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Original Article

Gradual Decline in the Potency of Antipseudomonas Drugs: Evaluated by Retrospective Comparative studies of Susceptibility and Resistance Pattern of Antimicrobials against Pseudomonas Aeruginosa Isolates Obtained from Intensive Care Unit

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ABSTRACT

Infectious disease accounts for the major cause of mortality and morbidity in past decays which create a serious and growing concern to treat communicable disease. P. aeruginosa 'superbug' responsible for hospital acquired nosocomial infections is versatile gram negative aerobe and pathogenecity caused by this ubiquitous organism is quite difficult to treat due to its rapid developing inherent resistance mechanism against antimicrobials and disinfectants. Variety of antimicrobials available to treat this notorious bacterium and there's a gradual decline in sensitivity pattern upon development of new evolution of antimicrobials which reflects inadequate utilization of antimicrobial. The purpose of this study is to estimate the degree of potency of antipseudomonas drugs by retrospective comparative analysis of susceptibility and resistance pattern in public and private health sector of intensive care units and community acquired infections in past two years. About more than 200 isolates tested against wide range of antimicrobials. In past two years, there's a gradual decline in susceptibility of amikacin (41%), gentamycin (45%), tobramycin (51%) and ciprofloxacin and emergence of initiation of resistance against carbapenems (23-29%), piperacillin (23%) & colisthemetate (33%) respectively. Quantitative estimation of this comparative studies we conclude antimicrobial resistance and susceptibility has close association with irrational utilization and inadequate evaluation of adaptive resistance mechanism against superbug called p.aeruginosa.

Keywords: P.aeruginosa infection, source, susceptibility pattern, Resistance adaptive mechanism, multidrug resistance pattern.

INTRODUCTION

Communicable disease accounts for major reasons of anguish and fatality rate in past decays but after extension with the antimicrobials during last decay necessitate the call of infectious disease expert but it has near to obsolete. On the other hand, in past 3 centuries, revival of communicable pathogenicity bring to a halt its emergent confidence seriously. moreover the variation and capacity of infections microbials developed/create emergence of challenges against gr+ve and gr-ve bacteria particularly MRSA,VRSA,E.COLI, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa[1]. Pseudomonas aerogenosa accounts for most Oppurtunistic disease producing hospital acquired infection by gr-ve aerobe responsible for secondary disorder [2] .Infection caused by this serious pathogen is quite complicated to treat with antiseptic and antimicrobials [3]. In 1850 sedillot reported its consequence of transmission later on change in colour of surgical dressing .upon culture it formed characteristics feature of blue-green pigments and it was isolated by Fordos at 1860 and its correlation with bacterium was identified by lucke in 1862[4]. P.aeruginosa identification responsible for discoloration of wounds was not isolated before 1882, P.aeruginosa (Bacillus pocyaneus) identified as causative agent for the greenish blue pus surgical wound betwixt 1889 and 1894[5, 6]. Marshes, soil and marine coastal are origin of this life opportunistic gram-ve infectious pathogen in addition to animal and plant group of cells [7]. This notorious pathogen isolated from variety of environment and possess the tendency to strive with inadequate nutrition and make suitable to grow in variety of condition permit it to stick with hospital and environment surroundings .variety of sources including surgical equipments, disinfectants, ophthalmic solutions soaps, wash tube, medications ,and water pools constitute its

