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## Occurrence of blaTEM among Pseudomonas aeruginosa Strains Isolated from Different Clinical Samples in Erbil City

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### Abstract

*Pseudomonas aeruginosa* was a significant source of nosocomial infection with the presence of extended-spectrum  $\beta$ -lactamases (ESBLs) and metallo- $\beta$ -lactamases (MBL) genes in *P. aeruginosa* being progressively documented globally. The purpose of this investigation was to detection the occurrence of MBL and ESBL in *P. aeruginosa* isolates acquired from diverse clinical samples, as well as the prevalence of blaTEM genes producing ESBLs. A total of 227 samples were obtained from various clinical specimens (wound, urine, sputum, bronchial wash, and burn) at public hospitals in Erbil region. Microorganism was obtained, treated, and recognized using normal microbiological culture procedures, biochemical testing, DNA extraction, and polymerase chain reaction to identify the presence of the blaTEM gene. Out of 227 specimens, 40 isolates (17.6%) were positive for *P. aeruginosa*, while 32 (80.6%) were considered as ESBL positive and 72.5% were positive for MBL manufacture by *P. aeruginosa* and the results of ESBL genes detection clarify that most of ESBL and MBL producer isolates of *P. aeruginosa* carried blaTEM gene which found in more than half (52.5%) of the isolates strains. The current study demonstrates that the development of MBL and ESBL in *P. aeruginosa* is increased and making these infections more challenging to cure. For the decrease of death rates and the propagation of multidrug-resistant organisms by genes, early identification of MBL and ESBL synthesis is critical. As a result, a variety of measures must be used to control the spread of these diseases.

### Keywords

blaTEM gene, Extended-spectrum  $\beta$ -lactamases, Metallo- $\beta$ -lactamases, *Pseudomonas aeruginosa*

RESEARCH ARTICLE

# Occurrence of *bla*<sub>TEM</sub> among *Pseudomonas aeruginosa* Strains Isolated from Different Clinical Samples in Erbil City

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## ABSTRACT

*Pseudomonas aeruginosa* was a significant source of nosocomial infection with the presence of extended-spectrum  $\beta$ -lactamases (ESBLs) and metallo- $\beta$ -lactamases (MBL) genes in *P. aeruginosa* being progressively documented globally. The purpose of this investigation was to detection the occurrence of MBL and ESBL in *P. aeruginosa* isolates acquired from diverse clinical samples, as well as the prevalence of *bla*<sub>TEM</sub> genes producing ESBLs. A total of 227 samples were obtained from various clinical specimens (wound, urine, sputum, bronchial wash, and burn) at public hospitals in Erbil region. Microorganism was obtained, treated, and recognized using normal microbiological culture procedures, biochemical testing, DNA extraction, and polymerase chain reaction to identify the presence of the *bla*<sub>TEM</sub> gene. Out of 227 specimens, 40 isolates (17.6%) were positive for *P. aeruginosa*, while 32 (80.6%) were considered as ESBL positive and 72.5% were positive for MBL manufacture by *P. aeruginosa* and the results of ESBL genes detection clarify that most of ESBL and MBL producer isolates of *P. aeruginosa* carried *bla*<sub>TEM</sub> gene which found in more than half (52.5%) of the isolates strains. The current study demonstrates that the development of MBL and ESBL in *P. aeruginosa* is increased and making these infections more challenging to cure. For the decrease of death rates and the propagation of multidrug-resistant organisms by genes, early identification of MBL and ESBL synthesis is critical. As a result, a variety of measures must be used to control the spread of these diseases.

