GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

STUDY FOR PLASMID CONTENT OF MULTI-DRUG RESISTANCE *PSEUDOMONAS AERUGINOSA* ASSOCIATED WITH URINARY TRACT INFECTION

Kareem A. Hammadi & Amur Al Najar
Department of microbiology, Bagdad University, Iraq

ABSTRACT
Isolation and identification for *Pseudomonas aeruginosa* was carried out from (n = 620) urine samples obtained from patients in main teaching hospitals, visitors to diabetic center and private labs in Baghdad during the period between January 2014 – October 2014. The percentage for positive culture for this bacteria in examined urine samples was (n = 112/ 620, 18.06 %) While those urine samples with no growth or mixed culture was (n = 508 l 620, 81. 93 %). Among a total of positive culture (n= 112). There were (n= 72/420, 17.3 %) represented isolates urine samples from hospitalized patients in dialysis, and burn unit. While, there was (n = 40 / 200, 20 %) represented isolates urine samples obtained from out patients visited to the diabetic center, and private labs. The bacterial isolates were screened for their resistance to Gentamicin, Tobromycin, Ciprofloxacin, Amikacin, Ceftriaxone, Carbenicillin, Aztreonam, Norfloxacin, Ceftazidime, Piperacillin, Piperacillin tazabactam, Ticarcillin clavulant, Cefotaxime, Meropenem and imipenem by using disc method. Those tested isolates with highest resistance to Gentamicin (87.5%) and lowest resistance to meropenem, imipenem with (16.7 %, 8.0 %) percentage respectively. Screening of plasmid content by agarose gel electrophoresis for this bacterial isolates revealed the presence of plasmids in (40 %) were carried plasmids with different sizes.

KEYWORDS: Ceftriaxone, Carpenicillin, Aztronam, Norfloxacin, Ceftazidime, Piperacillin, *Pipracillin tazabactam*, Ticarcillin clavulant, Cefotaxime, Meropenem, *Pseudomonas aeruginosa* , Urinary tract infection, Plasmid content.

INTRODUCTION

It is widely presents in the environment such as soil, water and sewage[1,2]. In cystic fibrosis, it shows the ability to live in micro aerobic enviroments[3]. It is able to form a biofilms which is an expolysaccharide alginate, that play a role in protection from phagocytosis, complement activation, and antibiotic action[4]. Many of the antibiotic resistance genes are located on plasmids. These plasmids play a major role in genetic plasticity, degradation of antibiotics resistance genes and the adaptability of this organism in different environments[5]. It maintains antibiotic resistant plasmid and able to transfer these genes by means of transduction and conjugation[6]. This could focus and pay attention on the role of plasmid in resistant phenomena, and help in the selection of drug of choice for this bacterial infections treatment.

For the above aims, the following steps were performed.

1. Isolation and identification of *Ps. aeruginosa* from urine speciemens.
2. Investigation of the occurrence of multi drug resistant in *ps. aeruginosa*.
3. Using plasmid profiling to ralate the types, number and size of plasmids with antibiotics resistance as molecular markers.

MATERIALS & METHODS

A total of (n = 620) urine samples were collected from two main Teaching hospitals, main diabetic center and privates labs in Baghdad through period from January 2014 to October 2014. These urine samples were collected in a sterile plastic container and culture on blood agar, Macconkay agar, the conventional laboratory procedures were used for isolation of (n = 112) *Ps . aeruginosa*[6,7]. These (n= 112) of bacterial isolates were tested with disc diffusion method for susceptibility to (15) antimicrobial agents (Himedia, India, and Binanlyse Turkey). The antimicrobial agents include: Carbencillin 100mg. Aztreonom 30mg, Amikacin 30mg, Cefataxion 30mg, Ceftazidime 30mg, Imipenem 10mg, Meropenem 10mg, Piperacillin 100mg, piperacillin tazobactam 100/10mg, Ticarcillin clavulanat 75/10mg, Gentamicin 10 mg, Tobramycin 10 mg, Ciprofloxacin 5 mg and Norfloxacin 10 mg, Ceftriaxone 10mg .

Plasmid DNA Extraction

It was carried for selected (n = 20) bacterial isolates by using the Accu. prp plasmid mini extraction kit (Bioneer, Korea) with modifications of alkaine lysis method Brinboimetal[8].

Agarose gel electrophoresis

Plasmid DNA samples were resolved by agarose gel electrophoresis[9]. Agarose gel was prepared by adding (1) gm of agarose to (100) µl of IXTBE buffer, power was turned on at (70) V for (1.5) hr . (3) ml of loading dye (ethidium bromide for staning the bands was used) . The plasmids were visualized by using ur transilluminator at (350) nm and photographed by uv scanner [10].

