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MOLECULAR STUDY OF ESBL GENES AND ANTIMICROBIAL RESISTANCE PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM THE BURN PATIENTS IN AL-NAJAF AL-ASHRAF, IRAQ

Ihsan Kadhum Humady[1*], Oday Mitib Hadi[2]

[1, 2]Al-Furat Al-Awsat Technical University/ College of Health And Medical Techniques/Kufa, Iraq.

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Corresponding Author:

Ihsan Kadhum Humady

Email:
ihsantag1975@gmail.com
kuh.aud@atu.edu.iq

ABSTRACT

Background: *P. aeruginosa is a bacterium that causes numerous some systemic illnesses. Identification of Pseudomonas aeruginosa phenotypic and genotypic Extended Spectrum Beta Lactamase, including bla$_{CTX-M}$, bla$_{OXA}$, and bla$_{TEM}$ genes in the burn infections.* ***Materials & Methods:*** *One hundred and sixty-one burn swab samples were randomly gathered from patients with symptomatic wound infections at the burn center in Al-Najaf Governorate, Iraq, between January and April 2023. Results: Out of the total isolates, 42 (26%) were confirmed as P. aeruginosa based on microscopic, cultural, and biochemical features. These isolates were then assessed for their capacity to generate ESBLs using the Double Desk Synergy Test. 16 (38.1%) isolates producing extended-spectrum β-lactamases, previously identified using phenotypic methods, were analyzed for the presence of β-lactamase genes using polymerase chain reaction. Results show that all 16 out of 42 ESBL-positive burn patients isolates carried the bla $_{CTX-M}$ gene, 93.7% of them (n = 15) carried the bla $_{TEM}$ gene, and 87.5% of them (n = 14) carried the bla $_{OXA}$ gene. This study was conducted at the Burn Center in AL-Najaf AL-Ashraf, Iraq. The purpose of this study was detected the prevalence of ESBL genes and antimicrobial resistance pattern of Pseudomonas aeruginosa isolated from the burn patients in AL-Najaf AL-Ashraf, Iraq.* ***Conclusion:*** *The current research findings show a higher occurrence of multi-drug resistance and a greater percent of ESBL- associated genes in P. aeruginosa isolates from burned patients in AL-Najaf AL-Ashraf City.*

INTRODUCTION

P. aeruginosa is a corporate source of infections in people. It is an adaptable, Gram -ve, non-lactose fermenter bacteria. It is found in many disorders such as bacteremia, dermatitis, burn wound infections, respiratory system infections, and soft tissue infections, particularly in hospitalized patients and immunocompromised persons[(1)]. Furthermore, persons with burns are especially subject to this bacterial contagion during their hospitalization, leading to significant mortality and morbidity rates. Some studies have connected a greater mortality rate in *P. aeruginosa* contaminations to the bacteria's ability to rapidly adapt to the environment, swiftly develop antibiotic resistance, and produce various virulence factors [(2)].

Further investigation showed that the pathogen's cell walls have reduced permeability to anti-pseudomonal medications and possess a greater genetic capacity for quickly developing drug

resistance [3]. state that multi drug resistance isolates of this bacterium can lead to life-threatening and often incurable illnesses, making them a significant concern in infection control [4].

Even though several beta lactamase enzymes have been identified, the betalactam resistance of *bla*$_{TEM}$, *bla*$_{OXA}$,and *bla*$_{CTX-M}$ has led to their decreased frequency of production and subsequent clinical importance [5].

Pseudomonas aeruginosa secretes a variety of enzymes and exotoxins. It also possesses significant biofilm-forming capabilities. The various biomolecules including proteins and polysaccharides in the biofilm Shielded bacteria from antimicrobial agents and the host's immune system [6]. Alginate is a prevalent polysaccharide found in biofilm structures. Due to this situation, treating infections caused by biofilm-forming bacteria is challenging and can lead to serious issues in burn hospitals [7]. The current study focused on identifying the genes responsible for encoding class A (ESBLs) of *bla*$_{TEM}$, *bla*$_{OXA}$, *and bla* $_{CTX-M}$ as well as studying the type of antimicrobial drug resistance amongst *P. aeruginosa* collected from the burn wounds in AL-Najaf Al-Ashraf Province, Iraq.

