GENETIC STUDY OF RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATED FROM DIFFERENT CLINICAL SOURCES TO IMIPENEM [+]

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Abstract:

The antibiotic resistance is now a major clinical problem all over the world . In this study eighty swabs were collected from two different clinical sources; forty samples from wound infections and forty from otitis infections. Out of (23) *Pseudomonas aeruginosa* isolates there were (14) isolates from wound infections and (9) isolates from otitis infections. There were (5) isolates resistant to imipenem. The plasmid DNA were studied to these five isolates. The primary step were DNA extraction to showing DNA plasmid. The secondary step was curing DNA plasmid with acridin orange by using different concentrations. The results showed that the isolates lost DNA plasmid in 512µg/ml of acridin orange and disappear resistance to the imipenem. The genes that encoded to the resistance *Pseudomonas aeruginosa* to imipenem may be located on plasmid .These character may led to distribution of resistance to other bacteria by bacterial conjucation.

Key words: Resistant *Pseudomonas aeruginosa* to Imipenem , Otitis infections , Wound infections.

دراسة وراثية لمقاومة بكتريا *Pseudomonas aeruginosa* المعزولة من مصادر سريرية مختلفة لمضاد الأمييبنيم

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المستخلص :

تعد مقاومة البكتريا لمضادات الحياة من المشاكل السريرية الرئيسية في العالم لذلك جاءت هذه الدراسة لتتضمن جمع (80) عينة سريرية من مصدرين مختلفين، حيث قسمت العينات الى قسمين، القسم الأول تضمن (40) عينة سريرية من التهاب الجروح والحروق والقسم الثاني تضمن (40) عينة سريرية من التهاب الأذن الوسطى. تم الحصول من القسمين اعلاه بعد التشخيص البكتريولوجي على (23) عزلة بكتيرية تعود لبكتريا *Pseudomonas aeruginosa* كانت (14) عزلة تعود لألتهاب الجروح والحروق و(9) عزلات تعود لألتهاب الأذن الوسطى. بعد اجراء فحص الحساسية لمضاد الاميبينيم تم الحصول على خمسة عزلات مقاومة لهذا المضاد. جاءت الدراسة الوراثية على هذه العزلات الخمسة من اجل تحديد صفة المقاومة وراثياً، تضمنت الدراسة الوراثية استخلاص الدنا البلازميدي ومن ثم تحييده (فقدان الحزم البلاميدية) بأستعمال العامل المحييد (acridin orange) وبتراكيز مختلفة. بينت النتائج ان العزلات البكتيرية قد فقدت الحزم البلازميدية عند التركيز512µg/ml من هذه المادة المحييدة. اختبرت مرة أخرى قابلية

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العزلات البكتيرية الخمسة المقاومة لمضاد الأميبينيم بعد تحييدها لفحص الحساسية لهذا المضاد وبينت النتائج فقدان صفة المقاومة اذ اصبحت العزلات حساسة لهذا المضاد مما يشير الى أن الجينات المشفرة لصفة المقاومة قد تكون بلازميدية الموقع وبالتالي سهولة انتقالها الى الأنواع البكتيرية الأخرى السالبة لصبغة كرام بعملية الأقتران البكتيري.

Intruduction :

The bacteria *Pseudomonas aeruginosa* is a classic opportunistic pathogen especially because of its innate resistance to many antibiotics and disinfectants; and also due to its armoury of putative virulence factors plus additional acquired resistance due to plasmids [1]. It is also the most common Gram negative bacterium found in nosocomial infections causing various spectra of infections [2,3]. Carbapenems, such as imipenem and meropenem, remain one of the best drugs to treat infections caused by *Pseudomonas aeruginosa*. Increasing usage of these drugs and other expanded-spectrum antibiotics has resulted in the development of carbapenem-resistant *P. aeruginosa*. The clinical utility of these antimicrobials is under the emergence of acquired genes for β-lactamase, particularly those coding metallo-beta-lactamases (MBLs). Acquired MBLs expression in gram-negative pathogens is becoming a therapeutic challenge since these enzymes are capable of hydrolyzing all β-lactams except the monobactams [4,5]. The genes responsible for the production of MBLs are typically part of an integron structure and carried on transferable plasmids but can also be part of the chromosome [6] , The *bla*IMP genes were often located in *Pseudomonas aeruginosa* rods on large size plasmids.

Meterials and methods :

Specimens:

Eighty samples were collected from somehospital in Baghdad city including 40 samples from wound infection and 40 samples from otitis infections . The bacterial isolates were culturedon MacConkey agar and blood agar as well as usingAPI20E for the identification at species level .

Antibiotics susceptibility testing

Antibiograms were tested according to) [7] . as the following:

1. Preparation of bacterial supernatant by the use of normal saline and comparing the turbidity of bacterial supernatant with standard turbidity (McFarland's stantard) that refer to about(1.5×10^8) cell/ml.
2. The cotton swabs were used to spread part of bacterial supernatant on the plates of Muller-Hinton agar. The antibacterial discs were put on the isolated cultured on Muller-Hinton agar (five discs on one plate) by using sterile forceps.
3. The plates of Muller-Hinton agar were incubated at (37c°) for (24) hours. The inhibition zones were measured. The results were expressed as susceptible or resistant according to [8].

