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Research Article

Frequency and antimicrobial sensitivity patterns of AMP-C and metallo-beta-lactamase producing Pseudomonas Aeruginosa

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ABSTRACT

Objective: To determine the frequency of AmpC beta-lactamase and metallo-beta-lactamase producing Pseudomonas aeruginosa in clinical isolates and to study the antibiotic susceptibility patterns of these Pseudomonas aeruginosa strains.

Methodology: It was a descriptive cross-sectional study conducted at Department of Pathology (Microbiology) Pakistan Railways Teaching Hospital (PRH) Islamic International Medical College, Rawalpindi during October 2023- September 2024. 112 confirmed Pseudomonas aeruginosa strains were collected from clinical samples. Detection of AmpC and MBL production was performed using phenotypic methods, including the disc antagonism test, Amp-C disk test, EDTA disc synergy test, and the Modified Hodge test. SPSS version-26 was used for descriptive and inferential statistics.

Results: Our data comprised on 112 P. aeruginosa strains, mostly specimens were collected from urine c/s (30.4%) and pus c/s (27.7%) samples. 27(24.1%) isolates of P. aeruginosa were inducible chromosomal mediated Amp C beta-lactamase producers and 12(10.7%) were non-inducible plasmid mediated Amp C beta-lactamase producers. Amongst MBL producing strains, 37(33.0%) were positive by Imipenem-EDTA combined disc test and 21(18.8%) were by Modified Hodge test. 35(31.2%) showed β-lactamase production and 48(42.8%) showed MBL production. However, 14(12.5%) strains were co-producers of both beta lactamase and MBL production.

Conclusion: The study demonstrated significant increase in antibiotic resistance due to expression of diverse beta-lactamases owing to increased MDR P. aeruginosa strains. The sensitivity patterns showed that amikacin, imipenem and piperacillin tazobactam can be used for treatment of Amp-C and MBL producing strains. In addition to this cefoperazone-sulbactam can also

be used for Amp-C producing isolates.

Keywords: AmpC beta-lactamase, Antibiotic susceptibility pattern, Metallo-beta-lactamase (MBL), Pseudomonas aeruginosa.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium that is a common opportunistic pathogen found in many environments, including soil, water, and human- and animal-impacted environments. It's also found in common foods, especially vegetables, and in medical facilities.1 It predominantly affect immunocompromised patients and is a multidrug resistant (MDR) pathogen, innately resistant to an extensive variety of antimicrobials.2,3 P. aeruginosa is a leading cause of healthcare-associated infections, accounting for 7.1% to 7.3% of such cases globally, with its prevalence increasing in intensive care units, where it represents up to 16.2% of infections.4

P. aeruginosa is notable for its ability to create metallo-β-lactamases (MBLs) and AmpC β-lactamases, among other types of β-lactamases.5 The prevalence of inducible and non-inducible Amp C beta-lactamase and metallo-beta-lactamases (MBLs) producing P. aeruginosa species was determined to be 47.5%, 4%, and 19%, respectively.6 The presence of AmpC and MBL-producing strains further complicates treatment strategies and necessitates alternative approaches to combat the infections effectively.7 Antimicrobial resistance (AMR) is a serious global health concern, which leads to higher medical costs, prolonged hospital stays, and increased mortality, making it a critical issue in modern medicine.8 AMR development is driven by a number of complicated mechanisms, including genetic mutations, horizontal gene transfer, selective pressure from antibiotic use, and inadequate infection control measures, all contributing to its spread.9 Amongst the inducible and non-inducible Amp C beta-lactamase and metallo-beta-lactamases (MBLs) producing P. aeruginosa species, the highest resistance were observed against cephalosporins (44-56.5%)10, the lowest levels of resistance was against colistin (2%), imipenem (19.5%), and amikacin (21.5%). Resistance rates to piperacillin-tazobactam, ciprofloxacin, gentamicin, and cefoperazone-sulbactam were reported at 24%, 31.5%, 32%, and 36.5%, respectively.6 However, the relatively low sensitivity highlights the limited treatment options available for these strains, emphasizing the urgent need for continued surveillance and development of novel therapeutic strategies to combat multidrug-resistant Pseudomonas infections in clinical practice.3 The rapid identification of metallo-β-lactamase (MBL) producing pathogens is very important to control their extensive distribution. Antimicrobial susceptibility testing (AST) determines the effectiveness of antibiotics against specific pathogens. Common phenotypic methods are; disc antagonism test, AmpC disc test, EDTA disc synergy test, and the Modified Hodge test.11

