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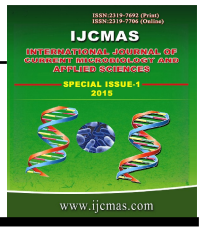

Original Research Article

Antibiotic Resistance Pattern in *Pseudomonas aeruginosa* Strains Isolated at Era's Lucknow Medical College and Hospital, Lucknow, India

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Keywords

Pseudomonas aeruginosa, Antimicrobial resistance, Disk diffusion technique, MDR strains, Reserve drugs

A B S T R A C T

Currently antibiotic resistance in bacterial populations is one of the greatest challenges to the effective management of infections. *Pseudomonas aeruginosa* is one of the most common gram-negative micro-organism identified in clinical specimens of admitted patients. The present study was undertaken to assess antibiotic resistance pattern in clinical isolates of *P. aeruginosa* in our hospital. This study was conducted from July 2013 to June 2014. Total of 236 *P. aeruginosa* isolates were identified in various clinical specimens. The samples were selected on the basis of their growth on MacConkey and Nutrient agar with oxidase test positive. Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method according to CLSI guidelines (2014). The majority of specimens from which *P. aeruginosa* was isolated consisted of urine, pus and sputum. The acid resistant penicillin such as piperacillin combination (R=16.1 %) had greater antibacterial activity against *P. aeruginosa*, when compared to its monotherapy (R=57.6%). Maximum resistance was seen against Ciprofloxacin (71.18 %) followed by gentamicin (52.11%), ceftazidime (22.03%) and imipenem (11.01 %). minimum resistance with amikacin and no resistance to meropenem were seen. Infections by *P. aeruginosa* are life threatening and difficult to treat because of its intrinsic resistance to various antimicrobials and its ability to acquire adaptive resistance during a therapeutic course. Strict adherence to the concept of "reserve drugs" is required to minimize irrational antibiotic usage. Therefore, amikacin and meropenem should be used only in severe nosocomial infections, to avoid rapid emergence of resistance.

Introduction

The Pseudomonads are a diverse bacterial group of established and emergent pathogens (Govan, 1998). Members of the genus are major agents of nosocomial and community acquired infections, being widely distributed in the hospital environment where they are particularly difficult to eradicate. *Pseudomonas aeruginosa* is the species amongst the Pseudomonads most commonly associated with human diseases (Hugbo and Olurinola, 1992).

Being an opportunistic human pathogen, it is the leading cause of nosocomial infections. It has been implicated in diverse nosocomial infections like nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections, in severe burns and in infections in immune-compromised individuals.

Of particular concern is that infections caused by *P. aeruginosa* are frequently life threatening and often difficult to treat, due to the constitutive low level resistance to several anti-microbial agents and the multiplicity of mechanisms of resistance (Babay, 2007). Its general resistance is due to a combination of factors (Lambert, 2002). It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages and become resistant during a therapeutic course. In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P. aeruginosa* has been noticed; complicating decisions on treatment with antibiotics and its relation to high morbidity and mortality (Ergin and Mutlu, 1999; Babay, 2007).

Regional variations in the antibiotic resistance exist for different organisms, including *P. aeruginosa* and this may be related to the difference in the antibiotic prescribing habits.

According to different studies, the term MDR *P. aeruginosa* has been described as resistance to at least three antibiotics from a variety of antibiotic classes, mainly aminoglycosides, penicillins, carbapenems, cephalosporins and quinolones (Falagas *et al.*, 2006).

Current study followed the definition of MDR *P. aeruginosa* as stated by European Center for Disease Prevention and Control (ECDC) and Centre for Disease Control and Prevention (CDC), where MDR *P. aeruginosa* was defined as the one that has acquired non susceptibility to at least one agent in three or more categories of antimicrobials (Magiorakos *et al.*, 2012).

The periodic testing and analysis of antibiotic resistance would enable the physicians to detect trends in resistance pattern to the commonly prescribed antibiotics in a given organism.

The aim of the study was to retrospectively analyze and determine the distribution rate and antimicrobial resistance pattern in *P. aeruginosa* among clinical specimens for a period of 1 year.

