Molecular Analysis of *Pseudomonas aeruginosa* Strains Isolated from Hospitalized Patients in Erbil

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Abstract

A total of 10 *Pseudomonas aeruginosa* isolates was collected from burns and wounds patients in different Erbil hospitals.

Nine of these isolates were resistant to Ampicillin, Gentamycin, Ciproflaxacin, Cephalexin, Erythomycin, and Chloramphenicol, but sensitive to Naladixic acid, Cortimoxazol and Polymexin-B. While the isolate P. E 10 is resistant to Naladixic acid in addition to above.

The bacterium was found to contain two plasmid bands on agarose gel.

Transformation revealed that the resistance to the antibiotics was encoded by non-conjugative plasmids while no correlation was found with virulence determinants.

الخلاصة

جمعت 10 عزلات من *Pseudomonas aeruginosa* من حالات الحروق والجروح من مختلف مستشفيات اربيل وجد انها منتجة لليوريز والهيمولايسين.

درست حساسية جميع عزلات البكتريا *P.aeruginosa* لعدد من المضادات الحياتية واظهرت النتائج ان 9 عزلات مقاومة لامبيسيلين , جينتامايسين ,سايبرفلوكساسين , سيفاليكسين , اريثرومايسين , كلورامفينيكول , بينما كانت حساسة لنالدكسيك اسيد , كورتيموكزازول , بوليميكسين – ب . اما لعزلة العاشرة التي درست لاحقا P.E 10 كانت مقاومة لنالدكسيك اسيد بالاضافة الى مقاومتها للمضادات الحيوية المذكورة اعلاه .

أظهرت نتائج الترحيل الكهربائي في هلام الاكاروز احتواء العزلة على بلازميدين ذو وزن جزيئي صغير . ولم تظهر نتائج الاقتران البكتيري امكانية انتقال اي من صفات البكتريا المظهرية الى سلالة مختبرية من ايشرشيا القولون , في حين اظهرت نتائج التحول الوراثي انتقال صفات المقاومة للمضادات الحيوية الى ايشرشيا القولون المهيأة لالتقاط الدنا البلازميدي المستخلص من بكتريا *P.aeurgisano* . واظهرت النتائج ان المورثات المشفرة عن صفتي اليوريز والهيمولايسين هي كروموسومية الموقع .

Introduction

Pseudomonas aeruginosa is a ubiquitous, gram negative, opportunistic pathogen that is capable of acute infection in neutropenic and burn patients[1][2][3]. The bacterium is widely studied by scientists who are interested in not only its ability to cause disease and resist antibiotics, but also its metabolic capability and environmental versatility[4].

These bacteria are clinically important because they are resistant to most antibiotics and they are capable of surviving in conditions that few other organisms can tolerate. They also produce a slime layer that is resistant to phagocytosis[5]. The opportunistic pathogen *P. aeruginosa* has the capacity to produce large variety of virulence factors that play a role in the infection of injured or immunocompromised host[6][7]. The production of virulence factors is regulated in response to the

environmental conditions, such as iron and oxygen availability[8][9][10]. Infection with *P.aeruginosa* may result in death of patient with burns[11] and nosocomial pneumonia in intubated[12][13].

Clinical samples , in general , yield one or another of two smooth colony types . One type has a fried-egg appearance which is large ,smooth , with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence [14].

In this work we present the results of plasmid profile, conjugation and transformation from clinical isolate of *P . aeruginosa* (P.E10).

Materials and methods

- Bacterial strains

Clinical isolates of *P . aeruginosa* was obtained from a case of burns and wounds from 10 patients . Microorganisms from pure cultures were then stored in trypticase soy broth supplemented with 15 % glycerol at -30 C until used.

E . coli MM 294 (hsdR , hsdM , thi,Rif) was used as a recipients in conjugation and transformation experiments .

- Microbiological analysis

Sensitivity of *P. aerugionsa* isolates against 9 antibiotics (ampicillin, Gentamysin, Ciprofloxacin Cephalexin , Erythromycin , Chloramphenicol, Naladixic acid , Cortimoxazol , Polymexin –B) was conducted by the disk diffusion test[15].

