ANTIBIOGRAM OF METALLO-BETA- LACTAMASE AND EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* FROM ICU PATIENTS.

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ABSTRACT

INTRODUCTION: *Pseudomonas aeruginosa*, is one of the important pathogens responsible for infection in hospitalized patients. *P. aeruginosa* is considered stubborn pathogens in any hospitalized setup as it is adaptable to multiple drug-resistant mechanisms and capacity to remain alive in most environments. Therefore, it is important to have prior knowledge of susceptibility patterns in particular area, as it becomes easy to choose appropriate antimicrobial against these resistant strains.

AIM: To detect antimicrobial susceptibility pattern of extended spectrum beta-lactamase and metallo- beta-lactamase producing *P. aeruginosa* from ICU patients.

MATERIAL METHODS: *P. aeruginosa* were isolated from various clinical specimens by standard methods. Antibiogram was obtained by Kirby–Bauer disc diffusion methods. Combined Disc Diffusion technique was used for detection of metallo- beta-lactamase and extended spectrum beta-lactamase producing *P. aeruginosa*

RESULT: Prevalence of metallo-beta-lactamase and extended spectrum beta-lactamase producing *P.aeruginosa* was 46.25% and 33.75% respectively. Aminoglycosides and antipseudomonal penicillins like piperacillin and combination drugs like amoxicillin and clavulanic acid were the most sensitive antimicrobials against them.

CONCLUSION: The study underlines the unique problem of *P. aeruginosa* infections in ICU setup. Also, it is very important to have routine surveillance studies for drug resistance mechanism by phenotypic detection method, especially for *P. aeruginosa* isolated from ICU.

Key words: MBL, ESBL, Imipenem-EDTA test, Antibiogram, Susceptibility
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INTRODUCTION

Pseudomonas aeruginosa, is one of the important pathogens responsible for infection in hospitalized patients [1]. It is responsible for infections of skin and soft tissue, ocular infections, burn wounds, urinary tract infections, respiratory infections like pneumonia specifically ventilator associated pneumonia (VAP) in ICU patients, bacteremia, secondary meningitis [2,3].

Intensive Care Unit (ICU) patients, are of immunocompromised status, and invasive procedures like catheterization which may be central venous, intracath, or urinary, and other interventions in the form of intubation, mechanical ventilation, and tracheostomy procedures are usually done as a part of management of patient. Because of this, patient is at high risk of infections by *P. aeruginosa* infections [4]. Further because of increased use of antibiotics in ICU patients to treat these infections results into development of multidrug resistant (MDR) strains [5]. As a result ICU becomes important source for development, transmission and multiplication of such resistant microbes [6].

P. aeruginosa shows natural resistance to many antimicrobials and antiseptics. *P. aeruginosa* is one of the stubborn pathogens in any hospitalized setup as it is adaptable to multiple drug resistant mechanism and capacity to remain alive in most environment [6,7]. Natural resistance mechanism for *P. aeruginosa* includes presence of excess of efflux pumps, and reduced outer cell membrane permeability [8]. The organism can acquire resistance by gene transfer or by mutation in genes which encodes for chromosomal β- lactamase, penicillin- binding–proteins, efflux pumps and outer membrane porins. These are the basic mechanism of resistance for *P. aeruginosa* against β-lactams, carbapenems, aminoglycosides and quinolones[9]. Most common enzymes responsible for resistance produced by *P. aeruginosa* includes metallo-β-lactamses (MBLs). Extended spectrum β-lactamases enzymes (ESBL) are also frequently produced by them. *P. aeruginosa* infections results in therapeutic failure scenario which are more commonly seen in Intensive Care Units [10]. With prior knowledge of susceptibility pattern in particular area, it becomes easy to choose appropriate antimicrobial against these resistant strains. The present study therefore was aimed to determine antimicrobial susceptibility profile of clinical isolates of *P. aeruginosa* from ICUs in a tertiary reference center with objective of providing effective empirical therapy for infections caused by them. The phenotypic test will further enlighten the resistant mechanisms which are prevalent in the ICU set up.

