JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 981-988

Frequency, Susceptibility and Co-Existence of MBL, ESBL & AmpC Positive *Pseudomonas aeruginosa* in Tertiary Care Hospitals of Peshawar, KPK, Pakistan

Muhammad Ilyas[1], Muhammad Khurram[1], Shabeer Ahmad[2] and Irshad Ahmad[3]

[1]Department of Microbiology, and Biotechnology, Sarhad, University of Science and Information Technology, Peshawar, KPK, Pakistan.
[2]Department of Microbiology, Abasyn University, Peshawar, KPK, Pakistan.
[3]Department of Life Sciences King Fahd University of Petroleum and Minerals PO Box 2001, Dhahran 31261, Saudi Arabia.

(Received: 12 February 2015; accepted: 10 April 2015)

Pseudomonas aeruginosa **responsible for nosocomial infections prevail in hospitals, producing β-lactamase enzymes that hydrolyze β-lactam drugs and reduce their efficacy. This study was carried out to determine prevalence, susceptibility and production of different β-*lactamases* by *P. aeruginosa*. The isolates were screened and characterized using standard protocols. Among isolates 32%, 10%, 6% and 6% were obtained from pus swabs, sputum, urine and body fluids, respectively. Prevalence of MBL positive strains was 25.7%, AmpC was 17.1% and ESBL was 8.5%. The co-production of MBL + AmpC, MBL + ESBL and ESBL + AmpC was 10%, 5.7% and 2.8% respectively. MBL + AmpC positive strains were 100% resistant to ceftriaxone, amoxicillin + clavulanic acid, norfloxacin. ciprofloxacin, cefaperazone + sulbactam, pipracilline + tazobactum, cefotaxime and imipenem, While MBL + ESBL positive stains were 100% resistant to ceftriaxone, amoxicillin + clavulanic acid, norfloxacin, ciprofloxacin, ceftaxime, ceftazidime, imipenem, cefaperazone + sulbactam and pipracilline + tazobactum. Only least resistant (25%) observed for amikacin and cefepime. Co-production of AmpC + ESBL positive strains were 100% resistant to ceftriaxone, amoxcilline + clavulanic acid, ciprofloxacin, norfloxacin and cefotaxime, cepaferazone/sulbactum and pipracilline/ tazobactum. Production of MBL was higher than ESBL and AmpC β-lactamase. Co-production of these enzymes was responsible for multidrug resistance. Production of MBL + AmpC was higher than ESBL + AmpC and ESBL + MBL. Least resistance was noted against cefepime and amikacin by MBL, ESBL and AmpC co-producers.**

Key words: *Pseudomonas aeruginosa*, Metallo β-lactamase, Extended Spectrum β-lactamase, AmpC β-lactamase.

Pseudomonas aeruginosa belongs to the family *Pseuodomonadaceae* is a non-fermenting, aerobic, motile and Gram negative bacilli are widely distributed in nature and more prevalent in the hospital settings, responsible for nosocomial infection[1, 2]. The hospital environment is more suitable for its growth; therefore it becomes a challenging pathogen worldwide having imperfect treatment options due to the acquisition of resistance[1].

Inspite of advance therapeutic regimes the incidence of nosocomial infections is increasing day by day. The emergence of different β-lactamases e.g. metallo β-lactamase (MBL), extended spectrum β-lactamase (ESBL) and AmpC β–lactamase (AmpC) by *P. aeruginosa* hydrolyzes all β-lactam drugs reducing their efficacy by conferring resistance to these antimicrobial agents[3].

The first metallo beta-lactamase enzyme was reported in Japan in 1991 which is dependent

* To whom all correspondence should be addressed.
Tel.: +966-03-860-8393; Tel.: +92-91-5230931-3;
Fax: +92-91-5230930;
E-mail: milyas_1978@hotmail.com

on zinc cofactor, a divalent required for its enzymatic activity. In vitro activities of MBL enzyme were inhibited by the action of chelating agents such as ethylene diamine tetra acetic acid (EDTA)[4]. MBL enzyme has the ability to hydrolyze nearly all β-lactam drugs including pencilline, cephalosporin and carbapenems [5]. MBL producing *Pseudomonas* spp: have the ability to form biofilm especially in the chronic chest infection and catheterization for prolonged period of time. These organisms have the ability to resist phagocytosis and change the permeability of membrane to inhibit the penetration of drugs into the bacterium[6]. Multidrug-resistant bacteria have developed resistance to carbapenem, antibiotic of last resort to combat infection. The genes responsible for production of MBL and AmpC β-lactamase are often found on plasmid and transferred horizontally among other Gram negative organisms[7].

