

## Original Research Article

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## Antibiotic Profile of *Pseudomonas aeruginosa* Isolated from Patients Visiting Grecian Super Specialty Hospital, Mohali, India

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### ABSTRACT

Antimicrobial resistance especially in *Pseudomonas* spp. is an increasing problem across the world and therefore, its trend of antimicrobial resistance requires to be studied. The objective of current study was to determine the prevalence of *Pseudomonas aeruginosa* in various clinical samples collected during study and to analyze the antibiotic susceptibility patterns of various drugs against it. During the study, a total of 231 *P. aeruginosa* isolated from 870 clinical samples like broncho-alveolar fluid (BAL), blood, endotracheal secretion, Foleys tip culture, pleurinal flurineid, pus, spurinetrinem, sputum, swab curinelturiner, urine and wound. Antibiotic susceptibility testing were carried out according to the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines. The highest number of *Pseudomonas* infections was found in urine, followed by endotracheal, blood, BAL, spurinetrinem, wound, pus, foley's tip, pleurinal flurineid, sputum and swab. Tobracef was the most active drug tested (82.25% susceptible), followed by piperillin+tazobactam (74.89% susceptible), cefoperazone+sulbactam (67.10% susceptible), meropenem (64.94% susceptible) and ceftazidime (60.17% susceptible). From the present study, it appears that lower respiratory tract infection was the most common hospital acquired infection caused by multidrug resistant (MDR) *P. aeruginosa*. Moderate rates of drug resistance among *P. aeruginosa* isolates were observed against ceftazidime and meropenem. This pattern of resistance indicates probable overuse of broad-spectrum antibiotics without the having knowledge of sensitivity patterns of drugs. So there is a continuous need of conduction of surveillance programmes to recommend appropriate therapy to patients. Moreover, our data also demonstrated that Tobracef has enhanced *in-vitro* antibacterial activity and exhibited 17% superiority over meropenem. Therefore, it can be a better choice to treat the infections caused by multi-drug resistant *P. aeruginosa*.

#### Keywords

*Pseudomonas aeruginosa*, clinical isolates, Resistant, Susceptible.

#### Article Info

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### Introduction

*Pseudomonas* is an aerobic, motile, opportunistic problematic Gram-negative bacterium and has been implicated in diverse healthcare associated infections (HCAs) such as lower respiratory tract infection, urinary tract infection, skin and soft-tissue infections, in severe burns and in infections among immune compromised individuals (Tadvi et

al., 2017; Siddiqua et al., 2018; Rao et al., 2017; Prasad et al., 2017). It is responsible for 10-15 % of nosocomial infections across the world (Hancock and Knowles, 1998; Maniatis et al., 1997; Blanc et al., 1998). In critically ill patients, *P. aeruginosa* contributes 3%–15% of blood stream infections with high mortality rate of about 27%–48%. In spite of recent advances in therapy, *P. aeruginosa* bacteremia remains

fatal in more than 20% of cases. Over 50% of deaths happen within a few days of infection (Pragasam *et al.*, 2018). It is the most common pathogen recovered from lower respiratory tract infection (LRTIs).

LRTI is one of the leading causes of morbidity and mortality worldwide (Alter *et al.*, 2011). In addition, of total 3,941,000 deaths in the world, LRTI accounts for 34.60% deaths in the South-East Region (WHO, 2002). In developing countries, the situation is more complicated, and management is often difficult due to the problem associated with the identification of the etiological agents and the administration of an appropriate treatment in cases requiring antibiotic therapy (Khan *et al.*, 2015).

Earlier studies have reported that *P. aeruginosa* to be most common isolate (35-59%) from patients with LRTIs (Goel *et al.*, 2009; Khan *et al.*, 2015; Ozyilmaz *et al.*, 2005; Gladstone *et al.*, 2005; Imani *et al.*, 2005). Infections with multidrug-resistant *Pseudomonas* are not only associated with considerable morbidity and mortality, but it also presents an economic burden as these are associated with high treatment costs and longer duration of hospital stay when compared to those associated with their drug-susceptible counterparts (Livermore, 2002; Mauldin *et al.*, 2010).

The prime reason for morbidity, mortality and healthcare cost is the rapidly growing antibiotic resistance (Camell *et al.*, 1993; Medina and Pieper, 2016). *Pseudomonas* exhibits a high level of intrinsic resistance to antimicrobial drugs and an ability to become even more drug resistant due to production of ESBL enzymes, AmpC hyper expression, repression or inactivation of oprD, over expression of efflux pumps and low permeability of its outer membrane (Aloush *et al.*, 2006; Livermore, 2002; Livermore, 1984).