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major source of transmission [4,8,9]. P.aeruginosa accounts an important part of normal flora on skin and mucous membrane with increasing susceptibility hospital acquired infections specifically on part exposed to open wounds infections of burns, purulent, post-surgical open wounds, equipment exposed to mucous lining catheterization and ventilation [4,10,11,12,13,14,15]. It possess the ability to make biofilm in weak immunocompromised patients like common skin flora staphylococcus epidermidis on wet surface accounts for distressing severe infections particularly in patients with cystic fibrosis patients. [16, 17].patients with immunod efficient are more prone to infection caused by this ubiquitous pathogen and it destroy the common flora after exposure to antibiotic make condition favorable for its infection expansion [4,10,11,18,19]. Although, this Life opportunistic pathogen create emergence of multi-drug resistance which severely affect living cells after invention of wide range of antimicrobials in health practice [20]].it severely affect patient with cystic fibrosis and quite difficult to treat which may leads to pulmonary failure after comparative studies with other bacterium it acquire highly resistance to diverse group of antimicrobials which interfere with cell wall and protein synthesis because of low penetration to external surface[21,22,23]. For this reason it confines antimicrobials with very low permeability to outer membrane resulting in incapable to produce antibacterial response. Alternatively they combat bacterial response after modification by variety of actions inclusive of bacterial cell wall enzymatic production or either by enhance emanation of beta-lactamase. Life opportunistic bacterium produced innate-modification basically constituted from the part of its hereditary frame work, which govern elevated threshold for least inhibitory concentration which yield group of antimicrobials resistant across pseudomonas aeruginosa. They modify their action more pronouncedly by alteration of genetic mechanism. These genetic alterations render wide variety of antimicrobials inefficient against this life opportunistic bacterium [24, 25, 26, 27, 28]. Various studies showed there's a gradual decreased in pseudomonas sensitivity counter to variety of antimicrobials.[29,30,31].Gradual decline in pseudomonas aeruginosa sensitivity revealed by Centers for Disease Control and Prevention National Nosocomial In-factions Surveillance System with reported events of carbapenems 56%, quinolones 50% and 10% broad spectrum cell wall inhibitor correspondingly in comparison vigilance between 1994-1998 to 1999, infection acquired by hospital in intensive care units[2]. Choice of suitable antibacterial therapy necessitate call to encounter Multi-drug resistance emergence with rational practice education in different healthcare settings and practitioner.[32]

The **objective** of this study were to estimate the potency of antimicrobials against P.aeruginosa and to analyze data obtained from the intensive care units of the critically ill patients to evaluate the comparative susceptibility and multi drug resistance pattern among different antimicrobials against this opportunistic pathogen pseudomonas aeruginosa from public and private healthcare settings of Pakistan from retrospective studies of past 2 years with emerging view of the fact to appraise rational practice of empirical therapy to encounter emergence associated with this so called 'superbug' pseudomonas aeruginosa.

METHODOLOGY

Study designed basically comprised of retrospective data of past two years from Intensive Care Units of public and private Health Care Sector of Critically Ill patients. For that purpose Susceptibility and resistance pattern of most ubiquitous pathogen pseudomonas aeruginosa, more than 200 isolates were obtained from antibiograms of hospitals. Isolates obtained were mostly hospital acquired with little community acquired. Duplicate isolate meet the exclusion criteria and nonduplicate isolates antibiogram were interpreted on results. Although testing of repeated isolates warranted by hospital. Method for susceptibility testing Perfomed by participating hospital was based on: Disk Diffusion Method of Kirby-Bauer; VITEK (biomerieux vitek,Hazlwood MO) ; with microbroth dilution of cation adjusted Mullier-Hinton broth (CAMHB) and colonies suspended on them were equivalent to a 0.5 McFarland Standard. data interpreted on reports were based on accordance to performance standard for antimicrobial disk susceptibility test, CLSI (NCCLS).[33] Approximately more than 30 antimicrobials were tested against standard microdilution system. Only antimicrobials sensitive to pseudomonas isolates were interpreted on final result like quinolones, cephalosporins, aminoglycosides, beta-lactamase inhibitors, carbapenems, monobactams, anti-infectives, macrolides and colisthemetate respectively. It has been noted that isolates susceptible with third generation cephalosporins may became resistant within three or four days after initiation of therapy. ZONE size inhibition measurement for interpretation of susceptibility and resistance pattern were based on standard content disc of piperacillin/tazobactam 100/10µg, parenteral cephalosporins 30µg (ceftazidime, cefepime), Aztreonam 30µg, Carbapnems 10µg, Colisthemetate 10µg, Amikacin 30 µg, Gentamycin

and tobramycin 10µg with ciprofloxacin 5µg respectively with reference standard according to CLSI of zone of sensitivity and resistance measurement. Intermediate zone size has excluded in this criteria. Resulting outcomes were compared with ATCC standard strain 27853 of pseudomonas aeruginosa.

RESULT

For the determination of antimicrobials susceptibility and resistance pattern, about 210 isolates of pseudomonas aeruginosa were included in this study and detailed study of this opportunistic pathogens based on retrospective studies of past two years. Samples collection for isolation of this bacterium includes Blood, Pus, Ear Swab, Urine, Rectal, Nasal, Mucous and bronchial secretions. Blood and Ear Swab found to be most common source of isolation of this pathogen in hospital acquired infection shown in Table 1 & percentage in Figure 4.