AmpC beta-lactamase, a cephalosporinase is poorly hydrolyzed by clavulanic acid conferring resistance to large number of antimicrobial agents including beta-lactam, a-methoxy-beta-lactam (cefoxitin), narrow, expended, broad spectrum cephalosporins, beta-lactamase inhibitor combinations and aztreonam[8]. A standard disc diffusion method was used for the screening of AmpC in which the break points for cefoxitin zone diameter <18 mm and a confirmatory test were performed in three dimensional extract tests[9].

Extended spectrum beta-lactamase (ESBL) is an extracellular enzyme produced by large number of bacteria causing breakage of amide bond in beta-lactam ring of pencilline. It is capable of inactivating oxyimino-cephalaosporin, pencilline and aztreonam, but cannot hydrolyze cephamycin and carbapenems [10]. This enzyme is commonly found in *E. coli* and *K. pneumonia*, but recently they have been detected in *P. aeruginosa*. Co-existence of the studied enzymes restricts the treatment option. MBL+AmpC have shown resistance to carbapenems is of major concern because it is the drug of last remedy used to treat such infections [11].

Different methods were used for in-vitro determination of these β-lactamases like AmpC by using modified three dimensional test, while MBL by imipenem-EDTA disc test and ESBL by combined disc diffusion method[11]. There is very little awareness among the diagnostic laboratory services particularly in this region about these enzymes, its detection and their co-existence. The phenotypic detection of these enzymes is very significant to know the exact therapeutic agent in order to control and eradicate the spread of resistant organisms among the patients. Keeping in view the above scenario the current study was designed to find out the frequency and co-existence of MBL, ESBL and AmpC β-lactamase positive *P. aeruginosa* in tertiary care hospitals of Peshawar.

MATERIALS AND METHODS

This study was conducted at City Medical Laboratory Peshawar from August, 2013 to August, 2014. A total of 550 clinical samples were collected aseptically from urine, sputum, blood, pus and different body fluids of the admitted patients in different wards at Post Graduate Medical Institute Lady Reading Hospital Peshawar KPK, Pakistan.

Isolation and identification of Pathogens

The pathogens were isolated on sterile bacteriological media including blood agar, MacConky agar and CLED agar. All the samples were inoculated on the plates and were incubated aerobically at 37 ^{0}C for 24 hours. Identification of the organism was done on the basis of Gram staining and routine biochemical test including triple sugar iron, oxidase, urease, citrate utilization test and pigment production.

Antimicrobial Susceptibility Testing

P. aeruginosa isolates were subjected to in vitro testing for the determination of their susceptibility to various antibiotics by using a disc diffusion method (Kirby Baur,s method) on Muller Hinton agar according to the clinical laboratory standard institute, 2011 [12]. Eleven antibiotics were used in this study included; cepaferazone/sulbactum (105µg), pipracilline/ tazobactum (110µg), amoxicillin/clavulanic acid (20+10µg), ceftazidime (30µg), cefotaxime (30µg), norfloxacin (10µg), ciprofloxacin (5µg) amikacin (30µg), imipenem (10µg), ceftriaxone (30µg) and cefepime (30µg) discs were obtained

from Oxoid UK. The zone of inhibition showed by bacterial pathogen around the discs is measured in mm and susceptibility interpretation was made according to the CLSI, 2011 [12].

Detection of metallo β-lactamase by imipenem-EDTA double-disc synergy test

P. aeruginosa strains having diameter ≤13 mm around imipenem disc were considered carbapenem resistant and further subjected for MBL detection. Muller Hinton agar plates were inoculated with tested organisms. Two imipenem (10µg) discs were placed at a distance of 25 mm a part from each other on the inoculated plates. To obtain 750 µg concentrations, about 4 µl of 0.5M EDTA solution was added to one of the discs. Incubate the plates at 37^0C for 24 hours. After incubation the zones of inhibition around imipenem and imipenem+EDTA discs were compared. The zone of inhibition was ≥7 mm around imipenem+EDTA disc was compared with imipenem without EDTA, considered MBL positive as shown in figure 1(a) [13].