Besides, *Pseudomonas* species acquires class B metallo- $\beta$ -lactamases (MBLs) which are another antibiotic resistance determinants and can be disseminated horizontally through transfer of mobile genetic elements coding for MBLs (Livermore, 2002).

Over the years, increases in the rate of antibiotic resistance to *P. aeruginosa*, in particular to beta-lactams, aminoglycosides, and fluoroquinolones, has been reported from many parts of the world (Jones *et al.*, 2002). However, such problems are of great concern in developing countries particularly due to easy availability of antibiotics over the counter (Martinez, 2002).

A study by Gupta *et al.* (2016) from Aligarh, India reported an average 76.1%, 70.2% and 65-68% of the isolated *Pseudomonas* species were resistant to fluoroquinolones, aminoglycosides and cephalosporins, respectively. Several other studies from India as well as Indian Council of Medical research (ICMR) also highlighted the resistance of *Pseudomonas* species against monotherapy of penicillins, cephalosporins, fluoroquinolones, tetracyclines and macrolides (Javiya *et al.*, 2008; Walia *et al.*, 2019; Kumari *et al.*, 2019; Ramana and Chaudhury, 2012; Chaudhary and Payasi, 2013; Prakash *et al.*, 2014). A decreased susceptibility rate of *P. aeruginosa* to  $\beta$ -lactams, carbapenems, quinolones and aminoglycosides has been reported in various countries (Khan and Faiz, 2016; Mansoor *et al.*, 2009; Féria *et al.*, 2002).

Therefore, there is a need to conduct surveillance studies of *Pseudomonas* for its resistance pattern. The present study was undertaken to find out the drug resistance and antibiotic susceptibility patterns in *Pseudomonas* species isolated from different clinical specimens of patients at a tertiary care hospital of India.

## Materials and Methods

### Sample processing

Two hundred and thirty one samples collected aseptically from broncho-alveolar fluid (BAL), blood, endotracheal secretion, Foleys tip culture, pleurinal fluid, pus, sputum, swab curinelturiner, urine and wound were reported the presence of bacterial infection. The samples were inoculated on different media like Nutrient Agar, Blood Agar and MacConkey Agar for isolation as per the Standard Operative Procedure. *P. aeruginosa* were further identified by use of Indole, Citrate, Triple sugar iron (TSI), Catalase, Oxidase and physiologically by Urea hydrolysis and Mannitol motility (Koneman, 2006).

### Antibiotic susceptibility testing

The antimicrobial susceptibility testing was performed by Kirby Bauer's disk diffusion method in accordance to the guidelines published by Clinical Laboratory Standards Institute (CLSI) (CLSI, 2018). Inoculum of 0.5 McFarland standards turbidity was prepared from isolated colony of pathogens selected from 18-24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller-Hinton agar (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60° over the agar surface. After 3–5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated for 16-18 hrs aerobically at 37° C within 15 minutes

of disc application and zone of inhibition measured in millimetres and the results were interpreted as Sensitive, Intermediate and Resistant.

Paper disks impregnated with following antibiotics were used in the study: Tobracef (ceftazidime+tobramycin) (40 µg), ceftazidime (30 µg), cefoperazone +sulbactam (105 µg), piperacillin+tazobactam (110 µg) and meropenem (10 µg). Discs of these drugs were purchased from Indian market on behalf of sponsor.

## Results and Discussion

The increasing of infections caused by multi-drug resistant bacteria has now become a major threat in medical world. Now-days, *P. aeruginosa* is considered a leading cause of gram negative bacterial infections especially in immuno-suppressed patients who need prolonged hospitalization (Arora *et al.*, 2011).

A total 870 different clinical samples were collected from patients visiting Grecian Super Specialty Hospital, Mohali and processed for isolation of pathogenic bacteria. Eleven types of clinical samples included broncho-alveolar fluid (BAL), blood, endotracheal secretion, Foleys tip culture, pleurinal fluid, pus, sputum, swab curinelturiner, urine and wound.

A total of 231 *P. aeruginosa* isolates were obtained from 870 clinical samples. The maximum *P. aeruginosa* was recovered from urine samples (39.83%) followed by endotracheal section which contributed to 23.38%, indicating urinary tract and endotracheal infections are most common hospital acquired infections. These results are in line with earlier study (Raman and Chaudhary, 2012). Each of blood, BAL and sputum samples contributed to 5.63%. Share of wound and pus sample was 5.19%

each. However, samples from foley's tip, pleurinal flurineid, sputum and swab curinelturine contributed to <5% (Table 1; Figure 1). Rajkumari et al. (2014) reported that the most common sample from which *P.aeruginosa* was recovered was from urine samples (29.0 %), followed by tracheal aspirates (24.4 %), pus/wound swabs (20.0%), blood (8.0 %), bronchalveolar lavage (8.0 %), tissues (1%), and sputum (0.1%). Another study by Javiya et al. (2008) reported highest number of *P. aeruginosa* from urine followed by pus and sputum.

