Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(2):56-61

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Prevalence of CTX-M beta-lactamase of *Pseudomonas aeruginosa* in Al-Diwaniya City

Abeer Hamoodi Jabbar and Syoof Khowman Alramahy*

Biology Department, University of AL- Qadisiyah, AL-Diwanyia province, Iraq

---

ABSTRACT

Atotal of360 clinical isolates were routinely collected. isolates were classified according to source of collection to threesets(169 swab ear ,102 swab burns, and 89 sample urine), results of cultural and biochemical tests showed that 50 isolate belong to Pseudomonas aeruginosa, all study showed isolates contain the gene 16s-ribosomal RNA which represents the gene diagnostic designed,In the present study results indicate to ability these isolates to produce the broad- spectrum bla$_{CTX-M}$β- lactames enzymes groups by detecting the presence of bla$_{CTX-M-1}$,CTX-M-2, CTX-M-15, CTX-M-43 enzymes among those isolates by the (polymerase chain reaction)seriesthe results show the proportion to presence of geneb la$_{CTX-M-2}$ that was Highest rate, amounting to 54.16% in the 13 isolation followed by the gene bla$_{CTX-M-43}$as it was his presence a 33.3% rate in the eight isolates of either gene of bla$_{CTX-M-1}$as Seen by 20.83% in the five isolates of the present study indicated that isolates all of which did not give the result of amplification to bla$_{CTX-M-15}$Which recorded rate 0%. the nucleotide sequence for nucleic acid DNA in this study was detected by using the DNA Sequencing to isolates that appeared resistance to antibiotics ofβ-lactam groups.

Keywords: Pseudomonas aeruginosa; Antibiotics resistance mechanisms; β- lactam antibiotic; Beta-lactamase enzymes, DNA sequencing

---

INTRODUCTION

*Pseudomonas aeruginosa*are one of profiteer pathogens that have many of the virulence factors and is an important cause of hospital-acquired infections [1]. These bacteria can live in inactive environments with little nutritional requirements [2].The infection with this bacteria is an tricky to treat due to the Possession of natural impedance against several drugs (MDR) [3].In recent decades there has been a universal spread of bacterial resistance to antibiotics, which declared a state of emergency and the World Health Organization considered a public health problem in 1998 [4]. Thus, the extensive use of wide spectrum antibiotic increased bacteria *Pseudomonas aeruginosa* resistance to drugs Used in therapy [5].And resistant of antibiotics occur through the acquisition of several distinct mechanisms including alteration of the target site of anti-foreign or modulating membranes, producing enzymes beta-lactamase, active Efflux- systems. Bacterial resistance to antibiotics has increased, and the reason is due to the misuse todrugs, such as the β-lactam antibiotics and aminoglycosides antibioticin burn patientsIn addition to lack Provide and high costs of another efficient drugs [6]. This study aimed to: Knowledge spread to enzymes beta-lactamase type *bla$_{CTX-M}$* in isolates of *P. aeruginosa* strains that are isolated of clinical situations in different hospitals in the Diwaniyacity.

METHODOLOGY

Samples collection

During the period from November 2015 to April 2016, aggregate to 360 non duplicate Clinical specimens were Gathered from patients visited/or admitted to Al-Diwaniya hospitals in Al-Diwaniyacity.Samples which were collected by sterile swabs and containers had been cultured on macConkey agar and bloodagar, to get pure colonies subculture done on macConkey agar, incunated for overnight at 37C^{o}. The sampleswasInsulated from ear swab (46.9), burn (28.3%), and urine (24.7%). And conducted out biochemical tests, then isolates stored at –76°C in milk broth glycerol.

