Distribution of resistance plasmid among clinical and environmental isolates of Pseudomonas aeruginosa

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الخلاصة:
صممت الدراسة لتوضيح دور البلازميد في انتشار المقاومة للمضادات الحيوية بين عزلات الزوائف الزنجارية التي تم الحصول عليها من الحالات السريرية والبيئية . تم جمع ٦٠ عزلة من الزوائف الزنجارية هذه العزلات كانت ذات درجة عالية لمقاومة ١٢ مضاد حيوي متوفر في الاسواق. تم دراسة النسق البلازميدي لـ٢٠ عزلة ماخوذة سريريا وبيئيا ولوحظ وجود مختلف البلازميدات في هذه العزلات. ان التشابه بنمط المقاومة للمضادات الحيوية والنسق البلازميدي بين العزلات السريرية والبيئية يؤكد دور البلازميد في انتشار الجينات المقاومة.

Summery
The present study has been designed to explain the role of plasmid in distribution of antibiotic resistance among clinical and environmental isolates of *Pseudomonas aeruginosa.* A total of 60 clinical and environmental isolates of *P.aeruginosa* has been collected . All isolates showed highly antibiotic resistance to 6 commercial available antibiotics. Plasmid profile of 20 isolates from clinical and environmental source showed the presence of different plasmid in these isolates. Similarity in antibiotic resistance pattern and plasmid profile between clinical and environmental isolate confirmed the role of plasmid in distribution of resistance genes.

Introduction

Pseudomonas aeruginosa is an opportunistic bacteria that lives in soil, water and even in environmental like hot tubes [1,2]. *P. aeruginosa* is ranking the second among gram negative hospital acquiring pathogens and one of leading cause of burn infection reported to the National Nosocomial in Infection Surveillance System [3,4], and most nosocomial infection was caused by *P. aeruginosa* that are often resistant to multiple antibiotics[5].

Natural resistance of *P. aeruginosa* to antibiotics was due to the permeability barrier offered by its outer membrane Lipopolysaccharide , formation of biofilm, presence of effluex pump system and harboring of antibiotic resistance genes located on plasmid[6]. Natural isolates of *Pseudomonas* are often harboring plasmids which are currently classified in to 14 incompatibility groups[7,8,9], most of these plasmids encoded traits contributed to the genetic plasticity , degradation , antibiotics resistance gene and adaptability of Pseudomonads in various ecological nichs[10,11].

In the present study we tried to explain the role of plasmid in distribution of antibiotic resistance among clinical and environmental isolates of *P. aeruginosa*.

Materials and methods

Bacterial isolates: 30 environmental isolates of *P. aeruginosa* collected from contaminated soil at garbage disposal site in different regions in Al- Najaf Al-Ashraf city and 30 clinical isolates of *P. aeruginosa* obtained from patients with burns in Al-Sadder Teaching Hospitals were collected during February – May 2011 and identified according to the standard microbiological procedures[12].

Antibiotic susceptibility test

The antibiotic resistance pattern of clinical and environmental isolates of *P. aeruginosa* was carried out by method of Bauer *et.al.*[13]. On Mueller Hinton agar by using 6 available antibiotics which include (Ceftazidim, Ceftriaxon,Amikacin, Gentamicin, Neomycin and Cefotaxim).

Plasmid profile

Large scale alkaline lysis method described by Kado and Liu [14] was carried out as the following:

*10 ml of LB broth inoculated with *P. aeruginosa* was centrifuged for 10 min. at 5000 rpm.

* The cell pellet was washed 2- times in Saline-EDTA buffer (NaCl 0.15M, EDTA 0.1M) .

*0.4ml of Glucose buffer (Tris-base0.01M , EDTA0.025M ,Glucose0.05M PH 8)was added.

* 0.4 ml of lysisbuffer (0.4 gmNaOH 0.2M in 1% SDS) was added and incubated on ice for 10 min.

