



Diversity of Extended Spectrum β -lactamases among Multi Drug Resistant Clinical Isolates of *Pseudomonas aeruginosa* Collected from Tertiary Care Hospitals of Peshawar, Pakistan

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen and the leading cause of nosocomial infection worldwide. This bacterium produces enzymes known as extended spectrum β -lactamases which render broad spectrum cephalosporins and penicillins inactive. This study reports antibiotic susceptibility pattern, multiple antibiotic resistance (MAR) index and prevalence of extended spectrum β -lactamases among clinical isolates of *P. aeruginosa* collected from tertiary care hospitals of Peshawar, Pakistan. A total of 187 *P. aeruginosa* isolates were collected. Antibiotic susceptibility was evaluated by Kirby Bauer disc diffusion method using nineteen different antibiotics and multiple antibiotic resistance (MAR) index was determined. Prevalence of extended spectrum β -lactamases was studied by double disc synergy test. The ESBL genes blaCTX-M, blaOXA-10, blaPER-1, blaSHV and blaTEM were analyzed by PCR amplification among the isolates. Susceptibility to antibiotics was: imipenem (85.02%), meropenem (82.88%), cefepime (76.47%), piperacillin-tazobactam (76.47%), colistin (74.86%), ciprofloxacin (74.33%), piperacillin (72.19%), ceftazidime (68.98%), ofloxacin (68.44%), amikacin (66.84%), cefoperazone (66.31%), carbenicillin (66.31%), gentamicin (64.7%), tobramycin (64.7%), aztreonam (52.4%), ticarcillin (42.78%), ceftriaxone (32.08%), cefotaxime (15.5%), amoxicillin-clavulanic acid (6.41%). A total of 36.89% (n=69) isolates showed multi drug resistance. The MAR index of 34.22% (n=64) isolates was higher than 0.2. Phenotypic ESBL production was observed in 21.39% (n=40) isolates. Prevalence of blaOXA-10, blaCTX-M, blaTEM and blaSHV was 36.89% (n=69), 20.85% (n=39), 5.34% (n=10) and 3.2% (n=6) respectively. PER-1 gene was not detected. Resistance to antibiotics is increasing in *P. aeruginosa* which is a matter of concern and needs proper management. Non-selective and over use of antibiotics should be avoided and proper control measures should be taken to avoid the spread of these multi-drug resistant strains.

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Authors' Contribution

AA conducted the experiments, compiled the data and wrote the manuscript. SR contributed towards manuscript writing and proof reading. KA designed the project, supervised the work, contributed towards data analysis, manuscript writing and proof reading.

Key words

Pseudomonas aeruginosa, MDR, MAR Index, ESBL, blaOXA-10, blaCTX-M, blaPER-1, blaTEM, blaSHV

INTRODUCTION

Pseudomonas aeruginosa is one of the main causes of nosocomial infections like burn infections, wounds infections, urinary tract infections, pneumonia, bacteremia, otitis externa, endophthalmitis, meningitis and infections in cystic fibrosis patients (Branski *et al.*, 2009). These infections could develop into more severe form in immune compromised patients like cancer and neutropenic patients (Bodey *et al.*, 1983). The global emergence of multi-drug resistant *P. aeruginosa* is serious health issue as *P. aeruginosa* resistant to several classes of antibiotics such as penicillin, cephalosporin, aminoglycoside, quinolone and carbapenem have been reported (Dundar and Otkun, 2010). Antimicrobial resistance mechanisms in *P. aeruginosa* include multidrug efflux pumps, outer

membrane impermeability to antibiotics, enzymatic degradation of antibiotics and target site modification (Lambert, 2002; Mesaros *et al.*, 2007). Production of extended spectrum beta lactamases (ESBLs) by *P. aeruginosa* is an important mechanism to inactivate antibiotics. These enzymes could hydrolyze penicillins, extended spectrum cephalosporins such as, ceftriaxone, ceftazidime, cefotaxime and the monobactam aztreonam (Paterson and Bonomo, 2005; Khanfar *et al.*, 2009). However, they have no effect on cephamycins or carbapenems and their activity is inhibited by clavulanic acid (Paterson and Bonomo, 2005). The global spread of ESBL producing *P. aeruginosa* pose a serious health threat.

Extended spectrum β -lactamase producing bacteria were first reported in Germany in 1983 (Knothe *et al.*, 1983). Different variants of ESBLs such as TEM, PER, SHV, GES, VEB and CTX-M have been reported in *P. aeruginosa* of different geographical origins (Aktas *et al.*, 2005; Al Naiemi *et al.*, 2006; Celenza *et al.*, 2006; Zhao and Hu, 2010). Cefotaximase-Munich (CTX-M)