RESULTS & DISCUSSION

Pseudomonas is widely presents in the natural enivorment with an adaption to live ranging from surface water,

disinfectants and even in the humidifiers of respirators[11]. This bacteria was reported to be the most commonly isolated gram negative bacteria (18. 1%) from nosocomial phenmonia, and it is second in related to urinary tract infection[12].

TABLE 1: Bold the distribution of urine samples (n = 620) according to the Gender and Location of collection .

Patients status	No of urine samples	%	Collected Location
Male	334	53. 80	
Female	286	46. 20	Dialysis Units
Total	620	100	
Inpatients	280	45. 25	
	140	22. 50	Burn Units
Out patients	200	32. 25	Diabetic private Center and labs

Results from table (1) revealed that there were (n = 420/ 620, 67.75%) of urine samples collected from hospitalized patients including (280/ 620, 45.25 %) from dialysis units and (n = 140/ 620, 22.50) from burns units. There were (n = 200/620, 32. 25 %) represented urine samples from out patients visited diabetic enters and privates labs. Table (1) also showed that there were (n = 334/ 620, 53. 80 %) urine samples from male, while there were (n = 280/620, 46.20 %) urine samples from female. These results showed that male were predominance among patients.

TABLE 2: the distribution of *Ps* . *aeruginosa* isolates among (n = 620) urine samples .
Distribution of Bacterial isolates Obtained from Urine Samples According to Isolation Sources.

Urine Samples according to isolation sources	No. of Urine Sample	No. of *Ps. aeruginosa* Isolates	% *of Ps. aeruginosa* isolates
Dialysis unit	280	29	10.35%
Burns unit	140	43	30.7%
Diabetic center and private Labs (Patient)	100	28	28.0%
Diabetic center Private Labs Healthy (Person)	100	12	12.0%
Total	620	112	18.06%

Table (2) showed the distribution of this bacterial isolates according to isolation sources. There were (n=43/ 140, 30.7%) bacterial isolates from urine samples obtained from patients in burns units, from dialysis units there were 29/280, 10.35%) bacterial isolate, from diabetic center and private labs there were (28/100, 28%) bacterial isolates. While there were (12/100, 12%) represented bacterial isolates from non-diabetic patient.

TABLE 3: The Distribution of Number and Percentage of Resistance *ps. aeruginosa* isolates by Disc Diffusion Method (n=112)

No.	Antimicrobial agent	Symbol	No. of resistance isolates	Percentage %
1	Gentamicin	CN	98	87.5
2	Tobramycin	TOB	86	76.6
3	Ciprofloxacin	CIP	83	74.1
4	Amikacin	AK	75	66.9
5	Ceftriaxone	CRO	70	62.5
6	Carbenicilin	PY	69	61.6
7	Piperacilin	PRL	67	58.9
8	Ceftazidime	CAZ	65	58.1
9	Norfloxacin	NX	64	57.1
10	Cefataxime	CTX	64	57.1
11	Ticarcilin clavalant	CTC	61	54.4
12	Aztreonam	ATM	60	53.5
13	Piperacillin tazobactam	PTZ	42	37.5
14	Meropenem	MEM	18	16.7
15	Imipenem	IMP	9	8.0

Result from table (3) showed that piperacillin resistance was (58.9 %) which was close to findings in Najaf (60.5 %) [13, 14] in comparison with (65.1 %) in India, in Pakastan was (32.0%)[15]. In related to carbenicillin resistance result was (61.6%) in comparison with (80 %) in some British hospitals, in related to ceftazidme and cefataxime the results were (58.1%, 54.4%) respectively, these results correlated with increase antibiotics resistance worldwide [17].

Table (3) showed different levels of resistance to Gentamicin (87.5%) which represented the highest resistance, while the lowest resistance, while was imipenem (8. 0 %)

Table 4: Plasmid Profile and Antimicrobial Resistance for (n = 20) *Ps . aeruginosa* isolates .

