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Original Research Article

A study on susceptibility patterns, resistance mechanisms and cross- resistances of antibiotics against *Pseudomonas aeruginosa* in a teaching hospital at PuducherryHemalatha Gurumurthy^{1,*}, G K Poongothai², K Bhaskaran²¹Dept. of Microbiology, Melmaruvathur Adhiparasakthi Institute of Medical Sciences, Melmaruvathur, Tamil Nadu, India²Dept. of Microbiology, Aarupadaiveedu Medical College and Hospital, Puducherry, India

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ABSTRACT

Background: *Pseudomonas aeruginosa*, a gram negative bacteria causes lung and nosocomial infections, mostly infect the body after surgery or invasive techniques. There has been a increasing prevalence in drug resistant variants in the recent years.

Objectives: 1. To determine the antibiotic susceptibility patterns of *P. aeruginosa*; 2. To assess the antibiotics used against *P. aeruginosa* and the cross-resistance pattern existing between them; 3. To evaluate the possible resistance mechanisms of *P. aeruginosa* by phenotypic techniques.

Materials and Methods: Thirty six consecutive, nonduplicate *P. aeruginosa* isolates were collected between January to July in the year 2018 from the hospital pus samples. The isolates showed synthesis of pyocyanin and a oxidase positive reaction. Kirby bauer's disc diffusion method (HIMEDIA). was used for assessing the sensitivity of drugs. Disk approximation test was done to check the prevalence of inducible β -lactamases. Modified Hodge test was done to assess the metallo- β -lactamase activity. Double disk synergy method had been preferred to evaluate the extended-spectrum beta-lactamase (ESBL) activity.

Results: The most sensitive antibiotic was found to be ciprofloxacin which is followed by amikacin and ceftazidime ($p < 0.05$). 36% of the samples were resistant to more than one antibiotic groups. Cross-resistance was observed between the antibiotics. 53% of the samples had Inducible β -lactamases. Eighty percent of the samples which were non-resistant to ceftazidime showed positive reaction for inducible beta-lactamase. 2% isolates by DDS method showed the presence of ESBLs. The study samples did not show the presence of Metallo- β -lactamases.

Conclusion: Strict adherence to the recent trend of "reserve drugs" concept and minimizing the misuse of antibiotics can bring down the drug resistance and morbidity. The addressal of irrational and inappropriate use of antimicrobials among the clinician is the need of the hour.

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1. Introduction

Pseudomonas belonging to the family of Pseudomonadaceae is a Gram-negative bacterium which can cause serious infections.¹ *Pseudomonas aeruginosa* is an aerobic and rod-shaped bacteria which colonize the human host and this opportunistic organism adapts to the inhabiting environment.^{2,3} These organisms can inhabit

even in water sources present in hospital utilising the minimal nutrients in them e.g. tap water. They can dwell well in the soap solutions with hexachlorophene, detergents and also in certain antiseptics.

Nearly 10% of the general population had *P. aeruginosa* in the colonic bacterial flora and it is also present in water, skin and soil. In hospitalised patients, it inhabits the moist skin and can invade the upper respiratory passages. It can survive even in intravenous fluid, distilled water,

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and anaesthesia equipment. *P. aeruginosa* can multiply in simple aqueous solutions which in turn can cause the contamination of respiratory therapy.

The prime cause for morbidity and mortality in patients with cystic fibrosis are the respiratory infections due to *P. aeruginosa*.⁴ There has been a global challenge for clinicians as there is rising trend in antibiotic resistance for *P. aeruginosa*. As it is ubiquitous bacterium of environmental origin, the organism survive in wound, skin, urinary tract and respiratory passage and great difficulty is faced to treat the infections caused by them.^{5–13}

The exotoxins, endotoxin, releasing enzymes are the various virulence factors that play an major role in pathogenesis of the organism. The endotoxin released by them is the prime factor for the development of bacterial septicaemias and septic shock.

Few strains have a protective mechanism to prevent the antibody neutralization, as they have “type III secretion system” that directly transmit the bacterial exotoxin into the adjacent human cell. Few other strains which cause cystic fibrosis have a predominant glycocalyx- slime layer that helps in its adherence to human mucous membrane.

Production of cephalosporinase, antibiotic inactivating enzymes, the constitutive expression of efflux pumps in them and reduced permeability of its outer membrane are the major causes for its resistance to various antibiotics. outer membrane with restricted uptake of antimicrobials, Energy-dependent efflux and β -lactamases are the secondary resistance mechanisms.^{14–16}

Due to its dynamic genome that has genetic plasticity and metabolic versatility, this highly robust and adaptive robust organism can survive in a greater environmental variations.

