



## EMERGING ANTIBIOTIC RESISTANCE IN PSEUDOMONAS-A CHALLENGE

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## ABSTRACT

The present study was undertaken to assess the antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Punjab, India. Due to significant changes in microbial genetic ecology, as a result of indiscriminate use of anti-microbials, the spread of anti-microbial resistance is now a global problem.

**Keywords:** *Pseudomonas aeruginosa*, Antibiotic susceptibility

## INTRODUCTION

Owing to its physiologic versatility *Pseudomonas aeruginosa* is considered one of the pathogens difficult to treat in practice<sup>1,2</sup>. It continues to be the major pathogen in patients with immunosuppression, cystic fibrosis and malignancy<sup>3</sup>. In a survey conducted by the Center for Disease Control in the United States from 1976 to 1980, its frequency of occurrence as a nosocomial pathogen has increased<sup>3</sup>. With the widespread use of antibiotics and the increase in number of immunosuppressed host. *Pseudomonas aeruginosa* has become a leading cause of gram negative bacterial infections especially in immunosuppressed patients who need prolonged hospitalization<sup>4,5,6</sup>. It was also noted that *Pseudomonas aeruginosa* bacteremia is associated with higher mortality than other gram negative bacteremia<sup>7</sup>. The underlying immunosuppression as well as the resistance of *Pseudomonas aeruginosa* to several antibiotics could be a contributory factor. To overcome the latter, several studies indicate that a combination of antibiotics is the preferable therapy for severe *Pseudomonas aeruginosa* infections<sup>8</sup>. Antimicrobial agents, treatment of Pseudomonal pneumonia is often challenging<sup>9,10</sup>. The diversity of clinics and the regional variations in antibiotic protocols result in the different resistance profiles<sup>11,12</sup>. Patients hospitalised are at particular risk of acquiring nosocomial infections due to serious underlying disease, compromised membrane and skin barriers following the use of invasive devices, and extended length of hospital stay, among other factors. Exposure to various antimicrobial agents may further complicate such hospitalisation and create conditions conducive to resistance selection among host bacterial flora or nosocomially-transmitted pathogens. Studies have demonstrated that rates of antimicrobial resistance are greater in bacteria isolated from ICUs compared with other hospital wards and outpatient clinics<sup>13</sup>. *Pseudomonas aeruginosa* frequently displays resistance to multiple antimicrobial agents<sup>14</sup>. Serious infection due to strains of *Pseudomonas aeruginosa* that exhibit resistance to all common antipseudomonal antimicrobials is an increasingly serious problem<sup>15</sup>. In this study done at Adesh Medical College Bathinda. we aimed to establish the prevalence of *Pseudomonas aeruginosa* in our hospital and to compare their antibiotic susceptibility patterns.

## MATERIALS AND METHODS

The study was conducted over a period of one year (March 2009 to March 2010) at Bathinda. Samples were obtained from patients who were hospitalized for more than one week duration. The various specimens obtained were urine, tracheal aspirate, blood and exudate from any lesion which was present (e.g. Burn wound, non-healing ulcer, post-operative wounds). A total of 500 samples were obtained. From different sources out of which 193 were *Pseudomonas aeruginosa*. These specimens were inoculated onto the primary isolation media like blood agar, MacConkey, eosin-methylene blue and other selective differential media. Colorless colonies, characteristic of pseudomonas, were transferred to triple

sugar iron (TSI) agar slants for presumptive identification. *Pseudomonas aeruginosa*, a glucose non-fermenting gram-negative rod produced an alkaline red slant and alkaline red or no change in the butt indicator after 24 hours of incubation. A grape-like odor of the growing colonies was also recognized. An isolate presumptively identified in TSI as a glucose Non-fermenter was confirmed by inoculating to oxidative fermentative glucose medium, which yielded positive results. The isolates, if inoculated to PAF agar slants, produced the characteristic greenish pigment. A total of 193 samples of pseudomonas were obtained from various sources *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain. i.e. cefotaxime, ceftriaxone, ceftazidime, amikacin, gentamicin, ciprofloxacin, piperacillin and imipenem. The Kirby Bauer Method using the disc diffusion technique was the procedure of choice for antibiotic sensitivity testing. A sensitive result is defined as a zone of inhibition that meets the interpretive standards recommended by the American Society for Testing and Materials as shown below for inoculation Mueller Hinton Agar was done using the standard method.

## RESULTS

A total of 193 samples of pseudomonas were obtained from various sources *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain shown in Table 1.

Each isolate was evaluated for susceptibility to different antibiotics i.e. cefotaxime, ceftriaxone, ceftazidime, amikacin, gentamicin, ciprofloxacin, piperacillin and imipenem. Out of 193 isolates, 136 (70%) were from male patients and 60 (30%) were from female patients. Maximum resistance was seen to third generation cephalosporins-116 (60%) to cefotaxime, 141 (75%) to ceftriaxone, 121 (63%) to ceftazidime, Amikacin showed resistance in 81 (41.5%) and Gentamicin in 153 (79%) of the isolates. Ciprofloxacin resistance was seen in 143 (73.2%) isolates while piperacillin resistance was seen in 85 (44%) of the isolates. Minimum resistance was seen to imipenem -5 (3.7%).