Fig.1 shows that the Prevalence percentage of clinical pathogens among different clinical samples. In the present study 82.25% of isolated multi-drug resistant (MDR) bacteria were observed to be susceptible to Tobracef which is consistent with previous studies (Chaudhary and Payasi, 2015). The higher susceptibility of Tobracef in *P. aeruginosa* results from enhanced level of drug uptake and higher outer membrane permeability (Chaudhary et al., 2014). This observation highlights the importance of Tobracef in MDR Gram-negative pathogens in causing LRTI.

The ureidopenicillin group represented by piperacillin+tazobactam appeared to be second most susceptible drugs exhibiting 74.89% susceptibility this result corroborate a previous study of Arumugam et al. (2018). Amongst third generation cephalosporins, ceftazidime is the drug used to treat Pseudomonal infections. In the current study, it showed 60.17% susceptibility to *P. aeruginosa*. However, over the past few years, Pseudomonal resistance to this drugs has increasingly been reported (Arora et al., 2011; Valenza et al., 2010).

Studies carried out by previous authors (Chitnis et al., 2003; Laura et al., 2000) in cephalosporins showed that the overall resistance to various generations of

cephalosporins was high on MDR pathogens (i.e. resistance to at least three antibiotics) due to various mechanism including production of antibiotic inactivating enzyme. Pathogens maintain their MDR phenotype and spread throughout the community. Addition of beta-lactamase inhibitor (sulbactam) to cephalosporins monotherapy significantly reduced the percentage resistance and increased the percentage susceptibility against all the organisms. In the current study, cefoperazone+sulbactam (a combination of beta-lactam and beta-lactamase inhibitor) showed 67.10 % susceptibility. Amongst the beta-lactam and beta-lactamase inhibitor group, cefoperazone+sulbactam showed resistance of 32.90%. Increasing resistance of *P. aeruginosa* against  $\beta$ -lactamase inhibitor antibiotics may be due to excessive  $\beta$ -lactamase production and/or active efflux mechanism may also contribute to the full expression of  $\beta$ -lactam resistance in *P. aeruginosa*. Multi drug efflux pumps in the inner and outer membrane of *P. aeruginosa* may protect the bacterium from to  $\beta$ -lactam agents (Srikumar et al., 1997).

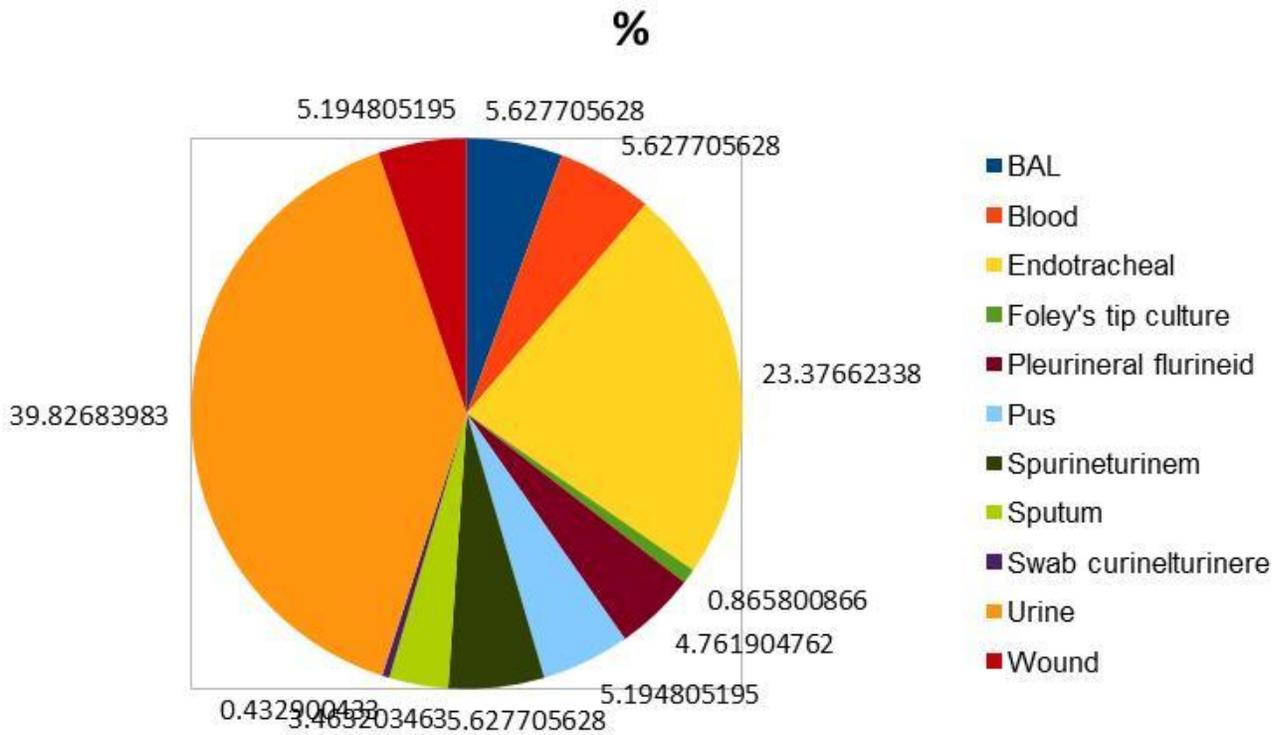
Carbapenems such as meropenem is often used as last choice for treatment of infection by Pseudomonas. In the current study showed a percentage susceptibility of 64.94 % against meropenem. Supporting this observation, Arumugam et al. (2018) reported 73.6 % susceptibility against meropenem. Another study by Javiya et al. (2008) reported 69.64% susceptibility against meropenem.