METHODOLOGY

Isolation and Identification of Pseudomonas aeruginosa

One hundred and sixty-one clinical isolates were taken from burned patients at the Center of the burn in AL-Najaf AL-Ashraf, Iraq, between January and April 2023. Before beginning work, permission and ethical approval were received from the AL-Najaf health directorate (number: 61938, on January 28, 2022). Every patient who was part of the study was told that their information will be utilized for research (all patient phone numbers were obtained from the microbiology unit archive). We can isolate the *Pseudomonas aeruginosa* by using Cetrimide Agar which contain the cetrimide, a quaternary ammonium compound that inhibits the growth of most other bacteria, making it selective for Pseudomonas species. The Cetrimide Agar also contains malachite green, which causes Pseudomonas aeruginosa to produce a characteristic blue-green pigment, aiding in its identification [8]. 42 (26%) samples of *P. aeruginosa* were diagnosed by morphological, culturing, chemical, analytical profile index, and Vitek-2 analysis.

Antimicrobial susceptibility experimentation:

Pseudomonas aeruginosa resistance to 17 antimicrobials, including Gentamicin, Amikacin, Clavulanic acid, Cefotaxime, Ceftazidime, Ceftriaxone, Norfloxacin, Cefepime, Aztreonam, Tobramycin, Meropenem, Imipenem, Piperacillin-tazobactam, Levofloxacin, Colistin, Piperacillin, and Ciprofloxacin, was determined following (CLSI) guidelines [9].

We used the reference strain of At ATCC 27853 as the control. Extended Spectrum Beta Lactamase was identified using the double disk diffusion method using cefotaxime (30µg) and ceftazidime (30µg) disks alone and in combined with clavulanic acid (10µg) on Mueller-Hinton media (10).

A positive reaction result is defined as Zone of inhibition around the antibiotic-inhibitor disk but not around the antibiotic disk [11].

Phenotypic detection of production of ESBLs

Double Discs Synergy Test (DDST)

The disc diffusion process was conducted by inoculating a few new colonies from each *P. aeruginosa* isolate into nutritional broth. If the inhibitory zones extended more than 5 mm towards the Amoxicillin/Clavulanate disk, it was considered positive for ESBL development [12].

Genotypic Detection

Genotypic Detection of ESBLs Production

PCR was conducted to amplify the *bla*$_{TEM}$, *bla*$_{OXA}$ and *bla*$_{CTX-M}$ genes using specific primers as detailed in table (1) and a master mix from Promega USA.

Table (1) Primers that were used to detect ESBLs encoding genes in the study

β-lactamase type	Primer	Gene name	Oligo sequence (5'-3')	Product size (bp)	Reference	Company
ESBL	CTX-M	*bla*$_{CTX-M}$	F:ATGTGCAGYACCAGTAA R:CCGCTGCCGGTYTTATC	512	(13)	Biocop Canada
	TEM	*bla*$_{TEM}$	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	643	(14)	Biocop
	OXA	*bla*$_{OXA}$	F:ATATCTCTACTGTTGCATCTCC R: AAACCCTTCAAACCATCC	619		

Electrophoresis on Agarose Gel:

The Bartlett and Stirling technique was used to conduct all technical aspects, preparation, and criteria for DNA agarose-gel electrophoresis analysis and detection in 2003.

Ethical Consideration

This study received approval from the Postgraduate Studies Committee in the College of Health and Medical Techniques at Al-Furat Al-Awsat Technical University in Kufa. Prior to collecting clinical samples, patients provided consent, and staff at the burn center supervised the sample collected.

Statistical Analysis

The data from the current study was analyzed using IBM Corporation's SPSS v20.0 statistical software based in Armonk, New York, USA.

RESULTS AND DISCUSSION

Out of 161 swab samples obtained from burned patients, 42 (20%) were identified as P. aeruginosa based on microscopic, culture, and biochemical features. Among these isolates, 33 out of 42 (78.6%) were found to be multidrug-resistant (MDR) as shown in Table (2). (Table 2). Imipenem and ciprofloxacin are the most effective antimicrobial against isolates of P. aeruginosa.