Detection of AmpC β-lactamase by three dimensional methods

P. aeruginosa strains having diameter ≤ 5 mm around cefoxitin disc were considered AmpC positive were further subjected for inducible AmpC detection by modified three dimensional tests with some little modification[8]. The surface of the Muller Hinton agar plates were inoculated with *E. coli* sensitive to cefoxitin. Then 30 µg cefoxitin disc was placed at the centre of the inoculated plate. Take a sterile surgical blade and making a slit beginning from the edge of the disk cutting the agar in an outward radial direction. Take a single colony from overnight culture and inoculate the slits near the disc. The plates were incubated at 37 ^{0}C for 24 hours. A clear distortion in zone of inhibition appeared near the slits was considered positive for inducible AmpC production (figure 1b).

Detection of ESBL by combined disc diffusion method

P. aeruginosa isolates were phenotypically screened for ESBL detection. Bacterial suspension equal to 0.5 M McFarland solution was inoculated on the surface of the Muller Hinton agar plate. Two discs of ceftazidime (30µg) were placed at a distance of 15 mm apart from amoxcilline+clavulanic acid disc in centre on Muller Hinton agar. Incubate the plates at 37°C for 24 hours. A zone of inhibition around each ceftazidime disc was extended on the side nearest to amoxicillin+clavulanic acid. This synergistic effect was considered as ESBL positive as shown in figure 1(c) [14].

Statistical analysis

Data was analyzed statistically by using GraphPad Prism 5 (GraphPad Software Inc. San Diego CA, USA). The Chi-square test was used to analyze the qualitative data. A *p* value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

A total of 70 positive samples of *P. aeruginosa* isolated from admitted patients, were identified and tested for antimicrobial susceptibility to different antibiotics and presence of different beta-lactamases, i.e. MBL, ESBL and AmpC. In Table 1, Out of 550 clinical samples *P. aeruginosa* was more prevalent in the pus swabs (32%) followed by sputum (10%), urine (6%) and body fluids (6%).

Productions of different β-lactamases by *P. aeruginosa* in various clinical samples were studied in the current experiment. It is evident from the Table 2, that in 70 *P. aeruginosa* isolates MBL, AmpC and ESBL were noted in 18(25.7%), 11(15.7%) and 6(8.5%) respectively. It was found that 33.3% of *P. aeruginosa* were MBL producers in the pus samples followed by body fluids (16.6%) and sputum (10%). Among the tested isolates, 08(16.8%) were AmpC β-lactamase producer in the pus samples followed by 02(20%) in sputum and 01 (16.6%) in body fluids, whereas 05(10.4%) *P. aeruginosa* were ESBL producers observed in pus samples followed by 01(16.6%) in urine samples.

The co-existence of MBL and AmpC reported in seven (10%) isolates, whereas MBL and co-production of ESBL was shown by 4 (5.71%) of the isolates and the co-production of AmpC and ESBL was noted in 2 (2.85%) of the isolates shown in Table 3.

Antimicrobial susceptibility profile against different beta-lactamase producer's strains of *P. aeruginosa* showed in Table 4. All MBL positive isolates were 100% resistant to β-lactams tested including, ceftriaxone, ceftaxime, imipenem, while ceftazidime showed (83.4%)

resistance against MBL producers. High resistance was noted against combination of β-lactams and β-lactamase inhibitors tested were amoxicillin + clavulanic acid (100%), tazobactum+pipracilline (88.9%), salbactum +cefaperazone (88.8%). Among quinolones, ciprofloxacin and norfloxacin showed (100%) resistance against MBL producers. The antimicrobial agents which showed maximum sensitivity were cefepime (100%), followed by amikacin (55.5%).

Antibiotic susceptibility pattern of AmpC positive isolates showed 100% resistance to ciprofloxacin, norfloxacin, ceftaxime, ceftriaxone, amoxicillin+clavulanic acid, cefaperazone+sulbactum, pipracilline + tazobactum and 81.8% to ceftazidime. It was noted that AmpC producers were multidrug resistant and the most effective agents which showed maximum sensitivity were cefepime (91%), amikacin (81.8%) and imipenem (36.4%).