DNA plasmid extraction :

Boiling method was used to obtain DNA plasmid according to [9] as the following:

1. Bacterial supernatant (1.5ml) was transported to Eppendrof tube for each isolate and separated by microcentrifuge at speed (5000 rpm/minute) for (5) minutes.
2. Three hundred and fifty µl of sucrose Tris HCl EDTA solution (STET) and 25 µl of 10mg/ml lysozyme solution were added to the deposit of the centrifuged sample and were mixed for 3 second by vortex .
3. The solution was put in a water bath at (100 c°) for (40 seconds) and then was seperated by microcentrifuge at13000 rpm/minute for (10) minutes.
4. The viscouse pellet was removed and 40µl of potassium acetate solution and 420µl of isopropyl alcohol were added . These were mixed and saved at (-20 c°) for (1-2) hours.
5. The mixture was separated by microcentrifuge at 13000 rpm/minute for 15 minutes, and then 50µl Tris-Hcl EDTA solution was added to be ready for electrophoresis .

DNA plasmid electrophoresis in gel agarose :

The gel electrophoresis used in detection of plasmid DNA according to [10] as the following:

1. Agarose gel at concentration of 0.7% was prepared using Tris-Hcl boric acid EDTA (TBE) . The agarose gel solution was heated to boiling and then cooled down to 45-50 °c . Thus way folled by adding 10µl of ethidium bromide in concentration of 0.5µg /ml.
2. The comb was fixed in the slab to create wells that contianinig the sample and agarose gel solution was added carefully and abandoned for (30 minutes) to solidify.
3. The comb was removed from agarose gel carefully and the slab was fixed in electrophoresis chamber and Tris-Hcl boric acid EDTA was added to cover the surface of the agarose gel .
4. A volume of 10µl of the sample under test was put in Appendorf tube and 5µl of loading buffer was added and mixed carefully .
5. The samples were put in the created wells and electricity was passed at (5 volt/cm^2) for (1-2) hour until the pigment arrive to the other side of the agarose gel.
6. The agarose test was done by using ultraviolate transilluminator in wave length (360) nanometer.

Curing DNA plasmid :

The curing DNA plasmid was used according to [11] as the following:

1. Different concentrations of acridin orange (16, 32, 64, 128, 256, 512, 1024, 2000 ,2500, 3000) µg/ml were prepared and (0.1) ml of bacterial growth on nutrient broth,was added to each concentration .
2. The tubes that contain these concentrations were incubated at 37c° for 24 hours.
3. The subminimal inhibitory concentration was detected and decimal simplifications (10^{1-} - 10^{10-}) of this concentration was worked out .
4. A volume of (0.1) ml from the simplifications (10^{4-} - 10^{8-}) was taken and cultured on nutrient agar and then incubated at 37c° for 24 hours.
5. Gel electrophoresis of the isolates after curing process was used to ensure that the isolates were lost bounds of plasmid .
6. Antibiotic susceptibility test of imipenem was used to detect character of the resistance of the isolates that had lost bounds of plasmid .

Results and Discussion :

Eighty clinical samples were collected and examined including forty samples from wound infection and forty samples from otitis infection. There were (23) isolates of *Pseudomonas aeruginosa* comprising (14) isolates from wound infection and (9) isolates from otitis infection. The antibiogram were tested on Muller-Hinton agar by using imipenem. The result showed that (5) isolates from the total were resistant to imipenem (Table 1) . The DNA plasmid of the (5) isolates that were resistant to imipenem were studied, These isolates had lost the plasmid at 512µg/ml concentration of acridin orange and also lost the ability for resistance to imipenem.

Table (1) : Distribution of *Pseudomonas aeruginosa* in clinical samples sources and their resistance to imipenem

clinical samples	Source of sample	isolates of *Pseudomonas aeruginosa*	Resistance to the imipenem	Percent of resistance
40	Wound infection	14	3	21%
40	Otitis infection	9	2	22%