Drugs such as polymyxin B, cefoperazone, amikacin, cefepime, meropenem and piperacillin, were considered to have high efficacy *anti-P. aeruginosa* antibiotics with less resistance potentials before twenty years and it is not true today.¹⁷ Several biocides such as preservatives, antiseptics and disinfectants are considered less effective against *P. aeruginosa* as these organisms exhibit a wide range of intrinsic antimicrobial resistance as well as the tolerance.^{18–20}

Recently several antibiotic combinations are found to have the action against the multi drug resistant strains of *P. aeruginosa* and they are readily available in the market. In vitro and clinical case reports have proved that drug resistance was appreciable even with the newer antibiotics.¹⁷

The World Health Organization has recognised that *P. aeruginosa* must be given priority on the view of development of newer drugs. This is due to the world wide emergence of multidrug-resistant (MDR) and high-risk clones in *P. aeruginosa*, which are resistant to almost all the antimicrobials.²¹

There is only a limited knowledge on the antibiotic sensitivity pattern in the southern part of India. In a situation like this, our study was planned to assess the current antibiotic susceptibility forms of *P. aeruginosa*, to evaluate the cross-resistance patterns among widely used antipseudomonal antibiotics in our hospital and to determine the possible resistance mechanisms by phenotypic techniques.

2. Materials and Methods

This cross-sectional study was carried out in a tertiary care set up in south India between January 2018 and July 2018 using purposive sampling technique for *P. aeruginosa*. Prior Institutional ethical committee approval was obtained. Thirty six consecutive, nonduplicate isolates of the same were collected from pus samples.

Almost all the isolates showed synthesis of pyocyanin and an oxidase positive reaction. Kirby bauer's disc diffusion method (HIMEDIA) was used to assess the sensitivity of cefoperazone/sulbactam, ciprofloxacin, gentamycin, amikacin, imipenem, cefepime, meropenem, piperacillin/tazobactam and ceftazidime. Disk approximation test was done to check the prevalence of inducible β -lactamases.²² Modified Hodge test was done to assess the metallo- β -lactamase activity.²³ Double disk synergy method had been preferred to evaluate the extended-spectrum beta-lactamase (ESBL) activity.²⁴ *P. aeruginosa* ATCC 27853 was used as reference strain. All the data was recorded in Microsoft excel sheet and was analysed using SPSS software. The antibiotic sensitivity pattern was expressed as frequencies and percentages. Kappa statistics was used find out the agreement between different antibiotics. $P < 0.05$ was considered statistically significant.

3. Results

The most sensitive antibiotic was found to be ciprofloxacin which is followed by amikacin and ceftazidime ($p < 0.05$). 36% of the samples were not susceptible to more than one group of antibiotics. Cross-resistance was observed between the antimicrobials (Table 1).

About all of meropenem resistant isolates were also resistant to imipenem ($\kappa = 0.92$, $p < 0.001$) and 75% of them were resistant to piperacillin/tazobactam ($\kappa = 0.464$, $p < 0.001$). Seventy-five percent of carbapenem resistant isolates were susceptible to ciprofloxacin and amikacin. 50% percent of ceftazidime resistant isolates were also resistant to other -lactams, especially cefepime ($\kappa = 0.28$, $p < 0.043$) and also cefoperazone ($\kappa = 0.320$, $p < 0.030$). (Table 2)

53% of isolates showed positive reaction for Inducible β -lactamases. Eighty percent of the samples which were non-resistant to ceftazidime showed positive reaction for

Table 1: Distribution of the study samples according to their sensitivity pattern

Drug	Sensitive		Resistant	
	Frequency	%	Frequency	%
Imipenem	7	19.4	29	80.6
Meropenem	8	22.2	28	77.8
Cefepime	18	50	18	50
Ceftazidime	23	63.9	13	36.1
Ciprofloxacin	28	77.8	8	22.2
Cefoperazone/Sulbactam	14	38.9	22	61.1
Piperacillin/Tazobactam	16	44.4	20	55.6
Gentamicin	6	16.7	30	83.3
Amikacin	26	72.2	10	27.8

inducible beta-lactamase. Only 2% isolates by DDS method showed the presence of ESBLs. Metallo- β -lactamases were not identified in the current isolates.