Antibiotic susceptibility pattern of AmpC positive isolates showed 100% resistance to ciprofloxacin, norfloxacin, ceftaxime, ceftriaxone, amoxicillin+clavulanic acid, cefaperazone+sulbactum, pipracilline + tazobactum and 81.8% to ceftazidime. It was noted that AmpC producers were multidrug resistant and the most effective agents which showed maximum sensitivity were cefepime (91%), amikacin (81.8%) and imipenem (36.4%).

A multidrug resistant pattern was observed against *P. aeruginosa* having co-production of different beta-lactamases in Table 5. A co-production of MBL+AmpC positive *P. aeruginosa* has shown 100% resistance against ceftriaxone, cefotaxime, amoxcilline+clavulanic acid, norfloxacin. ciprofloxacin, cefaperazone +sulbactum (SCF), pipracilline+tazobactum (TZP) and imipenem. Low resistant observed against amikacin 42.8% and cefepime 28.5%. A co-production of MBL+ESBL positive *P. aeruginosa* showed 100% resistance against ceftriaxone, amoxcilline+clavulanic acid, norfloxacin, ciprofloxacin, ceftaxime, ceftazidime, imipenem cefaperazone+sulbactum (SCF) and pipracilline+tazobactum (TZP). Least 25% resistance was noted for cefepime and amikacin against MBL+ESBL producers. A co-production of AmpC+ESBL positive *P. aeruginosa* were 100% resistant to ceftriaxone, amoxicillin+clavulanic acid, ciprofloxacin, norfloxacin, ceftaxime, cepaferazone+sulbactum and pipracilline+tazobactum.

P. aeruginosa is widely distributed in

Table 1. Percent prevalence of *P. aeruginosa* isolates in various clinical samples.

Specimens (n)	*P. aeruginosa* n (%)
Wound swab (150)	48 (32)
Urine sample (100)	6 (6)
Blood sample (100)	-
Sputum sample (100)	10 (10)
Body fluid (100)	6 (6)
Total	70 (12.7)

Table 2. Percent prevalence of MBL, AmpC and ESBL positive *P. aeruginosa* in different clinical samples.

Specimen	*P. aeruginosa*	MBL n (%)	AmpC n (%)	ESBL n (%)
wound swab	48	16 (33.3)	8 (16.7)	5 (10.4)
Sputum	10	1 (10)	2 (20)	-
Urine	6	-	-	1 (16.6)
Blood	-	-	-	-
Fluids	6	1 (16.6)	1 (16.6)	-
Total	70	18/70 (25)	11/70 (15.7)	6/70 (8.5)

Table 3. Co-existence of MBL, AmpC β-lactamase and ESBL positive *P. aeruginosa*

Total isolates	MBL+AmpC n (%)	MBL+ESBL n (%)	AmpC+ESBL n (%)
70	7 (10)	4 (5.71)	2 (2.85)

nature and more prevalent in the hospital settings responsible for nosocomial infection. It carries the biggest genome and has potential to demonstrate high variability; like acquisition of resistance to multiple classes of antibiotics, emergence of different β-lactamases which hydrolyze all β-lactam drugs, thereby reducing their efficacy and conferring resistance against these antimicrobial agents. [2,3]

In the present study the overall prevalence of *P. aeruginosa* was 12.7% isolated from different clinical samples which is an agreement with the report of Mahmoud *et al*. (2013)[11]. Previously Ullah *et al*. (2009)[16] reported 27% prevalence of *P. aeruginosa* in wound swabs, however in current study it increases to 33.3%.

Emergence of multiple beta-lactamases in *P. aeruginosa* particularly of hospital environment can cause major therapeutic failures and pose a significant clinical challenge if remain undetected. Therefore, early detection of these bacterial enzymes is necessary as appropriate treatment might reduce the spread of resistant strains. In the study of Kumar *et al*. (2014)[17], production of AmpC β-lactamase positive *P. aeruginosa* was 32.7%, the co-production of AmpC+ESBL was 24.5%, while AmpC+MBL was 48.5%. In the present study AmpC β-lactamase positive *P. aeruginosa* was 15.7% and co-production of AmpC+MBL was 10%. This slightly differences can be attributed to the

Table 4. Antibiotics susceptibility pattern of MBL, ESBL and AmpC β-lactamase producing *P. aeruginosa.*