Despite the studies investigating the incidence or prevalence of AmpC and MBL-producing Pseudomonas aeruginosa strains using phenotypic methods, there remains a room of study regarding the antibiotic sensitivity patterns particularly in these strains. The few regional studies were focused on prevalence of these strains. This study aims to determine the frequency of Amp-C beta-lactamase and metallo-beta-lactamase producing Pseudomonas aeruginosa with their antibiotic susceptibility patterns. The evaluation of frequency in this study will contribute to the comparison and better understanding of existing trend at local tertiary care facility. In this study the resistant strains is identified with their respective antimicrobial sensitivity patterns which is crucial for guiding empirical therapy decisions and effective

infection control measures.

Methodology

This descriptive cross-sectional study conducted at Department of Pathology (Microbiology) Pakistan Railways Teaching Hospital (PRH) Islamic International Medical College, Rawalpindi over a period of one year (October 2023- September 2024). A sample size of 112 Pseudomonas aeruginosa isolates was calculated by considering the 8% prevalence of Amp-C and metallobetalactamase producing P. aeruginosa12 by using WHO sample size calculator.

Confirmed non-duplicate Pseudomonas aeruginosa isolates from patients without recent antibiotic intake obtained from various clinical specimens (including pus, HVS swabs, various body fluids like CSF, pleural fluid, urine and sputum) for culture and sensitivity submitted to the microbiology laboratory of PRH, within the study period were included through non-probability convenient sampling technique. Isolates with missing or incomplete patient data, samples identified as contaminated during laboratory processing, isolates from patients hospitalized within the preceding 30 days and isolates from immunocompromised patients or those with specific comorbidities (e.g., HIV/AIDS, organ transplantation) were excluded. Data comprised on demographics details, clinical details including detection of AmpC Beta-lactamase and Metallo-beta-lactamase (MBL) and antibiotic Susceptibility Patterns. The clinical samples from all the hospital departments were collected with proper aseptic technique and precautions then transported immediately to the microbiology laboratory for processing. Each specimen was processed using standard microbiological techniques, including inoculation onto Blood agar and MacConkey agar followed by Antibiotic susceptibility pattern of all P. aeroginosa strains is mentioned in table-3 & incubation at 37°C for 18-24 hours. Identification of Pseudomonas aeruginosa involved examining colonial morphology, gram staining, positive motility test, and standard biochemical tests such as positive catalase, positive oxidase, and positive citrate utilization test. Detection of AmpC and MBL production was performed using phenotypic methods, including the disc antagonism test, AmpC disc test, EDTA disc synergy test, and the Modified Hodge test. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following CLSI guidelines.

The data was analyzed using SPSS version-26. Descriptive statistics, including frequencies and percentages, were calculated to determine the frequency of AmpC and metallo-beta-lactamase producing P. aeruginosa isolates. Chi-square test was employed to assess the association between AmpC and metallo-beta-lactamase producing P. aeruginosa strains and their antibiotic sensitivity patterns. Statistical significance was determined at $p<0.05$ level.

