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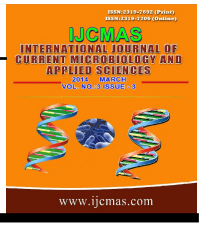

Original Research Article

Prevalence and Resistance pattern of *Pseudomonas* strains isolated from ICU Patients

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Keywords

Antibiotic susceptibility; Carbapenems; Intensive care units; *Pseudomonas aeruginosa.*

A B S T R A C T

Intensive care units (ICUs) are often lodged with a high frequency of nosocomial infections caused by multi drug resistant nosocomial pathogens. Among the different pathogens, during the recent years *Pseudomonas aeruginosa* has emerged as one of the most problematic and important gram negative pathogens. The present study was conducted with an aim to identify the frequency of *Pseudomonas aeruginosa* from various clinical samples in ICUs and to investigate their resistance pattern against various antibiotics widely used for treatment – mainly antipseudomonal drugs and against Carbapenems. The study was carried out from April 2011 to March 2013. Antibiotic susceptibility testing was done by disc diffusion method according to NCCLS guidelines. *Pseudomonas* was isolated from 17.36 % (274/1578) of the patients in ICUs. Most effective antibiotic was the carbapenem namely Imipenem even though it showed 20.8% resistance. Our results show high alarming resistance to Aminoglycosides, 3rd generation cephalosporins and quinolone. To curb the emergence and spread of the resistance in ICUs, judicial use, regular and periodic monitoring of drug resistance should be considered.

Introduction

Antibiotic resistance is a major concern in contemporary medicine. The ongoing emergence of resistant strains that cause nosocomial infections contributes substantially to the morbidity and mortality of hospitalized patients (Acar JE et al., 1997, Goldmann DA et al., 1996). Bacteria from intensive care units (ICUs) have the highest proportion of resistance (Fridkin SK et al.,1999). *Pseudomonas aeruginosa* is one of the main organisms responsible for drug-resistant nosocomial infections. Aerobic, oxidase positive, non fermenter, *Pseudomonas aeruginosa* is becoming day by day a very common pathogen. It is being isolated from various clinical samples. It belongs to the genus Pseudomonas, which is widely distributed in nature. Although it is considered as a contaminant, it may colonize healthy humans without causing disease, but sometimes, it's potential to act as a pathogen can be identified without any doubt due to its isolation in clinical

samples with positive disease impact and as an agent of nosocomial infection. It is regularly a cause of nosocomial pneumonia, nosocomial urinary tract infections, surgical site infections, infections of severe burns and infectious patients undergoing either chemotherapy for neoplastic diseases or antibiotic therapy. Multiple factors contribute to make *Pseudomonas aeruginosa* a nosocomial pathogen, for example, injudicious administration of broad-spectrum antibiotics, instrumentation, and intrinsic resistance of microorganisms to numerous antimicrobial agents (Bonglio G et al.,1998). Not only that but this non fermenter is one of the β- lactamase producing bacteria which is responsible for multi drug resistance. This has fueled for the development of newer antibiotics. Carbapenems were the God sent agents in fighting these β lactamase producing bacteria. Carbapenems, due to their stability to hydrolysis by most ß-lactamases, have been the drugs of choice for treatment of infections caused by penicillin-resistant or cephalosporin-resistant gram-negative infections (Mendiratta DK et al.,2005). Carbapenems first introduced in 1980 are now frequently used as a drug in treating serious infections caused by multi drug resistant gram negative bacilli. These antibiotics are stable to β- lactamases including extended spectrum β- lactamases (ESBLs) and AmpC produced by gram negative bacilli. Unfortunately during the recent years resistance was also shown towards these Carbapenems (Deshpande LM et al.,2004).

Keeping in view the paucity of information on carbapenem resistant *P.aeruginosa* isolated from various samples collected from patients admitted in intensive care units, we undertook the present study to determine its incidence and sensitivity data with special reference to Carbapenems resistance.

Materials and Methods

The present study was conducted at the Department of Microbiology in a teaching hospital during the period from 01/04/2011 to 31/03/2013. Samples received from various ICU units were taken into study. All the samples received from ICU units were inoculated on 5% sheep blood agar and MacConkey agar plates and incubated overnight at 37°C aerobically. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques (Collec JC et al., 1996). A sample was included in the study only if it was positive for less than two types of bacteria.