After collection of Data from Intensive care units of eight hospital participated in this study, antimicrobial susceptibility and resistance pattern were analyzed according to their highest frequency of utilization in past two years and detailed study showed their sensitivity and resistance pattern percentages increasing day by day which create emergence of antibiotic multi-drug resistance and improper utilization of antibiotics against opportunistic bacterium pseudomonas aeruginosa shown in figure 1. Figure 1 Illustrate there is increasing frequency in consumption of antimicrobials which leads progression of resistance and shifting of trends to high class of antimicrobials. Antimicrobials resistance and sensitivity pattern shown in Table 2 clearly illustrate there is frequent rise in antibiotic resistance and susceptibility trends for pseudomonas aeruginosa. The percentage of pseudomonas aeruginosa susceptibility is incredibly higher with piperacillin/tazobactam 77%, imipenem 77%, meropenem 71%, ceftazidime 67%, flouroquinolones 66%, Colisthemetate 67% & Aminoglycosides 45% (Table 3).Percent frequency of antimicrobial resistance against pseudomonas aeruginosa to Carbapenems (23% & 29%) and pieracillin (23%) was lower compared to Cephalosporins (90% almost except cefoperazone, cefipime, ceftazidime & cefotaxime) Aminoglycosides (29% tobramycin, gentamycin 31% ,59% amikacin)and quinolones (ciprofloxacin 10%) respectively shown in Table 2.

Figure 3 & 4 Represent Antimicrobial resistance and sensitivity pattern with significant Association among Cephalosporins, Ciprofloxacin and Amikacin decreasing sensitivity and emergence of Carbapenems, piperacillin and colithemetate initiation of resistance with decreasing sensitivity create an emergence of multidrug resistance of antimicrobials against pseudomonas species.

Comparative analysis of Antimicrobials against Pseudomonas aeruginosa clearly reveals the fact there's very close association between antimicrobials utilization with sensitivity and resistance pattern in past two years in the intensive care units of chiefly hospital acquired infection which may create an emergence of antimicrobials resistance in pseudomonas aeruginosa (Fig 5).

DISCUSSION

Rationale cure of communicable disease create serious challenge in our society because it accounts for high rate of mortality and morbidity in past decays. This is alarming situation in healthcare sector in present situation because of high utilization of antimicrobials in particularly in community acquired and hospital acquired infection which is increasing gradually due to decline in susceptibility and hereditary alteration of microbials in patients with immunodeficiency. [1]. Pseudomonas aeruginosa accounts for opportunistic infections which correspond to serious challenge for both hospital and community acquired disease. Which create need for the selection of targated pathogens to encounter problem associate with multidrug resistance. [34,35].

Pseudomonas aeruginosa have highest susceptibility to develop resistance during therapeutic course of antimicrobials which pose a serious challenge regarding its safety and efficacy. Past studies shows Cephalosporins, quinolones and beta-lactame inhibitors were highly sensitive against pseudomonas aeruginosa islolates in past era.bt after many several studies it has been observed there's remarkable reduction in the sensitivity of flouroquinolones against pseudomonas aeruginosa gradually 18 to 37% in last 8 years of experience. Unfortunately inadequate availability of antibiotics with economic aspects of hospital cost cutting imparts greater factor for the development of resistance in past years particularly in ciprofloxacin [33]. Quinolones with their extensive consumption aim to cost effective available in wide range but this strategy is not appreciative with respect to rationale utilization of antibiotic [36]. This life opportunistic bacterium, Pseudomonas aeruginosa often called superbug after emergence of multi-drug resistance due to inadequate penetration on the outer membrane of

the bacterium by adaptive genetic modification make this bacterium highly resistance against variety of antimicrobials. This pathogen creates life threatening problems for immunocompromised patients particularly in hospital acquired infection of intensive care unit.