Results

A total of 112 specimens were collected from the patients of mean age 45.17±21.1 years, 64(57.1%) females and 48(42.9%) males. Mostly specimens were collected from urine c/s (30.4%) and pus c/s (27.7%) samples. Descriptive statistics is mentioned in table-1. 27(24.1%) isolates of P. aeruginosa were inducible chromosomal mediated Amp C beta-lactamase producers and 12(10.7%) were non-inducible plasmid mediated Amp C beta-lactamase producers. Amongst MBL producing P. aeroginosa strains, 37(33.0%) were positive by Imipenem-EDTA combined disc test and 21(18.8%) were by Modified Hodge test as mentioned in table-2. figure-1.

figure-2, which showed highest resistance with Ceftazidime 79(70.5%) followed by Aztreonam

53(47.3%), Gentamycin 46(41.1%) and Ciprofloxacin 46(41.1%). However Imipenem 84(75%), Cefoperazone-sulbactam 80(71.4%), Amikacin 78(69.6%) and Piperacillin-tazobactam 76(67.9%) showed highest sensitivity against P. aeroginosa strains.

Resistance pattern of β-lactamase producing P. aeruginosa strains showed statistical significance with gentamycin against both inducible (resistance: 51.9%; p=0.012) and non-inducible Amp-C (resistance: 16.7%; p=0.070) test, while Ceftazidime showed significance with non-inducible Amp-C (resistance: 50%; p=0.059) test (table-4).

Correspondingly, the resistance pattern of MBL producing P. aeroginosa strains showed higher resistance of 70.3% and 76.2% with Ceftazidime by Imipenem-EDTA combined disc test and modified Hodge test respectively (p>0.05). Although higher sensitivity was observed with Piperacillin-tazobactam (67.6% and 76.2%), Imipenem (73% and 76.2%) and Cefoperazone-sulbactam (62.2% and 71.4%) with both Imipenem-EDTA combined disc test and Modified Hodge test (p>0.05) (table-5).

Out of 112 P. aeroginosa strains, 35(31.2%) strains showed β-lactamase production. Out of 35 beta-lactamase producing strains, only 04(11.4%) strains were positive with both inducible and non-inducible Amp-C test. However, 48(42.8%) MBL producing P. aeroginosa strains were observed. Out of these, 10(17.2%) showed MBL producing activity with both tests (i.e. Imipenem-EDTA combined disc test and Modified Hodge Test). Amongst the 35 Beta lactamase producing and 48 MBL producing P. aeroginosa strains, 14(12.5%) strains were both Beta lactamase and MBL producers, as mentioned in table-6. Overall the prevalence of MBL producers (42.8%) was of serious concern as compared to Amp C beta-lactamase (31.2%) producing isolates.

Discussion

This study was aimed to determine the frequency of inducible and non-inducible AmpC beta-lactamase and metallo-beta-lactamase producing P. aeruginosa in clinical isolates and to assess their antibiotic susceptibility pattern by evaluating 112 specimens collected from all the hospital departments. 27(24.1%) isolates of P. aeruginosa were inducible chromosomal mediated Amp C beta-lactamase producers and 12(10.7%) were non-inducible plasmid mediated Amp C beta-lactamase producers. Amongst MBL producing P. aeroginosa strains, 37(33.0%) were positive by Imipenem-EDTA combined disc test and 21(18.8%) were by Modified Hodge test. Amongst 112 P. aeroginosa strains, 35(31.2%) showed β-lactamase production and 48(42.8%) showed MBL production. Our study reported 57.1% females and 42.9% males with the mean age of 45.17 years. Similar to our data, Muddassir et al., also reported female predominance in their study (145 females and 110 males) among 40–49 years of age group.13

In our study, antibiotic susceptibility pattern of P. aeroginosa strains showed highest resistance with Ceftazidime (70.5%) followed by Aztreonam (47.3%), Gentamycin (41.1%) and Ciprofloxacin (41.1%). In line with our data, Mp et al., reported highest resistance with Ceftazidime (56.5%).6 Similarly, Aneela et al., reported piperacillin/tazobactam as the most sensitive antibiotic (95.5%) against P. aeroginosa,12 which is also consistent with national antibiotic resistance data of Pakistan.14 On contrary, According to Muddassir et al., the percentage of resistance against imipenem was found to be 53%. He claims that the resistance against imipenem in clinical isolates is due to the MBL and ESBL enzymes.13