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beta lactamase was first isolated from *Escherichia coli* recovered from ear exudate of newly born baby in Munich, Germany (Bauernfeind *et al.*, 1990). These β lactamases have been divided into five groups based on amino acid sequence i.e. CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 (Paterson and Bonomo, 2005; Gupta, 2007). All these CTX-M β lactamases have been reported from different countries including Japan, Germany, Argentina, Poland, Taiwan, France, Spain, Brazil, China, Korea and Canada (Canton *et al.*, 2012). These enzymes possess hydrolytic activity against cefotaxime and have approximately 40 % or less identity with other β -lactamases such as TEM and SHV (Paterson and Bonomo, 2005). Oxacillinases (OXA) are β -lactamases that could hydrolyze oxacillin and have been reported in *P. aeruginosa* (Naas *et al.*, 2008; El-Shouny *et al.*, 2018; Odumosu *et al.*, 2016). The OXA type variants, OXA-10 and OXA-13, weakly hydrolyze cephalosporin (cefotaxime and ceftriaxone) and aztreonam (Naas *et al.*, 2008). The *Pseudomonas* extended resistance (PER-1) β -lactamase of *P. aeruginosa* can efficiently hydrolyze third generation cephalosporins, penicillins and aztreonam but has no effect on cephamycins and carbapenems (Aktas *et al.*, 2005; Nordmann and Naas, 1994; Opus *et al.*, 2017; Qing *et al.*, 2014). Sulfhydryl variable (SHV) type β -lactamases i.e. SHV-1 efficiently hydrolyze cefotaxime but slightly hydrolyze ceftazidime (Paterson and Bonomo, 2005). More than 50 SHV β -lactamases have been identified which are derived either from SHV-1 or SHV-2 (Paterson and Bonomo, 2005; Gupta, 2007; Peymani *et al.*, 2017). In 1965, TEM-1 β -lactamase was first confirmed in *Escherichia coli* isolated from a patient named Temoneira in Athens (Datta and Kontomichalou, 1965). Such β -lactamases could hydrolyze β -lactam antibiotics such as penicillins and cephalosporins (Salverda *et al.*, 2010; Hassuna *et al.*, 2015). The spread of multiple antibiotic resistant *P. aeruginosa* in hospital environments has been reported across the world (Krumperman, 1983; Paul *et al.*, 1997).

Infectious diseases are highly prevalent in Pakistan, however; there is scarcity of data on genotypic characteristics of locally prevalent bacterial pathogens. This study was aimed at investigating phenotypic and genotypic characterization of extended spectrum beta lactamases among clinical isolates of *P. aeruginosa* isolated from different clinical specimens in tertiary care hospitals of Peshawar, Khyber Pakhtunkhwa Pakistan.

MATERIALS AND METHODS

Bacterial isolates

A total of 187 *P. aeruginosa* isolates were collected from clinical specimens in tertiary care hospitals of

Peshawar, Pakistan during 2014-2016. Among these, 74 isolates were recovered from pus, 34 from urine, 24 from sputum, 21 from wound, 12 from bronchial wash, 8 from cerebrospinal fluid, 6 from blood, 5 from high vaginal swab and 3 from diabetic foot. The cultures were grown on MacConkey agar (Oxoid, UK). Pure isolates were identified as *P. aeruginosa* using morphological and biochemical tests (Parija, 2006).

Antibiotic sensitivity

Antibiotic sensitivity was evaluated using Kirby Bauer disc diffusion method as suggested by the Clinical Laboratory Standard Institute (Clinical Laboratory Standard Institute, 2007; Clinical Laboratory Standard Institute, 2014). The antibiotics (Oxoid, UK) used were: Amoxicillin-clavulanic acid (30 μ g), Cefotaxime (30 μ g), Piperacillin-tazobactam (110 μ g), Cefoperazone (75 μ g), Ceftazidime (30 μ g), Ceftriaxone (30 μ g), Gentamicin (10 μ g), Meropenem (10 μ g), Cefepime (30 μ g), Aztreonam (30 μ g), Carbenicillin (100 μ g), Imipenem (10 μ g), Ticarcillin (75 μ g), Piperacillin (100 μ g), Amikacin (30 μ g), Ciprofloxacin (5 μ g), Tobramycin (10 μ g), Ofloxacin (5 μ g) and Colistin (10 μ g). Bacterial colonies were suspended in normal saline and turbidity was adjusted by comparing with 0.5 McFarland standard. Bacterial suspension was inoculated on Muller Hinton agar (Oxoid, UK) plate and discs were placed on the medium at equal distances. The cultures were incubated at 37 °C for 18-24 hrs. and zones of inhibition were measured.

Determination of multiple antibiotic resistance index

Multiple antibiotic resistance index for each isolate of *P. aeruginosa* was determined using the formula, MAR index= a/b where 'a' represent the number of antibiotics to which isolate show resistant, where 'b' represent total number of antibiotics used (Krumperman, 1983; Sandhu *et al.*, 2016).

Phenotypic detection of extended spectrum β -lactamases

Double disc synergy test was used for detection of ESBLs production (Jarlier *et al.*, 1998). Bacterial lawn was made on Muller Hinton Agar and amoxicillin-clavulanic acid disc was applied in the center. Discs of aztreonam, cefepime, cefotaxime and ceftazidime were placed 15-20 mm away from the disc of amoxicillin-clavulanic acid. The plates were incubated at 37 °C for 18 h and inhibition zones were measured. Increase in size of inhibition zone around one or more cephalosporin discs and aztreonam towards amoxicillin-clavulanic acid disc showed presence of ESBL production.

DNA extraction

GeneJET Genomic DNA purification kit (Thermo Scientific, Lithuania, #K0721) was used for isolation of

bacterial genomic DNA. Isolated DNA was preserved at -20°C.

Molecular detection of ESBLs

Previously reported primers were used for the amplification of blaCTX-M, blaOXA-10, blaPER-1, blaSHV, and blaTEM genes (Farshadzadeh *et al.*, 2014; Peerayeh *et al.*, 2014). PCR reaction mix (25 µl) contained 12.5 µl SuperHot Master Mix (BIORON, Cat. No. 119102), 1 µl of each primer (0.5 µM), 1 µl genomic DNA and 9.5 µl molecular grade water (Sigma-Aldrich, US). Reaction conditions consisted of initial denaturation (95 °C for 5 min), followed by 30 cycles of denaturation (94 °C for 1 min), annealing (55 °C for blaCTX-M and blaTEM; 57 °C for blaOXA-10; 48 °C for blaPER-1; 60 °C for blaSHV) and extension (72 °C for 1 min). Final extension was carried out at 72 °C for 5 min. PCR products were analyzed using agarose gel (1.5 %) and 100 bp DNA ladder (BIORON, Cat. No. 304105) was used as size marker.

