Journal of Bioresource Management

Volume 9 | Issue 4 Article 8

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Recommended Citation

Ishfaq, R., Khan, H. R., Javeed, M., Tanveer, M. I., & Ashraf, A. (2022). Prevalence and Evaluation of Multidrug Resistance Pattern of Pseudomonas Aeruginosa among Critical and Non-Critical Areas at a Tertiary Care hospital of Multan, *Journal of Bioresource Management, 9* (4).
ISSN: 2309-3854 online
(Received: Feb 27, 2022; Accepted: Dec 14, 2022; Published: Dec 31, 2022)

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Cover Page Footnote

We would like to thank Dr. Asghar Javaid (Nishtar Medical Hospital Multan) for his help during the research work.

PREVALENCE AND EVALUATION OF MULTIDRUG RESISTANCE PATTERN OF *PSEUDOMONAS AERUGINOSA* AMONG CRITICAL AND NON-CRITICAL AREAS AT A TERTIARY CARE HOSPITAL OF MULTAN

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ABSTRACT

Pseudomonas aeruginosa is an extremely wide spread microorganism linked to nosocomial illnesses. Effective inspection of variations in antimicrobial resistance patterns of *P. aeruginosa* is vital for selecting suitable antimicrobial drugs for pragmatic treatment. The current research has been performed for assessing antimicrobial sensitivity profile of *P. aeruginosa* isolated as of a variety of medical specimens collected from critical and non-critical admitted patients of Nishtar Hospital, Multan. The isolates were detected utilizing standard lab practices, as well as the sensitivity was examined employing Kirby-Bauer disk diffusion method corresponding to Clinical and Laboratory Standard Institute (CLSI) recommendations 2019. Out of 373 samples, 110 (29.49 %) *P. aeruginosa* isolates were from admitted patients in different wards. 82 (74.5 %) came from non-critical units along with 28 (25.4 %) belonged to critical units. Prevalence of *P. aeruginosa*from the non-critical units was detected from surgical ward 35 (42.6 %) followed by medical ward 25 (30.48 %), gynecology 15 (18.29 %) and orthopedics 7 (8.5 %). The highest prevalence of *P. aeruginosa* among critical areas were from Medicine Intensive Care Unit 14 (50 %) followed by Surgery Intensive Care Unit 9 (32.14 %) and Respiratory Intensive Care Unit 5 (17.85 %). All were observed as multidrug-resistant against different antibiotics. The current research facilitates estimating the occurrence of MDR strains in intensive care units. Therefore, routine investigation of antibiotic sensitivity patterns is crucial for lowering the healthcare-linked infection levels as well as antimicrobial resistance.

Keywords: *Pseudomonas aeruginosa*, hospital acquired infections, antimicrobial resistance, nosocomial infection, multidrug resistance.

INTRODUCTION

Antibiotic resistance is the main apprehension of current medication. Nosocomial infections are caused by the emergence of resistant strains which contributes to the morbidity along with death of patients being hospitalized. *Pseudomonas aeruginosa* is the next greatest recurrent source of pneumonia, the fourth most frequent cause of urinary tract infections (UTI), and the sixth common cause of bacteremia in intensive care units (ICUs) due to the ubiquitous and versatile opportunistic organism (Bekele et al., 2015; Saeed et al., 2018). The evolutionary antimicrobial resistance tactics of bacteria have developed using a wide variety of antibiotics resulting in the emergence of resistance. Due to the frightening increase of drug resistance, the effectiveness of many antibiotics to treat infections has become moderately restricted. Thus, the threat from drug-resistant strains is accumulating and increasing day by day. Multidrug-resistant

(MDR) *P. aeruginosa*is considered as a pervasive increase in clinical dilemma, which is expected to be an alarming threat to public health around the world. It has an important role in the increased rate of mortality and morbidity along with healthcare costs (Bayani et al., 2013). MDR *P. aeruginosa*isolates are known to be resistant from three or more antibiotics of the subsequent classes of antibiotics: carbapenems, penicillins, cephalosporins, monobactams, aminoglycosides, and fluoroquinolones (Dash et al., 2014). MDR strains of *P. aeruginosa*arbitrated different mechanisms including bacterial efflux pumps, altered target sites, loss of membrane proteins and enzyme production or inhibition, etc (Odisha, 2012).