Antibacterial agent	MBL (Number/Percent)		ESBL (Number/Percent)		AmpC (Number/Percent)		
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	*p* value
Ceftriaxon	00(00)	18(100)	00(00)	06(100)	00(00)	11(100)	-
Augmentin	00(00)	18(100)	00(00)	05(83.3)	00(00)	11(100)	-
Tazobactum+Pipracilline	00(00)	18(100)	01(16.6)	00(00)	00(00)	11(100)	< 0.0001***
Salbactum+Cefaperazone	02(11.1)	16(88.8)	06(100)	00(00)	00(00)	11(100)	< 0.0001***
Amikacin	02(11.1)	16(88.8)	06(100)	01(16.6)	09(81.8)	02(18.1)	< 0.0001***
Norfloxacin	10(55.5)	08(44.4)	05(83.3)	06(100)	00(00)	11(100)	0.0091**
Ciprofloxacin	00(00)	18(100)	00(00)	06(100)	00(00)	11(100)	-
Ceftaxime	00(00)	18(100)	00(00)	06(100)	00(00)	11(100)	-
Ceftazidime	00(00)	18(100)	00(00)	06(100)	02(18.1)	09(81.8)	0.0989
Imipenem	03(16.6)	15(83.3)	00(00)	00(00)	04(36.3)	07(63.6)	-
Cefepime	00(00)	18(100)	06(100)	00(00)	10(90)	01(9.0)	< 0.0001***

Chi-square test was applied and $p < 0.05$ was considered as significant at 95% confidence interval***p and **p = value is significant

Table 5. Resistance pattern of various antibiotics tested against *P. aeruginosa* having co-production of MBL, ESBL and AmpC beta-lactamase (MBL+AmpC=7, MBL+ESBL=4, AmpC+ESBL=2).

Antimicrobial agents	MBL+AmpCn (%)	MBL+ESBL n (%)	AmpC+ESBL n (%)
Ceftriaxone	7 (100)	4 (100)	2 (100)
Amoxicillin+clavulanic acid	7 (100)	4 (100)	2 (100)
Tazobactum+pipracilline	7 (100)	4 (100)	2 (100)
Salbactam+Cefaperazone	7 (100)	4 (100)	2 (100)
Amikacin	3 (42.8)	1 (25)	-
Norfloxacin	7 (100)	4 (100)	2 (100)
Ciprofloxacin	7 (100)	4 (100)	2 (100)
Ceftaxime	7 (100)	4 (100)	2 (100)
Ceftazidime	6 (85.7)	4 (100)	1 (50)
Imipenem	7 (100)	4 (100)	-
Cefepime	2 (28.5)	1 (25)	-

variation in co-production rate of these enzymes due to differences in geographical distribution, species variation among the genera and nature of the specimen.

The production of metallo β-lactamase by *P. aeruginosa* was 25% [14], which is in conformity to our data, where the production of MBL was 25.7%. In the study of Peshattiwar and Peerapur, (2011)[18] the production of ESBL positive *P. aeruginosa* was 22.2%, whereas MBL positive organism was 7.8%, which are in contrast to present findings where production of ESBL and MBL were 8.5% and 25.7% respectively. This difference can be due to injudicious use of antimicrobial agents, species variation with respect to geographical distribution.

High prevalence (65.2%) of *P. aeruginosa* was observed in wound samples in Ghana[19]. They also reported production of AmpC (26.2%), with the co-production of ESBL+AmpC β-lactamase was 7.0%. The findings are more than present data. The comparative difference can be due to greater samples size and geographical variation. Furthermore socioeconomic status of Ghana is for more lower then this region that may be contributing factors. The co-production of ESBL+AmpC by *P. aeruginosa* in the study of Goel *et al*. (2013)[3] was 11.39%, which is higher than the current report, where the co-production of these enzymes was 2.85%. Production of multiple β-lactamases by *P. aeruginosa* has significant therapeutic consequences especially in the hospital settings. Upadhyay *et al*. (2010),[20] reported co-existence of AmpC+ESBL (3.3%) and AmpC+MBL (46.6%) in *P. aeruginosa*. In the present study co-existence of AmpC+ESBL was 2.8% and AmpC+MBL were 10%. It was observed that co-production of these β-lactamases in *P. aeruginosa* resulted in multidrug resistance.