Based on the findings, out of the 16/42 extended spectrum beta-lactamase -positive burned patients samples 100% of them (16/ 16) harbored *bla* $_{CTX-M}$ gene, 93.7% of them (15/16) harbored *bla* $_{TEM}$, 87.5% of them (14/16) contained *bla* $_{OXA}$ genes that observed in Center of burn In AL-Najaf AL-Ashraf / Iraq.

The ability of each isolate under investigation to create ESBLs was assessed using Double Discs Synergy Test 16/42(38.1%) ESBL. 16 (38.1%) ESBL producers were examined for ESBLs expressing genes using the PCR methodology after they had previously been assessed using the phenotypic method.

The molecular techniques detected ESBLs-encoding genes 69% of them (n = 29) harbored *bla* $_{CTX-M}$ gene, 61.9% of them (n =26) harbored *bla*$_{OXA}$ and 54.7% of them (n = 23) harbored *bla* $_{TEM}$ gene.

Detection of ESBLs-producers shown in Figure (1,2,3).

Table (2): Antibiotic susceptibility patterns of the isolated *P. aeruginosa*.

Antibiotic Type	Susceptible	Intermediate	Resistant
Cefotaxime	4.8%	0 %	95.2 %
Ticarcillin- Clavulanat	7.1%	0 %	92.9 %
Ceftriaxone	9.5%	0 %	90.5 %
Ceftazidime	14.3%	0 %	85.7 %
Gentamicin	14.3%	0 %	85.7 %
Norfloxacin	23.8%	0 %	76.2 %
Cefepime	28.5%	0 %	71.5 %
Piperacillin	26.2%	4.8 %	59 %
Aztreonam	35.7%	0 %	64.3 %
Tobramycin	38%	0 %	62 %
Piperacillin-tazobactam	31%	11.9 %	57.1 %
Levofloxacin	45.3%	0 %	54.7 %
Colistin	45.3%	0 %	54.7 %
Meropenem	50%	0 %	50 %
Amikacin	45.3%	4.7 %	50 %
Ciprofloxacin	54.7%	0 %	45.3 %
Imipenem	66.7%	4.8 %	28.5 %

