mg/ ml) Alum by stabbing the media and observe the motility after 24 hrs incubation at 37°C.

Results:

Seven bacterial isolates were tested against different concentrations of Alum.

The results showed that Minimum Inhibition Concentration (MIC) of Alum on agar to *P. mirabilis* is 0.8 mg/ml. Swarming was began to inhibit in all seven *P.mirabilis* isolates at concentrations 0.3 mg/ ml but 0.6 mg/ ml, completely blocked the swarming ability of *P. mirabilis* (figure 1) .The diameter of swarming was decreased with increasing the concentration of Alum (table1).

Motility was inhibited at concentration of 0.6 mg/ml for all isolates of bacteria after 18hrs and 24hrs incubation(table 2) .The result show no cloudy around the stabbing of bacteria in this concentration (negative result) while motility test give positive result (cloudy root-like region around the stabbing) in the following concentration(0.0, 0.05, 0.1, 0.3, 0.5) mg/ml .

Discussion

A wide variety of natural products has been under scrutiny for their clinical potential, both in terms of disease prevention and treatment . In this study Alum (Aluminum potassium sulfate) a naturally occurring was tested as swarming inhibitor and the motility in *P. mirabilis.* In this study is was found that ,Alum inhibits *P. mirabilis* swarming and motility at a concent

ration as low as 0.3 mg/ ml (Figs 1 and table 1), and significantly affect the growth rate of the bacteria at concentrations up to 0.1 mg / ml . This material hydrolyzes in water to form sulfuric acid, which is responsible for rising the acidity in the environment therefore precipitate the protein **(17)** so the flagella formation and growth rate of bacteria will inhibition **(18)** .

To our knowledge, it is the first report describing the inhibitory effect of Alum on bacterial swarming and motility.

Alum inhibit swarming by inhibition of motility. *Proteus mirabilis* immobilizes by inhibition of flagellum formation or by some lytic action on the flagella already synthesized. therefore the ability to motile will be effected because flagella, necessary for motility **(19).**As a result the swarming that closely related with motility decreases too .

It was observed that if the concentration of alum increased this will decrease the ability to swarming on agar .The effect begins from concentration of 0.1 mg/ml and the diameter of swarming will be 49 mm .In 0.3mg/ml the diameter decrease to 30mm ,in the concentration 0.5 mg/ml the diameter became 10mm and in the concentration 0.6 mg/ml the swarming inhibited completely because the bacteria appear as small colony but the bacteria growth inhibition in 0.8mg/ml and when we increase the concentration all isolates were killed on nutrient agar. It was showed that Alum inhibited *P. mirabilis* swarming and motility at 0.3-0.6 mg/ml . These concentrations were used in purification of potable water**(20)** .Alum has a low toxicity in experimental animals, and because the body doesn't absorb aluminum, it's harmless when swallowed. but ingestion of 30 grams (one ounce) has killed adult humans. Concentrated solutions have caused breakdown of gum tissues, kidney damage, and fatal intestinal bleeding **(21).** Oneda and others proved that administration of Aluminum potassium sulphate (alum) 1.0, 2.5, 5.0 and 10.0% (w/w) does not exert tumorigenic or any other toxic actions in B6C3F1 mice **(11).** But if Alum is to be used to treat or prevent *P. mirabilis* infection, its effect on human cells needs to be addressed.

The emergence of bacterial strains that exhibit resistance to various antibiotics possess a major threat to medicine and public health. As a consequence, there is renewed interest in antibacterial targets which, by attenuating

virulence, disrupt the capacity of pathogenic bacteria to cause infection. **Cheong** and others **(22)** proved that the combinations of aluminum with chlorhexidine or erythromycin, are potentially useful as antibacterial agent **.** It is therefore, more beneficial to develop antibacterial agent using aluminum salts. However, more studies on the effects of these salts on the physical properties as well as toxicity are necessary.