**Keywords:** *bla*<sub>TEM</sub> gene; Extended-spectrum  $\beta$ -lactamases; Metallo- $\beta$ -lactamases; *Pseudomonas aeruginosa*

## INTRODUCTION

*Pseudomonas aeruginosa* may colonize human body locations, preferring moist regions including the (axilla, perineum, ear, nasal mucosa, and throat; and feces). The incidence of *P. aeruginosa* colonization in healthy people are typically modest, but increased colonization rates might be found after hospitalization, particularly among issues preserved with broad-spectrum antimicrobial drugs specific for protracted spectrum cephalosporins. Establishment occurs often in mechanically ventilated patients' respiratory tracts, in individuals undergoing anticancer chemotherapy's gastrointestinal tracts, and on the skin of burn victims. In the hospital context, mop, sink, disinfectant solutions, respiratory equipment, food mixers, and other moist settings can all serve as reservoirs for *P. aeruginosa* (Pollack, 2000). *P. aeruginosa* is a hazardous and feared bug since it is one of the utmost common bacteria in clinical settings that cause difficulties due to its strong resistance

to antimicrobial treatments. With the widespread use of antibiotics, *P. aeruginosa* has evolved into a resistant to nearly all medications. Antibiotics that include a beta-lactam ring are known as beta-lactams. Extended-spectrum  $\beta$ -lactamases (ESBLs) are clavulanate-resistant enzymes that hydrolyze oxyiminocephalosporins and monobactams but not cephalosporins or carbapenems. There are around 300 different ESBL variants, which are classified into nine categories based on amino acid sequences (Goyal et al., 2009). They're frequently encoded by plasmids produced by Gram-negative (GN) bacteria and transferred from one species to the next (Eftekhar et al., 2012). Spectrum  $\beta$ -lactamases (SBLs) are frequently plasmid mediated, and the majority of the enzymes belong to the TEM and SHV families, which have been identified in a variety of nations. In Greece, the TEM was originally discovered in *Escherichia coli* isolated from a patient named Temoniera. The other beta-lactamase, SHV, gets its name from the sulfhydryl variable active site (Mansouri and Ramazanzadeh, 2009).

Molecular detection and type of ESBLs, on the other hand, can provide a quick and accurate diagnostic for the genes that cause these enzymes (Manoharan et al., 2011). Most ESBLs are generated from SHV and TEM, which exhibited resistance to penicillin and narrow spectrum cephalosporins due to mutations in one or more amino acids surrounding the active side. More than 70 SHV and 150 TEM kinds have been identified. These enzymes are encoded by the genes ( $bla_{SHV}$  and  $bla_{TEM}$ ) (Harada et al., 2008). Resistance to quinolones, imipenem, and third-generation cephalosporins all rose by 15.9%, 20%, and 20%, respectively. Similarly, from 1993 to 2002, a nationwide surveillance study of intensive care unit (ICU) patients found a substantial rise in multidrug-resistant (MDR) *P. aeruginosa* isolates (defined as resistance to at least three of four agents: Imipenem, ceftazidime, ciprofloxacin, and tobramycin) (Obritsch et al., 2004). MDR isolates with broad-spectrum antibiotic resistance have a limited number of viable treatment choices. Aminoglycosides and polymyxins are commonly used as last-resort treatments treating MDR pathogens. These drugs may or may not be as effective as first-line treatments, but they may also be linked with more substantial side effects (e.g., nephrotoxicity, ototoxicity, and neurotoxicity) (Li et al., 2006). Metallo- $\beta$ -lactamases (MBL)-producing isolates have developed in *P. aeruginosa* over the past decade. These isolates have been linked to the failure of carbapenem treatment and have been linked to severe illnesses including septicemia and pneumonia. MBL genes have recently migrated from *P. aeruginosa* to *Enterobacteriaceae*, resulting in a clinical situation (Marra et al., 2006). It is widely known that patients with severe infections caused by MBL-producing organisms have a bad prognosis when they are treated with antibiotics to which the organism is fully resistant (Marra et al., 2006).

### The Aim of this Study

This study aims to detect the occurrence of MBL and ESBL in *P. aeruginosa* isolates acquired from diverse clinical samples, as well as the prevalence of  $bla_{TEM}$  genes producing ESBLs.