Materials and Methods

This study was conducted at the Department of Microbiology in a tertiary care hospital; Era's Lucknow Medical College & Hospital, Lucknow during July 2013 to June 2014.The present study comprises 236 *Pseudomonas aeruginosa* positive samples: swab, urine, sputum, pus, pleural fluid, BAL, blood samples etc. submitted for microbiological diagnosis to the Microbiology Department. All these samples were obtained from various wards of hospital. The clinical data was obtained from the respective units and wards of the patients.

Sample processing

The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose non-fermenting pale

colonies with oxidase test positive and on Nutrient agar green pigmented (pyocyanin production) colonies with oxidase positive. A grape-like odor of the growing colonies was also recognized.

Confirmation of *Pseudomonas spp.*

After obtaining the pure strains, the strains were subjected to Gram staining (gram negative slender rods) and biochemical identification tests to identify *Pseudomonas* spp. For this purpose samples were inoculated in Triple Sugar Iron media (TSI), Citratemedia, Peptone water, and Urease media and kept in an incubator for 18 hrs at 37°C. Next day the results were noted on TSI, Citrate media and Urease media. Part of growth on Peptone water was subjected to Indole test with Kovac's Reagent and part for motility test by 'Hanging drop' method.

A strain of *Pseudomonas* in the TSI medium showed alkaline slant, no reaction in butt. It showed negative reaction for indole test, negative urease test and positive citrate test. Glucose is utilized oxidatively, forming acid only. Also Nitrate reduction test was positive (Koneman, 2006). *P. aeruginosa* ATCC 27853 strain was used as the quality control.

Antimicrobial disc: susceptibility test

Application of antibiotic discs to the inoculated agar plates:

Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby-Bauer disc diffusion method) and the results were interpreted according to the latest CLSI guidelines (2014).

All the clinical isolates and a standard strain of *Pseudomonas aeruginosa* ATCC 27853 were tested for their sensitivity against a panel of anti-pseudomonal antimicrobials including:

Piperacillin (100 µ-gm)
Piperacillin - tazobactam (100/10 µ-gm)
Gentamicin (10 µ-gm)
Amikacin (30 µ-gm)
Ciprofloxacin (5 µ-gm)
Ceftazidime (30 µ-gm)
Cefepime (30 µ-gm)
Imepenem (10 µ-gm)
Meropenem(10 µ-gm).

Result and Discussion

A total 236 samples of *P. aeruginosa* were obtained from various sources. Isolation rate of *P. aeruginosa*, in our study, was comparable with other studies.

In the present study, sex wise prevalence of clinical isolates (Table 1) shows that infections caused by *P. aeruginosa* are more common in males (61%) compared to females (39%). This is comparable with the studies of Javiya *et al.* (2008), Jamshaid *et al.* (2008), Rashid *et al.* (2007) and Prakash *et al.* (2014) who also reported in their study that male patients had a higher isolation rate.

Also, in our study, the age wise prevalence of clinical isolates (Table 2) shows that most of the patients were aged between 21 and 40 years (n = 102; 43.22%). This is comparable with the study of Rashid *et al.* (2007) and Mohanasoundaram (2011). This may be due to maximum occupational exposure to the organism.

In our study, maximum clinical isolates of *P. aeruginosa* (Table 3; Fig. 1) were isolated from pus/swab (53.8%), followed by urine (16.1%) which is in line with the study of Jamshaid *et al.* (2008).

Also, highest percentage of *P. aeruginosa* infections was observed in the surgical

ward, followed by pediatric ward and medical ward. Prevalence of infection was higher in surgical ward as maximum isolates were isolated from pus/swab samples.

MDR strains of *P. aeruginosa* (Table 4; Fig. 2) were predominantly isolated from pus 40 (51.94%), sputum 13 (16.88%) followed by urine samples 8 (10.38%). Prakash *et al.* (2014) also reported a high prevalence of MDR *P. aeruginosa* in clinical samples of pus and urine, in their study.

Murase *et al.* (1995) in their study showed that there is distinct difference in the sensitivity pattern of isolates of *P. aeruginosa* from specimen to specimen.

