Antimicrobial resistance pattern of *Pseudomonas aeruginosa* in a tertiary level hospital in Southern India

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Abstract

Introduction: Pseudomonas have now a days become a major cause of nosocomial and community acquired infections. They are widely distributed in the hospital environment which makes them difficult to eradicate. *P.aeruginosa* has become the leading cause of hospital acquired infection because of its recent trend to gain resistance to most of the drugs used against it causing a multidrug resistance. Therefore it is necessary to know the changing pattern so as to achieve a good therapy for pseudomonas infection. Therefore this study was conducted to determine the antimicrobial resistance pattern of *P.aeruginosa* with different parameters from the patients attending the tertiary level hospital in Chennai.

Objective: The aim of the study was to enumerate the antimicrobial sensitivity pattern of Pseudomonas infection from various samples in a tertiary care hospital.

Materials and Methods: The study was carried out in Microbiology department of a tertiary care hospital during the period of February 2016 to January 2017. A total of 93 non repetitive clinical isolates of *P.aeruginosa* were collected from different samples.

Result: In the present study the males 51(54.8%) were more affected than females 42(45.2%). Age distribution showed more to be affected among adults of age 40 and above. Amikacin, Ciprofloxacin and Gentamicin shows 96.8% sensitivity. Among Cephalosporins highest resistance was observed among 1st generation cefazolin (83.9%) followed by 2nd generation Cefotaxime (17.2%) and Ceftazidime (23.7%) the least was seen among 4th generation Cefepime which showed 6.5% resistance. All isolates were sensitive to Imipenem.

Conclusion: Restriction of antibiotic usage must be formatted by each institution, to combat the emergence of multi drug resistant *P. aeruginosa*. Imipenem was the only anti-pseudomonal drug against which all isolates of *P. aeruginosa* were fully sensitive. We recommend a restricted and a more rational use of this drug in this hospital setting

Keywords: *P.aeruginosa*, Antimicrobial resistance, Hospital acquired infection.

Introduction

Infection by Pseudomonas has now a days become a major threat among the nosocomial and community acquired infectionsas they are widely distributed in the hospital environment and are difficult to eradicate. The clinically important species of pseudomonas are *P.aeuroginosa*, *P.putida*, *P. fluroscence*, *P.stutzeri*, *P. alcaligenes*, *P.pseudoalcaligenes*, *P.putrefaciens*, and *P.mendocina*. However the most important among these is *P.aeruginosa*, as the rate of infection produced by other species are far less when compared to the infections produced by the *P.aeruginosa*.

The resistance produced by Pseudomonas is complex and has varied mechanisms. *P.aeruginosa* is notorious as it is resistant to many structurally unrelated antimicrobial drugs, this property may be attributed to low permeability of its outer membrane, acquired resistance through gene from other organism via the plasmids, transposons, bacteriophages, and biofilm production.^{2,3} Despite the advances in medical and surgical care and availability of wide range of antipseudomonal agents, treating the infections have become a challenge. Infections produced by ESBL(Extended spectrum beta lactamases), MDR (Multi drug resistance), MBL(Metallo beta lactamases)

producing *P.aeruginosa* strains is rising in an alarming rate. This creates a serious health problem resulting in the increased burden of mortality, morbidity, and high health care cost.

The resistance pattern shown by pseudomonas is not the same in all countries. The highest is seen Turkey(70-93%), followed by India (43-68%), Bangladesh(40-86%), Pakistan (35-40%), Iran (36-75%) but low in Singapore(10-23%), West Indies(2.6-12.3%) and Malaysia (6.7-23%). There is also a trend in which the resistance percentage is increasing in some countries as seen in United Kingdom where the resistance percentage was 8% in 2001 and has raised to 43% in 2003, in Saudi Arabia it has increased from 10% 1998 to 40 % in 2004.

P.aeruginosa has become the major cause of hospital acquired infection because of its recent trend to gain resistance to most of the drugs used against it causing a multidrug resistance. Therefore it is necessary to know the changing pattern so as to achieve a good therapy of pseudomonas infection. Therefore this study was conducted to determine the antimicrobial resistance pattern of P.aeruginosa with different parameters from the patients attending the tertiary level hospital in Chennai.