Polymerisation chain reaction (PCR)

Polymerisation chain reaction (PCR) has been done polymerization chain reaction examination so as to conduct confirmatory diagnosis of isolates to bacterium *Pseudomonas aeruginosa*, In addition to investigate some of the types of resistant genes to drugs and Extended spectrum beta-lactamase (bla-$_{CTX-M}$) [7]. PCR amplification was performed using polymerization reaction mixture by using a series of several PCR PreMix processed by the Korean Bioneer the company, according to the company's instructions as follows reaction mixture was prepared in a series ofPCR tubes required with a kit containing polymerization chain reaction components and other components were added to the reaction mixture [8]. After the completion of the preparation of a mixture polymerization chain reaction tubes and then closed the tubes and mix carefully to a vortex mixer for 10 seconds.. The tubes transported to a PCR Thermocycler for cases of PCR thermocycler conditions [9]. Amplicon then Conceive on a UV transilluminator system and photographed.

DNA sequencer method

DNA sequencer method It was conducted way DNA sequencing to identify the genetic sequence of the resistance genes life for antibiotics class of beta-lactamase (*bla-$_{CTX-M}$*) at the bacterium diagnosed manner examine the PCR through conducting genetic tree analysis Phylogenetic tree analysis using the Mega programs 6, where he was initially caused by the interaction of the PCR for all genes *bla-$_{CTX-M}$* genes were then send the result of the PCR reaction to Macrogen company in South Korea and to conduct DNA sequencing using the device AB DNA sequencing system.

Table 1: DNA Primer which purchased from Bioneer (Korea) company

Genbank code	Sequence		Amplicon	Primer
FM881781.1	F	CGCCGTTAAACGATGTCGAC	**510bp**	16S rRNA
	R	CAGACTGCGATCCGGACTAC		
X92506.1	F	GGAAGACTGGGTGTGGCATT	682bp	CTX-M-1
	R	CCTTAGGTTGAGGCTGGGTG		
X92507.1	F	ACCGCCGATAATTCGCAGAT	424bp	CTX-M-2
	R	GATTTTTCAGGGTCTGCGCC		
DQ102702	F	CCTTCCGTCTGGACAGAACC	329bp	CTX-M-43
	R	CTGCTCCGGTTGGGTAAAGT		
AY044436.1	F	CGCGTGATACCACTTCACCT	279bp	CTX-M-15
	R	CCTTAGGTTGAGGCTGGGTG		

RESULTS

A total of 360 clinical samples have been collected, the distribution of clinical samples was explained in table 1. 169 (46.9%) were collected from ear while102 (28.3%) and 89 (24.7%) samples were collected from burn and urine respectively. According to the gender 162 (45%) samples were collected from female while 198(55%) samples were collected from male. Outpatient constituted 226 (62.7%) and134 (37.2%) samples from inpatient. The study recorded a significant difference in infection rates between arrivals and those who are asleep and patients as the sample source, while significant differences between males and females did not register in the abstract level of P <0.05 .

Table 2: Distribution of 360 clinical samples according to the type, gender and hospitalization

Sample	Type	Total number	Percentage
Source	Ear	169	46.9
	Burn	102	28.3
	Urine	89	24.7
gender	Male	198	55
	Female	162	45
Hospitalization	Outpatient	226	62.7
	Inpatient	134	37.2

Only 50 (13.8%) isolates were belonging to *P. aeruginosa* in which 15.3% of them were isolated from ear swab while 13.7% and 11.2% were isolated from burn and urine respectively. The study recorded a considerable difference atbacterial isolatestobacterial isolates were negative and the positive and gram by source of collecting samples at a moral level of <0.05 .P.