## MATERIALS AND METHODS

### Isolation and Identification of *P. aeruginosa*

This research took place in Erbil province's Rizgary, Teaching hospital, Laboratory Centre, Raparin, and "Nanakaly Hospitals" between September 2019 and March 2020. The 227 specimens were collected and inoculated into agar medium and MacConkey agar plates included sputum, urine, wounds, burns, and bronchial wash. Plates were then incubated at 37°C for 24–48 h under aerobic conditions. *P. aeruginosa* was identified provisionally using microscopic characteristics, culture traits, and biochemical

tests and then definitively using Vitek 2 Compact: Colorimetric technology for the identification of bacteria; a GN card was utilized for *P. aeruginosa* documentation. All of the actions below completed according to the manufacturer's instructions (France, Biomerieux). In a smooth examination pipe, 3 ml of normal saline was added and a scuttle full of cell suspension was injected. The colony was standardized to McFarland average solution ( $1.5 \times 10.8$  cfu/ml) using the test tube in the Dens Check machine. After the standardized inoculum was placed into the cassette, a sample papers number was uploaded into the processor software through barcode. After reading the Vitek 2 card type from the barcode positioned on the postcard during manufacture, the card was connected to the sample proof of identity port. In the filter module, the cassette was put, then transferred to the reader/incubator module when the card was full. The instruments were in-charge of all subsequent procedures, including controlling the incubation temperature, optical card scanning, and continually monitoring and transferring test results to the computer for analysis. The device automatically ejected the cards into a trash receptacle when the test cycle was completed.

### Antimicrobial Predisposition Test and ESBL by Vitek 2 Arrangement

Antimicrobial monitoring, evaluation system, and ESBL of the isolates were assessed to using the Vitek 2 compact system, an automated ID, and susceptibility antimicrobial susceptibility testing (AST) system (bioMerieux, USA). The Vitek 2 system includes an Advanced Expert System (AES) with high specificity and sensitivity values (94–100%) that's also widely used for identifying and/or antimicrobial susceptibility, which typically employs different antimicrobial Susceptibility Test cards (AST cards) based on the pathogens expected. The cards were inoculated and cultured in the machine according to the manufacturers' instructions. The results of the Vitek 2 susceptibility test subsequently changed to minimum inhibitory concentration values and categorized as susceptible, moderate, or resistant to use the Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI, 2011). The AES was used to interpret the final results (software version VT2- R05.04) (Eigner et al., 2005).

### Phenotypic Detection of ESBL Enzyme

The Vitek 2 classification with the antimicrobial sensitivity quiz extension was used to screen all bacterial species for the production of ESBL enzyme "AST-EXN8 card." On the same plate, this technique can implement both screening and assenting examinations for phenotypic identification of ESBL. Six bores contain ceftazidime 0.5 mg/L, cefepime 1.0 mg/L, and cefotaxime 0.5 mg/L, whereas the remaining three wells have the same three

antibiotics in conjunction with acid as clavulanic (4.4 and 10 mg/L, correspondingly).

An optical reader was used to measure growth in each well quantitatively. The proportionate reduction in development in cephalosporin plus clavulanate-treated wells compared to cephalosporin-treated wells was thought to indicate ESBL formation. Positive ESBL screening results were reported for all phenotypic interpretations of ESBLs. When the AES offered phenotypic interpretations other than ESBLs, strains were described as ESBL negative (Eigner et al., 2005).

The polymerase chain reaction (PCR) method was used to detect lactamase enzymes in generating isolates.

### Plasmid Isolation

The genomic DNA Mini kit was used to purify plasmid DNA, and the manufacturer's instructions were followed (Korea, Geneaid).

### Suspension of Primers Preparation

The DNA sequences are resuspended as stock solution by melting the lyophilized textbook after spinning them down with TE buffer according to the manufacturers' instructions. TE buffer was used to dilute the working primer tube. The final picomoles were determined by each primer's process.