Nosocomial *P. aeruginosa*isolates demonstrate high level of drug resistance. ICUs of most hospitals cover maximum number of critically ill patients from all the wards. Among these patients nosocomial infections transmission rate is approximately 20 % (Mythri and Kashinath, 2014). Patients admitted in ICU are further prone to Hospital-acquired infections (HAIs) by *P. aeruginosa*in contrast to patients staying in non-critical units (Bayani et al., 2013). The preference of empiric administration in ICUs is extremely complex. It is necessity to strike a equilibrium between narrow spectrum as well as broad spectrum antibiotics (Harris et al., 2016). In critical units, MDR *P. aeruginosa*is limiting the existing therapeutic choices for bacterial infections (Qadeer et al., 2016).

It is the need of hour to recognize and respond the problem of evaluation of the *P. aeruginosa*sensitivity pattern against regularly approved antimicrobial agents. It would guide the physicians for the justified use of currently available antibacterial management choice. Thus, the goal of the research was to assess frequency along with antibiotic-resistance profile of *P. aeruginosa*of hospitalized patients from critical and non-critical units at tertiary care hospital, Multan.

MATERIALS AND METHODS

Ethical Consideration

The investigation was carried out after authorization from the Ethical Committee of Nishtar Hospital Multan.

Study Duration

A cross-sectional investigation was carried out in Nishtar Hospital, Multan in the duration of January 2019 to August 2019.

Sample Size and Sample Collection

During this period of study, a total of 373 samples of blood, pus, and urine (from catheterized and non-catheterized) were collected from critical and non-critical units of the hospital. Critical units include the surgery intensive care unit (SICU), respiratory intensive care unit (RICU) as well as the medical intensive care unit (MICU), and Non-critical units include gynecology, general medicine, respiratory medicine, surgery, and orthopedic departments.

Isolation and Detection of P. aeruginosa

There were 110 *P. aeruginosa* isolates found in total via a variety of clinical specimens as of non-critical as well as critical units at Nishtar Hospital Multan. Gram staining followed by various biochemical tests were performed as per standard protocols (Colle et al., 2007) for the identification of *P. aeruginosa*.

Antimicrobial sensitivity Analysis

The disc diffusion method of Kirby–Bauer was used to perform antibiotic sensitivity evaluation. Antimicrobial sensitivity testing was

carried out via Muller Hinton Agar (Hudzicki, 2009). Profiles of antimicrobial susceptibility were obtained by following antibiotics: piperacillin (100 mcg), ceftazidime (30 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), aztreonam (30 mcg), gentamicin (10 mcg), imipenem (10 mcg) andmeropenem (10 mcg). The results were noted in accordance with the Clinical and Laboratory Standards Institute's guidelines (CLSI protocols) (Balouiriet al., 2016).

Statistical Assessment

MS Excel was used to clean all categorical data and statistical analysis was done by XL-stat software 2010. All tests were completed to explore the antibiotic resistance of *P. aeruginosa.*

RESULTS

Distribution of P. aeruginosa

A total of 373 samples of blood, pus, and urine (from catheterized & non-catheterized) were analyzed from hospitalized patients at Nishtar Hospital Multan. Out of 373 samples, 110 (29.49 %) samples were positive for *P. aeruginosa*. The isolates of *P. aeruginosa*were categorized according to the wards from which samples were obtained; about 82 (75 %) isolates were from non-critical areas and 28 (25 %) *P. aeruginosa* strains were isolated via critical areas of the hospital as represented in Table 1.

Table 1: Distribution of *P. aeruginosa* (N= 110)

Specimen	**Non-Critical Sectors**		**Critical Sectors**	
	No. of *P.aeruginosa*isolates	% Age of *P. aeruginosa* isolates	No. of *P. aeruginosa*isolates	% Age of *P. aeruginosa* isolates
Pus	33	30 %	10	9.0 %
Urine (Catheterized)	11	10 %	5	4.5 %
(Non – Catheterized)	18	16.4 %	5	4.5 %
Blood	20	18.2 %	7	7.3 %
Total	82	75 %	28	25 %

Table 2: Distribution of *P. aeruginosa*based on non-critical areas.