Antimicrobial susceptibility studies reveal that resistance in *P. aeruginosa* is increasing to the commonly used antibiotics. Multiple factors responsible for acquisition of resistance in *P. aeruginosa* include permeability barriers of the cell surface, multidrug efflux pumps and production of different β-lactamases such as AmpC β-lactamase, ESBL and MBL.[21]

The antimicrobial agents such as cefaperazone+sul bactum and aminoglycoside tested against MBL positive *P. aeruginosa* showed 62% resistance[4], while in the present study we observed 100% resistance to above mentioned agents. This high resistance pattern can be due to the acquisition of resistance by bacteria, poor hygienic environment in the hospitals, carelessness in post operative wounds especially in the surgical wards and indiscriminate use of antibiotics particularly in Peshawar, Pakistan.

The most frequently prescribed agents, amoxicillin+clavulanic acid is finding high resistance (94.4%) from *P. aeruginosa* reported [22], however in this report it rose to 100% for this drug. This high resistance can be due to its overuse in the community, where it can be easily obtained without prescription. Production of MBL in *P. aeruginosa* causing inactivation of carbapenem, which is the only therapeutic option against such infection, is an alarming situation. In the current study MBL producing isolates were multidrug resistant. The present study revealed high resistant pattern of antimicrobial agents against *P. aeruginosa* producing β-lactamases. Poor hygienic condition in the hospitals, indiscriminate uses of antibiotics and quacks practices in this

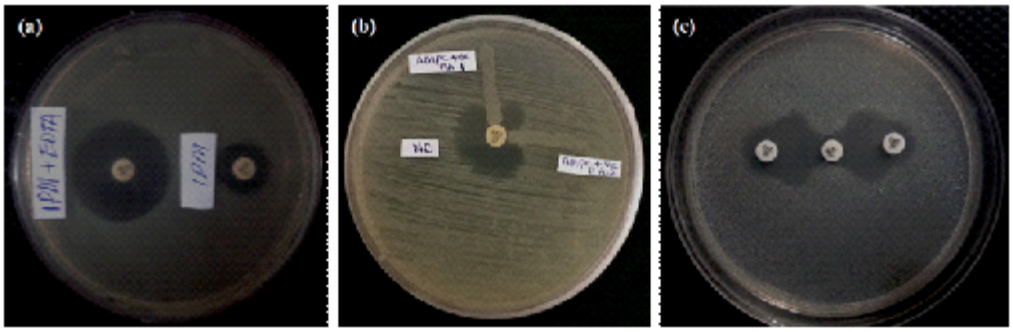

Fig. 1. Comparison of inhibition zones produced by discs with imipenem and imipenem+ EDTA by MBL producer (a), Organism showing a clear distortion zones, where P.A1 and P.A2 are AmpC producers (b) and Combined disk diffusion method for ESBL producer (c)

region is the major factors responsible for acquisition of resistance against antimicrobial agent.

CONCLUSION

We have found high prevalence of *P. aeruginosa* in pus samples. The production of MBL was higher than ESBL and AmpC β-lactamase. The co-production of these enzymes may responsible for multidrug resistance. We have observed higher co-existence of MBL+AmpC than ESBL+AmpC and ESBL+MBL co-producers. Imipenem, amikacin and beta-lactamase inhibitors combination were the drug of choice against ESBL producers, while least resistance was noted against cefepime and amikacin by MBL, ESBL and AmpC co-producers.

ACKNOWLEDGEMENT

The authors highly acknowledge the provision of lab facilities by City Medical Laboratory, Peshawar, Pakistan for the conduction of this study.