### "ESBLs" are being studied

Attendance of blaESBL genetic factor was discovered utilizing PCR using bla<sub>TEM</sub> directing sequence and under the identical surroundings as previously labeled (Pagani et al., 2003). The "bacterial DNA" was extracted using the boiling technique. The PCR mix was made up of ultrapure water, primer, template DNA, and master mix. For PCR, the following criteria were used: 3 min at 94°C, then 35 cycles of 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C, with a final extension at 72°C for 5 min. Briefings castoff in this study was: For detection of "TEM β-lactamases" F: TTCTGCTATGTGGTGC GGTA and R: TTATCCGCCTCCATCCAGTC. Gel electrophoresis was used to examine the PCR results, which were put on a 2% (w/v) agarose gel with 0.5 mg/mL ethidium bromide.

### Detection of MBL by Disc Potentiating Test

The imipenem-EDTA double-disc synergy test was used to evaluate the imipenem resistance isolates, as pronounced by Li et al., 2006. CLSI advised inoculating the examination organism on top of Mueller-Hinton agar (MHA) plates. With overnight incubation at 37°C, an imipenem 10 g disc was put 10 mm edge to edge from a blank disc containing 10l of "EDTA" (750 g). A good result was interpreted as an increase in the zone of inhibition between the imipenem

and the EDTA discs as compared to the zone of reserve on the drug's far lateral.

### Ethical Statement

The "Hawler Medical University – College of Health Sciences, Institutional Ethics Committee approved the study studies."

### Statistical Analyses

The study initiatives were authorized by the College of Sciences as Health/Medical of Hawler University's Institutional Ethics Board. The statistic work for the sciences of social Statistical Package for the Social Sciences (SPSS) software was done to conduct statistics investigates (SPSS-19). The Fisher's exact check or the two check was done to analyze categorical data.  $P > 0.05$  was considered to be statistically significant.

## RESULTS AND DISCUSSION

Out of 227 samples 40 (17.6%) isolates were positive for *P. aeruginosa*, the highest isolation rate (2.6%) was observed in burn specimens as it showing in Table 1. While 32 (80.6%) of isolated *P. aeruginosa* were found to have the ability to produce ESBL and they known as ESBL producer and 72.5% were have the ability to produce MBL so they known as MBL producer from *P. aeruginosa* isolates and Maximum ESBL and MBL positive isolates of *P. aeruginosa* were observed among burn samples (71.4%) and maximum antibiotic resistance patters were detected in urine (64.3%).

Due to its capacity to thrive on minimum nutritional needs and to endure a variety of physical circumstances, *P. aeruginosa* persists in both community and hospital settings (Lister et al., 2009). It has the ability to infect nearly all tissues and is a leading source of illness and death. *P. aeruginosa* accounted for 40 (17.6%) of isolates from clinical samples in our investigation, which is lower than the percentage reported by Bunyan et al., 2018, in Hilla, Iraq (21%). However, the largest proportion of *P. aeruginosa* was found in burns 14 (35%) surveyed by wounds (22.5%) and urine (17.5%). There was no significant difference in prevalence across scientific specimens ( $P > 0.05$ ). These findings are consistent with a research conducted by Latif in Iraq in 2009, the Recognized Ethic Board of the College of Healthy Sciences in Hawler of Medical University approved the research projects. Our findings about bronchial wash and sputum 30% coincided with Hadadi et al., 2008, who reported that lower respiratory tract infection in the ICU constituted 29.5% (Agnihotri et al., 2004) as well as that of Izquierdo-Cubas et al., 2004. The amount of dispersion with isolates, which different agreeing on the

**Table 1: The prevalence of *Pseudomonas aeruginosa* in various clinical samples**

Culture results of <i>P. aeruginosa</i>	Urine	Bronchial wash	Sputum	Wound	Burn	Total	P-value
Positive	7	4	6	9	14	40	<0.001
Negative	113	18	12	37	7	187	
Total	120	22	18	46	21	227	

49.4716 is the Chi-square statistical 0.00001 is P-value. At  $P = 0.05$ , the outcome is substantial.