The current study reported 31.2% Amp-C beta-lactamases producers, showed statistical significance with gentamicin against both

inducible (resistance: 51.9%; p=0.012) and non-inducible Amp-C (resistance: 16.7%; p=0.070) test, while Ceftazidime showed higher resistance with both inducible (resistance: 77.8%) and non-inducible Amp-C (resistance: 50%; p=0.059) test. However Mp et al., revealed 47.5% of inducible and 4% of non-inducible plasmid mediated Amp C beta-lactamases.6 While the findings of Chanu et al, corroborates with our results by reporting Amp-C producers to be 20.4%.15 High cephalosporins resistance in our study (77.8% and 50% with inducible and non-inducible Amp-C) and in the study of Mp et al., (44% - 56%) possibly indicates pressure of cephalosporins as an inducing antibiotic in the hospital environment.6 Further studies are needed to understand the relation between use of inducing antibiotics and the occurrence of high inducible resistance among P aeruginosa.

In the present study, amongst 48(42.8%) MBL producing P. aeroginosa strains, 37(33.0%) were positive by Imipenem-EDTA combined disc test and 21(18.8%) were by Modified Hodge test. They showed higher resistance with Ceftazidime by Imipenem-EDTA combined disc test and Modified Hodge test respectively (70.3% and 76.2%) and higher sensitivity with Piperacillin-tazobactam, Imipenem and Cefoperazone-sulbactam with both tests. Similarly, a study in Pakistan has described the incidence of ESBL and MBL in clinical isolates of P. aeruginosa as 23.94% and 40.84% respectively.16 However, in another study, MBL-producers indicated 100% resistance towards applied antibiotics except piperacillin and piperacillin /tazobactam combination where 100% sensitivity was observed.12 Consecutively, Muddassir et al., reported high prevalence of 61.5% and 81.5% by combination disc test and Modified Hodge test respectively.13 In contrast, Chanu et al, reported 15.3% MBL producing isolates15, and Saleem et al., reported 18.1% MBL strains.17 The difference might be due to the difference in the study population, sampling technique, sample size, geographical difference, antibiotic usage etc.

In our study population, the prevalence of MBL producers (42.8%) was of serious concern as compared to Amp C beta-lactamase (31.2%) producing isolates. However, the prevalence of coproducing strains is 12.5%. In a study the prevalence of the P. aeruginosa isolates is 56.9%, 32.3%, and 61.5% with ESBL, MBL, Amp-C producing strains, respectively.18 Consecutively, Chanu et al., reported that 9.7% isolates were AmpC producers, 15.3% were MBL and AmpC - MBL co-production was seen in 3.9% isolates.15 Correspondingly, Bharty et al., reported 10.81% AmpC producers and 75.67% MBL. The coproduction of both MBL and AmpC enzymes was 3%.19 Shrestha et al., reported sensitivity to Imipenem, amikacin and piperacillin tazobactam among beta-lactamase, while, MBL producers show higher resistance to nearly all the used drugs and sensitive to amikacin only, so it is a matter of great concern.20 This difference in prevalence rates might be attributed to geographical variations, climatic features, antibiotics misuse etc. Therefore, there is a need to institute correct antibiotics for patients infected with MBL producers. The laboratories need to prepare for detection of antibiotic resistance mechanisms and timely reporting to the clinicians. The clinical team to be cautious in opting antibiotics depending on the resistance mechanism revealed.6

Empirical drug therapy and over the counter usage of antimicrobial agents is common practice in Pakistan.13 P. aeruginosa which produces AmpC, and MBL is becoming more common; this might be a warning indication that an increasing number of organisms are developing resistance mechanisms, making antimicrobial drug's fewer effective prescriptions inefficient.13,19 Though phenotypic tests for AmpC and MBL detection

can be conveniently performed in most laboratories, the results of these tests must be confirmed by molecular techniques. It is critical to identify coproducing strains of Amp C, and MBL as soon as possible because the tip of the iceberg is only being detected compared to their actual prevalence.19

Study limitations:

The potential limitation of this study is that molecular, epidemiologic, and characterization of beta-lactamases (Amp-C and MBL) was not carried out. The early detection of beta-lactamases producing isolates in a routine lab could help avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility testing. Further strict antibiotic policies and measures could be implemented to limit the indiscriminate use of antibiotics and to minimize the emergence of multiple beta-lactamases (Shrestha et al., 2022).

