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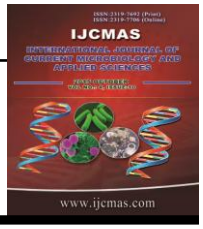

Original Research Article

Isolation, Identification and Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* from Clinical Isolates in a Tertiary Care Centre

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Keywords

Antimicrobial sensitivity testing, Drug resistance, *Pseudomonas aeruginosa*

A B S T R A C T

Pseudomonas aeruginosa is emerging as a nosocomial pathogen. It has intrinsic as well as acquired resistance to many antimicrobial drugs. This study investigated the antimicrobial resistance pattern of *P. aeruginosa* from clinical isolates. It is a retrospective study carried out from June 2014–May 2015 in Department of Microbiology at Shri B.M. Patil Medical College, H&RC, Vijaypur. Out of 792 culture positive samples, 156 were identified as *P. aeruginosa* (by standard bacteriological identification procedures). These isolates were recovered from various specimens like pus, urine, sputum, broncho-alveolar lavage (BAL) and tracheal aspirate. Antimicrobial sensitivity testing was done by Kirby-Bauer disc diffusion method. Out of 792 cultures positive, 156 were positive for *P. aeruginosa*. The isolation rate was 19.69%. *P. aeruginosa* were more sensitive to combination drugs like piperacillin+tazobactum (93.5%) and cefoperazone+sulbactum (92.3%) followed by imipenem (88.2%), meropenem (87.1%). Sensitivity to amikacin, tobramycin, gentamicin and ceftazidime ranges from 35% to 55%. Highest resistance rate was seen for amoxicillin followed by doxycycline. From our study, we concluded that *P. aeruginosa* is one of the most common nosocomial pathogen. It is sensitive to combination drugs like piperacillin+tazobactum and cefoperazone+sulbactum. It is also sensitive to carbapenems like imipenem, meropenem and aminoglycosides like amikacin, tobramycin, gentamicin, and cephalosporins like ceftazidime. Rational use of these drugs is necessary to prevent further spread of antimicrobial resistance among *P. aeruginosa* strains and also emergence of multi drug resistance.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. It is found in moist environment and disinfectant solution due to its ability to utilize different organic compounds and survive in nutrient deficient conditions. *P.*

aeruginosa is a notable cause of nosocomial infection of the respiratory tract, urinary tract, wound, blood stream and central nervous system. For immunocompromised patients, such infections are often severe and life threatening.

Mechanisms that cause antimicrobial drug resistance in *P. aeruginosa* is due to acquisition of resistance genes (e.g.: those encoding betalactamase), and aminoglycoside modifying enzymes via horizontal gene transfer and mutation of chromosomal genes (target site efflux mutations). Last one is the mechanism of resistance in fluoroquinolones particularly ciprofloxacin. Biofilm production especially in case of pulmonary infections in patients with cystic fibrosis contributes to its resistance to antimicrobial agents.

Multiple antibiotic resistances in bacterial population are a growing clinical problem which is a threat to public health. Hence there is a need to conduct studies to profile different pathogens responsible for specific infections and their resistance patterns so as to generate data that would help clinicians to choose correct antibiotics for treatment.

Materials and Methods

It is a retrospective study carried out from June 2014 to May 2015 in Department of Microbiology at Shri B.M. Patil Medical College, H&RC, a tertiary health care centre, Vijaypur, Karnataka.

Specimen

A total of 1087 non-duplicate samples from hospitalized patients admitted in different wards of hospital were investigated for bacterial culture and identification. Specimens were taken from various sources like sputum, urine, pus, catheter, broncho alveolar lavage and tracheal aspirate.

Laboratory identification of isolates

Identification of bacterial isolates was done by standard microbiological procedure. Specimens were inoculated on nutrient agar, blood agar, MacConkey agar and were studied for colony morphology. A battery of tests were performed that included Gram stain, oxidase test, motility test, production of pyocyanin, growth at 42°C, oxidative metabolism of glucose and arginine hydrolase.

Antibiotic susceptibility tests

Antibiotic susceptibility test was done by Kirby-Bauer disk diffusion method as per CLSI guidelines. Paper discs were impregnated with antibiotics of standard strength as below:

Penicillins: Amoxycillin (20µg), Piperacillin (100µg),
Ticarcillin (>75µ g) Fluoroquinolones: Ciprofloxacin (5µ g), Levofloxacin (5µ g)
Tetracyclines: Doxycycline (30µ g)
Macrolides: Azithromycin (15µ g)
Cephalosporins: Cefoperazone (75µ g), Ceftazidime (30µg), Cefepime (30µ g), Ceftriaxone (30µ g)
Carbapenems: Imipenem (10µg), Meropenem (10µg)
Aminoglycosides: Gentamicin (10µ g), Tobramycin (10µg), Amikacin (30µ g)
Combination drugs: Piperacillin+tazobactum (100/10µg), Cefoperazone+Sulbactum (75/30 µ g)

They were incubated overnight at 37^0C. The diameter of the zone of inhibition was measured and results were interpreted as sensitive, intermediate and resistant strain.