Pseudomonas aeruginosa was identified by colonial morphology, a positive oxidase reaction, pyocyanin production, motility. Colonies which displayed a positive oxidase test were further subjected to biochemical reactions.

Antimicrobial sensitivity was performed on Muellar-Hinton agar (Hi-Media , India) by Kirby – Bauer disk diffusion method as per National Committee for Clinical Laboratory Standards (Wayne PA, 2002).

The routine antibiotic sensitivity tests were put up for the carbapenem, Imipenem (10µg disc). Isolates were considered carbapenem resistance when the zone of inhibition around Imipenim disc was ≤ 13mm. Sensitivity was also investigated for Amikacin, Gentamycin , Tobramycin, Ceftazidime, Cefotaxime, Ciprofloxacin, Chloramphenicol, Norfloxacin, Cefoperazone - sulbactam and Piperacillin.

Results and Discussion

Pseudomonas was isolated from 274 (17.36 %) out of 1578 patients whom various clinical samples (such as urinary, surgical site, tracheal aspiration etc.,) were taken. Of them 57 (20.8%) were found to be resistant to Carbapenems. The highest isolation rates *Pseudomonas* strains were observed in among the following departments in descending order: surgical ICU, casualty, internal cardiovascular surgical and so on. Figure 1 shows the distribution of isolates from various ICU.
The age group of 20-40 showed majority culture positives. Table 1 shows the distribution of positive cultures according to different age groups.

Of these 183 patients (66.78 %) were males and 91(33.21 %) were females. Among the different antimicrobials employed, Imipenem was the best followed by Norfloxacin, Amikacin and Piperacillin. Majority of resistance were shown by Gentamicin and Tobramycin.
Carbapenem resistance was found to be 20.8%. The overall resistance of *Pseudomonas aeruginosa* to different antimicrobials was 40.87 % *Pseudomonas aeruginosa* is an important opportunistic and nosocomial pathogen which is becoming a great threat in treating infections. Despite advances in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life threatening infections caused by *Pseudomonas aeruginosa* continue to be hospital infections. ICU patients are particularly susceptible to nosocomial infection because the normal skin and mucosal barriers to infection are commonly compromised by the use of invasive devices (Jarvis WR et al., 1996). *Pseudomonas aeruginosa* is a β-lactamase producing bacteria which is responsible for multi drug resistance. Multi drug resistance is a major treat for the physicians in prescribing suitable drug treatment. Carbapenems were able to combat theses β-lactam bacteria. They are one of the essential antibiotics in the armamentarium against serious nosocomial infections. Development of resistance against these Carbapenems has become a major concern. Among physicians, fear of litigation and perception of patients expectations contribute to antibiotic misuse and, therefore, bacterial resistance. Inappropriate duration of antibiotic therapy is also another cause for the development of resistance (Philipee E et al., 1997).

In this study, the highest *Pseudomonas* isolation rate was 26.64% from SICU followed by COT (20.43%) and cardiovascular surgical ICU (17.15%). The surgical units are a very susceptible habitat for bacterial colonization (Cookson BD et al., 1999). ICUs are generally considered epicenters of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria.

The most important risk factors are obvious, such as excessive consumption of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population with severe underlying diseases (Weber DJ et al., 1999). Nowadays, the prevalence of *Pseudomonas* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections (Maniatis AN et al., 1997). The overall isolation rate of *Pseudomonas* from various ICUs was 17.36% .

This study was observed that the incidence of resistance against carbapenem, Imipenem was 20.8%. This study shows slightly low percentage and correlates with other study which showed 30% (Gupta et al.,2006).The resistance to carbapenem is due to reduced levels of drug accumulation or increased expression of pump efflux. The resistance may also be due to the production of metallo-β-lactamases (MBL) which can be chromosomally encoded or plasmid mediated(Hancock REW et al., 1998). Most of these MBL confer resistance to not only Carbapenems but also to other β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam (Karlowsky JA et al., 2003).Limited literature is available regarding the prevalence of resistance to Carbapenems in various clinical isolated obtained from ICU in our country.