Conclusion

The study demonstrated significant increase in antibiotic resistance due to expression of diverse b-lactamases owing to increased MDR P. aeruginosa strains. According to the sensitivity patterns, amikacin, imipenem and piperacillin-tazobactam can be used for treatment of Amp-C and MBL producing strains. In addition to this cefoperazone-sulbactam can also be used for Amp-C producing isolates. Further research is required to explore the specific factors responsible for increased Amp- C and metallobetalactamases in P. aeruginosa strains.

Recommendations:

• Implement advanced molecular techniques like PCR or sequencing for more accurate detection of AmpC and Metallo-β-lactamase (MBL) genes.

• Advocate for more stringent antimicrobial stewardship programs in healthcare settings to control the overuse of antibiotics, especially broad-spectrum agents. Reducing the misuse of antibiotics is crucial to curbing the spread of resistant P. aeruginosa strains.

• Advocate for national or regional surveillance programs to monitor antimicrobial resistance trends in P. aeruginosa. Which can help in early detection and rapid response.

• Reinforcement of hospital infection control practices is required, particularly in high-risk areas like ICUs. Effective infection control strategies can help prevent the spread of resistant P. aeruginosa within healthcare facilities.

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Table-1: Descriptive statistics of study sample (n=112)

Study variables		Frequency	Percentage %
Gender	Female	64	57.1
	Male	48	42.9
Type of Specimen	Blood C/S	9	8.0
	Catheter tip C/S	3	2.7
	ETT for C/S	2	1.8
	HVS C/S	5	4.5
	IUD tip C/S	2	1.8
	Pseudocyst of pancreas C/S	2	1.8
	Pus C/S	31	27.7
	Sputum C/S	11	9.8
	Throat swab C/S	3	2.7
	Tissue C/S	5	4.5
	Urine C/S	34	30.4
	Wound C/S	5	4.5

Table-2: Detection of Amp-C B-lactamase and Metallo- beta-lactamase producing P. aeroginosa strains (n=112)

Tests for detection			NS	NS%
ß-lactamase	*Inducible AMP-C*	*Negative*	*85*	*75.9*
		Positive	*27*	*24.1*
	Non-inducible AMP-C	*Negative*	*100*	*89.3*
		Positive	*12*	*10.7*
Metallo- beta lactamase	*Imipenem-EDTA combined disc test*	*Negative*	*75*	*67.0*
		Positive	*37*	*33.0*
	Modified Hodge Test	*Negative*	*91*	*81.3*
		Positive	*21*	*18.8*