Results and Discussion

A total of 2174 nonduplicate samples from hospitalized patients were processed, of which 792 were culture positive. Out of 792 culture positive samples, 156 were identified as *P. aeruginosa* by standard microbiological procedures. The rate of isolation of *P. aeruginosa* is 19.69%. Out of 156 culture identified *P. aeruginosa*, 72 (46.1%) were from male and 84 (58.4%) from females (Table 1).

The rate of isolation was more in the age group of 41–50 years. Major source of these isolates were from wound/pus (37.17%)] followed by urine, sputum, catheter, broncho-alveolar lavage and tracheal aspirate (Table 2).

In our study, 156 isolates were isolated and identified as *P. aeruginosa* by standard biological procedures. The rate of isolation is 19.69%. The rate of isolation was more in the age group of 41–50 years (25.6%) followed by elderly age group. This may be due to decreased immunity, prolonged hospitalization and associated co-morbidities. A study by Rajat *et al.* (2012) shows 29% isolation rate in the age group of 31–45 years which is similar to our study and study done by Chander Anil and Raza (2013) shows 20% in age group of 21–40 years. Out of 78, 36 (46.1%) were from male and 42 (53.84%) from female which is same as Chander Anil and Raza (2013).

In our study, 37.17% of isolates were obtained from pus/wound followed by 30.76% from urine specimen. In a study done by Anuradha *et al.* (2014) shows 39.39% were from pus samples and 37.87% from urine samples. Another study by Javiya *et al.* (2008) reported highest number of *P. aeruginosa* from urine followed by pus and sputum. In a study Anupurba *et al.* (2006) showed 32% isolation rate from pus. Similar results are also reported by Mohana Soundaram (2011) and Arora *et al.* (2011). This indicates that wound infections and urinary tract infection are most common hospital acquired infections. These are most common cause for morbidity in hospitalized patients. *P. aeruginosa* is a common cause of wound infection especially in burns patients because burns have large exposed area of dead tissue free of any defence, so ideal for *P. aeruginosa* infection.

The resistance profile of *P. aeruginosa* to the antimicrobial agents tested varied among the isolates investigated. Highest sensitivity was seen to combination drugs like piperacillin+tazobactum (93.5%) and cefoperazone+sulbactum (92.3%). Sensitivity to carbapenems like imipenem (88.2%) and meropenem (87.1%) was comparatively high. Similar studies like Al-Jasser and Elkhizzi (2004) showed sensitivity to meropenem (91.6%), imipenem (90.2%) and piperacillin+tazobactum (81.3%). Raja and Singh (2007) showed sensitivity to imipenem (90.1%) and piperacillin+tazobactum (90.6%). Ansary *et al.* (1994) showed sensitivity to cefoperazone+sulbactum (82%) which is similar to our study.

Sensitivity to aminoglycosides (gentamicin, tobramicin, amikacin) and cephalosporins (cefoperazone, ceftazidime, cefipime, ceftriaxone) ranges from 45–55% which is same as study conducted by Garba *et al.* (2012). Highest resistance was seen to amoxicillin (97.4%) followed by doxycycline (88.46%) which is similar to study conducted by Garba *et al.* (2012). Resistance to quinolones (ciprofloxacin & levofloxacin) ranges from 50–60% and azithromycin was 53.8%.

Table.1 Age and gender wise distribution of *P. aeruginosa* isolates

Age (In Years)	No. of Males	No. of Females	Total (In %)
<10 Years	2	4	6
11-20	12	8	20
21-30	10	4	14
31-40	16	6	22
41-50	14	26	40
51-60	8	14	22
>60	10	22	32

Table.2 Frequency of distribution of *P. aeruginosa* from specific sites

Source of specimen	Number of Samples	Percentage (%)
Pus/wound	58	31.17%
Urine	48	30.76%
Sputum	28	17.9%
Catheter	10	6.41%
BAL	08	5.12%
Tracheal aspirate	04	2.56%

BAL=Broncho Alveolar Lavage

The selective pressure from use of antimicrobial agents is a major determinant for emergence of resistant strains. The sub-inhibitory antibiotic concentration in wounds, due to administration of inappropriate dosage of beta-lactum antibiotic or regular administration of aminoglycosides in combination with beta lactum, provides optimal conditions for selection and persistence of multidrug resistant strains.

In conclusion, *P. aeruginosa* is a leading cause of nosocomial infection. Indiscriminate use of antibiotics has led to emergence of multidrug resistant strains. In our study, strains are more sensitive to combination drugs like piperacillin+tazobactum and cefoperazone+sulbactum and carbapenems like imipenem and meropenem. A more restricted and rational use of these drugs is necessary. A regular monitoring of antimicrobial susceptibility pattern is essential to guide the physicians in prescribing right combination of drugs and emergence of multidrug resistance strains of *P. aeruginosa*.

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