Several studies reveals the fact this 'superbug' have been resistant adjacent to antimicrobials [2,29,30] accountable for 16% resistant isolates of pseudomonas aeruginosa with multiple drug resistance cases of 1% pseudomonas isolates is of grave matter at the United states of America.[37].pseudomonas aeruginosa accounts for 10% pyelonephritis infections,21% alveolar infection ,13% ENT infections with 3 % septicemia illness including pharyngitis ,laryngitis and tonsillitis after Statistical evaluation by National Nosocomial Infections Surveillance from 1992-1997 at united states acquired in intensive care units.[38].collateral studies of western countries reported 'superbug' pseudomonas aeruginosa secondary leading cause of infectious diseases with reported cases of 19% pylonephritis illness,10% septicemia with 30% of pulmonary infections at the intensive care units (ICUs).[39]

Target therapeutic goal may be achieve with less chances of resistance development by treating this opportunistic infection with combination therapy, single therapy may not appropriate for cure of pseudomonas infection even prolong use may raise potential threat to develop resistance during the course treatment particularly in ciprofloxacin & amikacin observed in various detailed studies. Combination therapy of beta-lactam inhibitors with aminoglycosides may be helpful in optimizing therapeutic goal with aim to diminish the chances of resistance. Although this combination strategy does not always support AmpC- associated adaptive resistance mechanism in regaining therapeutic effectiveness [40,41,42].initiation of carbapenem resistance along with piperacillin create an emergence to control the resistance associated with these highly efficacious medication in our study. Combination therapy may not beneficial in support of hospital economical cost reduction strategy to treat pseudomonas infection but helpful in reduction of prevalence of multidrug resistance due to genetic mutation by this bacterium.

Irrational empirical therapy presents a contributing factor for the development of anti-pseudomonas resistance mechanism with decrease in efficacy. Appropriate selection of antimicrobials with awareness of these opportunistic pathogens among healthcare practitioner can prevent expansion of multi-drug resistance pattern. To encounter problem associated with decline in the sensitivity releveant to increase resistance is to evaluate the mechanism associated with antimicrobial decreases in sensitization with rationale utilization to enhance the efficacy of existing antimicrobials may effective in regaining potency of antimicrobial against pseudomonas isolates.

From the above discussion, it has been observed in detailed study of past two years in intensive care unit of Pakistan healthcare sector high utilization of antimicrobials with irrational practice are key factor responsible for decline in sensitivity with greater extent in adaptation of resistance mechanism should need further investigations to treat this most common opportunistic bacterium with complete surveillience studies required to determine evolution of decrease susceptibility and developed resistance mechanism .

CONCLUSION

Above retrospective finding demonstrate the facts significant rise in antimicrobial resistance correspond to gradual decline in sensitivity against so called superbug P.aeruginosa in detailed comparative evaluation. Prevalence of communicable disease accounts serious challenge against opportunistic bacterium p.aeruginosa particularly in immunocompromised patients is quite difficult to eradicate infections specifically in patient with cystic fibrosis. It has been observed that inadequate utilization of antimicrobial in hospital setting and treatment with single antimicrobial agent is economically cost effective but measure leading cause of reduction in sensitivity and adaptation of resistance mechanism due to genetic mutation alter the action of antimicrobials. Infection associated with p.aeruginosa is quite difficult to treat because of rapid developing of intrinsic resistance mechanism during the course of treatment. In conclusion, from above studies of past two years in hospital and community acquired nosocomial infection we can draw fact there's a continuous rise in multidrug resistance pattern in intensive care unit isolates of p.aeruginosa particularly against flouroquinolones, aminoglycosides and cephalosporins.

Potential strategy to treat nosocomial infection associated with this ubiquitous pathogen is treatment with combination therapy because single utilization of antimicrobials failed to achieve target therapeutic goal with emergence of rapid development of resistance mechanism alter the efficacy of antipseudomonas drugs which is grave concern for high class of antimicrobials particularly later on carbapenem initiation of resistance. Combination therapy may not only helpful to cure

infection also in decline resistance which is more frequent in pseudomonas infection during course of therapy. Combination therapy goal is quite difficult to achieve in hospital setting in economic aspects but very important in achieving target therapeutic goal correspond to decrease in chances of resistance.

After careful consideration we can conclude that there's urgent need for detailed surveillance studies of evolution of complexities of antimicrobial resistance mechanism with development of new drugs to combat the infection associated with p.aeruginosa in immunodeficient patients.

