

Inhibition of swarming and some virulence factors expression in *Proteus mirabilis* by amikacin in vitro

Abbas A.Al-Dulaimi

ABSTRACT:

The effect of amikacin on *proteuse mirabilis* virulence factors expression were examined. Exposure of bacterial cell to different concentrations of amikacin caused inhibition of swarming ability of *P. mirabilis*. The expression of virulence factors heamolysin was also inhibited by amikacin.

It was found that amikacin at concentration 2µ/ml could inhibit swarming activities, the production of urease and haemolysin was not affected by low concentration of amikacin but was inhibited by high concentration of it.

These results indicate the impact of amikacin on the virulence factors with *P.mirabilis* isolate.

INTRODUCTION:

Proteus mirabilis is a common causative agent of human urinary tract infection (U.T.I.) and a major cause of nosocomial infection(1).These bacteria also cause wound infection ,pneumonia and septicaemia(2).

Several pathogenic factors associated with *P. mirabilis* including fimbriae, flagella, urease, haemolysin, protease and outer membrane lipopolysaccharid(3)

Flagella necessary for motility are in involved in establishing infection(4).

The inducible urease is responsible for the formation of bladder and kidney stones at later stages of infection as a result of urea hydrolysis (5).

Furthmore, haemolysin secreted by *P. mirabilis* is cytotoxic for cultured Urinary tract epithelial cells(6).

Swarming behavior is characterized by the development of concentric rings of growth that are formed as cyclic events of swarmer cell differentiation (7 ,8). This cycle produces the bull's eye colony often associated with clusters of *P. mirabilis*(9). The ability of *p. mirabilis* to differentiate into swarming cells capable of rapid surface migration plays an important role in renal infections which involve colonization of the lower urinary tract followed by ascending migration of bacteria(10,11). This phenomenon is suppressed by using chemicals like sodium azide, boric acid, chloral hydrate and *p*-nitro phenyl glycerol (12).

The development of virulence factors can be inhibited by several methods such as growth medium or by substances added to the culture media for example antibacterial agents like B-jactam and aminoglycosides (13).

The aim of this study to investigate the effect of various concentration of amikacin (from aminoglycosides) on swarming activities of a clinical isolate of *P. mirabilis* and its ability to express virulence factors including urease and haemolysin.

Materials and methods:

1- Bacterial isolate: *Proteus mirabilis* isolate was isolated from a patient with U.T.I., and identified by using API 20 E system.

2- Swarming motility assays: was conducted to (12): the overnight culture (5ul) was inoculated centrally onto the surface of the dried swarming plates containing Luria-Bertoni (LB) broth, agar 1.5% and various concentration of amikacin and incubated overnight at 37C°.

3- Susceptibility testing: A minimum inhibitory concentration (MIC) of amikacin was determined by dilution testing on Muller Hinton agar (14).

4- Bacterial cells taken from swarming plates (in no 2) were suspended in P.B.S. after that the hemolysin & urease production was assayed:

- Blood agar plate method was used to detection of hemolysin Production
- Urea agar was used to detection of urease production.

Results & Discussion

After overnight incubation the swarming behavior of *P. mirabilis* was monitored. The swarming behavior was inhibited by amikacin at concentration 2 $\mu\text{g/ml}$ and was suppressed completely at 4 $\mu\text{g/ml}$ (Fig - 1).

(15) obtained similar result, when he reported the treatment of the bacterial cells with gentamicin changed significantly the cell morphology and caused inhibition of *Proteus* swarming'

(16) demonstrated a wide variety of morphological changes in clinical *Pseudomonas aeruginosa* isolates exposed to some B-lactam antibiotics.

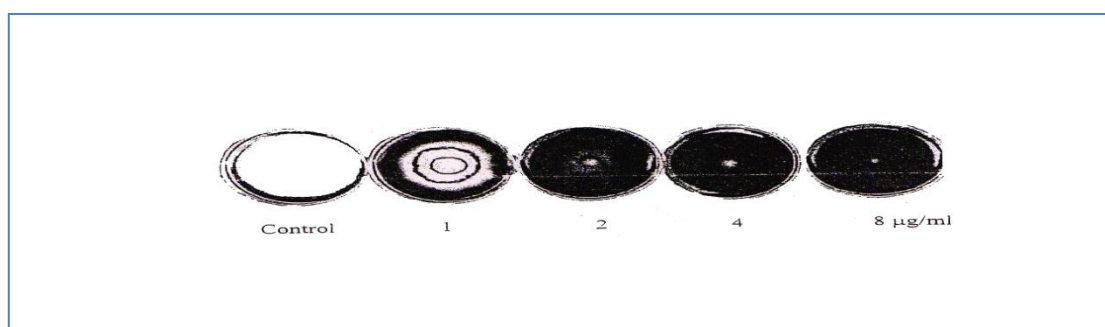


Fig - 1- effect of amikacin on the swarming activity of *P. mirabilis*,

* Numbers represent the amikacin concentration($\mu\text{g/ml}$) in L.B. agar.

(the MIC for amikacin against *P. mirabilis* was 16 $\mu\text{g/ml}$)

So, using amikacin in a concentration 4 µg/ml has a clear effect on the swarming activity without inhibiting of the cell growth. This considered is being important to decrease the virulence of pathogenic bacteria and help body immune system to work in right site. Several trails to decrease the dosage of antibacterial agent used in UTI were done and reveled successful results(17) successfully treated U.T.I in non pregnant women with amoxicillin 500mg three times daily for three days and it gave similar results to 10 days treatment.

Furthermore, sub MIC of some of these drugs may induce changes in Bacterial cells leading to better opsonization of the organisms. All these Changes could alter the pathogenicity and allow the host defense under certain circumstances to cope more readily with the infection(18).

To study whether production of virulence factors was also influenced by amikacin, the expression of urease and heamolysin in *P. mirabilis* isolate from L.B. agar plates containing various concentration of amikacin was determined.

The production of these factors was not affected by low concentration (0-4 µg/ml) of amikacin but was inhibited by high concentration (8 µg/ml) of it.

The amikacin inhibit organism by attaching to a specific receptor protein on the ribosome and prevent the synthesis of protein, this antibiotic could affect on the activity of regulators that control the expression of virulence factors and swarming activity.

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الخلاصة :

تم دراسة تأثير تراكيز مختلفة لمضاد الاميكاسين Amikacin على قابلية بكتريا *Proteus mirabilis* على الحركة المتموجة (الانثيال Swarming) وإنتاجها لبعض عوامل الضراوة مثل اليوريز والهيمولايسين والتي تلعب دورا مهما في أمراضية هذه البكتريا.

اوضحت نتائج هذه الدراسة ان لمضاد الاميكاسين تأثيرا واضحا على قابلية الانثيال ، فقد ثبتت هذه الحركة بتركيز 2 مايكروغرام /مل من مضاد الاميكاسين.

كذلك لوحظ ان هذا المضاد يمتلك تأثيرا على قابلية هذه البكتريا على انتاج اليوريز والهيمولايسين ولكن بتراكيز أعلى من التراكيز التي ثبتت بها ظاهرة الانثيال .