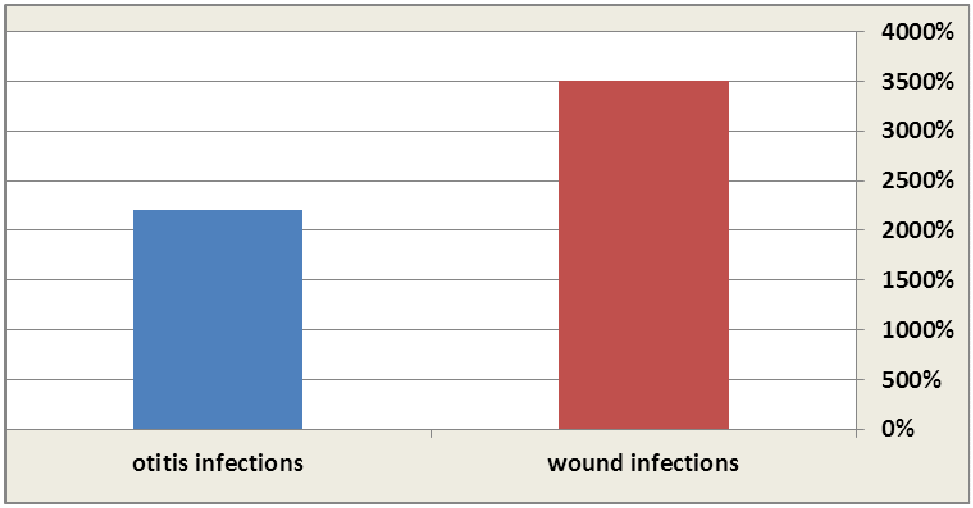

Figure (1) : Percent of the infections with *Pseudomonas aeruginosa*

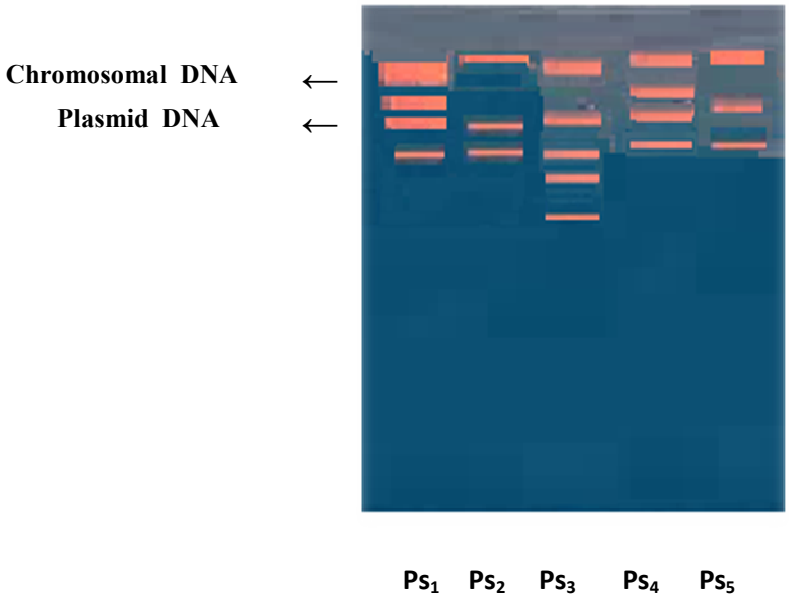

Figure (2) : Agarose gel electrophoresis showing DNA containinig of the isolates (Ps_1 , Ps_2 , Ps_3 , Ps_4 , Ps_5) that resist imipenem

In this study the results referd to that *Pseudomonas aeruginosa* was one of the major causes of otitis infections . A similar result was found by [12] . *Pseudomonas aeruginosa* is one of the major causes of infections with wound infections. A similar result was found by [13 , 14]. The percent of infection with *Pseudomonas aeruginosa* in case of wound infections was more than otitis infections. A similar result was found by [15] . Imipenem resistance by *Pseudomonas aeruginosa* was 21-22% of the total wound and otitis infection in this study respectively .

The ability to infect by this bacteria depend on virulence factors such as protease,hemolysin,exotoxin,pyocynin, biofilm information and resistance antibiotics. The resistance of *Pseudomona aeruginosa* to carbapenems (imipenem) increases with production of metalo-beta-lactamases [16]. *Pseudomonas aeruginosa* produces metalo-beta-lactamases , containinig metal ion that works as a cofactor for enzymes activity [17,18] . This can hydrolyse all metalo- beta- lactams [19]. The bacteria *Pseudomonas aeruginosa* may be resistant to antibiotic due to the mutation of efflux pumps or change permeability of outer membrane so that drug cannot penetrate through it , or hyper production of AmpC type beta-lactamases [20,21].

The resistance of *Pseudomona aeruginosa* to antibiotic may be as a result of genetic mutations, which occur either in the deoxyribonucleic acid (DNA) of the bacteria chromosomes or in the extrachromosomal transferable DNA called plasmids [22]. The antibiotic resistance can be transfered to other bacteria by the plasmid during conjugation [23,24] . The character resistance of *Pseudomona aeruginosa* to imipenem in this study was under plasmid controlingl. A similar result was found by [16 , 25].

There are studies shown the resistance gene can occur on transferable plasmid, transposons or jumping gene and specialized transposons [26] and The evolution of multidrug resistant plasmid often involves a site specific integration of antibiotic- resistance determinants [27]. These studies conclusion was the character of resistance antibiotic may be under plasmid controlling and these conclusion similar the conclusion was found in this study.

Conclusions :

The results of this study indicate that imipenem resistancce of *Pseudomonas aeruginosa* isolated from wound and otitis infections could be under plasmid control.

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