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TABLE #1 . ISOLATED SAMPLES ESTIMATION SOURCE OF

SAMPLES	No. of isolates obtained
Blood C/S	73
Pus C/S	10
Urine C/S	13
TRACHEAL C/S	2
EAR SWAB C/S	67
SPUTUM C/S	17
GLU. C/S	2
CENTRELINE	3
MOUTH C/S	7
NASAL C/S	4
RECTAL C/S	5
TOTAL	203

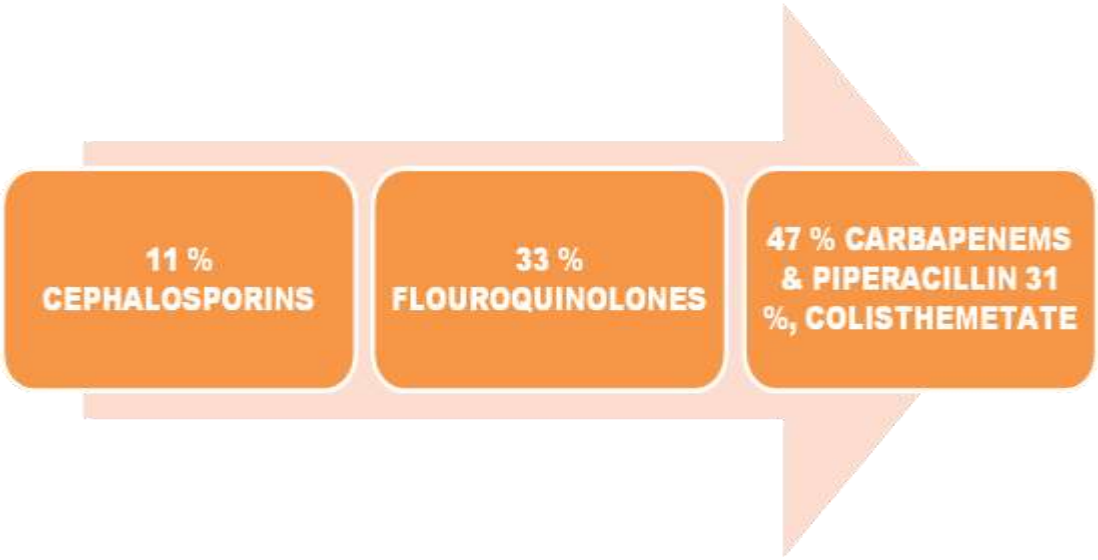

Figure 1 Increasing Frequency Of Utilization of Antimicrobials against P.aeruginosa