P. aeruginosa, in our study, showed high resistance rates in fluoroquinolones (ciprofloxacin), ureidopenicillin (piperacillin) followed by gentamicin, while minimum resistance was seen against amikacin and no isolate was found to be meropenem resistant (Table 5; Fig. 3).

Multidrug-resistant (MDR) *P. aeruginosa* is a growing menace. Altered target sites, bacterial efflux pumps, enzyme production or inhibition, loss of membrane protein, etc. are different mechanisms mediated by MDR *P. aeruginosa* (Elizabeth and Vincent, 2010).

The present study showed a 32.6% (n = 77) frequency of MDR *P. aeruginosa*, (Table 6; Fig. 4) while Gill *et al.* (2011) reported a 22.7% incidence in Islamabad. Another study conducted in Iran by Tavajjohi and Moniri (2011) reported 27.6% prevalence of MDR *P. aeruginosa*.

The prevalence of resistance to piperacillin in *P. aeruginosa* as reported by Javiya *et al.* (2008) is much higher (73.21%) than that reported in our study. Overall piperacillin resistance was found to be 57.6% and among the MDR isolates the resistance rate was found to be higher (76.62%). The ureidopenicillin combination (piperacillin-tazobactum), in our study, shows greater anti-bacterial activity against *P. aeruginosa* compared to its monotherapy (i.e. piperacillin alone). Resistance rates of piperacillin-tazobactum combination were considerably lower (16.1%) in comparison to piperacillin alone (57.6%) as concurrent administration of a β-lactamase inhibitor markedly expands the spectrum of activity.

Increasing resistance to beta-lactams in *P. aeruginosa* has become a serious threat, particularly against third and fourth generation cephalosporins. There are a lot of molecular mechanisms to develop resistance against these antibiotics; generation of extended-spectrum beta-lactamases (ESBL), by incorporation of *bla* genes in integrons and inability of porin genes to enhance their expression level and/or alteration of antibiotic target sites (Pfeifer *et al.*, 2010).

Ceftazidime and cefepime are the prescribed anti-pseudomonal third and fourth generation cephalosporins, respectively. The resistance to ceftazidime, in our study, was found to be22.03% and the resistance to cefepime was found to be 16.1%. This is comparable to a study from Iran by Tavajjohi and Moniri (2011) that showed Ceftadizime resistance at 25%, but is in contrast to high values of resistance which were reported from Gujarat (75%) (Javiya *et al.*, 2008). However, among the MDR isolates, in our study, the resistance rate was found to be higher (Cefatzidime; 55.84% & Cefepime; 35.06%).

Carbapenems are considered the most significant group of antibiotics against MDR *P. aeruginosa* and the treatment of choice against serious ESBL associated infections.

However, the development of carbapenem resistance is becoming a challenge for health care professionals, limiting the therapeutic options. The resistance to Carbapenems, especially in *P. aeruginosa*, results from reduced levels of drug accumulation or increased expression of pump efflux or production of metallo-β-lactamases (Kurokawa *et al.,* 1999; Navneeth *et al.,* 2002; Gupta *et al.,* 2006).

Prakash *et al.* (2014) in their study showed 22% imipenem resistance which is higher than that reported in our study (11%). Also, various studies have shown resistance to Imipenem up to 31.6% (Brown and Izundu, 2004). No isolate, in our study, was found resistant to Meropenem. Moreover, all the 26 Imipenem resistant strains, in our study, were found resistant to the remaining anti-pseudomonal antibiotics, making Imipenem resistance among the MDR isolates, 33.76%.

Fluroquinolone compounds are one of the important antimicrobial agents that have been used for a variety of infections. Ciprofloxacin, the most potent agent available in oral form for the treatment of *P. aeruginosa* infections, is in particular jeopardy. Present study showed an overall resistance of 71.18% and a resistance of 84.4% among the MDR isolates. This is comparable to a 3 year study from India that reports ciprofloxacin resistance of 63.1% (Mohanasoundaram, 2011). Tam *et al.* (2010) in their study claimed 100% resistance against Ciprofloxacin. High degree of resistance is probably because of the irrational approach of the clinicians of putting patients on quinolones straightway without going for antibiotic sensitivity. Because of the increasing resistance to fluoroquinolones in many hospitals, its empirical usage is either banned or restricted, to bring the developing resistance rates under control.

