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## Frequency and susceptibility pattern of Multidrug Resistant *Pseudomonas aeruginosa* in isolates of patients from a tertiary care hospital of Karachi, Pakistan

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### Abstract

**Objective:** To determine the frequency and susceptibility pattern of multidrug resistant *P.aeruginosa* isolates from clinical specimens in a tertiary care hospital in Karachi, Pakistan.

**Methods:** A cross sectional study was performed over a time frame of 2 years, from January 2013 to January 2015 at a tertiary care private hospital in Karachi. These specimens were collected from three different tertiary care units situated at three different locations of the city. Standard and specific microbiological methods were used to identify the clinical isolates. The isolates were cultured on chocolate and MacConkey agar. The susceptibility patterns were deduced by utilizing Kirby Bauer Disc diffusion method on Mueller-Hinton Agar.

**Results:** A total of 634 isolates of *P. aeruginosa* were cultured during this study's time frame ranging from January 2013 to January 2015. Positive cultures were then tested against 9 antibiotics from the following classes of drugs: B-Lactams, Carbapenems, Aminoglycosides and Fluoroquinolones. 36.5% of the samples were found to be resistant to 3 or more antibiotics, were labeled as MDR *P. aeruginosa*. The most sensitive drug was Colistin (100%), followed by Ciprofloxacin (66.8%), Piperacillin/Tazobactam (49.1%), Cefaperazone/Sulbactam (47.4%) and Cefepime (44%).

**Conclusions:** MDR *P. aeruginosa* isolates show a progressive trend as compared to previous studies. The increased resistance pattern of isolates to multiple drugs was noteworthy.

**Keywords:** *P. aeruginosa*; Multidrug resistance

### Introduction

*Pseudomonas aeruginosa* is a non-fermenter, pleomorphic gram negative rod. It is ubiquitous in nature i.e. it is widely distributed in nature as well as in the hospital environment and thus it is a prevalent cause of nosocomial infections, particularly amongst patients on ventilator support in intensive care units and high dependency units<sup>1</sup>. In United States *P.aeruginosa* is one of the most common causative pathogen for hospital acquired infections ranging from urinary tract infections to bacteremia<sup>2-3</sup>. Patients that are infected with drug resistant and multidrug resistant (MDR) *P.aeruginosa* have a 34% mortality compared to 22% mortality when infected with drug susceptible *P.aeruginosa*<sup>4</sup>. Resistant *P.aeruginosa* can also be considered as a marker for increased in-hospital mortality<sup>4</sup>.

Patients who have long hospital stays, who require ventilator support and who have previously used antibiotics are at a risk of developing ventilator associated pneumonia (VAP)<sup>5-7</sup>. VAP is mostly caused by multidrug resistant *Pseudomonas aeruginosa* and other multidrug resistant organisms, such as *E.Coli*, *K.pneumoniae* and *Acinetobacter species*<sup>8-8</sup>. Currently it is seen that MDR *P.aeruginosa* is evolving<sup>9</sup>. *P.aeruginosa* is a complicated pathogen due to the risk of emergence of resistance during treatment<sup>10</sup>. MDR *P. aeruginosa* is defined as isolates which are resistant to at least three drugs of the following classes: B-Lactams, Carbapenems, Aminoglycosides and Fluoroquinolones<sup>11</sup>. MDR *P.aeruginosa* produces inactivating enzymes such as extended spectrum beta lactamases and metalobetalactamases which render it resistant to the Beta lactam and Carbapenem group of drugs<sup>12</sup>. When patients are provided with initial therapy which is insufficient in covering resistant or MDR *P.aeruginosa*, it leads to poor clinical outcomes, high costs and extended hospitalization<sup>13</sup>. This study was aimed at observing the pattern of *P. aeruginosa* in the ICU and HDU departments of Ziauddin Hospital.