*NS=no. of strains, NS%: percentage of strains

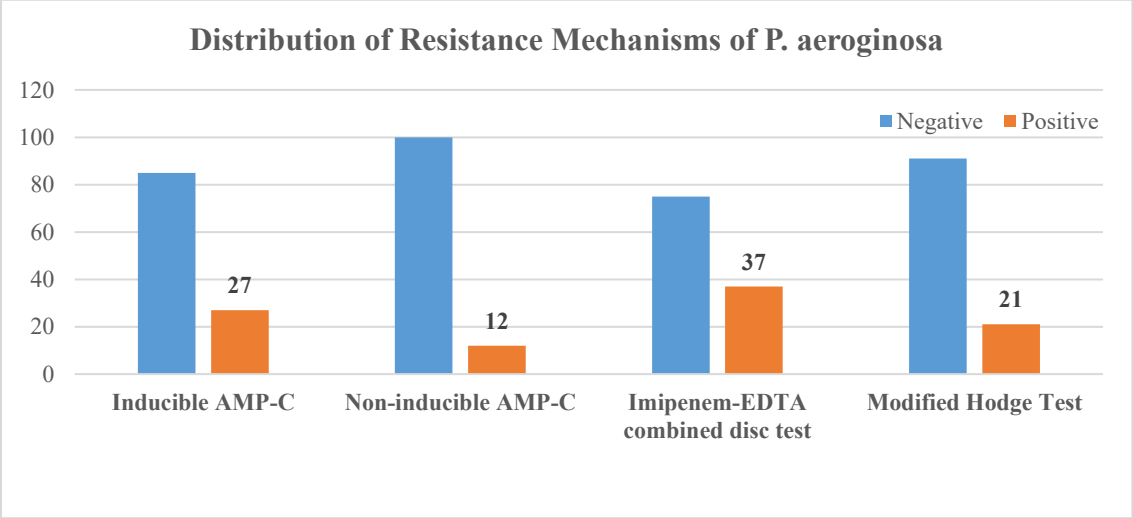

Figure 1: Distribution of resistance mechanisms for AMP-C β-lactamase and MBL producing P. aeruginosa strains

Table -3: Antibiotic susceptibility pattern of P. aeroginosa strains (n=112)

Antibiotics	Sensitive		Resistant	
	NS	S%	NR	R%
Piperacillin-tazobactam	76	67.9	36	32.1

Amikacin	78	69.6	34	30.4
Gentamicin	66	58.9	46	41.1
Imipenem	84	75.0	28	25.0
Ciprofloxacin	66	58.9	46	41.1
Ceftazidime	33	29.5	79	70.5
Cefoperazone-sulbactam	80	71.4	32	28.6
Aztreonam	59	52.7	53	47.3
Polymyxin B	22	19.6	--	--

*NS: no of sensitive strains; NR: no of resistant strains; S%: sensitivity percent; R%: resistance percent

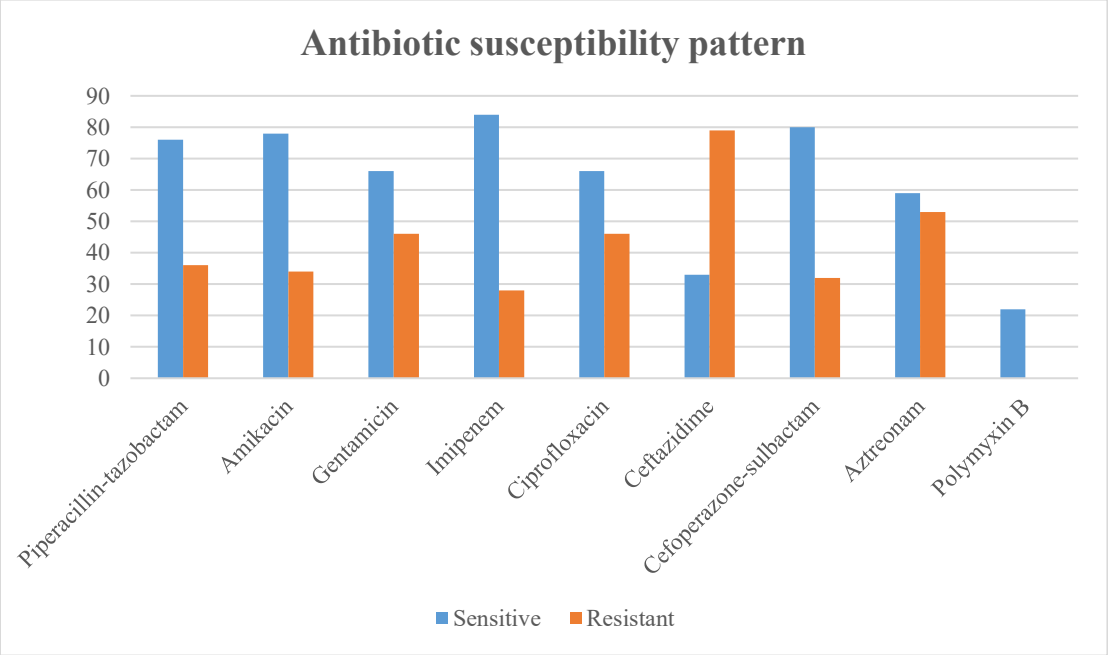

Figure-2: Antibiotic susceptibility pattern of P. aeroginosa strains

Table 4: Resistance pattern of Amp-C beta-lactamase producing *P. aeroginosa* strains