No.	Isolates Symbol	Antimicrobial agent	No. of Anti-Microbial agent	No. of plasmids	Size of plasmids
1	PS 3	AK, CIP, CTX, CTC	4	Free	-----
2	PS 111	CRO , AK	2	1	1.000
3	PS 19	CN, AK, CRO, PY , TOB	5	1	1.000
4	PS 112	AK, ATM, CN, CIP, CTC	5	Free	-----
5	PS 45	AK, CRO, CTX	3	Free	-----
6	PS 92	CRO, CTX, CN	3	Free	-----
7	PS 21	CN, PY, AK, CTX, CTC, CIP	6	2	1.000, 1.000
8	PS 77	AK, CTX, CRO, ATM	4	Free	-----
9	PS 2	---	--	Free	-----
10	PS 102	---	--	Free	-----
11	PS 75	CN, AK, PY, TOB, CRO	5	1	1.000
12	PS 106	AK, CTX, CIP, CN	4	1	1.000
13	PS 83	CIP, PY, AK	3	Free	-----
14	PS 88	---	--	Free	-----
15	PS 16	CIP, CTX, CN, CRO, TOB	5	Free	-----
16	PS 15	CTX, ATM, CW, CIP	4	2	1.000 0.900
17	PS 40	CTX, AK, PRL, ATM, TOB, CRO	6	3	650, 1000,2000
18	PS 82	---	--	Free	-----
19	PS 17	CRO , AK	2	Free	-----
20	PS 29	---	--	Free	-----

Results from table (4) , figures (1 , 2 , 3) showed that (12/20, 60 %) of this bacterial isolates had no plasmids .The remaining isolates (8/20, 40 %) had plasmids with different sizes .

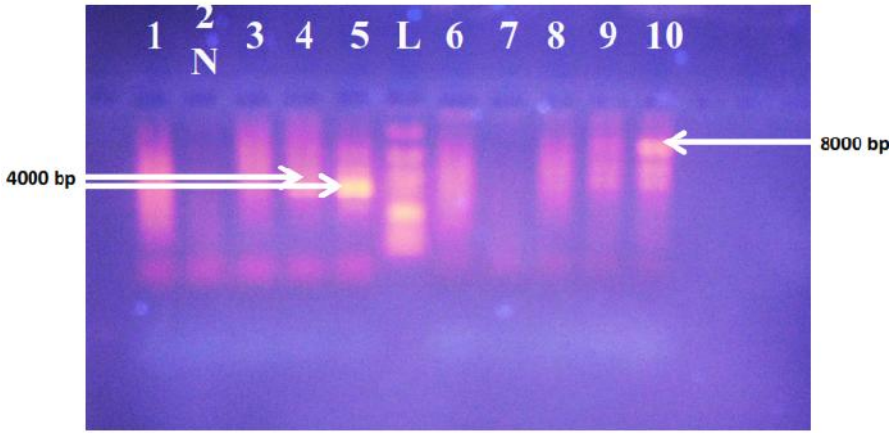

FIGURE 4 – 1: Gel electrophoresis (1% agarose, 70V, for 1.5 hour) using plasmid extracted DNA. Lane L : 10000 bp DNA ladder, Lane N : negative control (water). Lanes 4 (4000bp), 5(4000bp), 10(8000bp), show positive plasmid DNA.

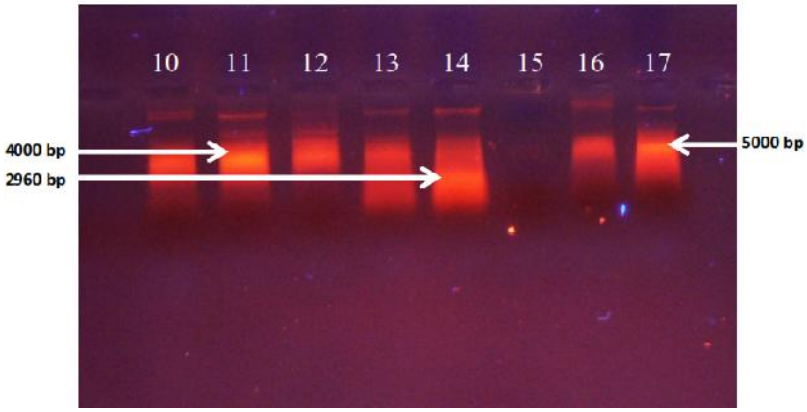

FIGURE 4 – 2: Gel electrophoresis (1% agarose, 70V, for 1.5 hour) using plasmid extracted DNA. Lane L : 10000 bp DNA ladder, Lane N : negative control (water). Lanes 11 (4000bp), 14(2960bp), 17(5000bp), show positive plasmid DNA.