Table I. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates.

S. No.	Antimicrobial	Susceptible, No. (%)	Intermediate, No. (%)	Resistant, No. (%)
1.	TZP	143 (76.47%)	26 (13.90%)	18 (9.62%)
2.	AMC	12 (6.41%)	20 (10.69%)	155 (82.88%)
3.	CTX	29 (15.50%)	80 (42.78%)	78 (41.71%)
4.	CAZ	129 (68.98%)	7 (3.74%)	51 (27.27%)
5.	CRO	60 (32.08%)	56 (29.94%)	71 (37.96%)
6.	CFP	124 (66.31%)	20 (10.69%)	43 (22.99%)
7.	FEP	143 (76.47%)	6 (3.20%)	38 (20.32%)
8.	ATM	98 (52.40%)	49 (26.20%)	40 (21.39%)
9.	IPM	159 (85.02%)	3 (1.60%)	25 (13.36%)
10.	MEM	155 (82.88%)	0 (0%)	32 (17.11%)
11.	TIC	80 (42.78%)	58 (31.01%)	49 (26.20%)
12.	PIP	135 (72.19%)	32 (17.11%)	20 (10.69%)
13.	CB	124 (66.31%)	12 (6.41%)	51 (27.27%)
14.	CN	121 (64.70%)	17 (9.09%)	49 (26.20%)
15.	AK	125 (66.84%)	16 (8.55%)	46 (24.59%)
16.	TOB	121 (64.70%)	14 (7.48%)	52 (27.80%)
17.	CIP	139 (74.33%)	7 (3.74%)	41 (21.92%)
18.	OFX	128 (68.44%)	11 (5.88%)	48 (25.66%)
19.	CT	140 (74.86%)	0 (0%)	47 (25.13%)

AMC, Amoxicillin-clavulanic acid (30 µg); CTX, Cefotaxime (30 µg); TZP, Piperacillin-tazobactam (110 µg); CFP, Cefoperazone (75 µg); CAZ, Ceftazidime (30 µg); CRO, Ceftriaxone (30 µg); CN, Gentamicin (10 µg); MEM, Meropenem (10 µg); FEP, Cefepime (30 µg); ATM, Aztreonam (30 µg); CB, Carbenicillin (100 µg); IPM, Imipenem (10 µg); TIC, Ticarcillin (75 µg); PIP, Piperacillin (100 µg); AK, Amikacin (30 µg); CIP, Ciprofloxacin (5 µg); TOB, Tobramycin (10 µg); OFX, Ofloxacin (5 µg) and CT, Colistin (10 µg).

RESULTS

Antibiotic sensitivity data is given in Table I. Resistance to cephalosporin third generation antimicrobials cefoperazone, ceftazidime, ceftriaxone and cefotaxime were 22.99%, 27.27%, 37.96% and 41.71%, respectively. Resistance to cephalosporin fourth generation cefepime was 20.32%. Resistance to penicillins antimicrobials i.e. piperacillin, ticarcillin and carbenicillin was 10.69%, 26.2% and 27.27%, respectively. Resistance to aminoglycoside antimicrobials amikacin, gentamicin and tobramycin was 24.59%, 26.2% and 27.8%, respectively. Resistance to fluoroquinolones antibiotics i.e. ciprofloxacin and ofloxacin was 21.92% and 25.66%, respectively. Resistance to carbapenem antimicrobials imipenem and meropenem was 13.36% and 17.11%, respectively. Resistance to monobactam antibiotic aztreonam was 21.39%. Resistance to polymyxin antibiotic colistin was 25.13%. All imipenem resistant isolates of *P. aeruginosa* were susceptible to colistin.

Table II. Prevalence of MDR isolates of *Pseudomonas aeruginosa* in different samples.

S. No	Specimen type	MDR <i>P. aeruginosa</i> (n, %), (n = 69)
1.	Pus	30 (43.47)
2.	Urine	11 (15.94)
3.	Sputum	06 (8.69)
4.	Wound	10 (14.49)
5.	Bronchial wash	05 (7.24)
6.	Blood	03 (4.34)
7.	Cerebrospinal fluid	04 (5.79)

Table III. MAR index of *Pseudomonas aeruginosa* isolates (n=187).

MAR index	Number of isolates, (%)
0	27 (14.43)
0.05	41 (21.92)
0.10	34 (18.18)
0.2	21 (11.22)
0.3	14 (7.48)
0.4	13 (6.95)
0.5	5 (2.67)
0.6	10 (5.34)
0.7	11 (5.88)
0.8	5 (2.67)
0.9	6 (3.2)

A total of 36.89% (n=69) isolates showed multiple drug resistance (MDR) having resistance against three or more drug classes (Table II). Multiple antibiotic resistance (MAR) index values for isolates of *P. aeruginosa* are given in Table III and Table IV. In total, 34.22% (n=64) isolates showed MAR index greater than 0.2 and 54.54% (n=102) isolates showed MAR index value less than 0.2. Source wise MAR index of higher than 0.2 for the isolates was: blood (4.68% isolates), cerebrospinal fluid (6.25%), bronchial wash 7.81%, sputum (9.37%), wound (14.06%), urine (15.62%) and pus (42.18%) as shown in Table IV. Highest multiple antibiotic resistance index (MARI) of 0.9 was observed for six isolates that were resistant to all tested antibiotics except colistin.

