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Research Article

Characterization of the antibiotic profile of *Pseudomonas aeruginosa* isolates from a tertiary care center

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ABSTRACT

Background: *Pseudomonas aeruginosa* has already gained its profile as a super bug and drug resistant clinical isolates are a challenge for the clinicians who treat those infections. The organism exhibits natural resistance to many of the commonly used antibiotics besides can acquire chromosomal as well as plasmid mediated resistance. Recent reports indicate the emergence of Multi drug resistant strains from various centres and there by underscore the importance of continuous monitoring of the sensitivity patterns. The present study was aimed to monitor the antibiotic profile of P.aeruginosa isolates from a tertiary care centre in South India and to understand the antimicrobial resistance pattern of the organism from this geographical region.
Methods: In this study we have screened various specimens like urine, sputum, broncho alveolar lavage (BAL), endotracheal aspirates (ETA), ascetic fluid, pleural fluid, pus and blood for the presence of *P. aeruginosa*. Identification was done by conventional methods and antimicrobial resistance patterns were analyzed.
Results: Eighty six isolates of P.aeruginosa were obtained from various clinical specimens over a period of one year. Most of the isolates were least susceptible to Ciprofloxacin and Meropenem (72 percentages each). All isolates were uniformly susceptible to colistin (100 %). The susceptibilities of isolates to other commonly used antibiotics were Ceftazidime 76%, Cefepime 77 %, Imipenem 94%, Amikacin 83%, Gentamicin 77%, Tobramycin 77 %, Netilmicin 77%, Piperacillin tazobactam 91 % and cefaperazone sulbactam 86%.
Conclusions: Increasing resistance in P. aeruginosa coupled with its ability to survive a variety of environmental conditions makes it a deadly pathogen, especially in the hospital environment. Constant surveillance of antimicrobial resistance trends, administration of appropriate antibiotics, use of combination therapy and simple measures like hand washing have become quintessential for the control of this organism.

Keywords: Pseudomonas aeruginosa, Super bug, Multi drug resistance

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous pathogen capable of surviving in a variety of environmental conditions. Its minimal nutritional requirements and tolerance to varied physical conditions allows it to persist on numerous living and non-living objects. In humans, it is capable of causing infections in people with a compromised immunity or breach of normal physiological barriers. It is strongly associated with wound infections in burns patients, respiratory infections in cystic fibrosis patients and severe life threatening infections like septicemia in neutropenic patients.[1]

P. aeruginosa is increasingly gaining importance as a multidrug resistant nosocomial pathogen complicating the treatment of inpatients in a hospital, especially in the ICUs.[2] It has many mechanisms by which it expresses

antimicrobial resistance and the rapid spread of these isolates can be a cause of concern to the treating physicians. The organism is intrinsically resistant to aminopenicillins, first and second generation cephalosporins, ertapenem, tigecycline, cotrimoxazole and macrolides. It can also acquire chromosomal as well as plasmid mediated resistance. Multidrug efflux pumps confer resistance to b-lactams, flouroquinolones and aminoglycosides. Enzymatic inactivation by means of b-lactamases and aminoglycoside modifying enzymes are also employed by the organisms. In addition, other mechanisms include target modification such as gyr A and par C conferring resistance to flouroquinolones and porin loss leading to impermeability in case of carbapenems.[3]

Continuous monitoring of the antimicrobial profile of *P. aeruginosa* isolates is becoming a necessity due to potentially rapid spread of resistance, its ability to persist in the environment and the implications of infections on patient morbidity, mortality and health care costs.

Objectives

This study was undertaken to monitor the antibiotic profile of *P. aeruginosa* isolates from a tertiary care centre in South India and to understand the current statistics of the antimicrobial resistance pattern of the organism from this geographical region.

METHODS

The present study was conducted at a 700 bedded tertiary care facility in South India. We have screened all clinical samples submitted to our diagnostic laboratory for *P. aeruginosa* over a time span of one year. The samples recruited for this study were urine, sputum, broncheoalveolar lavage (BAL), endotracheal aspirates (ETA), ascetic fluid, pleural fluid, pus and blood specimens. All specimens were processed by standard microbiological procedures which include culture, biochemical characterization and sensitivity testing.

The genus *Pseudomonas* was identified by colony morphology (Growth on MacConkey medium which showed non lactose fermenting pale colonies), gram staining, motility, oxidase test and failure to ferment glucose. Speciation was done by a battery of tests which included indole test, Methyl red test, Citrate test, Urease test, reactions on Triple sugar iron agar, arginine dihydrolase activity, and reduction of nitrate to nitrite and production of a classical bluish green pigment. A total of 86 isolates of *P. aeruginosa* were obtained and fully characterized. Antimicrobial susceptibility testing of all isolates was performed by Kirby Bauer disc diffusion method according to CLSI guidelines (4). The following panel of antipseudomonal antibiotics was tested: Ceftazidime, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Netilmicin, Ciprofloxacin, Piperacillin tazobactam, Cefaperazone sulbactam and Colistin.

RESULTS

All of the isolates were confirmed to be *P.aeruginosa* by the biochemical tests mentioned above. The sources of the various isolates are mentioned in Table 1. The isolates were most frequently obtained from respiratory samples which included sputum, BAL and endotracheal aspirates.

Table 1: Sources of *P. aeruginosa* isolates.