Table 3: Incidence of the bacterial typesisolated atvarious clinical samples

Source of Samples	No. of samples	No.(%) of P. *aeruginosa* Isolates	No.(%) of Gram positive and negative isolates	No.(%) of no growth or contaminated cultures
Ear	169	26(3.15%)	53(31.3%)	90(53.2%)
Burn	102	14(713.%)	36(2.35%)	52(9.50%)
Urine	89	10(11.2%)	32(9.35%)	47(52.8%)
Total	360	50(13.8%)	121(33.6%)	189) 5.52%)

Results appeared, all isolates of *P. aeruginosa* bacteria that are 24 isolate contain 16S rRNA gene, which is a diagnostic gene to the bacteria. Figure (1)

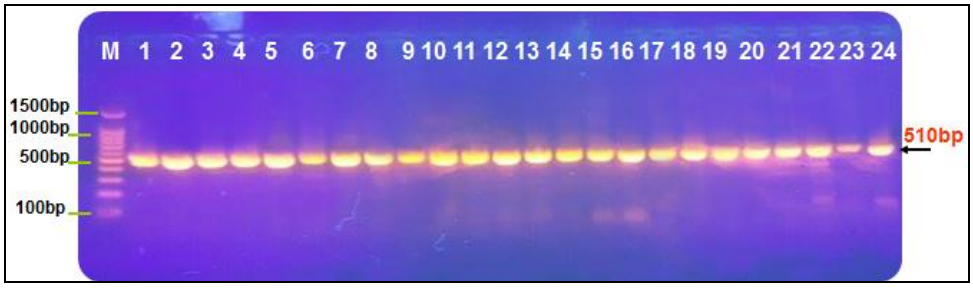

Figure 1: Electric deportation to gel agaroseand containing the results of the PCR assay for the gene 16SrRNA private gene diagnosis *Pseudomonas aeruginosa*. Where an M: Marker ladder 1500-100bp and drilling of the number (1-24) some positive bacterium Proteus mirabilis for testing an output length of 510bp. Using current (80) Amp and (100) volt for 1 hour

P. aeruginosa possess many of the mechanisms that enable them to resistance that include enzymes secretion analyst for antibiotic mechanism such as enzymes β-lactamases, and Cephalosporinases of the most important resistance mechanisms (10). To investigate the mechanism to β-lactam resistance Table (4) showed distribution to bacterial isolates and the presence of enzymes. The results showed presence to $bla_{CTX\text{-}M\text{-}2}$at 13 (54.16%) isolate of β-lactam resistance isolates. Figure (2)

Table 4: Apportionment of various resistance genes to β-lactam in *P. aeruginosa* isolate n=24

Occurrence of gene	No. of isolates	%
$bla_{CTX\text{-}M\text{-}1}$	5	%20.83-
$bla_{CTX\text{-}M\text{-}2}$	13	%54.16-
$bla_{CTX\text{-}M\text{-}43}$	8	%33.30-
$bla_{CTX\text{-}M\text{-}15}$	0	%0.00
Total	26	%52

Figure 2: Electric deportation to agarose gel and containing the results of the examination mPCR genes pharmaceutical antibiotics from Extended spectrum beta-lactamase group (bla-CTX-M genes) in isolates of the bacterium Pseudomonas aeruginosa. Where an M: Marker ladder 1500-100bp and drilling of No. (3 5-8o 13-15o18-20o 11 and 23) isolates were positive for the gene beta-lactamase (blaCTX-M-2) gene an output length of 424bp. And drilling of No. (9, 16, 17, 22 and 24) isolates were positive for the gene beta-lactamase ($bla_{CTX-M-1}$) gene) an output length of 682bp

Figure 3: Electric deportation to agarose gel and containing the results of the examination mPCR genes pharmaceutical antibiotics from Extended spectrum beta-lactamase group (bla-CTX-M genes) in isolates of the bacterium Pseudomonas aeruginosa. Where an M: Marker ladder 1500-100bp and drilling of the No. (8, 15, 16 and 24 19-22o) isolates were positive for the gene beta-lactamase (blaCTX-M-43) gene an output length of 329bp. And the lack of isolates positive for the gene beta-lactamase (blaCTX-M-15) gene) an output length of 279bp

There is a significant difference in spread to genes bla_{ctx-m} between bacterial samples sources of samples and gender of the patients at a moral level of P <0.05 .