Recently, increased resistance has been observed against 3th generation cephalosporins for gram negative bacilli, especially *Pseudomonas aeruginosa* (Holloway WJ et al., 1996). Cefepime and Ceftazidime are the commonest 3th generation antibiotics. Ceftazidime is also an antipseudomonal drug. It has a key role in resistance detection in ICUs. However in ICUs, antibiotic therapy protocols are different in almost all countries and there are also regional differences (Nathwani D et al., 1998).

Resistance to antipseudomonal drugs in our study was found to be ceftazidime (58.02%), ciprofloxacin (37.22%), piperacillin (28.46%), imipenim (20.8%). Among these anti-pseudomonal drugs, Imipenem and Piperacillin were found to be effective when compared to Ceftazidime and Ciprofloxacin. So, Imipenem which is both an anti pseudomonal drug and carbapenem was the best drug.

Among Aminoglycosides, Amikacin (74.45 % susceptible) was found to be quite effective when compared to the other two antibiotics (70.07% isolates were resistant to tobramycin, 71.89% isolates were gentamicin resistant). However, overall resistance of *Pseudomonas* aeruginosa was found to be very high (40.87%). In various studies, across the world varying resistance rates (4-60%) has been seen towards these drugs (Forster DH., 1998, Gonlugur U., 2004).

Thus, in ICUs, empirical antibiotic treatments should be avoided and treatment should be carried out using antibiotic susceptibility tests. ICUs should be regularly inspected for Pseudomonas colonization which shows a strong resistance pattern against the various antibiotics. Colonization of ICU patients with antimicrobial-resistant pathogens can lead to clinical infection because of breakdown of normal host defenses.

Measures to reduce antibiotic resistance include evidence-bases selection of antibiotics with adequate dosage, surveillance for resistance, preventing the spread of resistant organisms, development of new drugs to block resistance mechanisms. Steps need to be taken to prevent antimicrobial resistance or else this emerging menance would erode the strength of the life-saving antibiotics, leave them with the negligible effect of placebo and all the resources allocated to research and treatment will be a great waste in an already poor developing country like ours.

The high frequency of multidrug resistant Pseudomonas in ICUs suggest that, we

Figure.1 ICU wise distribution of *Pseudomonas aeruginosa*

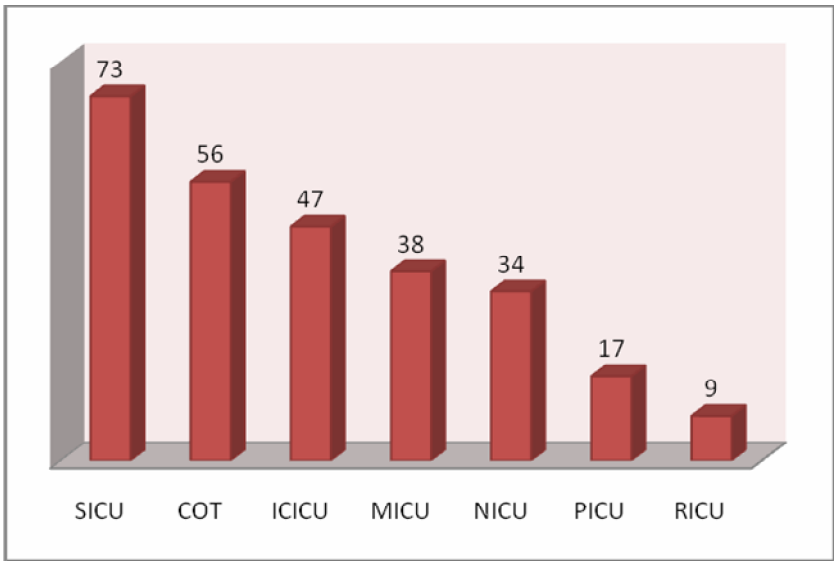


Table.1 Isolation of *Pseudomonas* strains in different age groups.