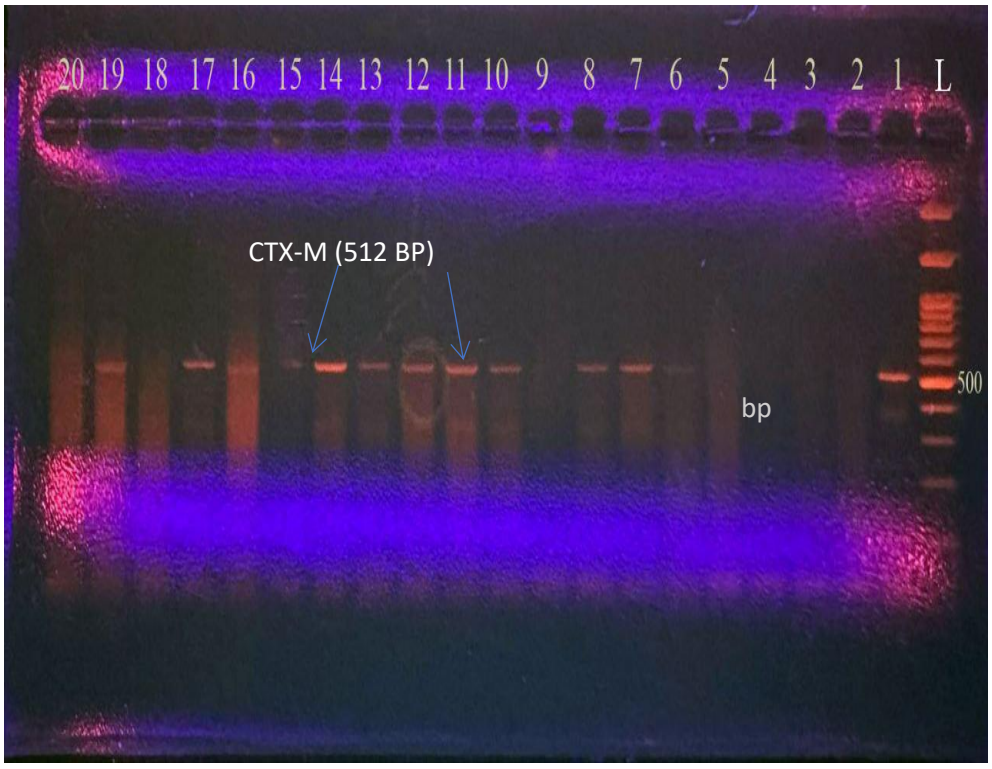

Figure (1). Detection of ESBLs-producers by PCR technique. The amplicon size of the *bla*$_{CTX-M}$gene is 512 bp [13]. Lane: L:100bp ladder marker (promega/ USA), Lane:1,6,7,8,10,11,12,13,1416,17 and 19 positive results, Lane: 2,3,4,5,9,15,18 and 20 negative results.

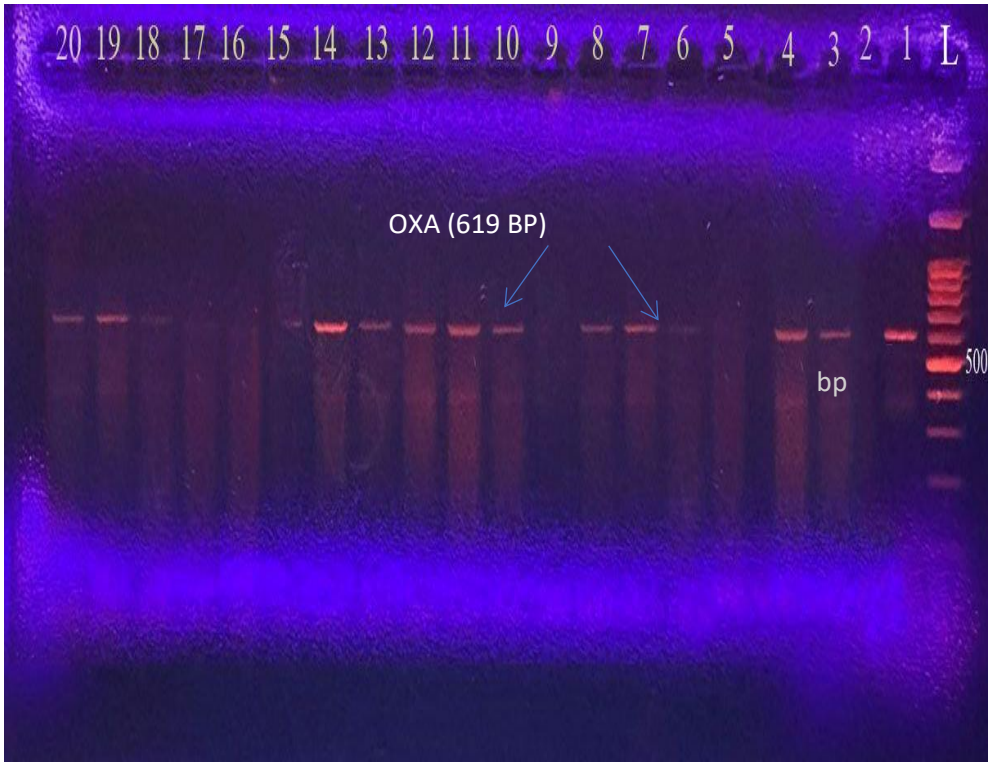

Figure (2). Detection of ESBLs-producers by PCR technique. The amplicon size of the *bla*$_{OXA}$ gene is 619 bp [14]. Lane: L:100bp ladder marker (promega/ USA). Lane:1,3,4,6,7,8,10,11,12,13,14,18,19and 20 positive results, Lane: 2,5,9,15,16 and 17 negative results.