MATERIALS AND METHODS

The laboratory based prospective study was carried out from January 2020 to May 2021 at ICUs, Krishna Hospital & Medical Research Centre (KH & MRC) and Department of Microbiology, Krishna Institute Medical Sciences, Karad. *P. aeruginosa* isolated from different clinical specimens from ICU, KH and MRC were included in the study. Repeat isolate of *P. aeruginosa* from same patient from repeat specimen was excluded from study, to avoid duplication of isolate. The study was approved by Ethics committee of Krishna Institute of Medical Sciences, Deemed to be University, Karad

Sample size: Raval et al. had found a prevalence of 27 % of *P. aeruginosa* in clinical specimens in ICU from Ahmadabad[11].

$$n = 4pq/L^2$$

$$= 4 \times 27 \times 73/10^2 \quad \text{where } p = 27,\ q = 100-p = 73,\ L = 10$$

= 79

So, in the present study, minimum 80 *P. aeruginosa* isolates, from clinical samples received from intensive care unit were studied.

SAMPLE PROCESSING –

A total of 80 non replicate isolates of *P. aeruginosa* from clinical samples were taken in the study. All samples were collected under aseptic precautions by standard procedures and processed according to standard guidelines. All specimens were inoculated on to nutrient agar, 0 MacConkey agar, blood agar and chocolate agar. The plates were incubated at 37 C for 24 hours.

Interpretation of the culture –

The plates were read after 24 hours of incubation for any growth.

Identification of isolates of *P. aeruginosa* was done on the basis of colony morphology and biochemical reactions using standard methodology [12].

Antimicrobial Sensitivity Testing

According to the CLSI guidelines the antibiotic susceptibility testing was done on all *P. aeruginosa* isolates by Kirby Bauer disc diffusion method [13]. The zone size was recorded and interpreted as per the CLSI guidelines 2020. *P. aeruginosa* ATCC 27853 was used as quality control strain [13]. The antibiotic disc used for antimicrobial sensitivity testing of *P. aeruginosa* were amikacin 30µg, gentamicin 10 µg, piperacillin/tazobactam100/10 µg, imipenem 10 µg, ceftazidime 30 µg, cefoperazone75 µg, ceftriaxone 30 µg, cefotaxime30 µg, cefepime 30 µg, amoxicillin/ clavulanic 30 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, piperacillin 100 µg, co-trimoxazole 1.25/23.75 µg.

Phenotypic detection of MBL activity:

The imipenem-resistant *P. aeruginosa* isolates were investigated for MBL production by Imipenem-Ethylene diamine tetra acetic acid combined disc test (IMP-EDTA CDT) as described previously by Young et al. with modification [14]. Briefly, overnight culture of the test organism was prepared and its turbidity was adjusted to 0.5 McFarland standard and surface inoculated on Mueller Hinton agar (MHA plate) (9 cm in diameter). Imipenem discs (10 µg) and Imipenem/EDTA (10/750 µg) (Hi-Media) were placed at a distance of 4-5 cm from each other on the plate. The inhibition zones of the Imipenem and Imipenem-EDTA discs were compared after 18 hrs. of incubation at 35 °C. Isolates were considered as MBL positive if, the zone diameter of Imipenem-EDTA disc is larger by more than or equal to 7 mm.

Phenotypic detection of Extended spectrum β-lactamases (ESBL) production:

A lawn culture of the organisms was made on a 9 cm-diameter MHA plate, as recommended by CLSI [15]. A disc of ceftazidime-clavulanate (30/10 µg) and ceftazidime disc (30 µg) was placed at a distance of 20 mm. After overnight incubation at 37°C, an increase of 5 mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone, was considered to be ESBL producer.

RESULT

Out of 80 isolates of *P. aeruginosa* 37(46.25%) were MBL positive and 43(53.75%) were MBL negative isolates.

Figure No.1 shows sample wise distribution of MBL positive *P. aeruginosa*. Majority of the isolates were from urine 17 (45.94%) followed by pus 10 (24.32%), blood 3(8.10%), ETT 3(8.10). The antibiotic sensitivity of the MBL positive was as shown in Table 1

For MBL positive *P. aeruginosa*, piperacillin (56.76 %) followed by amoxicillin/clavulanic acid (37.84 %) were the most sensitive antimicrobials. (Table 1).

MBL positive *P. aeruginosa* were having more resistance for the various antimicrobials which ranged from 43 % (piperacillin) to 97 % (co-trimoxazole) as compared to MBL negative isolates of *P.aeruginosa* for which resistance ranged from 23 % to 78 %. (Figure 2)

Out of 80 isolates of *P. aeruginosa,* 27(33.75%) were ESBL positive and 53 (66.25 %) were ESBL negative.