In this paper, it was demonstrated that Alum, an antibacterial used in industrial processes, vaccines, Hepatitis A: 250 mcg and DTaP (for Diphtheria, Tetanus, and Pertussis): 170-625 mcg of Aluminum **(23)** and in water treatment , has an inhibitory effect on *P. mirabilis* swarming and virulence factor expression. This finding opens up the opportunity to develop drugs that slow down *P. mirabilis* infection, allowing the host to gain valuable time to activate defense mechanisms, and to stop and eliminate pathogenic invaders. Also these material can be used in the laboratory to inhibit swarming on media cultures

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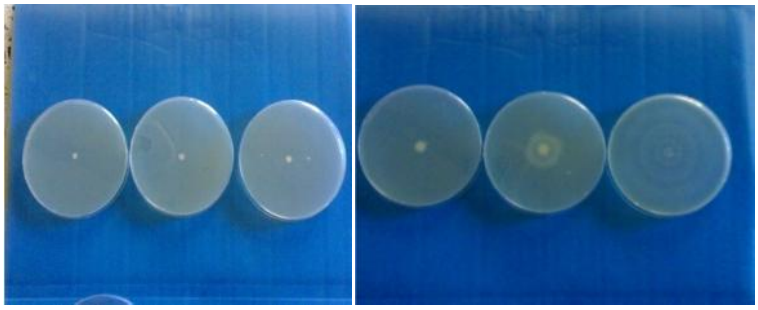
Table-1- Inhibition swarming of seven P. mirabilis isolates by various concentration of Alum on nutrient agar after 18 hrs incubation at 37.

Concentration of Alum mg/ ml	Swarming in diameter/mm mean	Mean ± SD
0.0	58	±0.5
0.05	57	±1.2
1	49	± 2.7
0.3	30	± 1.4
0.5	10	± 0.8
0.6	4	±2.3
0.7	3.5	±1.8

Table -2-Inhibition of motility of seven *Proteus mirabilis* isolates in semi-solid media after 18 and 24 hrs incubation

Concentration of Alum in mg/ ml	Motility test	Number of isolates after 18 hrs incubation	Number of isolates after 24 hrs incubation
0.0	+	7	7
0.05	+	7	7
0.1	+	7	7
0.3	+	5	7
0.5	+	6	7
0.6	-	7	7
0.7	-	7	7



F E D C B A

Figure (1) images of swarming on nutrient agar plates containing different concentrations of Alum (Aluminum potassium sulfate) at 37°C after 18 hrs inoculation.

A= (0.0 mg/ml) normal swarming phenomenon.

B = 0.3 mg/ml began the inhibition of swarm.

C=(0.5 mg / ml) decrease the diameter of swarming .

D= (0.6 mg / ml) Complete inhibition of swarming .

E=(0.7 mg/ ml) colony without swarming.

F-(0.8 mg/ ml) inhibition of growth.

تثبيط ظاهرة الانثيال في بكتريا *Proteus mirabilis* بواسطة الشب (كبريتات الألمنيوم البوتاسيوم الهيدروجينية)

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الخلاصة

اختبرت فعالية الشب(كبريتات الألمنيوم البوتاسيوم) ضد بكتريا المتقلبات Proteus mirabilis المسببة لالتهابات المجاري البولية وذلك باستخدام عدد من التراكيز المختلفة فوجد من خلال التجربة إن التراكيز التالية (٠,٠ ، ٠,٠٥ ، ٠,١، ٠,٣، ٠,٥، ٠,٦، ٠,٧، ٠,٨، ٠,٩) ملغم/ مللتر تعطي تدرجا في تثبيط ظاهرة الانثيال المرتبطة بالحركة . استخدمت هذه التراكيز لتثبيط ظاهرة الانثيال والحركة وهما من عوامل الضراوة المرتبطة باختراق البكتريا للخلايا الطلائية المبطنة للمجرى البولي . أثبتت الدراسة إن تأثير الشب المثبط لظاهرة الانثيال يبدأ عند التركيز ٠,٣ ملغم/مللتر لجميع العزلات السبعة المختبرة حيث يظهر نقص في قطر الانثيال بينما يختفي قطر الانثيال عند التركيز ٠,٦ ملغم/مللتر وتظهر بكتريا المتقلبات على هيئة مستعمرات صغيرة منفصلة لذا اعتبر هذا التركيز مثبط تام لظاهرة الانثيال وذلك من خلال قياس القطر على اطباق الاكار المغذي بالمليمتر . كما و أثبتت الدراسة انه كلما زاد تركيز الشب زاد تثبيط الانثيال و وجد أن التركيز المثبط للحركة في الوسط نصف الصلب هو ٠,٦ملغم / مللتر بعد أربع وعشرين ساعة من الحضن كما وأظهرت النتائج أن التركيز المثبط الأدنى للنمو هو ٠,٨ ملغم/مللتر . من نتائج الدراسة يظهر إن مادة الشب مادة فعالة ضد بكتريا المتقلبات P. mirabilis ومثبطة لظاهرة الانثيال والحركة .