Materials and Methods

The study was carried out in a tertiary care hospital during the period of February 2016 to January 2017. A 93 total of non repetitive clinical of P.aeruginosa were obtained from various specimens like high vaginal swab, sputum, CSF, body fluids, pus, ear swab and eye swab of patients attending the out patient departments & in-patients from various wards including Intensive care units. Ethical committee clearance was obtained from the Institute and informed consent was obtained from all the patients. It is a prospective study and data was analysed statistically using SPSS version 23. All the samples were inoculated onto nutrient agar plate, blood agar plate, Maconkey agar plate and incubated at 37°C overnight. The colonies were tested for oxidase test and other biochemical tests like Indole test, Citrate test, Urease test, Triple Sugar iron agar test and Mannitol motility media test were done for identification.

The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available discs (Hi-Media) on Muller Hinton Agar using Gentamycin (10mcg), Amikacin (30mcg), Ciprofloxacin (5mcg), Cefazolin, Ceftazidime (30mcg), Cefotaxime (30mcg), Imipenem (10mcg), Cefepime (30mcg).

Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

In this study among various clinical samples a total of 93 isolates of *P.aeruginosa* were identified.

Table 1: Distribution of age among the clinical isolates of *P. aeruginosa*

Age	Frequency	Percentage		
0-5	2	2.2		
5-20	7	7.5		
20-40	27	29.0		
Above 40	57	61.3		
Total	93	100.0		

Fig. 1:Sex distribution among clinical isolates of P.aeruginosa

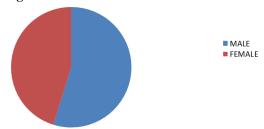


Table 2: Distribution of sample among clinical isolates of P.aeruginosa

Sample	Frequency	Percentage
Sputum	46	49.5
HighVaginal Swab	14	15.1
Pus	21	22.6
CSF	1	1.1
Ear swab	5	5.4
Eye swab	3	3.2
Suction tube	1	1.1
Drainage Tube	1	1.1
Throat Swab	1	1.1
Total	93	100.0

Fig. 2: Antibiotic Susceptibility patterns of *P. aeruginosa* clinical isolates

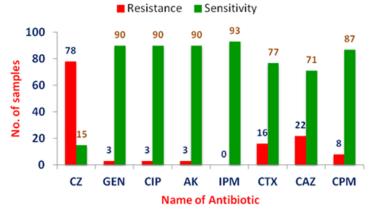


Table 3: Age Vs Sample using chi-square test

	Sample									
Age		High Vaginal			Ear	Eye	Suction	Drainage	Throat	Total
	Sputum	Swab	Pus	CSF	swab	swab	tube	Tube	swab	
< 5	0	0	1	0	0	1	0	0	0	2
05-20	1	1	0	0	4	0	0	0	1	7
20-40	9	7	9	0	1	0	1	0	0	27
>40	36	6	11	1	0	2	0	1	0	57
Total	46	14	21	1	5	3	1	1	1	93

Fisher Exact test value is: 56.536

P value: .000 (It is statistically significant)

Table 4: Sex Vs Sample using chi-square test

Sex	Sample							Total		
	Sputum	High Vaginal	Pus	CSF	Ear swab	Eye swab	Suction tube	Drainage Tube	Throat swab	
		Swab								
Male	26	0	16	0	4	3	1	1	0	51
Female	20	14	5	1	1	0	0	0	1	42
Total	46	14	21	1	5	3	1	1	1	93

Fisher Exact test value is: 30.077

P value: .000 (It is statistically significant)

Discussion

Pseudomonas aeruginosa has emerged as one of the significant causes of morbidity and mortality among in-patients. In this study, a total of 93 isolates of *P.aeruginosa* was isolated and identified from various clinical samples, from the hospitalized patients and their antimicrobial susceptibility patterns were determined. Most of them belonged to older age group of above 40 (61.3%) and found to be least 2(2.2%) among children 0-5 years. A similar finding was observed in another study which showed 1.78% of pseudomonas infection among children which increased with age of 40 and above.⁴

A study conducted in Chennai showed the age distribution of pseudomonas infection among diabetics to be ranging between 36-75 years which is comparable to our study.⁵ Another study by Golshani Z *et al* in the year 2012 in Iran showed the mean age of all the patients infected with *Pseudomonas aeruginosa* to be 45 years.⁶ Another recent study conducted in 2016 also shows majority of patients with *P.aeruginosa* infection were above 60 years of age.⁷