Figure 3 shows sample wise distribution of ESBL positive *P. aeruginosa*. Majority of the isolates were from urine10 (37.03%), followed by, pus 8 (29.6 %), ETT 4(14.81%).

As shown in Table 2, ESBL positive *P. aeruginosa* showed maximum sensitivity to gentamicin (48.15 %) followed by piperacillin (44.45%)

The resistance patterns of ESBL positive and negative *P. aerugonosa* were almost similar which ranged between 50 % to 90 % for different antimicrobials. (Fig.4)

DISCUSSION

P. aeruginosa infection in ICU is of constant concern. Colonization with this organism often proceeds to infection and the prevention is therefore extremely important. The choice of empiric antibiotics in the ICU setting is difficult. There needs to be balance between excessively broad coverage and too narrow coverage.

P. aeruginosa exhibits intrinsic resistance to various antimicrobials. It also shows acquired resistance to anti pseudomonal beta-lactams such as piperacillin, cephalosporins and carbapenems. The rise in the resistance to the last resort drug carbapenem has become a major challenge in treating the *P. aeruginosa* infections. Several mechanisms are responsible for the acquired resistance to the β- lactam antibiotics in *P. aeruginosa* which includes the production of β-lactamases, up regulation of the efflux pump systems and decreased outer membrane permeability. With respect to β-lactamases, the metallo-beta-lactamases are the emerging resistance mechanism in *P. aeruginosa*. Whenever empiric therapy does not cover *P. aeruginosa,* may lead to poor outcome for ICU patients, and eventually found to have *P. aeruginosa* infection.

As a broad-spectrum empirical therapy, there is indiscriminate use of 3rd generation cephalosporin resulting in secretion of ESBL enzymes. This mediates the resistance by breaking or hydrolysis of β-lactam ring of the β-lactam antibiotics.

In current study, prevalence of MBL producing *P. aeruginosa* was 46.25%. Comparative study showing prevalence of MBL for *P. aeruginosa* in various Indian studies ranged at higher level in the study by Goel V. et al. [16] and Umadevi s et al. [17] with 53.85 % and 65.7 % rate respectively. On the other hand the MBL prevalence rate was low in studies carried out by Peshattiwar PW et al. [18] (7.8 %), Varaiya A et al. [19] (20.8 %), Kaur A. et al. [20] (21.8 %), Present study showed higher prevalence MBL in comparison to above study. This may be due to overuse of carbapenem group of antimicrobials which is used as a last resort in management of ICU infections. Also, MBL producing genes are more rapidly spread in gram negative bacilli via horizontal transmission, which is more likely to happen in ICU setup [21].

In our study, prevalence of ESBL producing *P. aeruginosa* accounted 33.75%, out of 80 isolates. Similarly, findings of Agarwal R et al [22] showed number of isolates which were ESBL producing as 20.27% while Goel V. et al. [16] had found 42.30 % of ESBL prevalence. In the study conducted by Peshattiwar P.et al. [18] and Kaur A. et al. [20] it varied to 22.22 % and 17.7 % respectively. In the above studies, variation in the ESBL prevalence rate may be due to the study environment, where the studies were carried out. Indiscriminate use of cephalosporins may promote increased resistance to the antimicrobial group itself [23]. Also, Krishna Hospital & Medical Research Centre being a tertiary care hospital, where in patients are most of the time referred cases, many of whom had already received antimicrobials before getting admitted. This may be one of the reasons for higher prevalence of ESBL (33.75 %) among *P. aeruginosa* clinical isolates.

Maximum isolates of MBL and ESBL producing *P. aeruginosa* were from urine specimen. *P. aeruginosa* is one of the leading uropathogen which is responsible for urinary tract infections worldwide [24]. Urinary catheterizations, long term admission in ICU are a few of the predisposing factors responsible for *P. aeruginosa* infections [25].

In the present study the antibiogram of MBL producing *P. aeruginosa* showed resistance to

commonly used antimicrobial groups like cephalosporins, quinolones, carbapenems. Aminoglycosides and antipseudomonal penicillins like piperacillin showed comparatively higher sensitivity. Similar findings were found in the study of Choudhari V. et al. [26] Also, ESBL-producing isolates showed high resistance to ceftazidime, ciprofloxacin and imipenem while comparatively more sensitivity was seen to gentamicin, amikacin and piperacillin. These results match the findings in earlier studies [27, 28]. In the study by Farooqi A et al, other antibiotics, like amikacin, piperacillin/tazobactum have shown less resistance [29].