- Plasmid DNA extraction

Plasmid DNA was extracted from *P. aeruginosa* isolates according to [16]. Extracted plasmids were electrophoresed through 0.8 % agarose gel in Tris – borate – EDTA buffer (0.089 M Tris base , 0.089 M boric acid , 0.002 M EDTA).

The gel was run under an electric field of 4V/cm, stained with 5 mg/ml of ethidium bromide and was then analyzed on exposure to UV light.

- Conjugation and transformation

Conjugation experiments was performed as indicated[17]. While transformation experiment was performed as indicate [16].

- Plasmid endurance test

P . aeruginosa was alternately sub cultured in antibiotic – free LB- broth media every 48 hrs for one month . Plasmid profile analysis was performed once a week and compared with the original and patterns of the respective plasmids.

Results and discussion

Results of antibiotic sensitivity tests indicated that Cortimoxazol and Polymexin-B were most active , inhibiting all of the isolates. Resistance to different antibiotics is cause by many factors[11]. Resistance to Ampicillin is conferred by the production of β –lactamase[18]. The β – lactamase open the β– lactam ring of Ampicillin producing compounds devoid of antibiotic activity [19]. The bacterium is naturally resistance to many antibiotics due to the permeability barrier afforded by its outer membrane LPS .

Also its tendency to colonize surface in a biofilm form makes the cells impervious to therapeutic concentration antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycets and molds, it has developed resistance to a variety of their naturally – occurring antibiotics [14].

Plasmid profile analysis indicated that P.E 10 harbored two small plasmid bands on electrophoresed agarose gel (Fig.1).

Conjugation experiments using *E. coli* MM294 as a recipient revealed no selectable transconjugants indicating non conjugative nature of P.E. 10 plasmid. On the other hand, transformation experiments yielded *E. coli* transformants . No correlation was found between the presence or absence of plasmids in the isolate and the virulence factors of *P .aeruginosa* .it was concluded that the plasmid content of the strain is not linked to urease and haemolysin production .

Moreover *P.aeruginosa* maintains antibiotic resistance plasmids, both R-factors and RTFs , and its able to transfer these genes my means of the bacterial processes of transduction and conjugation .

Plasmid profile analysis indicated that *P.aeruginosa* plasmids were preserved after subsequent sub culturing 15 times in antibiotic-free media.

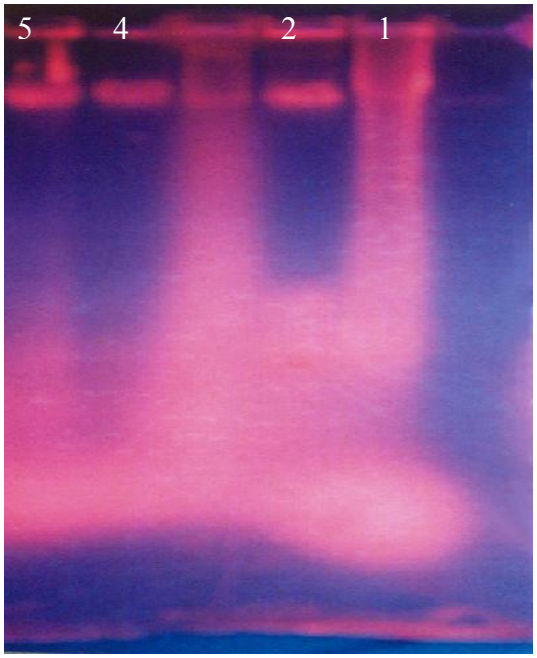

Fig. 1: Products of transformation and conjugation on agarose gel electrophoresis.
Lane 1. DNA *E. coli* after transformation.
Lane2. DNA of donor strain *Pseudomonas aeruginosa*.
Lane4. DNA of *E. coli* after conjugation.
Lane5. DNA of recipient strain *E. coli* MM294.

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