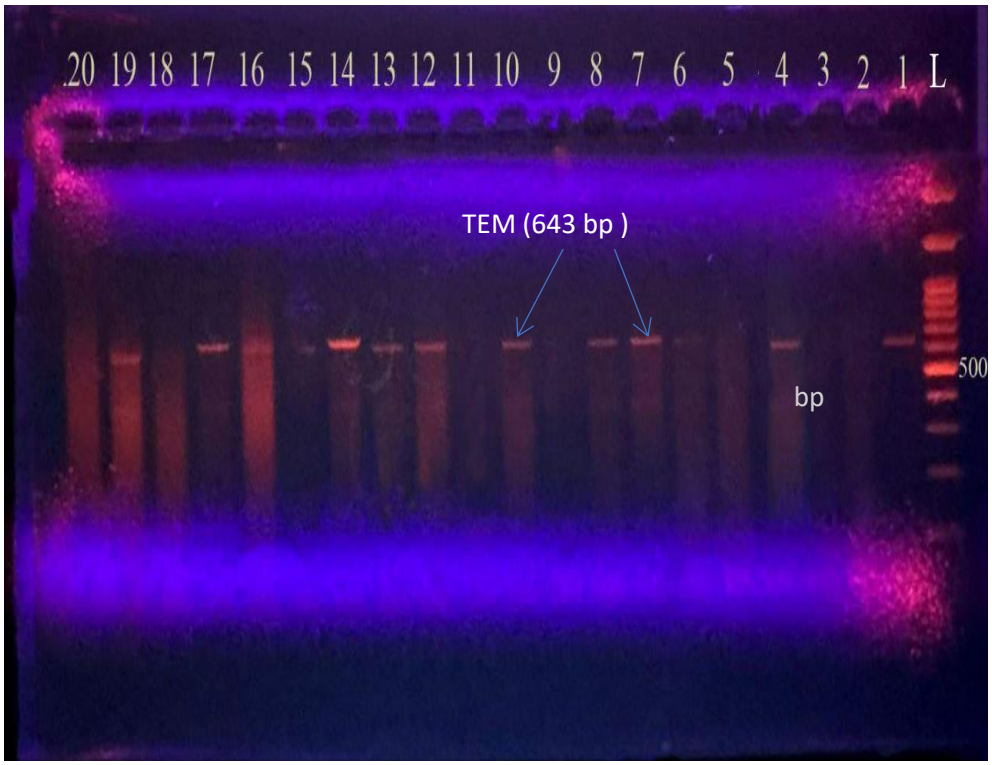

Figure (3). PCR-based detection of ESBL producers. The amplicon size of the *bla*$_{TEM}$ gene is 643 bp [14]. Lane: L:100bp ladder marker (promega/ USA), Lane:1,4,7,8,10,12,13,14,16,17 and 19 positive results, Lane: 2,3,5,6,9,11,15,18 and 20 negative results.

Discussion

Antimicrobial resistance is a significant challenge in managing infectious diseases globally. *Pseudomonas aeruginosa* naturally possesses resistance to several antimicrobials due to reduced exterior membrane permeability, persistent appearance of multiple efflux pumps, and production of various anti-microbial-inactivated enzymes [15].

Qin et al. [15] demonstrate that P. aeruginosa has a greater ability to produce biofilm, leading to reduced antibiotic permeability and limited access to the bacterium. When this study investigated resistance to antimicrobials and the existence of extended spectrum beta-lactamase and metalo-beta lactamase producing genes.

Results from 42 isolates indicated that 33 out of 42 (78.6%) are multidrug-resistant (MDR). Imipenem and Ciprofloxacin are two treatments with the high potential. Also, this study observed heightened resistance to meropenem, Cefotaxime, and Ceftriaxone. Some readings have shown increased multidrug-resistant P. aeruginosa strains that are resistant to carbapenems, which are often used to treat P. aeruginosa infections worldwide [2].

The prevalence of cephalosporin-resistant P. aeruginosa is increasing due to the greater usage of β-lactam antimicrobials. Adabi (16). discovered that Pseudomonas spp isolates from burned patients exposed the highest resistance to ceftazidime.

Increased occurrence of resistance to β-lactams may impact clinical consequences caused by these bacteria. Recent findings suggested a correlation between the higher death rate and the emergence of ESBL-producing bacteria producing community-acquired conditions such as *Pseudomonas aeruginosa* (17).

ESBL forming isolates show, high frequencies of *bla* $_{CTX-M}$ *(29/42, 69%)* in isolates is followed by *bla* $_{OXA}$ *(26/42 ,61.9%) and bla* $_{TEM}$ (23/42, 54.7%) respectively.