**Table 2: *Pseudomonas aeruginosa* isolates from clinical infections have antimicrobial resistance characteristics**

Antibiotics	Resistant		Sensitive	
	Frequency	Percentage	Frequency	Percentage
Penicillin	40	100	0	0
Piperacillin	40	100	0	0
Amikacin	29	72.5	11	27.5
Gentamicin	10	25.0	30	75.0
Tobramycin	2	5.0	38	95.0
Tetracycline	7	17.5	33	82.5
Ciprofloxacin	8	20.0	32	80.0
Azithromycin	8	20.0	32	80.0
Ceftriaxone	8	20.0	32	80.0
Chloramphenicol	40	100	0	0
Rifampicin	40	100	0	0
Imipenem	2	5.0	38	95.0
Meropenem	10	25.0	30	75.0

site of clinical sample groups, ecological aspects, feeding demands, and virulence characteristics, might be the source of these differences in all research.

As shown in Table 2, all isolates appear to be resistant to penicillin, piperacillin, and lincomycin, as well as chloramphenicol and rifampicin. These findings are consistent with those of Zhang et al., 2002, who originate that all separates of *P. aeruginosa* are resistant to the antibiotics and amoxicillin, with a resistance rate against piperacillin of 90.4% (Haran, 2012). *P. aeruginosa* also exhibits resistance to a range of antibiotics, including aminoglycosides, quinolones, and beta-lactams, according to those who discovered that resistance to the drug was high (88%) (Hancock, 2000). *P. aeruginosa*' adaptive resistance includes the development of biofilm in the lungs of infected individuals, which acts as a diffusion barrier to prevent antibiotics from reaching the bacterial cells (Drenkard, 2003).

Only 5% and 25% of the isolates were resistant to imipenem and meropenem, respectively. Imipenem and meropenem had good anti-pseudomonas action in our research. Some researchers in India (Jaykumar and Appalaraju, 2007) made a similar observation, whereas Varaiya et al., 2008. Patients from the ICU were originate to have a higher level of imipenem resistance (25%), which can be attributed to this study conditions in which this study was conducted, while a revision by Ullah et al., 2009, found that the lowest and highest levels of antibiotic resistance were against meropenem (5.66%) and ampicillin (5.66%),

respectively. Furthermore, amikacin resistance was found in 25.47% of the isolates.

Production of broad-spectrum  $\beta$ -lactamases is one of the ways through which *P. aeruginosa* becomes resistant to antibiotics of the  $\beta$ -lactam family. Since *P. aeruginosa* is an important cause of hospital-acquired infections, spread of ESBL-producing genes among *P. aeruginosa* strains can make treatment of infections more challenging (Shaikh et al., 2015).

Multidrug-resistant *P. aeruginosa* has complex treatment decisions and may result in treatment failures. A total of 40 strains isolated of *P. aeruginosa* were obtained from diverse clinical specimens and screened for ESBLs and MBLs in this investigation. According to the findings of this investigation, 30 (80.6%) of the isolates were confirmed to be ESBL positive [Table 3]. In contrast to a research demonstrating 20.27% of ESBL generation in *P. aeruginosa*, reports from various authors in other countries suggest a higher rate (Aggarwal et al., 2008) and also were higher than study done in Mosul by Al-Hasso, 2006, who recorded only 40% positive isolates while another study done by South Indian by Senthamatai et al., 2013, recorded lower proportion than our result that between 144 straining of *P. aeruginosa*, 51 (35.4%) presented ESBL manufacture in the united disc transmission check, wherein a study is done by Goel et al., 2013, presented occurrence of 42.31% ESBL fabricators. In the research (Amutha et al., 2009), 24 of 53 isolates were MDR, with 13 (25%) of them expressing ESBL. There was a significant connection in antibiotic resistance between ESBL-producing isolates and non-ESBL-producing isolates ( $P = 0.05$ ). Prevalence of ESBLs, which is significant since these strains frequently cause epidemics in the pediatric population, resulting in higher morbidity and mortality in patients with underlying illnesses or limiting treatment choices due to multidrug resistance (Amutha et al., 2009).