Specimen	No: of isolates n (%)
Urine	15(17)
Pus, body fluids	34 (40)
Respiratory samples (Sputum, ET aspirate, BAL)	35 (41)
Blood	2 (2)
Total	86

The overall susceptibility of all the isolates to the various antibiotics is demonstrated in Table 2. The isolates exhibit least susceptibility to Meropenem and Ciprofloxacin (72 percentages each) and all of them found to be sensitive to colistin.

Table 2: Percentage susceptibility of *P. aeruginosa* isolates from all clinical samples.

Antibiotic	Susceptibility (%)
Ceftazidime	76
Cefepime	77
Imipenem	94
Meropenem	72
Amikacin	83
Gentamicin	77
Tobramycin	77
Netilmycin	77
Ciprofloxacin	72
Piperacillin Tazobactam	91
Cefaperazone Sulbactam	86
Colistin	100

The susceptibility of the various isolates to the tested antibiotics with respect to their sources has been given in Table 3. The isolates from the urine sample showed the least susceptibility to almost all tested antibiotics except for colistin. All the isolates were susceptible to colistin.

DISCUSSION

P. aeruginosa has established itself as a significant pathogen in the hospital environment and is a dreaded complication in patients admitted in intensive care units. From the 86 strains of *P. aeruginosa* isolated in our laboratory, the majority was recovered from respiratory

samples which include sputum, broncheoalveolar lavage and endotracheal aspirate samples. This was followed by wound swabs and urine samples. Only two isolates were recovered from blood culture samples. These findings are consistent with the isolation rates from different samples as shown by different studies in India such as Mohanasoundaram and Arora et al.[5]

Table 3: Susceptibility (%) of *P. aeruginosa* isolates with respect to the source of isolates.

	CAZ	FEP	IMI	MEM	AMK	GEN	TOB	NET	CIP	P/T	CSL	COL
Respiratory samples	77	77	94	77	89	77	77	77	77	94	89	100
Urine	67	67	80	47	53	53	53	53	40	67	67	100
Pus, body fluids	77	79	100	77	88	82	82	85	79	97	91	100
Blood	100	100	100	100	100	100	100	100	100	100	100	100

(CAZ: Ceftazidime, FEP: Cefepime, IMI: Imipenem, MEM: Meropenem, AMK: Amikacin, GEN: Gentamicin, TOB: Tobramycin, NET: Netilmicin, CIP: Ciprofloxacin, P/T: Piperacillin Tazobactam, CSL: Cefaperazone Sulbactam, COL: Colistin)

The resistance patterns and isolation rates of *P. aeruginosa* varies regionally. Hence, increasing importance has been placed on the careful monitoring of antimicrobial resistance patterns of *P. aeruginosa* isolates for appropriate empirical as well as targeted treatment of the same. Being a member of the non-fermenter group of organisms, *P. aeruginosa* has a unique outer membrane that restricts the entry of macromolecules like antibiotics and hence confers it with a considerable degree of intrinsic resistance.[6] Other factors with secondary importance contributing to intrinsic resistance are efflux pumps and enzymatic inactivations.[7]

The overall resistance pattern of all isolates reveal the most resistance to flouroquinolones with only 72% of the total isolates showing susceptibility to ciprofloxacin. This finding correlates with the study results of Senthamarai et al from Kanchipuram in South India,[8] who showed resistance rates of 64% with ciprofloxacin and Chaudhari *et al* who demonstrated least susceptibility to ciprofloxacin among their isolates.[9] Principal modes of flouroquinolone resistance in *P. aeruginosa* is due to target modifications in DNA gyrase (gyr A) and topoisomerase IV(par C) or mutations in regulatory genes for efflux pumps that reduce intracellular concentrations of the antibiotic.[10]

All isolates were susceptible to colistin. Following colistin, the isolates were most susceptible to the b-lactam agents imipenem, cefaperazone-sulbactam and piperacillin-tazobactam. The relatively low resistance of *P. aeruginosa* to b-lactam agents is also demonstrated by the study conducted by Rakesh et al from a tertiary care centre in Ahmadabad. The main mechanism of resistance to b-lactams is due to the production of b-lactamase enzymes belonging to the various families.[11]

Among the aminoglycosides the isolates were most susceptible to amikacin (83% were susceptible) with comparable sensitivity for gentamicin, tobramycin and netilmicin. This is in comparison with the earlier published reports from India such as by Smitha et al[12] and Javiya et al.[13] The aminoglycoside modifying enzymes are classified into three major classes of drugs, namely, aminoglycoside phosphoryl transferase enzymes, aminoglycoside acetyltransferase enzymes and aminoglycoside nucleotidyltransferase enzymes.

The antimicrobial resistance pattern reveals a specimen to specimen variation. More resistance was seen among isolates from urinary infections. Both the isolates from the blood samples were susceptible to all tested antibiotics. The isolates from respiratory samples and pus samples had comparable sensitivity but the respiratory isolates showed more resistance to aminoglycosides than the isolates from pus samples.

Multidrug resistance in *P. aeruginosa* is increasing in prevalence as evidenced by surveillance studies. Our centre reported a prevalence of 24.4% multidrug resistant *P. aeruginosa* isolates among the total isolates. This is in compliance with the findings by Flamm et al who reported rates of multidrug resistance in *P. aeruginosa* ranging from 23 to 26%.

CONCLUSIONS

Increasing resistance in *P. aeruginosa* coupled with its ability to survive a variety of environmental conditions makes it a deadly pathogen, especially in the hospital environment. Constant surveillance of antimicrobial resistance trends, administration of appropriate antibiotics, use of combination therapy and simple measures like hand washing have become quintessential for the control of this organism.

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