REFERENCES

1. Fatima A, Naqvi SB, Khaliq SA, Parveen S and Jabeen S. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infections. *Springerplus*. 2012; **1**: 70.
2. Tripathi P, Banerjee G, Saxena S, Gupta M K, and Ramteke PW. Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection. *African Journal of Microbiology Research*. 2011; **5**(19): 2955-2959.
3. Goel A, Hogade S A, Karadesai S G. Prevalence of extended-spectrum beta-lactamases, AmpC beta-lactamase and metallo-beta-lactamase *producing Pseudomonas aeruginosa* and *Acinetobacter baumannii* in an intensive care unit in a tertiary care hospital. *Journal of the Scientefic Society.* 2013; **40**: 1.
4. Kaleem F, Usman J, Hassan A and Khan A. Frequency and susceptibility pattern of metallo-beta- lactamase producer in a hospital in Pakistan. *The Journal of infection in developing countries.* 2010; **4**(12): 810-813.
5. Rit K, Chakraborty B, Dey R, Chakaraborty P, Naha A and Saha R. Prevalence of *Pseudomonas aeruginosa* and *Acinetobacter spp* producing metallo beta-lactamase in a tertiary care hospital. *Journal of Dr. NTR University Health Sciences*. 2013; **2**(1): 18-21.
6. Perez L R R, Antunes A L S, Freitas A L P and Barth A L.When the resistance gets clingy: *Pseudomonas aeruginosa* harboring metallo-Beta-lactamases gene shows high ability to produce biofilm. *European Journal of Clinical Microbiology and Infectious diseases*. 2011; **31**(5): 711-714.
7. Kumar V, Sen M R, Nigam C, Gahlot R and Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing *Pseudomonas aeruginosa* in burn patients. *Indian Journal of Critical care Medicine*. 2012; **16**(3): 136-140.
8. Coudron PE, Moland ES and Thomson KS. Occurrence and Detection of AmpC Beta-lactamases among *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis* Isolates at a Veterans Medical Center. *Journal of Clinical Microbiology*. 2000; 1791-1796.
9. Manchandra V and Narendra PS. Occurrence and detection of AmpC ²-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. *Journal of Antimicrobial Chemotherapy*. 2003; **51**: 415–418.
10. Kumar VR, Mishra RK, Chandra W, and Gupta P. Incidence of Beta-Lactamases producing gram negative clinical isolates and their antibiotics susceptibility pattern in Allahabad. *International Journal of Research in pure and Applied Microbiology.* 2011; **1**(3): 36-39.
11. Mahmud RAB, Nehal HAA and Gomal FMG. Prevalence of Extended-Spectrum Beta-Lactamase, AmpC Beta-lactamase, and Metallo-Beta-Lactamase among Clinical Isolates of *Pseudomonas aeruginosa*. *Journal of Advanced Biotechnology and Bioengineering.* 2013; **1**: 22-29.
12. Performance standards for antimicrobial susceptibility testing. Twenty first informational supplement. *Clinical Lab Stand Inst*, 2011; **31**, 1.
13. Khakhkhar VM, Thangjam RC, Bhuva PJ and Ballal M. Detection of Metallo-Beta-Lactamase Enzymes producing *Pseudomonas aeruginosa* isolated from various clinical isolates. ***National Journal of Integrated Research in Medicine.*** 2012; **3(4): 4-9.**
14. Salimi F and Etekhar F. Co-existence of AmpC and Extended-Spectrum ²-lactamases in Metallo ²-Lactamase Producing *Pseudomonas*

aeruginosa Burn Isolates. *Jundishpur Journal of Microbiology.* 2013; **6**(8): 71-78.

15. Ullah F, Malik SA, Ahmad J. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan, *Journal of Burns*, 2009; 10.1016. **01**.005.
16. Kumar V, Sen MR, Nigam C, Gahlot R and Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing *Pseudomonas aeruginosa* in burn patients: A prospective study. *Indian Journal of Critical Care Medicine*. 2014; **16**(2): 136-40.
17. Peshattiwar PD and Peerapur BV. ESBL and MBL Mediated Resistance in *Pseudomonas aeruginosa* An Emerging Threat to Clinical Therapeutics. *Journal of Clinical Diagnostic Research*. 2011; **5**(8): 1552-1554.
18. Feglo P and Opoku S. AmpC beta-lactamase production among *Pseudomonas aeruginosa* and *Proteus mirabilis* isolates at the komfo Anokye Teaching Hospital, Kumasi, Ghana. *Journal of Microbiology and Antimicrobials*. 2014; **6**(1): 13-20.
19. Upadhyay S, Sen MR, Bhattachaejee A and Malay RS. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme. *The Journal of Infection in Developing Countries.* 2010; **4**(4): 239-242.
20. Lister PD, Walter DJ, Hanson ND. Antimicrobial resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanism. *Clinical Microbiology*. 2009; **22**(4): 582-610.
21. Asghar AH. Antimicrobial susceptibility and metallo-²-lactamase production among *Pseudomonas aeruginosa* isolated from Makah Hospital. *Pakistan Journal of Med Sciences*. 2012; **28**(5): 781-786.