Except for aztreonam, MBLs efficiently hydrolyze all beta-lactams. As a result, detecting MBL-producing GN bacilli are critical for providing effective patient care and limiting resistance dissemination (Lee et al., 2003). As shown in Table 3, 72.5% of MBL of *P. aeruginosa* were positive for MBL synthesis. The coexistence of various beta-lactamase-mediated resistance mechanisms in *P. aeruginosa* was investigated between ESBL and MBL (52.2%), several researchers in other countries have reported rates of MBL generating *P. aeruginosa* that are

**Table 3: In isolates of *Pseudomonas aeruginosa*, the number and proportion of antibiotic-resistant ESBL and MBL generation, as well as biofilm development**

Antibiotics	ESBL		MBL		Total resistant isolates (%)	P-value
	Producers, n=32 (%)	Non-producers, n=8 (%)	Producers, n=29 (%)	Non-producers, n=11 (%)		
Penicillin	32 (100)	8 (100)	29 (100)	11 (100)	40 (100)	0.001
Amikacin	25 (65.5)	4 (50)	21 (72.4)	8 (72.7)	29 (72.5)	0.0001
Gentamycin	8 (25)	2 (25)	10 (34.4)	0	10 (25)	
Piperacillin	32 (100)	8 (100)	29 (100)	11 (100)	40 (100)	0.001
Tetracycline	6 (18.7)	1 (12.5)	7 (24.1)	0	7 (17.5)	0.1
Ciprofloxacin	0	8 (100)	8 (27.5)	0	8 (20)	0.0001
Azithromycin	0	8 (100)	8 (27.5)	0	8 (20)	0.0001
Cefotaxime	0	8 (100)	8 (27.5)	0	8 (20)	0.0001
Chloramphenicol	32 (100)	8 (100)	29 (100)	11 (100)	40 (100)	0.001
Rifampicin	32 (100)	8 (100)	29 (100)	11 (100)	40 (100)	0.001
Imipenem	2 (6.25)	0	2 (6.8)	0	2 (5)	0.4
Meropenem	8 (25)	2 (25)	10 (34.4)	0	10 (25)	0.6

ESBL: Extended-spectrum  $\beta$ -lactamase, MBL: Metallo- $\beta$ -lactamases

lower than our results (Upadhyay et al., 2010), which were 20.8%, and also lower than our results (Upadhyay et al., 2010), which were 20.8% (Al-Grawi, 2011). According to Bashir et al., 2011, 114% and 11.6% of *P. aeruginosa* isolates were have been generating of MBLs enzymes by utilizing the combination disc technique, respectively, which is a lower proportion than this study. This difference in MBL prevalence might be linked to a distinct type of antibiotic therapy in two separate nations, or it could be linked to a specific mechanism that exists in several countries. However, as shown in Table 3, the majority of MBL producer isolates had a significant association ( $P < 0.05$ ) with antibiotic resistance.

Furthermore, 75% of ESBL-producing isolates and 66% of generating metallo were non-susceptible to meropenem, according to our findings. Because carbapenems are commonly used in hospital settings to treat infections caused by MDR GN bacteria, this discovery suggests that therapeutic options may be restricted in the future. This has clinical implications, particularly in the event that these strains become more common in the future. In keeping with prior research, this investigation was conducted (Santanirand et al., 2011).

Furthermore, the majority of ESBL-producing isolates were found to be MDR in our investigation, with greater resistance rates to the antibiotics examined. The current study found that resistance to six antibiotics (penicillin, vancomycin, bacitracin, erythromycin, piperacillin, chloramphenicol, and rifampicin) was 100% on average. According to Table 4, the most active medicines against ESBL- and MBL-positive isolates of *P. aeruginosa* are ciprofloxacin and azithromycin. In addition, the active susceptibility of chloramphenicol, rifampicin, penicillin, and piperacillin (100%) against ESBL- and MBL-positive *P. aeruginosa* was shown to be lower. While Peymani et al., 2017, reported that 71.5% of extended-