**Material and methods**

This is a cross sectional study was conducted in the Microbiology Department of Ziauddin Medical University Hospital Karachi, Pakistan from January 2013 to January 2015, over a period of two years. Tracheal aspirate, blood, pus, body fluids, urine and bronchoalveolar lavage samples were collected from inpatients who were admitted in the ICU and HDU at the hospital. The patients who were included spanned all age groups. The samples were cultured on Chocolate and MacConkey agar and incubated at 37C for a duration of 18 hours. Isolates were identified according to standard microbiological methods<sup>14</sup>. *P. aeruginosa* by its colony morphology on gram staining is a pleomorphic gram negative rod, non-lactose fermenter on MacConkey, pigment production with characteristics grape like odor, oxidase positivity, non motile along with its ability to reduce nitrate to nitrite and arginine decarboxylase and gelatin liquefaction. Api20 NE was used for confirmation of isolates.

Antibiotic sensitivity patterns of these isolates were studied by using Kirby Bauer Disc Diffusion method on Mueller – Hinton agar, by following CLSI 2014 Guidelines<sup>15</sup> and using Hi-media antibiotic discs. Antibiotics were tested, which included Amikacin (30mcg), Ceftazidime (30mcg), Aztreonam (30mcg), Cefepime (30mcg), Cefaperazone/sulbactam (75/30mcg), Ciprofloxacin (5mcg), Piperacillin + Tazobactam (100/10mcg), Meropenem (10mcg) and Colistin (10mcg). Strains which had the same types of resistance patterns (antibiotype) were considered to be from the same clone. *Pseudomonas aeruginosa* ATCC 27853 strain was used for quality control in the study. In our work, MDR *P. aeruginosa* was detected as a bacterium which was resistant to three or more anti-Pseudomonal antimicrobial classes (Piperacillin+ Tazobactam, Imipenem, Ceftazidime and Amikacin). Out of the 634 patients 232 isolates were found to be MDR (Table no.2).

Statistical analysis was performed by SPSS version 20. Frequency of MDR *P. aeruginosa* and percentage of resistant antibiotics were calculated.

**Discussion and Results**

*Pseudomonas aeruginosa* is a non-fermenter gram negative pleomorphic bacteria which is a well-recognized pathogen involved in causing a large variety of nosocomial infections in hospitalized patients especially in immunocompromised patients and patients with indwelling devices. There has been rapid emergence of MDR *Pseudomonas aeruginosa* recently, which is an alarming situation as therapeutic options for treating this MDR organism are very limited. Out of all the nosocomial infections caused by *Pseudomonas aeruginosa*, VAP is considered to be life threatening with the mortality as high as 40%<sup>16</sup>.

MDR *Pseudomonas aeruginosa* infections are prevalent in patients with cystic fibrosis, immunocompromised conditions and in patients with chronic obstructive pulmonary disease. Various out breaks have been reported in intensive care units<sup>17-21</sup> but recently MDR *P.aeruginosa* has been reported in critically ill patients in non-outbreak settings as well<sup>22-23</sup>. Bacterial efflux pumps, alteration in antibiotic target sites, loss of membrane proteins, porin channel mutations etc. are various mechanisms by which *P.aeruginosa* acquires resistance to different groups of antimicrobials<sup>24</sup>. A close relationship between the presence of certain plasmids and

characteristic pattern of antibiotic resistance in MDR *P.aeruginosa* was reported in a study conducted in Egypt<sup>25</sup>. In our study, *P.aeruginosa* showed resistance to Amikacin (76.7%), antibiotic susceptibility pattern of B-lactams showed resistance to Meropenem, Ceftazidime, 82.3% & 90.1 % respectively (Table no.1). Whereas B-Lactams and Beta Lactamase inhibitors Tazobactam and Piperacillin showed a resistive pattern of 50.9%. While Ciprofloxacin showed resistance pattern of 33.2 %. The only antibiotic which showed 100% susceptibility against MDR *pseudomonas aeruginosa* was Colistin (Table no.1). According to Srinivasan et al MDR *P. aeruginosa* showed a resistance pattern of 100% to Ceftazidime and Meropenem, whereas it was 98% to cephalexin<sup>26</sup>. Study conducted by Kaushik et al<sup>27</sup> and Ganesamoni et al<sup>28</sup> showed resistance of *Pseudomonas* in a range of 13.9-90 % to Amikacin, 4-90% to Ceftazidime, 50-77.7% to Gentamicin and 41-95% to Ciprofloxacin.