Table IV. Distribution of *Pseudomonas aeruginosa* isolates based on MARI value > 0.2 among different clinical specimens.

Specimen type	Isolates with MAR Index > 0.2 (n= 64)	Percentage (%)
Pus	27	42.18
Urine	10	15.62
Wound	9	14.06
Sputum	6	9.37
Bronchial wash	5	7.81
Cerebrospinal fluid	4	6.25
Blood	3	4.68

Table V. Phenotypically ESBL positive strains of *P. aeruginosa*.

S. No.	Specimen	No of <i>P. aeruginosa</i> isolates (n =187)	ESBL positive <i>P. aeruginosa</i> isolates (n = 40), n (%),
1.	Pus	74	14 (35 %)
2.	Urine	34	10 (25 %)
3.	Sputum	24	4 (10 %)
4.	Wound	21	5 (12.5 %)
5.	Bronchial wash	12	3 (7.5 %)
6.	Blood	6	2 (5 %)
7.	Cerebrospinal fluid	8	2 (5 %)
8.	High vaginal swab	5	0 (0%)
9.	Diabetic foot	3	0 (0%)

Out of 187 isolates, 21.39 % (n=40) were ESBL positive phenotypically (Fig. 1). Frequency of ESBL positive isolates was 5%, 5%, 7.5%, 10%, 12.5%, 25% and 35% from cerebrospinal fluid, bronchial wash, sputum,

wound, urine and pus samples respectively as given in Table V. Genotypically, blaOXA-10, blaCTX-M, blaTEM and blaSHV genes were detected in 36.89% (n=69), 20.85% (n=39), 5.34% and 3.2% (n=6) isolates respectively (Supplementary Table I, Fig. 2), however, blaPER-1 was not detected. Among phenotypically ESBL positive isolates (n=40), blaOXA-10, blaCTX-M, blaTEM and blaSHV were observed in 80% (n=32), 62.5% (n=25), 12.5% (n=5) and 7.5% (n=3) isolates respectively (Supplementary Table I). Among phenotypically ESBL negative isolates (n=147), blaOXA-10, blaCTX-M, blaTEM and blaSHV were observed in 25.17% (n=37), 9.52% (n=14), 3.4% (n=5) and 2% (n=3) isolates respectively (Supplementary Table I).

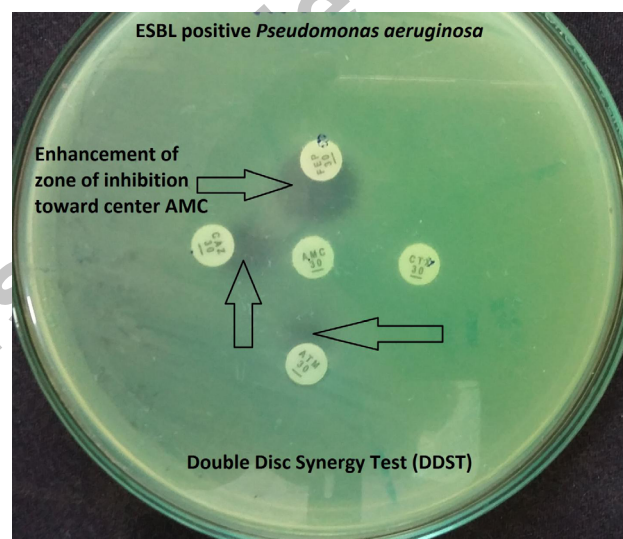


Fig. 1. Double Disc Synergy Test: ESBL positive *Pseudomonas aeruginosa*, the size of zones of inhibition around cefepime, ceftazidime and aztreonam are enhanced towards center amoxicillin-clavulanic acid disc as shown by arrows.

DISCUSSION

P. aeruginosa is rapidly developing resistance against the prevalent antibiotics. Previously, ESBLs producing *P. aeruginosa* has been reported from different geographical locations (Manchanda and Singh, 2003; Ghafourian et al., 2015). However, little data is available regarding ESBL producing *P. aeruginosa* prevailing in the region. Current study showed 21.39% prevalence of ESBL producing *P. aeruginosa* isolated from different clinical specimens. Isolates from pus had maximum frequency of ESBL production followed by urine, wound, sputum, bronchial wash, cerebro-spinal fluid and blood samples. These results are in harmony with the findings of Aggarwal et al.

(2008) and Shaikh *et al.* (2015) who reported 20.27% and 25.13% ESBL frequency respectively among *P. aeruginosa* isolates from various clinical samples. Imipenem and meropenem are broad spectrum carbapenems commonly used effectively against extended spectrum β -lactamase