In the present study the males 51(54.8%) were more affected than females 42(45.2%). A similar pattern was seen in the stuy by Golshani Z *et al* which showed 56% males and 44% to be females.⁶ Another study among diabetic patients also shows concordance to our study with Male:Female ratio of 2:1.⁵ A recent study also reveals that males were infected more than females with *P.aeruginosa* (59.3%).⁷

Regarding Clinical specimens majority of *P.aeruginosa* strains were isolated from sputum specimen (49.5%) followed by pus specimen (22.6%) and high vaginal swab(15.1%). A similar finding with majority of sputum specimen (38%) is seen in a study

by Ahmad OB *et al.*⁷ and on contrary certain study like Patel H *et al* shows sputum specimen(3%) to be among the minority specimens and wound or pus specimen(70%) to be the majority. Pus specimen is the second most common samplein our study.⁸ Another study by Chander*et al* showed pus specimen to be around 27.60% and sputum sample to be 24.10% and third common was urine sample with 20.70%. This study also shows high vaginal swab incidence of *P.aeruginosa* to be around 3.45 which is quite lower than our study.⁹ More than 80 % of sample is obtained from pus and sputum specimen in certain studies in India by Mohanasundaram*et al*,¹⁰ Arora *et al*¹¹ and Chander*et al*.⁹

There has been an alarming raise in resistance to different anti-pseudomonal drugs specifically among hospital strains world-wide. 12,13 This pose a serious problem in the management of disease due to these organisms. The antibiogramof *P. aeruginosa* to the eight anti-microbial agents tested shows a varied pattern among the isolates studied. In this study all the *P. aeruginosa* isolates were found to be sensitive to imipenem and this may be attributed to the limited use of imipenem in this hospital. This is consistent with a report published in 2002 in Mangalore, India. 14 Certain other recent studies show development of various levels of resistance to imipenem. 10,11,15,4

Amikacin, Ciprofloxacin and Gentamicin shows 96.8% sensitivity .These are the most effective drugs for routine use against *P.aeruginosa*. A study Nepal shows amikacin (81.4% sensitive) and ciprofloxacin (70.3% sensitive) among *P. aeruginosa* strains examined. A Study by Ahmed *et al* in 2013 shows sensitivity to Amikacin, Ciprofloxacin and Gentamycin to be 80.5%, 43.6% and 56.1% respectively. In

contrast another recent study shows sensitivity to Amikacin, Ciprofloxacin and Gentamycin to be 18.96%, 79.31% and 13.79% respectively. 18

From studies like Hancock et al, 19 Quinn et al, 20 Saderet al²¹ high rate of resistance against carbapenem, quinolones and third generation cephalosporin had been detected in Pseudomonas aeruginosa. Cephalosporins highest resistance was observed among 1st generation cefazolin (83.9%) followed by 2nd generation Cefotaxime (17.2%) and Ceftazidime (23.7%) the least was seen among 4th generation Cefepime which showed 6.5% resistance. This was comparable to a study by Ahmed OB et al.7 which showed 29.6%, 28.7%, 32.4% resistance to Cefotaxime, Ceftazidime and Cefepime respectively. In contrast a study by Fathimaet al shows 40% resistance among Cefepime²² and a study in Egypt showed increasing trends of resistance to cephalosporins like 77.2%, 91.2% and 98.2% to Cefotaxime, Ceftazidime and Cefepime respectively.¹⁷ Another study also revealed increase in trend of resistance pattern by showing 68% resistance to both Ceftazidime and Cefepime.⁶

In this study Colistin and Aztreonam were not used. They both are used as last resort of antibiotics in treatment of *P.aeruginosa*. The toxic effects of Colistin should be weighed before therapeutic use.

Conclusion

In conclusion, restriction of antibiotic usage must be formatted by each institution, to combat the emergence of multi drug resistant P. aeruginosa. Imipenem was the only anti-pseudomonal drug against which all isolates of *P. aeruginosa* were fully sensitive. We recommend a restricted and a more rational use of drug in this hospital setting. Amikacin, ciprofloxacin and semi-synthetic penicillin with betalactamase inhibitors are the preferred drugs for optimal management of infections caused by P. aeruginosa. The lack of newer antimicrobial agents with activities against P. aeruginosa, makes periodic studies on the antimicrobial resistance patterns very important.A solution can be brought by continuous efforts of an Infection control committee to promote greater understanding of this problem. Adequate hand washing to prevent spread of organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

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