Among all the drugs, Amikacin showed the least resistance against *P. aeruginosa*. (Overall resistance - 9.32% & resistance in MDR isolates - 23.37%). The current study explored that anti *P. aeruginosa* effect of Amikacin was higher than Gentamicin (Overall Gentamicin resistance; 52.11% & resistance among MDR isolates; 74%) and this correlates with the study done by Smitha *et al.* (2005), Poole (2005) and Javiya *et al.* (2008). Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation. The high activity of Amikacin observed in this study may be due to the presence of the aminohydroxybutyryl group, which generally prevents the enzymatic modification. However, some organisms have developed enzymes that inactivate this agent as well. Meenakumari *et al.* (2011), in their study, showed a higher resistance of 56.86% to amikacin. In our study, amikacin seems to be a promising therapy for pseudomonal infection. Hence its use should be restricted to severe nosocomial infections in order to avoid rapid emergence of resistance strains.

Our study thus indicated amikacin and meropenem as an efficient treatment of choice against MDR *P. aeruginosa* among all the tested antibiotics.

Also it brings to light that the clinical isolates of *P. aeruginosa* are becoming increasingly resistant to commonly used antibiotics and gaining more and more resistance to newer antibiotics. The antimicrobial agents are losing their efficacy because of the spread of multi-drug resistant strains due to indiscriminate use of antibiotics, lack of awareness, patient non-compliance and non-availability of anti-microbial testing facilities.

Antibacterial research is not sufficient to keep pace with the clinical challenges of MDR bacterial crises. Lack of new drug pipelines and other issues are leaving disastrous consequences on the health of the community. To overcome such issues, new therapeutic agents with maximum efficacy, lesser toxicity and cost effective in nature are urgently needed.

So in the present time, there is a need to emphasize rational use of antibiotics and strict adherence to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. Rigorous antimicrobial surveillance should be done periodically to monitor the current susceptibility patterns in local hospitals, to keep a tab on the development and spread of MDR strains.

An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics for better patient management. It is the need of the time that antibiotic policies should be formulated and implemented to resist and overcome this emerging problem. Every effort should be made to prevent the spread of multi-drug resistant strains.

Table.1 Sex wise distribution of cases

Sex	Total no	Percentage (%)
Male	144	61.1
Female	92	38.9
Total	236	100

Table.2 Age distribution of cases

AGE (YEARS)	NO. OF ISOLATES	PERCENTAGE (%)
0 – 20	85	36.01
21 – 40	102	43.22
41 – 60	36	15.25
> 60	13	5.5
TOTAL	236	100

Table.3 Isolation of *Pseudomonas aeruginosa* from different clinical samples

NATURE OF SAMPLE	NO. OF SAMPLE	PERCENTAGE (%)
PUS	127	53.8
SPUTUM	14	5.9
BAL	10	4.2
SWAB	20	8.4
URINE	38	16.1
CATHETER TIP	16	6.7
BLOOD CULTURE	10	4.2
CSF	1	0.4

Table.4 Isolation of MDR *Pseudomonas aeruginosa* from different clinical samples

Nature of sample	No. of sample	Percentage (%)
Pus	40	51.94
Sputum	13	16.88
Bal	4	5.19
Swab	7	9.09
Urine	8	10.38
Catheter tip	2	2.59
Blood culture	3	3.89
Csf	0	0

Table.5 Antibiotic resistance of *Pseudomonas aeruginosa* isolated from different clinical samples

Antibiotic	No. of Isolates Resistant	% Resistance
Piperacillin	136	57.6
Piperacillin - tazobactam	38	16.1
Gentamicin	123	52.11
Amikacin	22	9.32
Ciprofloxacin	168	71.18
Ceftazidime	52	22.03
Cefepime	38	16.1
Imipenem	26	11.01
Meropenem	0	0

Table.6 Antibiotic resistance of MDR *Pseudomonas aeruginosa* (n= 77) isolated from different clinical samples