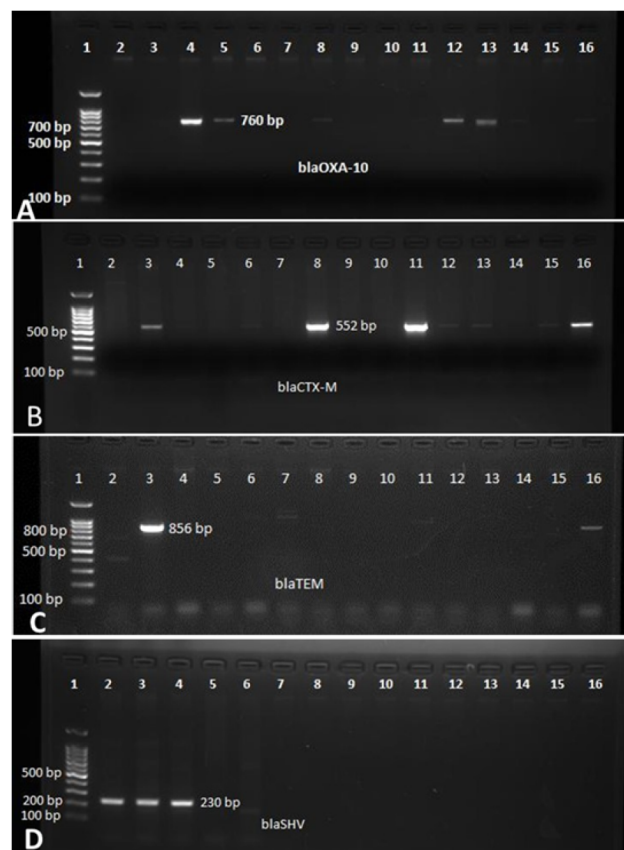


Fig. 2. A, PCR amplification of blaOXA-10 gene (A) Lane 1 = 100 bp DNA ladder. Lanes 4, 5, 8, 12, 13, 14 and 16 in A show blaOXA-10 (760 bp) blaCTX-M gene; (B), Lanes 3, 8, 11, 12, 13, 15 and 16 in B show blaCTX-M (552 bp) positive blaTEM gene (C), Lanes 3 and 16 show blaTEM (856 bp) positive blaSHV gene and (D) Lanes 2, 3 and 4 in D show blaSHV (230 bp) positive *Pseudomonas aeruginosa* isolates.

producing strains (Shaikh *et al.*, 2015). These two antibiotics were found effective against most of the isolates in current study. Similar results have been reported previously (Shaikh *et al.*, 2015; Alikhani *et al.*, 2014). Resistance to both imipenem (13.36%) and meropenem (17.11%) was also observed as reported previously (Pathmanathan *et al.*, 2009; Hong *et al.*, 2015). In current study, 36.89% isolates showed multi drug resistance (MDR). Ullah *et al.* (2009) observed 29.24% MDR frequency among clinical isolates of *P. aeruginosa* from burn patients. Alikhani *et al.* (2014)

observed 88.7% MDR frequency among *P. aeruginosa* isolates from west of Iran. In current study, ceftazidime was found to be the most effective (68.98% susceptibility) antibiotic among the third generation cephalosporins. (Shahid *et al.* (2003) reported 83.3% susceptibility to ceftazidime in samples from North India. A low level of resistance to the aminoglycosides amikacin, gentamicin and tobramycin was observed in the current study. In contrast, a study from Isfahan reported 60% resistance to gentamicin, 62% to tobramycin and 70% to amikacin (Golshani *et al.*, 2012). Resistance to aminoglycoside is due to acquisition of plasmids which produce aminoglycoside modifying enzymes (Hancock, 1998). These enzymes modify the aminoglycosides antibiotics by various mechanisms such as acetylation, adenylation, and phosphorylation which decrease the uptake or reduce the ribosomal interaction of enzymatically modified drugs (Hancock, 1998). In addition, the chromosome of *P. aeruginosa* has an aminoglycoside resistant gene *aphA* that is activated by certain mutations (Hancock, 1998). Among fluoroquinolones, ciprofloxacin showed good activity (74.33% susceptibility) in this study. A study from France reported 68% susceptibility to ciprofloxacin (Cavallo *et al.*, 2007). Resistance to ofloxacin and ciprofloxacin was 25.66% and 21.92% respectively in the current study. Golshani *et al.* (2012) reported high level of resistance to fluoroquinolones. Resistance to quinolone is because of mutations in regulatory gene *mexR* that regulates *mexAB-oprM* genes of efflux system and, hence, expression of efflux system genes is enhanced, and quinolone are extruded (Ziha-Zarifi *et al.*, 1999). Among penicillins, piperacillin showed maximum activity (72.19% susceptibility). This finding is in close agreement to the report of Llanes *et al.* (2013) who investigated *P. aeruginosa* isolates from cystic fibrosis in France. *P. aeruginosa* acquires resistance to penicillins mostly due to alteration in penicillin binding protein (Srikumar *et al.*, 1999).

In this study, 34.22% (n=64) isolates showed multiple antibiotic resistance (MAR) index value higher than 0.2. A higher MAR index value is an indication of over-use of antibiotics in a location that contributes to evolution of resistant bacteria (Krumperman, 1983; Paul *et al.*, 1997). A study from Turkey reported a high percentage (51.92%) of clinical isolates of *P. aeruginosa* with MAR index higher of than 0.2 (Guvensen *et al.*, 2017). Another study from India found that 39% *P. aeruginosa* collected from area with high use of antibiotics had MAR index of higher than 0.2 (Bhuvaneshwari, 2017). A high MAR index value (0.6 to 0.9) was observed for 22 isolates of *P. aeruginosa* recovered from clinical specimens and hospital environment in Nigeria (Chika *et al.*, 2017). This high MAR index was suggested to be linked to the development

of multi drug resistant isolates of *P. aeruginosa* because of intensive use of antibiotics in Nigeria (Chika *et al.*, 2017).