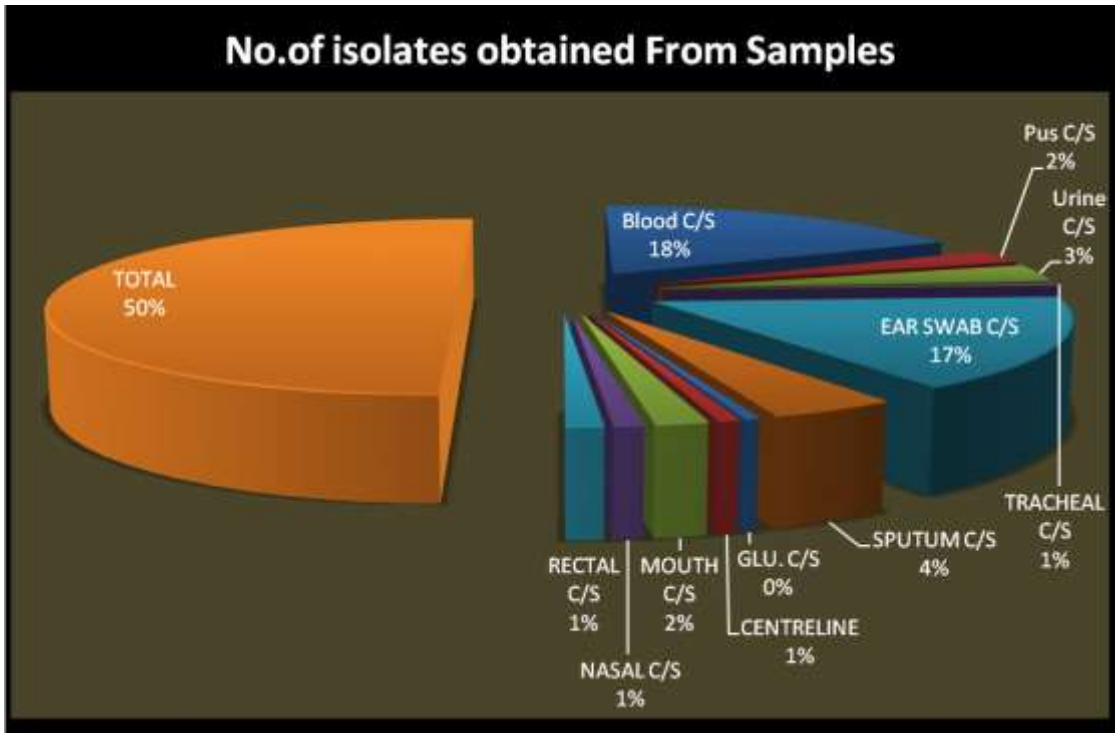

Figure 2 Percentage Frequency of Sample Collection Source

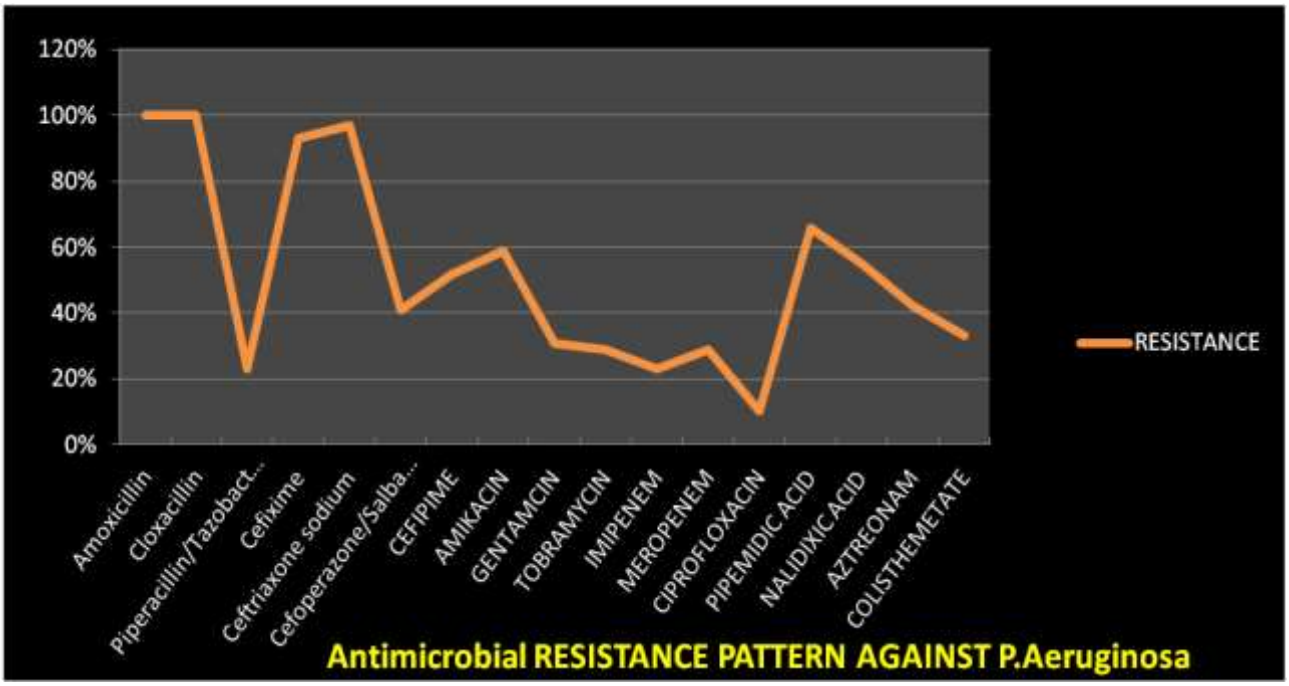

Figure 3 Resistance Pattern of Antipseudomonas Drugs

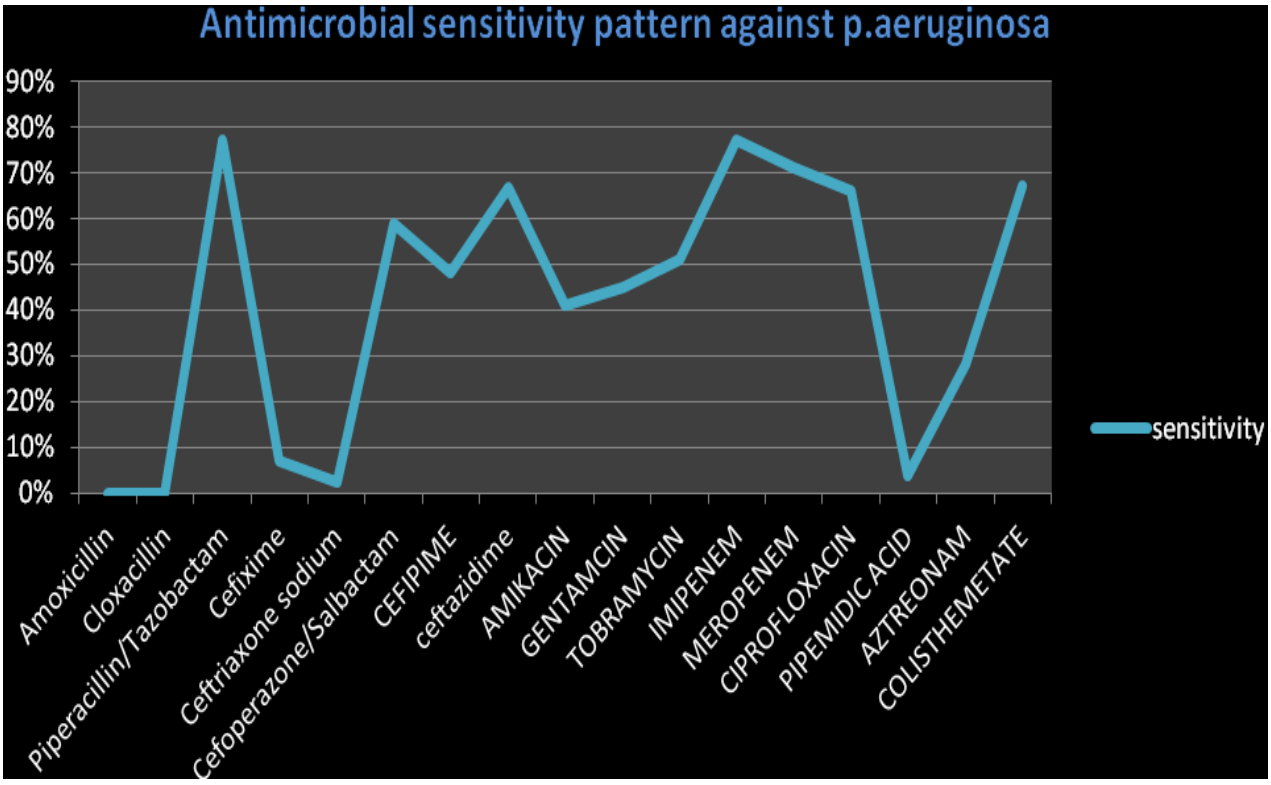

Figure 4 Susceptibilty Pattern against P.aeruginosa

DRUG	RESISTANCE	SENSITIVITY
PENICILLINS		
Amoxicillin	100%	0%
Cloxacillin	100%	0%
Piperacillin/Tazobactam	23%	77%
CEPHALOSPORINS		
Cefixime	93%	7%
Ceftriaxone sodium	97%	2.44%
Ceftazidime	33%	66.60%
Cefotaxime sodium	98%	1%
Cefoperazone/Salbactam	41%	59%
Cefipime	52%	48%
AMINOGLYCOSIDES		
Amikacin Sulphate	59%	41%
Gentamycin	31%	45%
Tobramycin	29%	51%
QUINOLONES		
Ciprofloxacin	10%	66%
Pipemidic Acid	66%	4%
Nalidixic Acid	55%	N.M.T
SULFONAMIDES		
SULFAMETHOXAZOLE	61%	9%
MACROLIDES		
Erthromycin	14%	N.M.T
MONOBACTAMS		
Aztreonam	42%	28%
CARBAPENEMS		
Imipenems	23%	77%
Meropenems	29%	71%
COLISTIMETHATE	33%	67%
ANTI-INFECTIVES		
Nitrofurantoin	8%	N.M.T

Table No.2 Percentage of Antimicrobial Resistance and sensitivity pattern against