Age (years)	Patients ή
< 20	74
20-40	82
40-60	77
>60	41
Overall total	**274**

Figure.2 Sex distribution

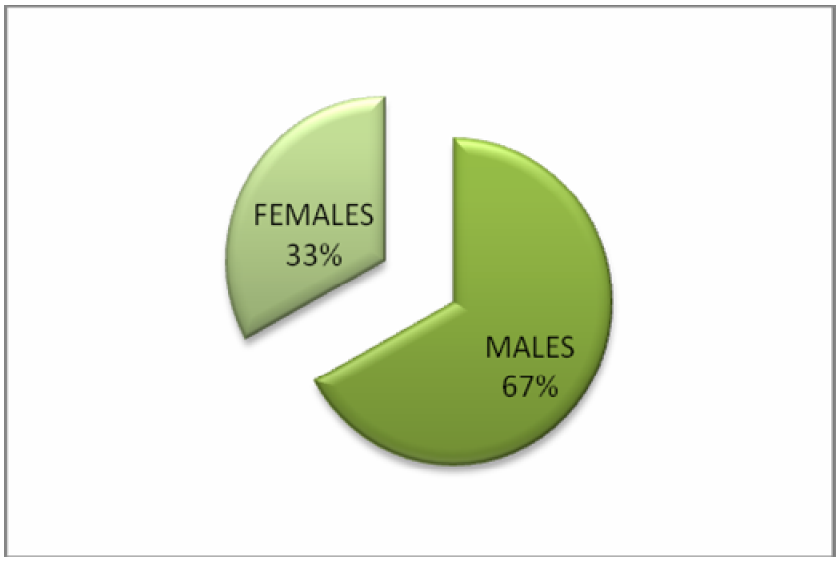
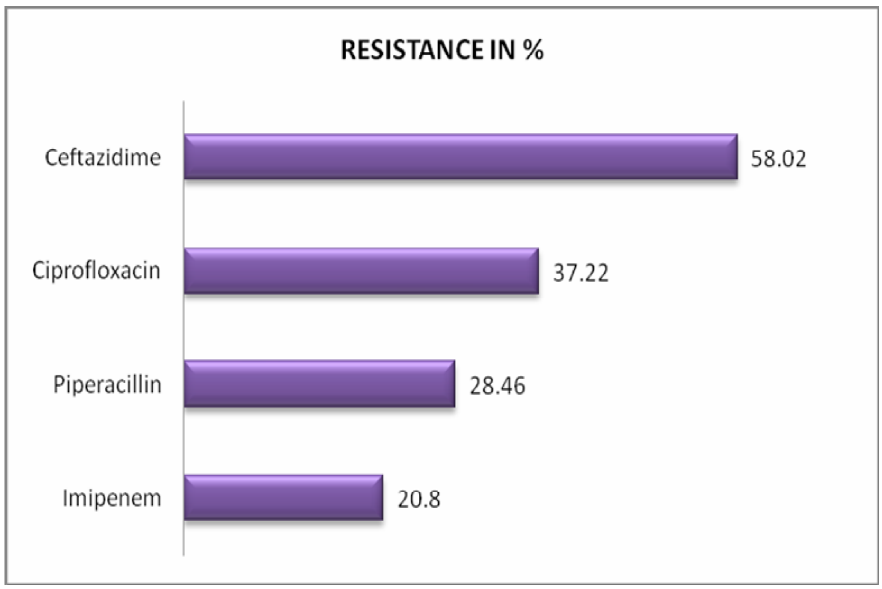


Table.2 Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Antibiotic	No of isolates susceptible	Susceptibility %	Resistance %
Amikacin	204	74.45	25.54
Gentamicin	77	28.10	71.89
Tobramycin	82	29.92	70.07
Ceftazidime	115	41.97	58.02
Ciprofloxacin	172	62.77	37.22
Cefotaxime	175	63.86	36.13
Norfloxacin	208	75.91	24.08
Cefoperazone salbactum	174	63.50	36.49
Imipenim	217	79.19	20.8
Piperacilline	196	71.53	28.46

Figure.3 Resistance rates of anti- pseudomonal agents.



need to prescribe broad spectrum antibiotics more wisely in order to reduce pressure on sensitive strains. This could be beneficial for saving ICU patients and preventing the spread of resistant isolates in these critical wards.

Even though in our study the carbapenem, Imipenim showed resistance, it was much better in effectiveness when compared to other anti pseudomonal drugs tested in this study. So, this drug remains as the choice of agent for treatment.

However, clinical efficacy of monotherapy or combined administration of these antibiotics remains to be assessed. Often than a single drug, combination of drugs are quite effective.

However to overcome inappropriate treatment of patients, periodical antibacterial susceptibility surveys for nosocomial infections in ICU wards are warranted. Regular monitoring can also aid infection control in determining how to focus efforts in reducing the emergence and spread of antimicrobial resistant pathogens.

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