High prevalence of ESBL and MBL mediated drug resistance is creating a therapeutic challenge for the clinicians and to microbiologists. Aminoglycosides and antipseudomonal penicillins like piperacillin and combination drugs like amoxicillin and clavulanic acid do give some hope as an effective therapy for MBL and ESBL producing *P. aeruginosa.*

CONCLUSION

The study underlines the unique problem of *P. aeruginosa* infections in ICU setup. To overcome the problem of these resistant *P. aeruginosa,* the microbiologists, the infection control team and clinicians need to play important role. Also, it is very important to have routine surveillance studies for drug resistance mechanism, especially for *P. aeruginosa* isolated from ICU.

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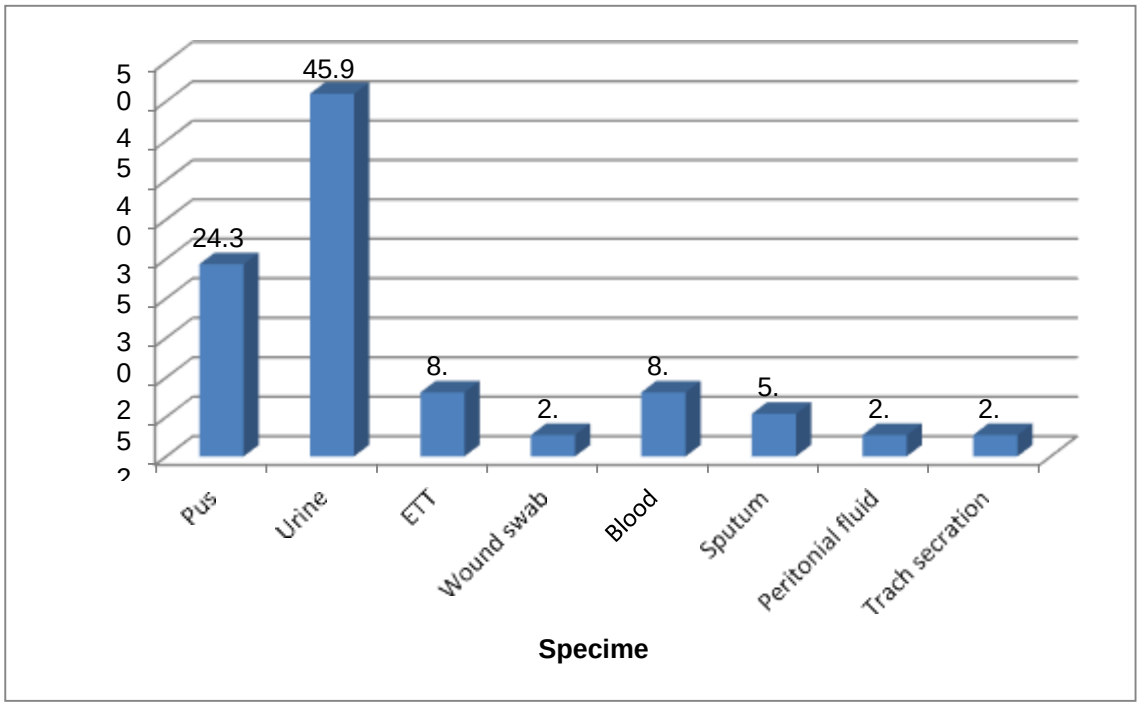

Fig No 1. Sample wise distribution of MBL producing *Pseudomonas aeruginosa*

Table 1: Antibiogram of MBL positive *P. aeruginosa*

Antibiotics	Sensitive (%)	Resistant (%)
Amikacin	7 (18.92)	30(81.08)
Gentamicin	10 (27.03)	27(72.97)
Piperacillin/Tazobactum	1(2.70)	36 (97.30)
Imipenem	0(0)	37(100)
Ceftazidime	4(10.81)	33(89.90)
Cefoperazone/sulbactam	3(8.11)	34(91.89)
Ceftriaxone	10(27.03)	27(72.97)
Cefotaxime	2(5.41)	35(94.59)
Cefepime	6(16.22)	31(83.78)
Amoxicillin Clavulanic	14(37.84)	23(62.16)
Ciprofloxacin	8(21.62)	29(78.38)
Levofloxacin	2(5.41)	35(94.59)
Piperacillin	21(56.76)	16(43.24)
Co-Trimoxazole	1(2.70)	36(97.30)