Antibiotic	No. of Isolates Resistant	% Resistance
Piperacillin	59	76.62
Piperacillin – tazobactam	30	38.9
Gentamicin	57	74.02
Amikacin	18	23.37
Ciprofloxacin	65	84.4
Ceftazidime	43	55.84
Cefepime	27	35.06
Imipenem	26	33.76
Meropenem	0	0

Fig.1 Sample-wise distribution of *P. aeruginosa* isolates (overall)

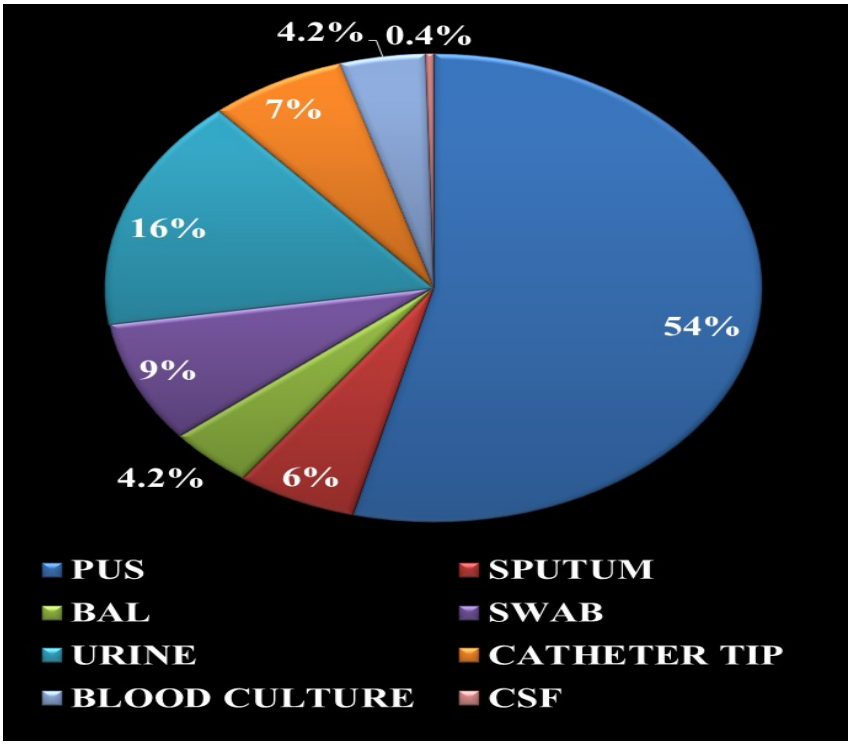


Fig.2 Sample-wise distribution of *P. aeruginosa* isolates (MDR)

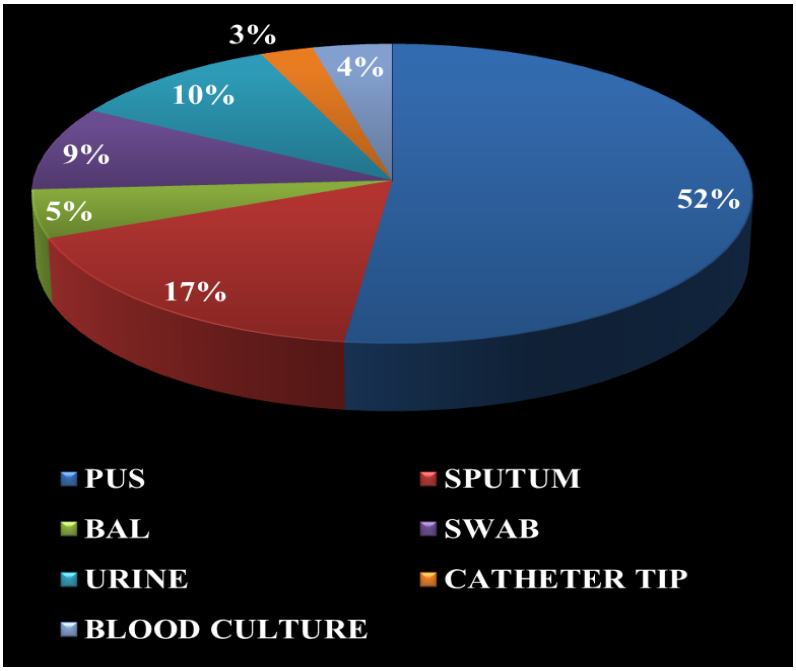


Fig.3 Antibiotic resistance pattern of *P. aeruginosa* isolates (overall)