Table 5: Distributiontobla_{CTX-M} enzymes according to samples sources

Genes	No.(%) of isolates	samples sources		
		Ear n=26	Burn n=14	Urine n=10
$bla_{CTX-M-1}$	5(20.83%)	3(11.53%)	2(14.28%)	-
$bla_{CTX-M-2}$	13(54.16%)	6(23.07%)	4(28.57%)	3(30%)
$bla_{CTX-M-43}$	8(33.3%)	(%519.23(	1(7.14%)	2(20%)
$bla_{CTX-M-15}$	0(0.0%)	-	-	-
total	(%52)26	14(53.84%)	7(50%)	5(50%)

Table 6: Distribution of the bla_{CTX-M} genes depending on gender

Genes	No.(%) of isolates	gender	
		Male n=19	Female n=31
$bla_{CTX-M-1}$	5(20.83%)	1(4.1%)	4(16.7%)
$bla_{CTX-M-2}$	13(54.16%)	4(16.7%)	9(37.5%)
$bla_{CTX-M-43}$	8(33.3%)	6(25%)	2(8.3%)
$bla_{CTX-M-15}$	0(0.0%)	----	----
total	26(52%)	11(57.8%)	15(48.3%)

DNA sequencer used in determining the sequence of nitrogenous bases of nucleotides which represents (adenine-guanine-cytosine-thymine), as it has been in this technique the analysis of phylogenetic tree. Figure (4)

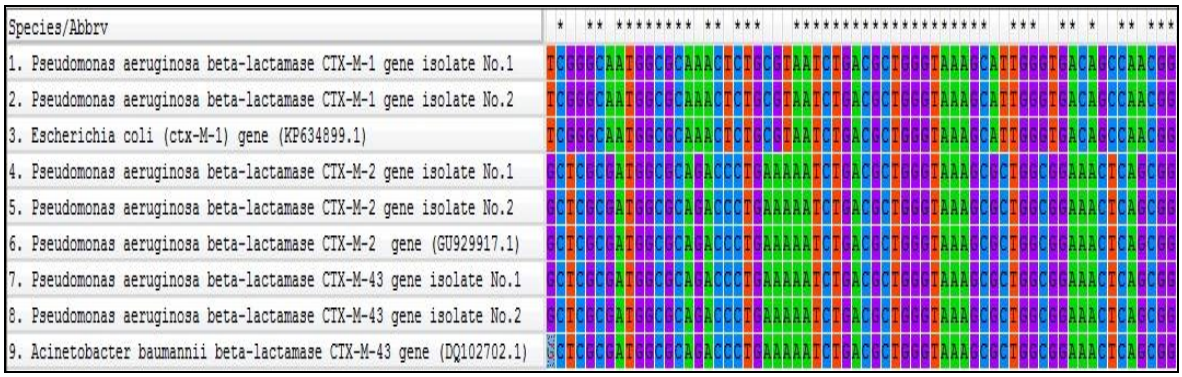

Figure 4: Multiple alignment analysis of the genetic bases sequence, using the program (MEGA6) for the results of PCR *bla*$_{CTX-M}$ gene in *Pseudomonas aeruginosa,* which show places the similarities for lining up sequence to bases of b-lactam$_{CTX-M}$ genes.representedby an asterisk

	1	2	3	4	5	6	7	8	9
1. Pseudomonas aeruginosa beta-lactamase CTX-M-1 gene isolate No.1									
2. Pseudomonas aeruginosa beta-lactamase CTX-M-1 gene isolate No.2	0.00								
3. Escherichia coli strain RIGLD-2D9-CRO-F1 CTX-M-1 (ctx-M-1) gene (KP634899.1)	0.00	0.00							
4. Pseudomonas aeruginosa beta-lactamase CTX-M-2 gene isolate No.1	0.75	0.75	0.76						
5. Pseudomonas aeruginosa beta-lactamase CTX-M-2 gene isolate No.2	0.75	0.75	0.76	0.00					
6. Pseudomonas aeruginosa strain PHB 53 beta-lactamase CTX-M-2 (blaCTX-M-2) gene (GU929917.1)	0.72	0.72	0.85	0.00	0.00				
7. Pseudomonas aeruginosa beta-lactamase CTX-M-43 gene isolate No.1	0.64	0.64	0.64	0.00	0.00	0.01			
8. Pseudomonas aeruginosa beta-lactamase CTX-M-43 gene isolate No.2	0.64	0.64	0.64	0.00	0.00	0.01	0.00		
9. Acinetobacter baumannii beta-lactamase CTX-M-43 gene (DQ102702.1)	0.73	0.73	0.87	0.00	0.00	0.00	0.00	0.00	