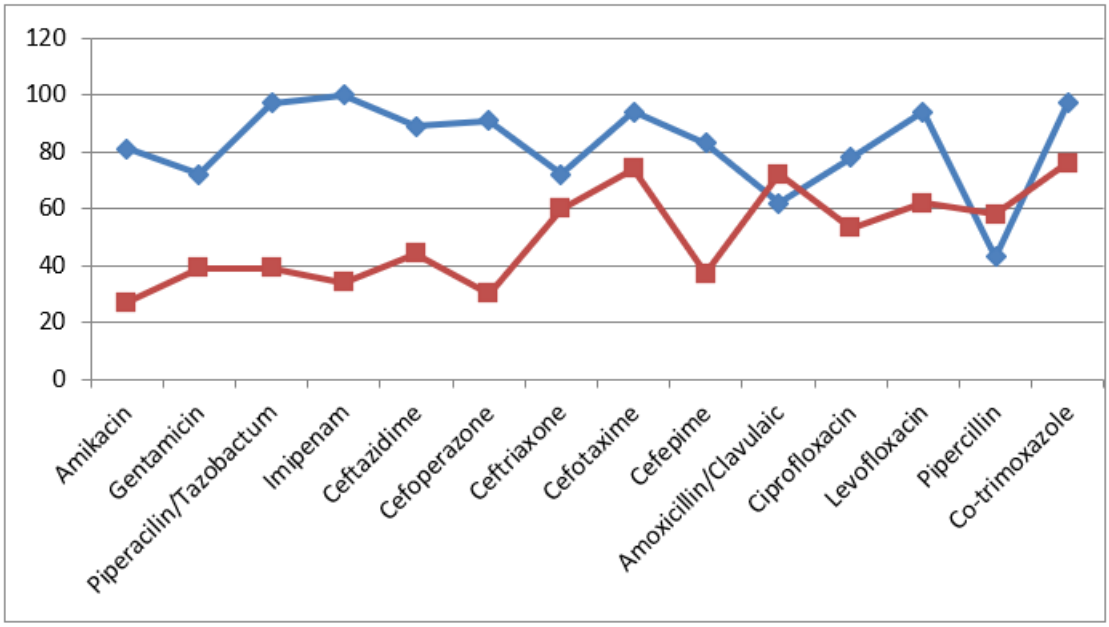

Fig. 2 Resistance pattern of MBL positive and negative *P. aeruginosa* isolates

Blue – MBL positive Red- MBL negative *P. aeruginosa* isolates

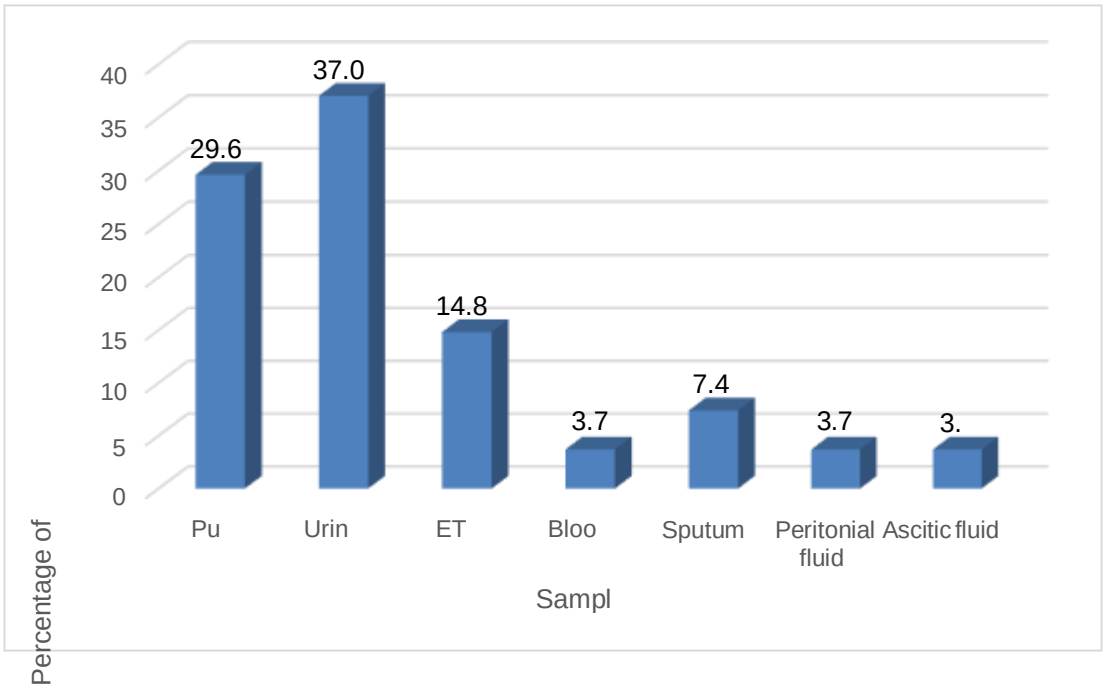

Fig. 3 Sample wise distribution of ESBL producing *Pseudomonas aeruginosa*

Table 2 : Antibiogram of ESBL positive *P. aeruginosa*

Antibiotics	**Sensitive (%)**	**Resistance (%)**
Amikacin	11(40.74)	16 (59.26)
Gentamicin	13(48.15)	14(51.85)