Depend on the outputs, the frequency of the *bla* $_{CTX-M}$ gene is consistent with the incidence found in burn infections of P. aeruginosa study published by Attarpour (18).

The gene frequency in our study (bla TEM and bla OXA) is similar to what other researchers found in studies such as Rajaee [19] and Mushtaq [20] respectively.

P. aeruginosa has been known to produce several β-lactamase enzymes, including ESBLs, in response to β-lactams, a resistance to which has been commonly observed.

P. aeruginosa strains that are capable of producing ESBLs pose a major threat to the world's health because these producers are resistant to a wide range of antibiotic classes, such as quinolones, aminoglycosides, and sulfa medications. Formerly, the primary ESBL genotypes identified in P. aeruginosa isolates were blaTEM, blaOXA, and *bla* $_{CTX-M}$ genes [21].

It was found in this investigation that the phenotypic approaches detected a higher prevalence of ESBL-producers than the molecular approach. These variations could be explained by the phenotypic approaches' sensitivity to ESBLs without requiring the identification of the type of encoded gene. In contrast to molecular technology, which is expensive, complex, and requires highly skilled technicians, phenotypic methods are frequently simple, quick, inexpensive, and do not require highly qualified experts. PCR is a common method for identifying genes, but it is only sensitive to the chosen primers; as a result, unless the necessary primers are supplied, it is unable to identify the isolates who make ESBLs.

Another cause for the variation between molecular and phenotypic detection techniques could be the existence of genes that code for the synthesis of ESBLs, which have not yet been found.This indicates that while ESBL-negative isolates are capable of expressing themselves, molecular technology necessitates providing the apparatus with significant data in order to provide acceptable outcomes and agree with the apparent detection. (22),(23),(24).

The blaCTX-M gene was the most dominant 29/42 (69.0%) according to the molecular results of the current investigation. This could be because blaCTX-M is easily transmitted amongst Gram-negative bacteria. Numerous studies revealed that blaCTX-M enzymes replaced other ESBLs in P. aeruginosa, including blaTEM and blaOXA variations [25].

The cause may have occurred as a result of the blaCTX-Mgenes spreading more quickly across transposons and plasmids, as well as the simplicity of transposon and plasmid connection and crossing over in successful clones. Additionally, co-resistance in blaCTX-M -generating organisms especially aminoglycosides and fluoroquinolones—is thought to be the source of the increased incidence of blaCTX-M. This could make co-selection easier to occur [26].

The remarkable spread of blaCTX-M that required an uncontrollable pandemic situation may have been facilitated by successful links between blaCTX-M genes and other resistance mechanisms.The future survival of the blaCTX-M genes within bacterial communities will be guaranteed by co-selection mechanisms because blaCTX-M-producing species frequently contain additional resistance genes, especially those that are still evolving like carbapenemases [27].

The genetic diversity of the isolates under investigation may have resulted from genetic changes and the ease with which genes can spread throughout strains of Gram-negative bacteria. In fact, P. aeruginosa has emerged as one of the most significant pathogenic bacteria in hospitals thanks to its genetic variety in producing ESBLs [28].

Antibiotic-resistant P. aeruginosa may become established in hospitals and become involved in nosocomial infections due to the diversity of β-lactamase and the high prevalence of β-lactam resistance. In this scenario, monitoring for β-lactam susceptibility will be required.Production of ESBLs have been connected to a number of detrimental effects, including higher costs, the cessation of long-term care, delays in the administration of proper care, longer hospital stays, and a rise in total infection-related mortality [27].Recent research, however, revealed that compared to other ESBLs-enzymes, the blaCTX-M enzyme was more prevalent and dominant among P. aeruginosa.

The predominance of blaCTX-producing bacteria has increased in Asian, American, and European nations, which is indicative of an ESBL shift away from the blaOXA or blaTEM lineage[29].

CONCLUSION

Unfortunately, P. aeruginosa isolates that produce ESBLs were common in AL-Najaf AL-Ashraf/Iraq, a pathogenic bacterium that contributes to burn injury complications. The results of this study showed how well phenotypic and genotypic approaches work to identify ESBLs, making it easier to identify resistant isolates, prescribe medication, and lower problems. bla CTX-M stands out from the other genes due to its higher level of dominance.

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