**Table 4: Relative between MBL and ESBL producers and resistance patterns**

Sample No.	Sample	Number	MBL %	ESBL %	Resistance patterns (%)
1	Burn	14	10 (71.4)	10 (71.4)	7 (77.8)
2	Wound	9	8 (88.8)	9 (100)	6 (85.7)
3	Urine	7	7 (100)	3 (42.8)	9 (64.3)
4	Sputum	6	3 (50)	6 (100)	5 (83.3)
5	Bronchial aspirate	4	1 (25)	4 (100)	3 (75)
	Total	40	29 (72.5)	32 (80)	30 (75)

ESBL: Extended-spectrum  $\beta$ -lactamase, MBL: Metallo- $\beta$ -lactamase

spectrum cephalosporin non-susceptible isolates were ESBL negative in his study, this can be linked to other mechanisms of resistance such as overproduction of chromosomal cephalosporinase (AmpC) and upregulation of the efflux system. MBL- and ESBL-producing *P. aeruginosa* were shown to have a greater prevalence of antibiotic resistance, according to Ilyas et al., 2015. *P. aeruginosa* isolates generating ESBL and MBL were shown to be fully. It is resistant to amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, and cefepime. They also had a higher MBL production rate than *P. aeruginosa* (25.7%) and a lower ESBL production rate (8.5%). All MBL-producing *P. aeruginosa* are sensitive to colistin, 37.5% resistant to aztreonam, according to Mirsalehian et al., 2017. Multiple beta-lactamases that have already been recognized as beta-lactamase producers should be detectable in laboratories so that appropriate therapy may be chosen for patient management. To track the source of infection, epidemiological investigations should be conducted. The detection of these beta-lactamase-generating isolates in a regular laboratory might aid in the prevention of treatment failure.

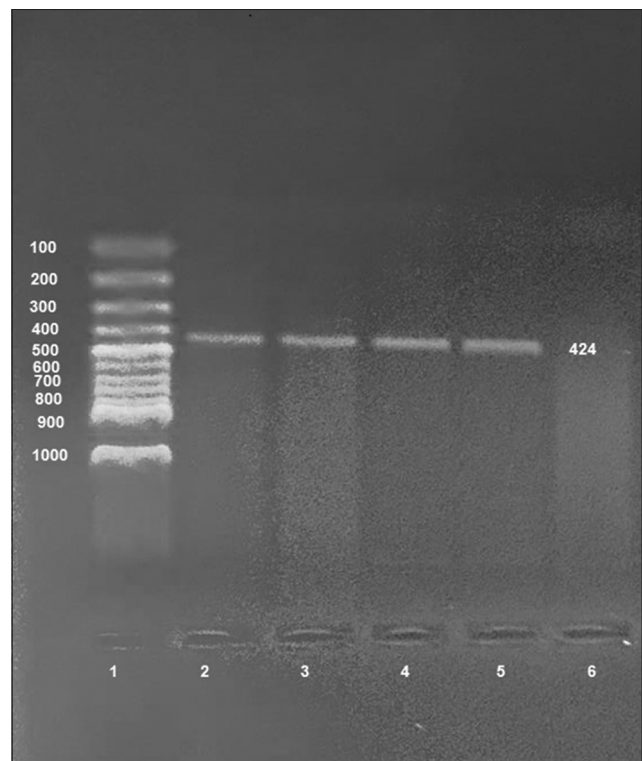
However, in the present study, most of our isolates *P. aeruginosa* ESBL and MBL producer were detected in

**Table 5: Frequency of bla<sub>TEM</sub> in not the same clinical examples**

PCR outcomes	Clinical samples						P-value
	Urine	Bronchial wash	Sputum	wound	Burn	Total	
PCR outcomes							0.2881
Positive							
No	4	1	3	5	8	21	
%	10	2.5	7.5	12.5	20	52.5	
Negative							
No	3	3	3	4	6	19	
%	7.5	7.5	7.5	10	15	47.5	
Total							
No	7	4	6	9	14	40	
%	17.5	10	15	22.5	35	100.0	