4. Discussion

The most important pathogen responsible for nosocomial infections is *P. aeruginosa*. It is one among the vital causes for morbidity and increased hospital stay among inpatients. The increase in resistance to common antiseptics and antibiotics has caused the prevalence of *pseudomonas aeruginosa* as a nosocomial pathogen.¹⁶

The most sensitive antibiotic was found to be Ciprofloxacin which was followed by amikacin and ceftazidime. All beta lactum antibiotics had a sensitivity of around 40%. The sensitivity for carbapenem were around 20%. Livermore²² and Carmeli et al²⁵ have discussed that the development of resistance to carbapenem are high compared to other class of antibiotics. Wadud et al²⁶ in his study in Bangladesh has also observed higher sensitivity to ciprofloxacin.

Hoque et al¹⁶ observed a higher resistance of 81.4%, while Nadeem et al²⁷ and Jamshaid et al²⁸ observed a prevalence of 6.73% and 24% respectively in Pakistan. Meenakumari et al²⁹ in India, observed 56.63% resistance to amikacin.

Higher resistance of betalactams was found in other studies done in India.^{30,31} Birru et al³² in their study also demonstrated similar cross-resistance patterns among different antibiotics. In the current study, ESBLs were detected in only 2% of the isolates. Similar results were found in study done by Gencer et al³³ in Turkey.

P. aeruginosa shows multi drug resistance which is attributed to the synergy between multi-drug efflux systems or a type 1 Amp C- β lactamase activity and reduced permeability to outer membrane.^{5,6,16} 80% of the samples showed Inducible Amp C -lactamase activity. The presence of inducible lactamase in 80% of isolates susceptible to ceftazidime shows that susceptibility may be reduced during treatment via selection of derepressed mutants from inducible populations. Acquisition of plasmids encoding

lactamases can also lead to the resistance

The Carbapenem is the major drug group active against ESBLs and the derepressed mutants. Recently resistance has been seen in the carbapenem group as well. During the treatment imipenem has showed the emergence of antibiotic resistance than the ceftazidime and ciprofloxacin.

Multi drug resistance was seen in One third of our isolates and cross-resistances was found between drugs. Most isolates showed resistance owing to the impermeability or multi-drug efflux or synergistic several resistance mechanisms. In vitro antibacterial activity was observed in ciprofloxacin which is followed by amikacin in our institution. Target gene mutations in quinolones is the major underlying reason for resistance mechanisms and regulatory gene mutations for drug efflux pumps can be the other reason. Cross-resistance to chemically unrelated antibiotics is called multiple antibiotic resistance (MAR) which resulted from the pump mutations.³³

Although the study populations were different, comparison between studies is difficult and the methods chosen for the trial is different, interestingly we have found out that higher level of resistance is appreciated in beta-lactams and a less resistance pattern to ciprofloxacin in recent studies.^{30,31} In contrast the previous trials between 1995–1999 emphasized the greater sensitivity pattern in beta lactamases.^{34–37} Inappropriate usage and mismanagement of drugs decide the incidence of resistance. The association between the development of resistance by beta lactamase-producing microorganisms and the previous use of broad spectrum cephalosporins is emphasised in the prior studies.³³

Reduced permeability to outer-membrane of these isolates, synergistic with the secondary resistance mechanisms like an inducible cephalosporinase or antibiotic efflux pumps take the advantage of low outer-membrane permeability and this can be attributed to the greater intrinsic resistance of *P. aeruginosa*. Minimal change in the antibiotic susceptibility of the isolates can lead to an increase in the Minimum inhibitory concentration (MIC) of a drug to a greater level than the expected.³⁸

Some isolates of *P. aeruginosa* shows resistance to all reliable antibiotics, and likely to increase more with the emergence of integrons that has gene cassettes encoding both amikacin acetyl transferases and carbapenemases.²²

5. Conclusion

Strict adherence to the recent trend of “reserve drugs” concept and minimizing the misuse of antibiotics can bring down the drug resistance and morbidity. The addressal of irrational and inappropriate use of antimicrobials among the clinician is the need of the hour.

Strict adherence to the antibiotic policies like dosage and duration of antimicrobial administration has to be undertaken to prevent the emergence and spread of these resistant bacteria. Infection control procedures and surveillance programmes for MDR organisms have to be implemented. Antibiotic susceptibility pattern of *P. aeruginosa* in intensive care units and clinical wards has to be monitored seriously and the results should be readily made available to clinicians so as to reduce the drug resistance and associated morbidities.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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