Currently the resistance towards this group of drugs is increasing. We observed the resistance of 35.06 % for meropenems. The resistance to carbapenems, especially in MDR *P. aeruginosa*, results from reduced levels of drug accumulation or increased expression of pump efflux (Gupta et al., 2006; Kurokawa et al., 1999).

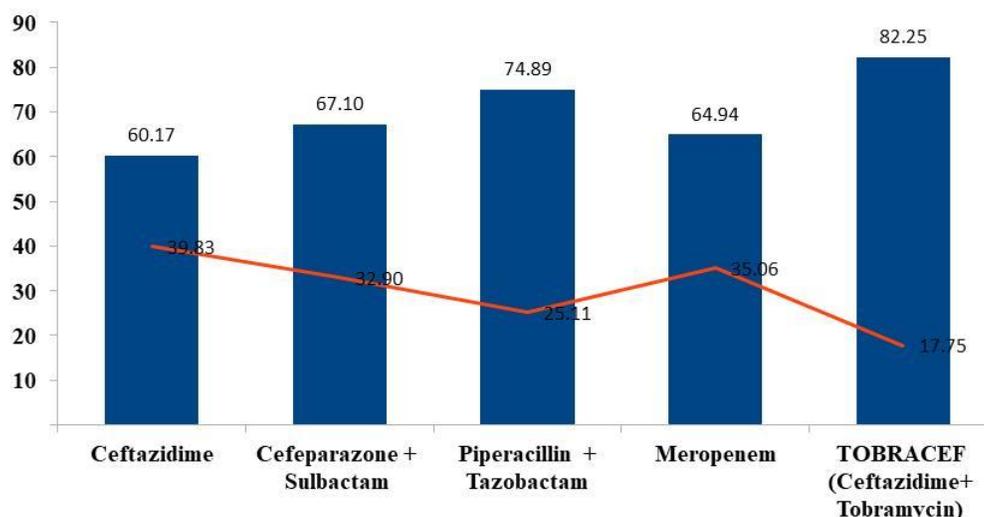
**Table.1** A profile of clinical samples used as a source of the pathogenic isolates

Sr. No.	Specimen	%	No.
1	BAL	5.63	13
2	Blood	5.63	13
3	Endotracheal	23.38	54
4	Foley's tip culture	0.87	2
5	Pleurineral flurineid	4.76	11
6	Pus	5.19	12
7	Spurineturinem	5.63	13
8	Sputum	3.46	8
9	Swab curinelturinere	0.43	1
10	Urine	39.83	92
11	Wound	5.19	12

**Figure.1** Prevalence percentage of clinical pathogens among different clinical samples.



**Figure.2** Susceptibility pattern of clinical isolates towards different antibacterial agents.



From the present study, it appears that lower respiratory tract infection was the most common hospital acquired infection caused by multidrug resistant *P. aeruginosa*. Moderate rates of drug resistance among *P. aeruginosa* isolates were observed against ceftazidime and meropenem. This pattern of resistance indicates probable overuse of broad-spectrum antibiotics without the having knowledge of sensitivity patterns of drugs. So there is a continuous need of conduction of surveillance programmes to recommend appropriate therapy to patients. Moreover, our data also demonstrated that Tobracef has enhanced *in-vitro* antibacterial activity and exhibited 17% superiority over meropenem. Therefore, it can be a better choice to treat the infections caused by multi-drug resistant (MDR) *P. aeruginosa*.

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