Antibiotics susceptibility pattern			**Inducible Amp-C (n=27)**		**Non-inducible Amp-C (n=12)**	
			Positive	**P value**	**Positive**	**P value**
Piperacillin-tazobactam	R	n	10	0.685*	3	0.476*
		%	37.0%		25.0%	

	S	n	17		9	
		%	63.0%		75%	
Amikacin	R	n	8	0.154*	1	0.216*
		%	29.6%		8.3%	
	S	n	19		11	
		%	70.4%		91.7%	
Gentamycin	R	n	14	0.012*	2	0.070*
		%	51.9%		16.7%	
	S	n	13		10	
		%	48.1%		83.3%	
Imipenem	R	n	09	0.081*	01	0.121*
		%	33.3%		8.3%	
	S	n	18		11	
		%	66.7%		91.7%	
Ciprofloxacin	R	n	11	0.216*	3	0.476*
		%	40.7%		25%	
	S	n	16		9	
		%	59.3%		75%	
Ceftazidime	R	n	21	0.186*	6	0.059*
		%	77.8%		50%	
	S	n	06		6	
		%	22.2%		50%	
Cefoperazone-sulbactam	R	n	7	0.661*	4	0.706*
		%	25.9%		33.3%	
	S	n	20		8	
		%	74.1%		66.7%	
Aztreonam	R	n	12	0.210*	3	0.463
		%	44.4%		25%	
	S	n	15		9	
		%	55.6%		75%	
Polymyxin B	R	n	--	1.000*	--	1.000*
		%	--		--	

	S	n	6		2	
		%	22.2%		16.7%	

*Fisher's exact test, S=sensitivity, R=resistance, n=no of strains

Table 5: Resistance pattern of metallo-beta lactamase producing *P. aeroginosa* strains

Antibiotics susceptibility pattern			Imipenem-EDTA combined disc test (n=37)		Modified Hodge Test (n=21)	
			Positive	P value*	Positive	P value*
Piperacillin-tazobactam	R	n	12	0.246	5	0.750
		%	32.4%		23.8%	
	S	n	25		16	
		%	67.6%		76.2%	
Amikacin	R	n	14	0.723	9	0.377
		%	37.8%		42.9%	
	S	n	23		12	
		%	62.2%		57.1%	
Gentamycin	R	n	16	0.741	10	0.560
		%	43.2%		47.6%	
	S	n	21		11	
		%	56.8%		52.4%	
Imipenem	R	n	10	0.415	5	1.000
		%	27%		22.9%	
	S	n	27		16	
		%	73%		76.2%	
Ciprofloxacin	R	n	17	0.733	11	0.382
		%	45.9%		52.4%	
	S	n	20		10	
		%	54.1%		47.6%	
Ceftazidime	R	n	26	0.720	16	0.366
		%	70.3%		76.2%	
	S	n	11		5	
		%	29.7%		23.8%	
Cefoperazone-sulbactam	R	n	14	0.136	6	0.764
		%	37.8%		28.6%	
	S	n	23		15	
		%	62.2%		71.4%	
	R	n	20	0.494	9	0.561

Aztreonam		%	54.1%		42.9%	
	S	n	17		12	
		%	45.9%		57.1%	
Polymyxin B	R	n	--	1.000	--	0.729
		%	--		--	
	S	n	8		5	
		%	21.6%		23.8%	

*Fisher's exact test, S=sensitivity, R=resistance, n=no of strains

Table-6: Resistance pattern of β-lactamase and MBL producing P. aeroginosa strains

		MBL positive		Total
		yes	no	
Beta lactamase positive	yes	14	21	35
	no	34	43	77
Total		48	64	112