Figure 5: genetic variations in the nitrogenous bases sequence of local bla$_{CTX-M}$ genes in *Pseudomonas aeruginosa* and compared it with the global isolates

DISCUSSION

The incidence of *P. aeruginosa* among the examined samples was 50 positive isolates with a percentage of 13.8 %.The result of current study agree with [11 ,12]The chronic ear infections was hit by humanity for a long time ago, to the present day, and the injury lead to complications and complexities variety and the Valmokhtchin in field of ear disease for several years ago tried to put or establish general terms to describe the clinical symptoms and pathological inflammation of the middle ear, caused by *P. aeruginosa* [13]. Also The result of current study didn't agree with [14,15] .The present investigation found that *P. aeruginosa*was most commonly isolated (15.3%) from the ear infection and this agree with some previous surveillance studies [16,17]. Then comes burns,infections which reach to isolation percentage of *P. aeruginosa* of which 13.7% [16], Present study revealedurinary tract infections rate of 11.2% [18]. Also the result of current study didn't agree with [19]. *P. aeruginosa* is a second pathogen and most frequent between gram negative bacteria by 16.3% of patients who suffering from Urethra infectionsat USA [20]. In present study β-lactam resistance rate in 24isolated of *P. aeruginosa* current study appeared most of isolates of bacteria under study showed a multi-resistant to antibiotics, largely the reason for multiple resistance to *P. aeruginosa* to possess a lot of resistance mechanisms, which include the production of enzymes modified β-lactam and enzymes Aminoglycosides and possession of automatic external pumping and other mechanisms by which learning together, causing the phenomenon of multiple antibiotic resistance (MDR) [21]**.** Present study showed presence of resistance enzymes type *bla*$_{CTX-M}$at isolates to*P. aeruginosa* with presence of both gene *bla*$_{CTX-M-1}$, *CTX-M-2*, *CTX-M-43* while the gene *bla*$_{CTX-M\ -15}$ did not record any Seen percentage at isolates, The present study had highest frequency of the gene *b-lactamase*$_{CTX-M-2}$, presence in 13 isolates of the *P. aeruginosa* by (54.16%), followed by gene *bla*$_{CTX-M-43}$, a presence at 8 isolates of the *P. aeruginosa* by (33.3%) In other performed Studies at Brazil it found that the percentage to presence gene *bla*$_{CTX-M-2}$ in clinical isolates of *P. aeruginosa*is (19.6%).[22] While the percentage of third gene *bla*$_{CTX-M-1}$ in the current study (20.83%), is a presence in 5 clinical isolates to*P. aeruginosa*, result of present study agree with [23] As for presence *bla*$_{CTX-M-15}$ Gene in the current study, percentage amounted to (0.0%), this result Do not agree with [24], The present study showed a similarity between nitrogenous bases

sequences to$bla_{CTX-M-2}$enzymeat local isolates to *P. aeruginosa* with isolates were registered in the world for the gene, while the nitrogenous bases sequence does not match$bla_{CTX-M-1}$enzyme local isolates to *P. aeruginosa* with isolates were registered in the world. as it has been compared to the results that have been reached for the gene $bla_{CTX-M-1}$of the *P. aeruginosa* with gene $bla_{CTX-M-1}$of isolates that belong to bacteria (634899.1KP) *Escherichia coli* that are globally registered and the results were identical. As for the gene $bla_{CTX-M-43}$ has scored match percentage with the gene $bla_{CTX-M-43}$ for bacterial isolates of (DQ 102702.1) *Acinetobacter Baumannii*.