Piperacillin /Tazobactum	8(29.63)	19 (70.37)
Imipenem	8(29.63)	19(70.37)
Ceftazidime	9(33.33)	18(66.67)
Cefoperazone/sulbactam	10(37.04)	17(62.96)
Ceftriaxone	11(40.74)	16(59.26)
Cefotaxime	7(25.93)	20(74.07)

Cefepime	10(37.04)	17(62.96)
Amoxicillin Clavulanic	10(37.04)	17(62.96)
Ciprofloxacin	10(37.04)	17(62.96)

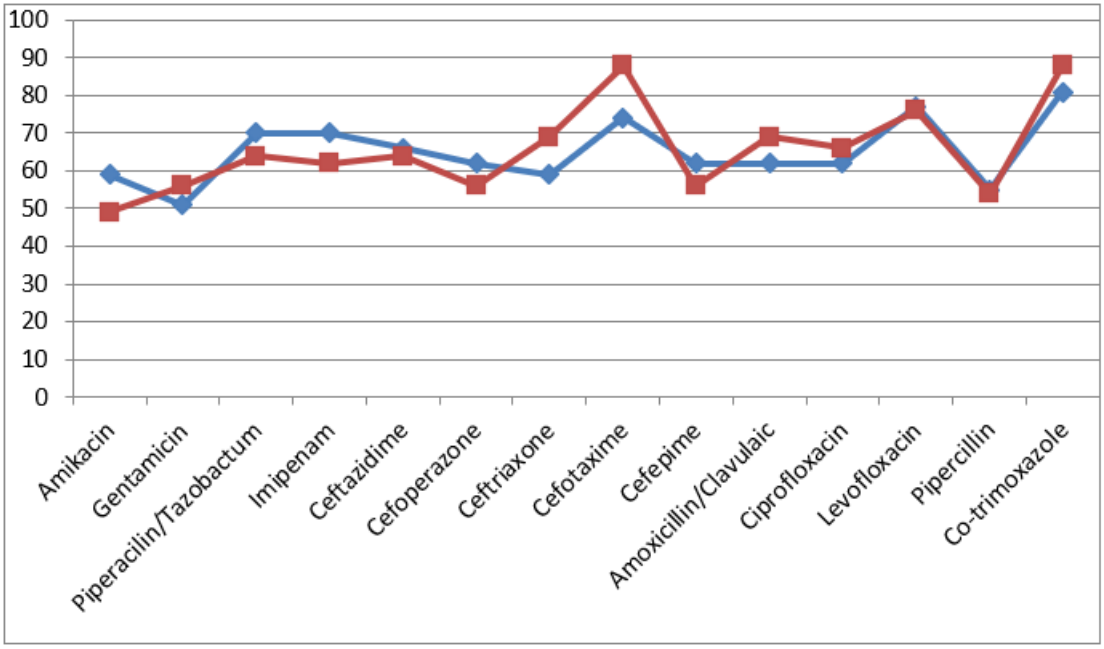

Fig. 4 Resistance Pattern of ESBL positive and negative *P. aeruginosa* isolates

Blue – ESBL positive, Red- ESBL negative, *P. aeruginosa* isolates.