According to current findings, prevalence of blaCTX-M was 20.85%. Prevalence of the gene was 10.7% among isolates of *P. aeruginosa* isolated from a hospital in Makkah, Saudi Arabia (Ahmed *et al.*, 2015) and 19.6% among isolates of *P. aeruginosa* collected from a Brazilian tertiary care hospital (Polotto *et al.*, 2012). A high frequency of blaOXA-10 gene was observed in this study that is in agreement with previous reports (Weldhagen *et al.*, 2003; Poirel *et al.*, 2001; Neyestanaki *et al.*, 2014). Low prevalence (5.34%) of blaTEM was observed in this study in contrast to previous reports from Iran (61%) and China (20.5%) (Neyestanaki *et al.*, 2014; Chen *et al.*, 2015). Low prevalence (3.2%) of blaSHV was observed in this study. Previous reports from Iran and India showed 36% and 1.78% prevalence of blaSHV respectively (Toupanlou *et al.*, 2015; Bharti *et al.*, 2016). According to our knowledge; this is the first report on prevalence of blaOXA-10, blaCTX-M, blaTEM and blaSHV in clinical isolates of *P. aeruginosa* collected from regional hospitals.

CONCLUSIONS

In conclusion, the prevalence of ESBL producing MDR *P. aeruginosa* of clinical origin was confirmed in the region. These findings demand for controlled use of antibiotics and proper management strategies both at community level and in hospital environments to prevent the dissemination of these resistant bacteria.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20190712160705>

Statement of conflict of interest

The authors declare there is no conflict of interest.

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Supplementary Materials

Diversity of Extended Spectrum β -lactamases among Multi Drug Resistant Clinical Isolates of *Pseudomonas aeruginosa* Collected from Tertiary Care Hospitals of Peshawar, Pakistan

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Table VI. Comparison of phenotypic antibiotic resistance, phenotypic ESBL production and genotypic detection of ESBL genes among *Pseudomonas aeruginosa* isolates. (+ indicates presence, - indicates absence).

S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
1	K	CRO, AMC	-	-	+	+	-	-
2	K22	AMC	-	+	-	-	-	-
3	K25	CTX, CRO, CAZ, CFP, FEP, ATM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	-	-	-	-
4	K26	AMC	-	+	+	+	-	-
5	K27	...	-	+	-	-	-	-
6	K29	OFX, AMC	-	-	-	-	-	-
7	K31	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	+	-	-
8	K32	CTX, CRO, MEM, TIC, CAR, CIP, OFX, AMC	-	-	-	-	-	-
9	K33	...	-	-	-	-	-	-
10	K34	AMC	-	-	-	-	-	-
11	K35	CTX, CRO, AMC	-	+	+	-	+	-
12	K36	CN, AK, CT, TOB, AMC	-	-	-	-	-	-
13	K37	CTX, CRO, CAZ, TIC, CAR, CN, AK, CT, TOB, AMC	+	-	+	-	-	-
14	K38	CTX, CRO, TIC, CAR, CN, AK, CT, TOB, AMC	-	+	-	-	-	-
15	K4505	CTX, CRO, CAZ, CFP, FEP, ATM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
16	K4506	AMC	-	+	-	-	-	-
17	K4507	CT, AMC	-	+	-	-	-	-
18	M1	CTX, CRO, CAZ, CFP, TIC, CAR, AK, OFX, CT, AMC	+	+	-	-	-	-

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S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
19	M2	AMC	-	-	-	-	-	-
20	M7	CT, AMC	-	-	-	-	-	-
21	M11	...	-	-	-	-	-	-
22	M12	AMC	-	-	-	-	-	-
23	M13	CTX, CRO, CN, CIP, OFX, TOB, AMC	-	-	-	-	-	-
24	M21	AMC	-	-	-	-	-	-
25	M23	CRO, AMC	-	+	-	-	-	-
26	M26	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, CAR, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
27	M28	AMC	-	+	-	-	-	-
28	M33	CTX, CRO, CFP, TIC, CAR, CN, CIP, OFX, TOB, AMC	+	+	-	-	-	-
29	M36	CTX, CRO, CAZ, FEP, CN, CT, AMC	-	-	-	-	-	-
30	M38	TZP, CTX, CRO, CAZ, CFP, FEP, MEM, TIC, CAR, CN, CIP, OFX, AMC	+	+	-	-	-	-
31	M39	TZP, CTX, CRO, CAZ, CFP, FEP, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
32	M41	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB	-	-	-	-	-	-
33	M42	CTX, CRO, CAZ, CFP, TIC, CAR, CIP, OFX	+	+	-	-	-	-
34	N1	AMC	-	-	-	-	-	-
35	N6	AMC	-	-	-	-	-	-
36	N7	AMC	-	-	-	-	-	-
37	N10	...	-	-	-	-	-	-
38	N11	ATM, CN, AK, CT, TOB, AMC	-	-	-	-	-	-
39	N12	...	-	-	-	-	-	-
40	N13	...	-	-	-	-	-	-
41	N16	CRO, CFP, AMC	-	-	-	-	-	-
42	N17	AMC	-	-	-	-	-	-
43	N20	TZP, CTX, CRO, CFP, MEM, TIC, PRL, CAR, AMC	-	-	-	-	-	-
44	N21	AMC	-	-	-	-	-	-
45	N23	AMC	-	-	-	-	-	-
46	N24	AMC	-	-	-	-	-	-
47	N25	AMC	-	-	-	-	-	-
48	N26	CTX, CRO, CFP, FEP, ATM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	-	+	+	-	-
49	N27	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
50	N28	...	-	-	-	-	-	-
51	N29	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	+	-	-
52	N30	CTX, CRO, AK, CT, TOB, AMC	-	+	-	+	-	-
53	N31	CTX, AMC	-	-	-	-	-	-
54	N32	...	-	-	-	-	-	-
55	N35	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-