**Table 1: Distribution of specimens of *Pseudomonas aeruginosa* isolates**

Sources of Specimen	Total number	Percentage (%)
Urine	70	36%
Wound discharge	40	20%
Ear discharge	10	5%
Sputum	8	4%
Tracheal Aspirate	17	8%
Blood	2	1%
Kidney Swab	2	1%
Pleural Fluid	4	2%
Lung Abscess	7	3%
CVP Catheter tip	5	2%
Others	17	8%

193 Strains of *Pseudomonas aeruginosa* were obtained (Table – 2). The rate of isolation of *Pseudomonas aeruginosa* was 20%. Exudates followed by urine accounted for the maximum isolate. The common sources of specimen are shown in Table 2 with the urine and wound discharges on the top list.

**Table 2: Antimicrobial sensitivity pattern of *Pseudomonas aeruginosa***

Antibiotic	Sensitive no. (%)	Resistant no. (%)
Cefotaxime	77(40)	116(60%)
Ceftriaxone	52(25)	141(75%)
Ceftazidime	70(37)	121(63%)
Amikacin	112(58.5)	81(41.5%)
gentamicin,	40(21)	153(79%)
Ciprofloxacin	50(26.8)	143(73.2%)
Piperacillin	108(56)	85(44%)
Imipenem	188(96.3)	5(3.7%)

## DISCUSSION

*Pseudomonas aeruginosa* is a major cause of nosocomial infection. Despite advances in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life threatening infections caused by *Pseudomonas aeruginosa* continue to be hospital infections. A critical factor in the survival of *Pseudomonas aeruginosa* in an unfavorable environment is its ability to transform from a mobile "swarmer" cell to a glycocalyx enclosed microcolony which serves to protect the organisms against the active phagocytes, surfactants, enzymes and high levels of specific antibodies. Nowadays, the prevalence of *Pseudomonas aeruginosa* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections.<sup>16</sup> Ceftriaxone and Ceftazidime are the commonest 3rd generation antibiotics in hospital protocols. Resistance to 3rd generation cephalosporins are significant in our study (60%-75%) similar to the study done by Holloway et al.<sup>17</sup> *Pseudomonas aeruginosa* detected significant resistant against aminoglycosides.<sup>18</sup> Reports of the susceptibility of *Pseudomonas aeruginosa* to gentamicin have ranged from as low as 49.8% and 77.7%, in Greece, to as high as 96.6% and 99.2%, respectively, in the United Kingdom.<sup>19</sup> In the our study, the rate of aminoglycoside resistance was also found to be relatively high (resistance to amikacin; 41.5%, and gentamicin; 79%). So, antipseudomonal effect of amikacin is higher than gentamicin. Consistent with these findings, resistance to amikacin of *Pseudomonas aeruginosa* was still lower than to gentamicin and this correlates with the study done by Smitha et al.<sup>19</sup> and Poole et al.<sup>20</sup> So, among the aminoglycosides, amikacin has the highest sensitivity. So, Amikacin seems to be a promising therapy for pseudomonas infection. Hence, its use should be restricted to severe nosocomial infections.<sup>21</sup> However, this data also suggests that resistance to amikacin is increasing progressively in our country. In various studies, it was reported that increased resistance rates have been detected against carbapenems, quinolones and third-generation cephalosporins for *Pseudomonas aeruginosa* worldwide.<sup>22,23,24</sup> In our study, resistance rates against imipenem were lower (3.7%) similar to study in Spain 14%.<sup>26</sup> The resistance of *Pseudomonas* to the antibiotics in the quinolone group is not consistent and variability has been reported in different centers.<sup>25,26,27</sup> In a prospective study, resistance to ciprofloxacin in ICU was reported as 8-31%.<sup>28</sup> In our study, resistance rates against ciprofloxacin as 73.2%. Quinolone resistance in our study is high as compared to study done by other studies 31.9% in Italy, and 26.8% in Latin America.<sup>29</sup> This is because of irrational approach of the clinicians of putting patients on quinolones straightway without going for antibiotic sensitivity. Overall we have observed that there is increased antibiotic resistance which may be due to the selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains.<sup>18,30</sup>

## CONCLUSION

*Pseudomonas aeruginosa* is one of the most important bacterial pathogens seriously contributing to the problem of hospital infection. Drug resistance to *Pseudomonas aeruginosa* is rapidly increasing.

irrational and inappropriate use of antibiotics is responsible for the development of resistance of *Pseudomonas* species to antibiotic monotherapy. Hence, there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.

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