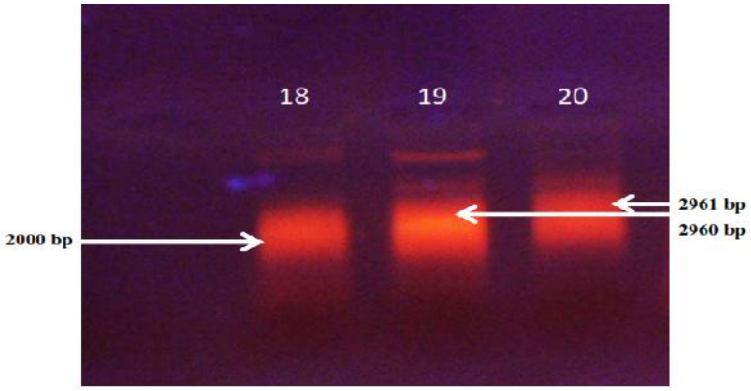

FIGURE 4 – 3: Gel Electrophoresis (1% agarose, 70v, for 1.5hour) using plasmid extracted DNA. Lines 18(2000bp), 19(2960 bp), 20(2961 bp). Showed positive plasmid DNA.

REFERENCES

[1]. Brooks , G.F., Butel, J.S. & Morse, S.. (2007) Enteric gram - negative rods (Enterobacteriaceae) . In Brooks G.F., Butel, J.S., Morse, S. A. Jawetz, Melnick and Adelberg`s medical microbiology 24 thed – MC – graw – Hill, U.S.A.

[2]. NeuHc (1983) The role of *pseudomonas aeruginosa* infection. J. Antimicrob, Chemother. 11 (suppl B). 1–13 .

[3]. Ulrich, M., Schwabu, Wortitzsch, D., Robert Talen et . al . , (2002) Effects of reduced mucous oxygen concentration in airway pseudomonas infections of cystic fibrosis patients. J. clin. Invest 109 (3): 317 – 325.

[4]. Horn , M.P., Zuercher, A. W., Imboden, M.A., Rudolf, M. P., Lazartt, W. U. H., Hoiby, N., Fas, S.C. & Lange , A.B. (2010) Pre clinical *In vitro* and *In vivo* characterization of the fully Human monoclonal Igm Antibody KbpA 101 specific for *pseudomonas aeruginosa* serotype IATs – OH – Antimicrob . Agents chemother, 54 (6) : 2338 -2344 .

[5]. IZ malkova , T. Y., Marrodi, D.V., Sokolov, S. L., kosheleva , I .A., Small, K., Thomas, C.M., Boronin, A.M . (2006) Molecular classifications of Inc p . 9 group naphthalene degradation plasmids. Plasmid, 56, 1– 10.

[6]. Todar, K. (2004) Pseudomonas and related bacteria. Todar`s online Text book of Bacteriaology . Available on line at: httP//tex book of bacteriology. net / pseudomonas. html. Acess 5th . July 2006.

[7]. Sagia, H. etal . Antimicrob . Agents chemother. 10, 573 - 578 (1976).

[8]. Hansen, J.B., Olsen, R.H. Nature 274, 715 – 717 (1978) .

[9]. Jacoby, G.A. (2009)Clin . microbial . Rev, 22(1) : 161 – 182 .

[10]. Thomas, L.C. (2007) Genetic methods for rapid detection of medically important nosocomial bacteria. Faculty of medicine, department of medicine, the University of Sydney, Australia.

[11]. Cornelis, P. (editor) (2008) pseudomonas genomics and molecular Biology (Isted). Caister Academic press. Is BNI – 9044 -55–19 –0.

[12]. Todars online Text book of Bcteriology : Text book of bacteriology . Net 2004. 06. 04 Retrived on 2011– 10 – 09.

[13]. Salam, K.B., Skander, G., Ahlem, J., Meriem, M., Chedlia, F., Abdellatif, B. and Maher, G . (2011) Epidemiology of *pseudomonas aeruginosa* in Intensive care unit and otolaryngology department of a Tunisian hospital. African J . Microbio . Res, 5 (19): 3005 – 3011 .

[14]. AL–Muhannak, F.H. (2010) Spread of some extend spectrum beta – lactomases in clinical isolates of gram – negative Bacilli in Najaf . M.Sc. Thesis college of medicine, university of Kufa .

[15]. Ali Khan , J . Zafari , I., Saeed, U. and Kalsoom, F . (2008) Prevalence and pattern of *pseudomonas aeruginosa* against various antibiotics. Pak . J . Pharm . Sci , 21 (3) : 311 – 315 .

[16]. Corona – Nakamura, A. L., Miranda – Novales, M.G. Leanos – Miranda, B., Partillo. Gomezl, Aguilar – Benavides, S., (2001) Epidemiological study of *pseudomonas aeruginosa* in critical patients and reservairs . Arch . Med . Res . 32 : 238 – 242

[17]. Clinical and laboratory standards institute (CLSI) (2010) Performance standards for antimicrobial susceptibility testing; 20th. Clin. and Labo. stand. Ins.