Where N.M.T Represent: No More Tested

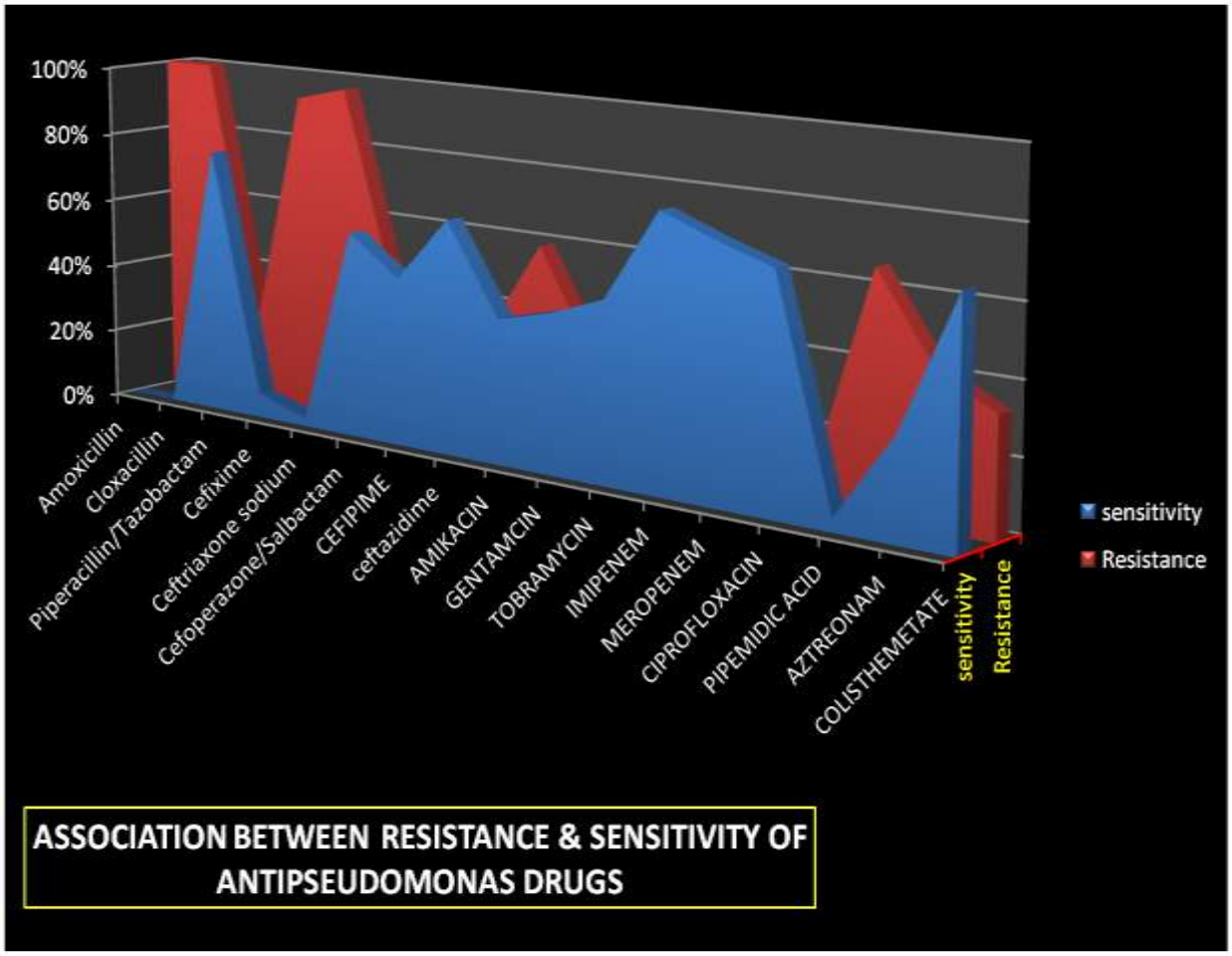

Figure 5 comparative analysis of susceptibility and resistance pattern of antimicrobials against pseudomonas aeruginosa

DRUG WITH SUCCESFUL SENSITIVITY		Percentages
Piperacillin/Tazobactam	↑	77%
Imipenem	↑	77%
Meropenem	↑	71%
Colisthemetate	↑	67%
Ceftazidime	↑	67%
Ciprofloxacin	↑	66%
Tobramycin	↓	51%
Gentamycin	↓	45%
Amikacin	↓	41%

Table 3 Antimicrobials with highest sensitivity pattern against

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