In our study MDR *P. aeruginosa* prevalence is 36.5%. Whereas a study done in Turkey by Unan et al<sup>29</sup> and one done in Pakistan by Sabir et al<sup>30</sup> determined the percentage of MDR *P.aeruginosa* strains as 36.2% and 60% respectively. A study conducted in Peshawar, Pakistan by Farhatullah et al<sup>31</sup> in 2009 reported 29% prevalence of MDR *P. aeruginosa*.

Table no.1 Antibiogram for MDR *P.aeruginosa*

Antibiotics	Sensitive n (%)	Resistant n (%)
Amikacin	54 (23.3)	178 (76.7)
Aztreonam	53 (22.8)	179 (77.2)
Cefepime	102 (44)	130 (56)
Ceftazidime	23 (9.9)	209 (90.1)
Meropenem	41 (17.7)	191 (82.3)
Ciprofloxacin	155 (66.8)	77(33.2)
Piperacillin/Tazobactam	114 (49.1)	118 (50.9)
Colistin	232 (100.0)	-
Cefaperazone/sulbactam	110 (47.4)	118 (50.9)

Table 2: Resistance pattern of *P.aeruginosa*

	N	%
Sensitive to all antibiotics	190	30
Resistant to one antibiotic	164	25.9
Resistant to two Abs	48	7.6
Resistance to 3	48	7.6
Resistance to 4	49	7.7
Resistance to 5	32	5.0
Resistance to 6	28	4.4
Resistance to 7	55	8.7
Resistance to 8	20	3.2
	634	100

The intrinsic susceptibility of *P. aeruginosa* leaves only a few antimicrobials that can be used as therapeutic agents against it, and now with the emergence of MDR *P.aeruginosa* on the rise, therapeutic options are even more limited. Furthermore, therapy at times is restricted to Colistin and Polymoxin B. Both of these drugs have serious side effects particularly renal toxicity, hence the serum levels of these agents and kidney function have to be monitored during drug therapy. Out of the 232 MDR isolates, 20 isolates were such in which only Colistin was sensitive, 60% of the patients (12) were older than the age of 65 years. Colistin is known to be insufficient for patients'

with osteomyelitis, biliary tract disease, endocarditis and pneumonia because the concentration of the drug at the site of infection may be suboptimal<sup>32</sup>. As most of the MDR organisms cause pneumonia specifically in patients on ventilators, at times treatment failure can occur. In a study 75% of the patients suffering from pneumonia failed to respond to colistin treatment<sup>33</sup>. Though colistin causes nephrotoxicity and neurotoxicity, it is useful when options are limited<sup>34</sup>. The most sensitive drug was Colistin (100%), followed by Ciprofloxacin (66.8%), Piperacillin/Tazobactam (49.1%), Cefaperazone/Sulbactam (47.4%) and Cefepime (44%).

MDR *P. aeruginosa* has emerged as a major concern in hospital settings. Strict adherence to infection control policies, judicious use of antimicrobial agents, along with surveillance and education of health care professionals regarding MDR *Pseudomonas* can play a role in limiting the incidence of MDR *Pseudomonas*. Moreover, typing of strains of MDR *Pseudomonas* is also essential in order to determine the epidemiology and designing strategies for controlling nosocomial MDR strains.

Finally, more clinical research studies are required to identify various factors which play a role in the spread and emergence of new strains of MDR *Pseudomonas aeruginosa*. Stringent efforts should be made to develop new therapeutic agents with fewer side effects and better efficacy to minimize both morbidity and mortality caused by *P. aeruginosa* infections.

### Conclusion

In conclusion judicious use of antimicrobial agents, strict adherence to infection control policies and continuous surveillance are key factors to combat the rapid emergence of MDR *P.aeruginosa*. More over research on new anti pseudomnal drugs is urgently required in order to treat patients with MDR pseudomonas effectively.

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