* 0.8 ml of ice sodium acetate buffer (60 ml of sodium acetate 5M , 11.5 ml of Glacial acetic acid and 28.5 ml D.W. PH 5.8) was added and incubated on ice for 15 min. Centrifuge at 10000 rpm for 10 min.

* Transfer the supernatant to a new sterile eppendorff tube and 0.6 -0.7 ml of iso-propanol was added. Incubated on ice for 24hrs.

* Centrifuge at 10000 rpm for 10 min. , discard the supernatant and wash the pellet in 70% ethanol.

*Dissolved the pellet in TE buffer(Tris-base0.05M, EDTA 0.001M PH7).

DNA samples were electrophoresed through 1% agarose supplemented with 0.01µl of Ethidium bromide(5mg/ml) in 1X TBE buffer(Tris-base0.089M, EDTA 0.002M , Boric acid 0.089M) at 50V for 3hrs[15].

Result and Discussion

A total of 60 isolates of *P. aeruginosa* belonged to clinical (30 isolates) and environmental (30 isolates) sources were examined for antibiotic resistance against 6 antibiotics. A highly multiple antibiotic resistance was showed among clinical and environmental isolates as explain in Table (1). The high percentage of antibiotic resistance was observed to Gentamycin (20,21)% in clinical and environmental isolates of *P. aeruginosa* respectively. While the antibiotic resistance to other tested antibiotics was ranged between (6-19)% in clinical and environmental isolates.

Table (1): Antibiotic resistance pattern of clinical and environmental isolates of *P. aeruginosa*.

Antibiotics	Antibiotic pattern of 30 clinical isolates				Antibiotic pattern of 30 environmental isolates			
	Susceptible		Resistance		Susceptible		Resistance	
	NO.	%	NO.	%	NO.	%	NO.	%
Ceftazidim	12	40	18	60	12	40	18	60
Ceftriaxon	14	46.6	16	53.3	13	43.3	17	56.6
Amikacin	13	43.3	17	56.6	14	46.6	16	53.3
Gentamicin	10	33.3	20	66.6	9	30	21	70
Neomycin	13	43.3	17	56.6	14	46.6	16	53.3
Cefotaxim	13	43.3	17	56.6	14	46.6	16	53.3

Only 10 multiple antibiotics resistance isolates of each clinical and environmental isolates were selected for plasmid profile. Figure (1 and 2) showed that most clinical and environmental have different plasmid bands that contributed in antibiotic resistance.