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S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
56	N36	...	-	-	-	-	-	-
57	N37	AMC	-	-	-	-	-	-
58	N38	CT, AMC	-	-	-	-	-	-
59	N39	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	-	-	-	-
60	N40	CTX, IPM, MEM, AMC	-	-	+	-	-	-
61	N41	...	-	-	-	-	-	-
62	N42	AMC	-	-	-	-	-	-
63	N43	CTX, CRO, CN, CT, AMC	-	+	-	-	-	-
64	N44	CN, AK, CT, TOB, AMC	-	-	-	-	-	-
65	N45	CN, AK, CT, TOB, AMC	-	-	-	-	-	-
66	N48	ATM, CT, AMC	-	+	-	-	-	-
67	N51	CT	-	-	-	-	-	-
68	N55	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, CAR, CN, AK, OFX, TOB, AMC	+	+	+	-	-	-
69	N56	...	-	-	-	-	-	-
70	N57	AMC	-	-	-	-	-	-
71	N58	CTX, FEP, ATM, MEM, AMC	+	-	+	+	-	-
72	N60	...	-	-	-	-	-	-
73	N63	...	-	-	-	-	-	-
74	N64	CTX	-	+	-	-	-	-
75	N65	AMC	-	-	-	-	-	-
76	N66	CTX, CRO, CAZ, CFP, TIC, CAR, AK, OFX, CT, AMC	+	-	+	-	+	-
77	N67	AMC	-	-	-	-	-	-
78	N68	...	-	-	-	-	-	-
79	N69	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, CAR, CN, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
80	N71	AMC	-	-	-	-	-	-
81	N76	...	-	-	-	-	-	-
82	N77	AMC	-	-	-	-	-	-
83	N78	TZP, CTX, CFP, TIC, PRL, CAR, AMC	-	-	-	-	-	-
84	N81	...	-	-	-	-	-	-
85	N82	CRO, CAZ, CFP, CT, AMC	+	+	-	-	-	-
86	N83	CTX, CRO, CAZ, CAR, CIP, OFX, TOB, AMC	-	-	-	-	-	-
87	N84	AMC	-	-	-	-	-	-
88	N85	CTX, CRO, CAZ, CFP, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
89	N86	CTX, CRO, AMC	-	-	-	-	-	-
90	N87	CTX, CRO, ATM, CN, CT, TOB, AMC	+	+	-	-	-	-
91	N88	CRO, AMC	-	-	-	-	-	-
92	N89	...	-	-	-	-	-	-
93	N90	TZP, CAZ, CIP, AMC	-	+	-	-	-	-
94	N91	CTX, CRO, CAZ, CFP, FEP, IPM, MEM, TIC, CAR, CIP, OFX, AMC	+	+	+	-	-	-
95	N92	CTX, CRO, ATM, AMC	-	+	+	-	-	-
96	N93	CTX, CRO, CAZ, CFP, TIC, CAR, AK, OFX, CT, AMC	+	-	+	-	-	-

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S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
97	N94	CRO, AMC	-	+	-	-	-	-
98	N95	TZP, CTX, CRO, CAZ, FEP, ATM, IPM, MEM, TIC, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
99	N96	CRO, CAZ, AMC	-	+	+	-	-	-
100	N98	CTX, CRO, CFP, FEP, AMC	+	-	+	-	-	-
101	N99	TZP, CRO, AMC	-	-	-	-	-	-
102	N100	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
103	N101	CAZ, CN	-	+	+	-	-	-
104	N102	CTX, CAZ, FEP, IPM, MEM, TIC, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
105	R4	AMC	-	+	-	-	-	-
106	R9	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CIP, OFX, AMC	+	+	+	-	-	-
107	R10	AMC	-	-	-	-	-	-
108	R11	AMC	-	-	-	-	-	-
109	R12	AMC	-	+	+	-	-	-
110	R14	AMC	-	+	-	-	-	-
111	R15	...	-	-	-	-	-	-
112	R16	AMC	-	-	-	-	-	-
113	R18	...	-	-	-	-	-	-
114	R19	CTX, CRO, CAZ, FEP, CAR, CIP, OFX, TOB, AMC	-	-	-	-	-	-
115	R20	TZP, CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	+	-	-	-
116	R22	CTX, CRO, CAR, OFX, AMC	-	-	-	-	-	-
117	R24	ATM, CT, AMC	-	-	-	-	-	-
118	R25	ATM, CT, AMC	-	-	-	-	-	-
119	R26	CN, AK, CT, TOB, AMC	-	+	-	-	-	-
120	R27	ATM, CN, AK, CT, TOB, AMC	-	-	-	-	-	-
121	R28	CTX, FEP, ATM, CT, AMC	+	-	+	-	-	-
122	R29	CTX, ATM, AMC	-	-	-	-	-	-
123	R30	CTX, AMC	-	-	-	-	-	-
124	R31	CTX, CRO, CAZ, CFP, FEP, IPM, MEM, TIC, CAR, CIP, OFX, AMC	+	+	+	-	-	-
125	R32	...	-	+	-	-	-	-
126	R33	...	-	-	-	-	-	-
127	R34	CTX, CRO, FEP, MEM, TIC, CAR, CIP, OFX, AMC	-	-	-	-	-	-
128	R35	AMC	-	-	-	-	-	-
129	R36	CTX, AMC	-	-	-	-	-	-
130	R37	...	-	-	-	-	-	-
131	R39	AMC	-	+	-	-	-	-
132	R40	ATM, CN, CT, TOB, AMC	-	+	-	-	-	-
133	R41	CTX, CRO, TIC, CN, AK, CT, TOB, AMC	-	+	-	-	-	-
134	R43	CTX, CAZ, CT, AMC	-	+	-	-	-	-
135	R45	CTX, CAZ, AMC	-	+	-	-	-	-
136	R46	CAZ, CFP, ATM, AK, CT, TOB, AMC	+	+	+	+	+	-