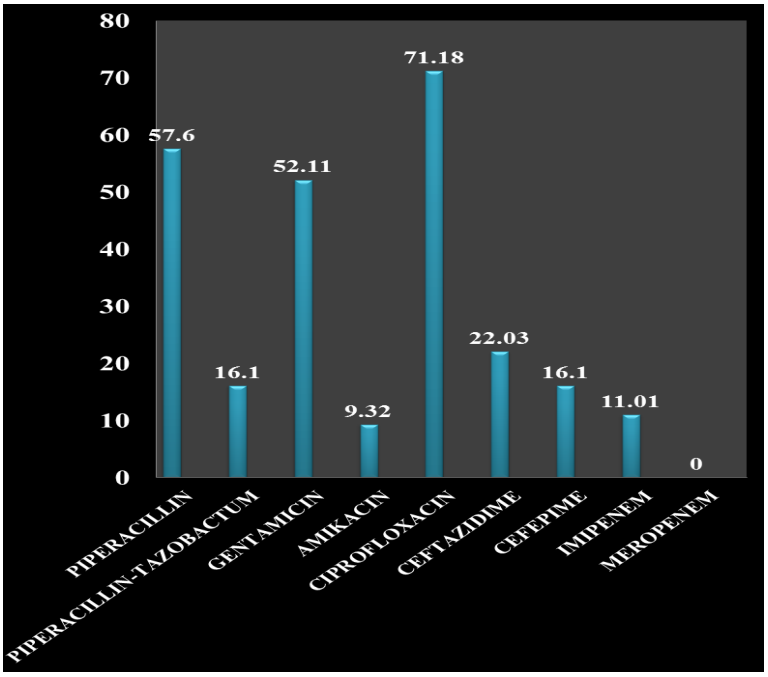
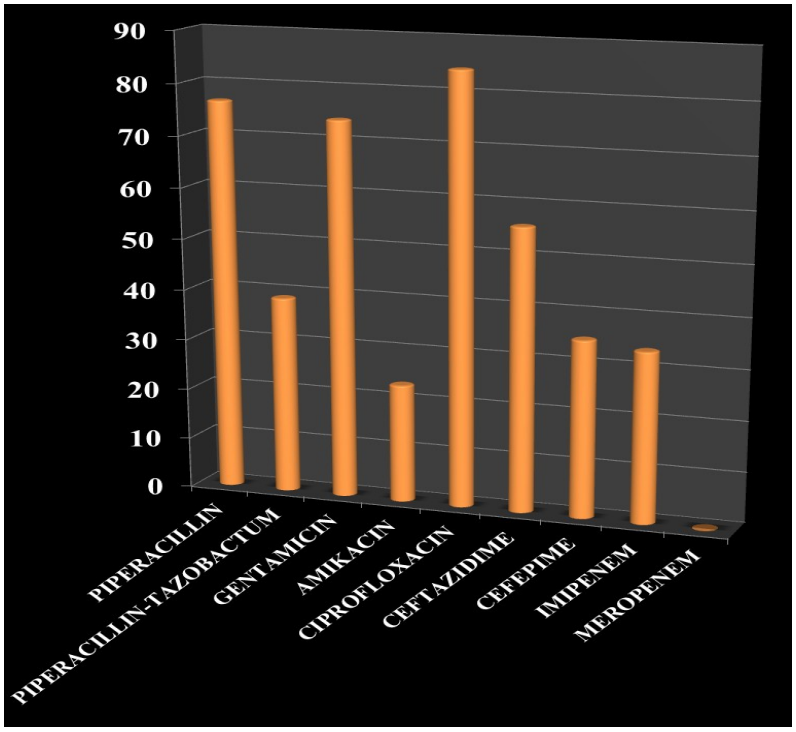


Fig.4 Antibiotic resistance pattern of *P. aeruginosa* isolates (MDR)



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