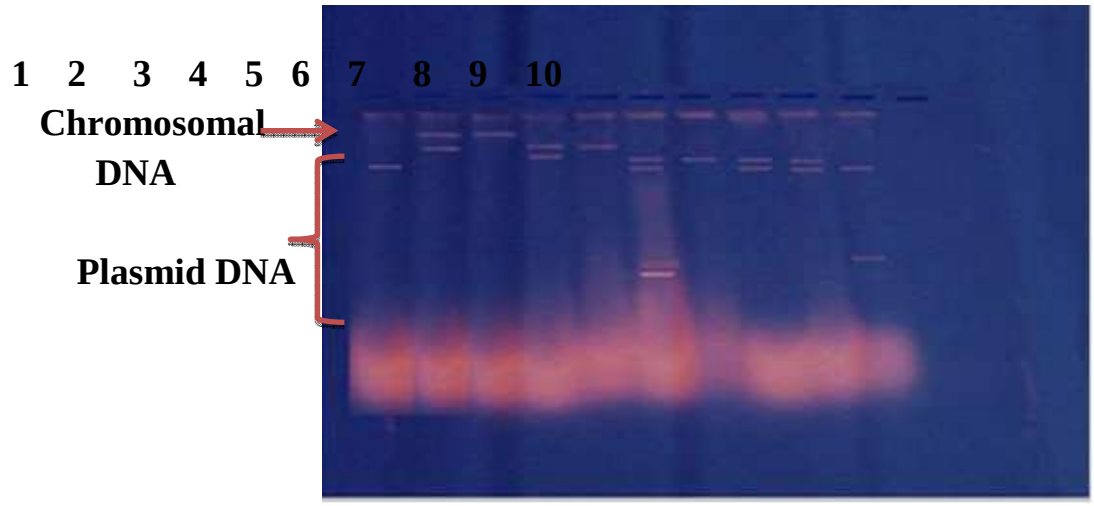

Figure 1: plasmid profile of 10 clinical multiple antibiotic resistance of *P. aeruginosa.*

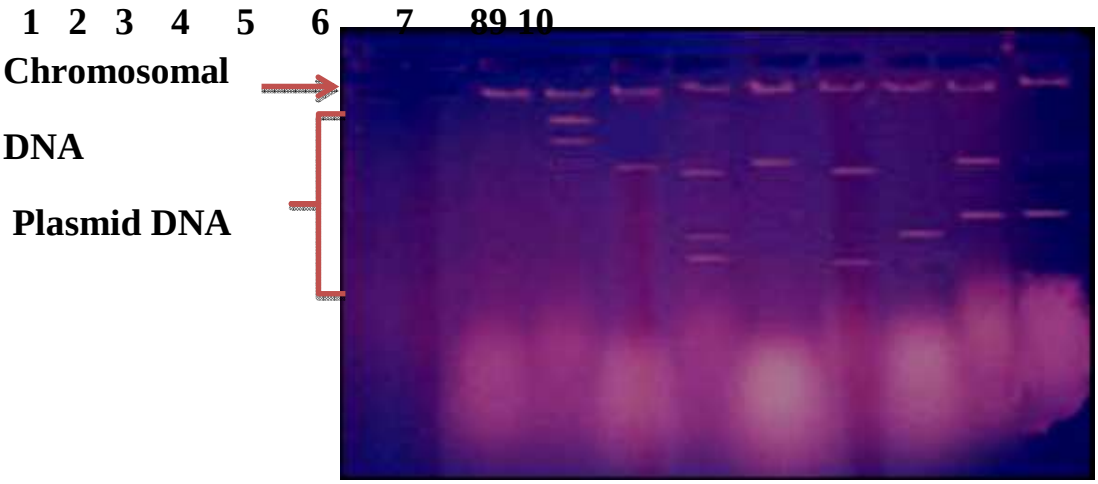

Figure 2: plasmid profile of 10 environmental multiple antibiotic resistance of *P.aeruginos*

Antibiotic resistance that showed in clinical and environmental isolates to certain antibiotic may be due to the ability of *P. aeruginosa* to produced certains enzymes that destroy the antibiotics such as chloramphenicol acetyl transferase[16],β-lactamase[17,18], aminoacyltransferase[19] or it may due to the effluex pump system[6]. Also *P. aeruginosa* harbors many R-plasmid that associated with antibiotics resistance [20,21,22]. Plasmids are extra-chromosomal DNA which carry resistance genes through generation which confers bacterial resistance[6]. The highly antibiotic resistance and plasmid profile showed in the present study confirmed that plasmids have been found to play an important role in developing of antibiotic resistance. The similarity in plasmid profile in clinical and environmental isolates explained that many plasmid may belong to the same family and may belong to the IncP-9 plasmid family which include large self-transmissible plasmid associated with degradation of antibiotic and toxic – metal

resistance marker[23,24,25]. On the other hand the similarity may revealed that *P. aeruginosa* is able to transfer R-plasmid by means of the bacterial processes of transformation and conjugation[6]. Horizontal gene transfers plasmid contribute considerably to bacterial evolution and adaptation.

In conclusion, *P. aeruginosa* associated with many problem in clinics as well as environmental pollution. Clinical and environmental isolates of *P. aeruginosa* showed highly antibiotics resistance.

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