burn samples (71.4%) followed by wound and maximum resistance pattern were in burn samples (64.3%) as shown in Table 4 (Nithyalakshmi et al., 2016). Previously reported results did not confirm this, they reported that the incidence of ESBL in *P. aeruginosa* isolates was 21.96%, obtained by most ESBL producers from urine samples (27.7%) followed by respiratory infections (23.68%) and then wound infections (22.95%). In contrast to our findings, a recent study by Pramodhini and Umadevi, 2015, reported high percentage from pus (Bunyan et al., 2018). This might be related to the fact that the types of illnesses linked with ESBL strains vary from place to location, underlining the importance of ongoing surveillance regardless of specimen type. Overall, there was a link between the synthesis of beta-lactamases and associated genes were found in the test isolates “ $P < 0.05$ .”

The existence of plasmid-mediated ESBL was examined further in all *P. aeruginosa* isolates. The attendance of ESBL genes that include bla<sub>TEM</sub> was detected by conventional PCR technique, the results have clarified that 21 of 40 *P. aeruginosa* isolates (52.5%) had bla<sub>TEM</sub> [Table 5] with size of amplified product (424 bp) as shown in Figure 1. The results of ESBLs genes detection clarify that most of ESBLs and MBLs producer isolates of *P. aeruginosa* carried bla<sub>TEM</sub> gene. Furthermore, a high frequency of bla<sub>TEM</sub> was identified in trauma patients (25%), as shown in Table 5, with no major difference in prevalence between clinical specimens ( $P > 0.05$ ), while the prevalence of bla<sub>TEM</sub> in 165 *P. aeruginosa* was 13.9%, respectively, in southwest of Iran (Alam et al., 2018), in another study, 45% of *P. aeruginosa* isolate harbored the gene TEM (Saeidi et al., 2014) while a study of Al-Kaabi, 2011, in Baghdad mentioned that percentage of bla<sub>TEM</sub> gene appeared in all isolates (100%) in *P. aeruginosa* isolates. They also discovered that isolates that produce both ESBL and MBL were extremely resistant. Toupanlou et al., 2015, discovered 87 cefotaxime-resistant GN bacilli among the 217 isolates. ESBL producers were discovered in 42 (48.3%) of them. In the 50 imipenem-resistant isolates of *P. aeruginosa* isolated from burn patients,



**Figure 1: Assenting (polymerase chain reaction) transmission study of expression of the bla<sub>TEM</sub> gene in symptomatic isolates of *Pseudomonas aeruginosa* (Lane 1: DNA ladder [BioLabs]); (lanes 2, 3, 4, and 5: Clinical isolates of *Pseudomonas aeruginosa*); (lane 6: bla<sub>TEM</sub> gene negative and amplified product [424 bp])**

the prevalence of bla<sub>TEM</sub> was 36% (Jaykumar and Appalraju, 2007). These frequencies are quite similar to the ones found in this research. The increased presence of ESBL genes in clinical specimens necessitates ongoing monitoring of antibiotic usage and infection levels. Physicians can use information on pathogen antibiotic resistance trends to help them choose the best antibiotic treatment. Overall, the TEM gene was found in more than half of the isolated strains in our investigation. Antimicrobial treatments are complicated by the increasing rise of ESBL-producing bacteria. As a result, a variety of measures must be used to control the spread of these diseases.



These enzymes could be carried on bacterial chromosomes, making them organism specific, or they can be plasmid mediated, allowing them to travel between bacterial populations. This has obvious consequences for infection transmission and control (Dhillon and Clark, 2012; Ali et al., 2020). If left undiagnosed, *P. aeruginosa* generating several beta-lactamases can cause substantial treatment failure and offer a considerable clinical problem. clinical problem. Because *P. aeruginosa* may include other drug-resistant genes.

## CONCLUSIONS

The current study demonstrates that the development of MBL and ESBL in *P. aeruginosa* is increased and making these infections more challenging to cure. For the decrease of death rates and the propagation of multidrug-resistant organisms by genes, early identification of MBL and ESBL synthesis is critical. As a result, a variety of measures must be used to control the spread of these diseases.

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