REFERENCES

[1] A Gawish; N Mohammed; G El-Shennawy; H Mohammed. *J Microbio Infec Dis*, **2013**, 3(3), 116-122.
[2] MC Fariñas; L Martínez-Martínez. *Enfermedades Infecc Microbiol Clínica.*, **2013**.
[3] DM Livermore. *Korean J Intern Med*, **2012**, 27(2), 128-142.
[4] H Iguarán; E Daibeth; L Castro, Aura L. *Boletin informativo GREBO*, **2010**.
[5] Y Peng; J Bi; J Shi; Y Li; X Ye; X Chen. *Am.J.Infect.Control*, **2014**, 42, 1308-1311.
[6] Z Tavajjohi; R Moniri; A Khorshidi. *Afr J. Microbiol Res*, **2011**, 5(20), 3223-3228.
[7] J Sambrook; EF Fritsch; T Maniatis. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, **1989**.
[8] JMS Bartlett; D Stirling. PCR Protocols: Methods in molecular biology. 2nd Edition. Humana Press Inc. Totowa, **1998**.
[9] S Upadhaya; R Shenoy; V Shetty; A Lamsal; P Lamichhane; S Pokhrel. *Int J Biomed Res*, **2014**, 5 (4), 0976-9633.
[10] JD Cavallo; P Plesiat; G Couetdic; F Letlanc; R Fabe. *J Antimicrob Chemoth*, **2000**, 50, 1039-1043.
[11] AA Abdul-Wahid. Dissemination of Aminoglycosides Resistance in Pseudomonas aeruginosa Isolates in Al-Nasseryia Hospitals. M.Sc. Thesis. College of Medicine. University of Kufa, **2014**.
[12] GF Gad; RA El-Domany; S Zaki; HM Ashour. *J Antimicro Chem*, **2007**, 60, 1010-1017.
[13] M Goycoolea; L Ruah. *Otol Clin. N Am*, **1991**, 24,757-761.
[14] M Magdy; LLA Afifi; MTA Suelam; Soliman; MGS El-Gohary. *Int J Biol Chem*, **2013**, 7 (2), 47-57.
[15] KL Hassan; SA Rafik; K Mussum. *J Adv Med Res*, **2012**, l.2 (3), 90-98.
[16] ZF Dakhl. *Int J Adv Res*, **2015**, 3 (11), 376-384.
[17] RM Salim, Prevalence of OXA beta-lactamase of Pseudomonas aeruginosa in Al-Diwaniya City., M.Sc. Thesis. College of Science. University of Al-Qadisiya, **2014**.
[18] Qasim KW. Effect of Some Chemical and Physical Factors on Pseudomonas aeruginosa Membrane permeability. M.Sc. Thesis. College of Science. University of Baghdad, **2006**.
[19] OH Haran. Detection of β-lactam Resistance Genes in Bacterial Isolates from Some Clinical Infections in Al_Diwaniyah City. M.Sc. Thesis. College of Science. University of Al-Qadisiya, **2012**.
[20] R Gaynes; JR Edwards. *Clin Infect Dis*, **2005**, 41, 848-854.
[21] M Shahid; A Malik. *India J Med Res*, **2005**, 122, 324-329.
[22] M Polotto; T Casella; MG de Lucca Oliveira; FG Rúbio; ML Nogueira; MT de Almeida; MC Nogueira. *BMC Infect Dis*, **2012**.
[23] Z Chen; H Niu; G Chen; M Li; Y Zhou. *Int J Clin and Exp Med*, **2015**, 8(10), 19400-19405.
[24] A Hashemi; F Fallah. *Brit Microbiol Res J*, **2013**, 12(4).