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S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
137	R47	...	-	+	-	-	-	-
138	R48	CTX, CRO, CAZ, TIC, CAR, CN, CT, TOB, AMC	+	+	-	-	+	-
139	R50	CTX, CRO, CFP, AMC	-	-	-	-	-	-
140	R53	CTX, AMC	-	-	-	-	-	-
141	R54	...	-	-	-	-	-	-
142	R55	CTX, CAZ, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	+	+	-	-	-	-
143	R58	CIP, OFX, AMC	-	+	-	-	-	-
144	R59	CTX, CRO, CAZ, FEP, ATM, IPM, CAR, AK, AMC	+	+	+	-	-	-
145	R60	TZP, CTX, CRO, CAZ, CFP, FEP, IPM, MEM, TIC, CAR, CIP, OFX, AMC	-	-	-	-	-	-
146	R61	CTX, CRO, CAZ, CFP, IPM, MEM, TIC, CAR, CN, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
147	R62	CTX, CRO, CAZ, FEP, ATM, IPM, MEM, TIC, CAR, CN, CIP, OFX, TOB, AMC	-	-	-	-	-	-
148	R63	TZP, CTX, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
149	R64	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, CAR, CIP, OFX, AMC	-	-	-	-	-	-
150	B1	AMC	-	-	-	-	-	-
151	B2	AMC	-	-	-	-	-	-
152	D1	ATM, CT, AMC	-	-	-	-	-	-
153	H7	CAZ, FEP, ATM, CT, AMC	+	+	-	-	-	-
154	H12	...	-	-	+	-	-	-
155	H23	CT, AMC	-	-	-	-	-	-
156	H30	CRO, AMC	-	-	-	-	-	-
157	H31	CN, AK, CT, TOB, AMC	-	+	-	+	-	-
158	H33	AMC	-	+	+	-	-	-
159	H35	MEM, OFX, AMC	-	-	-	-	-	-
160	H38	CTX, CRO, CAZ, CFP, FEP, ATM, IPM, MEM, TIC, PRL, CAR, CN, AK, CIP, OFX, TOB, AMC	-	-	-	-	-	-
161	H42	CRO, CAZ, CFP, TIC, CAR, AK, OFX, CT, AMC	-	+	-	-	-	-
162	H45	...	-	-	-	-	-	-
163	H49	ATM, CT, AMC	-	-	-	-	-	-
164	H59	TIC, CAR, CN, AK, TOB, AMC	-	-	-	-	-	-
165	H60	AMC	-	+	-	-	-	-
166	H62	CTX, AMC	-	-	-	-	-	-
167	H63	AMC	-	-	-	-	-	-
168	H64	CTX, CRO, CAZ, CFP, CIP, CT, AMC	+	+	-	-	-	-
169	H65	TZP, CTX, CRO, CAZ, CFP, FEP, IPM, MEM, TIC, CAR, CIP, OFX, AMC	+	+	+	-	-	-
170	H66	CRO, OFX, AMC	-	-	-	-	-	-
171	H68	CT, AMC	-	-	-	-	-	-
172	H71	CTX, CRO, CAZ, CFP, MEM, TIC, CAR, CN, CIP, OFX, TOB, AMC	+	+	-	-	-	-

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S. No	Isolate	Resistance pattern to antibiotics	Phenotypic ESBL	ESBL genes				
				blaOXA-10	blaCTX-M	blaTEM	blaSHV	blaPER-1
173	H75	AMC	-	-	+	+	+	-
174	H76	CTX, FEP, ATM, CT, AMC	+	+	+	-	-	-
175	H77	...	-	-	-	-	-	-
176	H78	CTX, CRO, CFP, FEP, TIC, PRL, CAR, AMC	+	+	-	-	-	-
177	H81	CTX, CRO, CN, CT, TOB, AMC	-	-	-	-	+	-
178	H83	AMC	-	+	+	-	-	-
179	H84	CTX, CRO, TIC, CN, AK, CT, TOB, AMC	+	-	+	-	-	-
180	H86	CTX, CRO, CN, CT, TOB, AMC	-	-	-	-	-	-
181	H87	CTX, CRO, CAZ, CFP, FEP, TIC, CAR, CN, AK, CIP, OFX, CT, TOB, AMC	+	+	-	-	-	-
182	H88	CTX, ATM, AMC	-	-	+	-	-	-
183	H90	CN, AK, CT, TOB, AMC	-	+	-	-	-	-
184	H95	ATM, CN, AK, CT, TOB, AMC	-	+	-	-	-	-
185	H96	CN, AK, CT, TOB, AMC	-	-	+	-	-	-
186	H97	ATM, CN, AK, CT, TOB, AMC	-	-	-	-	-	-
187	H99	CT, AMC	-	-	-	-	-	-

AMC, Amoxicillin-clavulanic acid (30 µg); CTX, Cefotaxime (30 µg), TZP, Piperacillin-tazobactam (110 µg); CFP, Cefoperazone (75 µg); CAZ, Ceftazidime (30 µg); CRO, Ceftriaxone (30 µg); CN, Gentamicin (10 µg); MEM, Meropenem (10 µg); FEP, Cefepime (30 µg); ATM, Aztreonam (30 µg); CB, Carbenicillin (100 µg); IPM, Imipenem (10 µg); TIC, Ticarcillin (75 µg); PIP, Piperacillin (100 µg); AK, Amikacin (30 µg); CIP, Ciprofloxacin (5 µg); TOB, Tobramycin (10 µg); OFX, Ofloxacin (5 µg) and CT, Colistin (10 µg).