Non–Critical Sectors N = 82	**Specimens N (%)**				**Total N (%)**
	Pus	Urine (Catheterized)	Urine (Non-Catheterized)	Blood	
Surgery	18 (21.9 %)	5 (6.09 %)	7 (8.53 %)	5 (6.09 %)	35 (42.6 %)
Medicine	5 (6.09 %)	3 (3.65 %)	7 (8.53 %)	10 (12.19 %)	25(30.48 %)
Gynecology	5 (6.09 %)	3 (3.65 %)	4 (4.87 %)	3 (3.65 %)	15(18.29 %)
Orthopedics	5 (6.09 %)	--	--	2 (2.43 %)	7 (8.5 %)

Table 3: Distribution of *P. aeruginosa* based on Critical areas

Critical Sectors N = 28	Specimens N (%)			Total N (%)
	Pus	Urine (Catheterized)	Blood	
S-ICU	4 (14.2 %)	3 (10.7 %)	2 (7.14 %)	9 (32.14 %)
M-ICU	3 (10.7 %)	7 (25 %)	4 (14.2 %)	14 (50 %)
R-ICU	3 (10.7 %)	-	2 (7.14 %)	5 (17.85 %)

Distribution of P. aeruginosaamong Non-Critical and Critical areas

Among non-critical areas 82(74.5 %), isolation rate of *P. aeruginosa*was found high in surgery ward 35(42.6 %) followed by medicine 25(30.48 %), gynecology 15(18.29 %), and orthopedics 7(8.5 %). The elevated incidence of *P. aeruginosa*was found in pus specimens 18(21.9 %) from the surgery ward in non-critical areas as shown in Table 2. Out of 28(25 %) isolates of critical sectors, the highest isolation of *P. aeruginosa*was observed from medicine intensive care unit (M-ICU) 14(50 %) followed by surgery intensive care unit (S-ICU) 9(32.14 %) and respiratory intensive care unit (R-ICU) 5(17.85 %).*P. aeruginosa*exhibited high incidence in urine specimens from MICU 7(25 %) in critical areas as represented in Table 3.

Antibiotic Susceptibility

The antibiotic sensitivity profile of *P. aeruginosa*isolates was determined through the Kirby-Bauer disk-diffusion method (Hudzicki 2009). Eight antibiotic discs piperacillin, ciprofloxacin, levofloxacin, aztreonam, ceftazidime, gentamicin, imipenem, andmeropenem were employed. All drugs have dissimilar resistant as well as sensitive patterns in accordance with their zone of inhibition. The antimicrobial susceptibility profile of *P. aeruginosa*isolated from non-critical areas compared to these drugs being described in Table 4. The current research demonstrates the fraction of *P. aeruginosa*being isolated from non-critical areas were mostly resistant to these drugs meropenem 54.8 %, levofloxacin 43.9 %, Gentamicin 36.5 %, ceftazidime 30.4 % (Figure 1), and 97.5 % sensitivity was observed against imipenem followed by piperacillin 95.1 %.

Table 4: Antimicrobial susceptibility profile of isolated *P. aeruginosa*as of non-critical regions (N=82).

Antibiotics	Resistant N (%)	Sensitive N (%)
Piperacillin	18 (21.9 %)	78 (95.1 %)
Ceftazidime	25 (30.4 %)	72 (87.8 %)
Ciprofloxacin	29 (35.3 %)	65 (79.2 %)
Levofloxacin	36 (43.9 %)	52 (63.4 %)
Gentamicin	30 (36.5 %)	77 (93.9 %)
Imipenum	10 (12.19 %)	80 (97.5 %)
Meropenum	45 (54.8 %)	42 (51.2 %)
Aztreonum	16 (19.5 %)	69 (84.1 %)

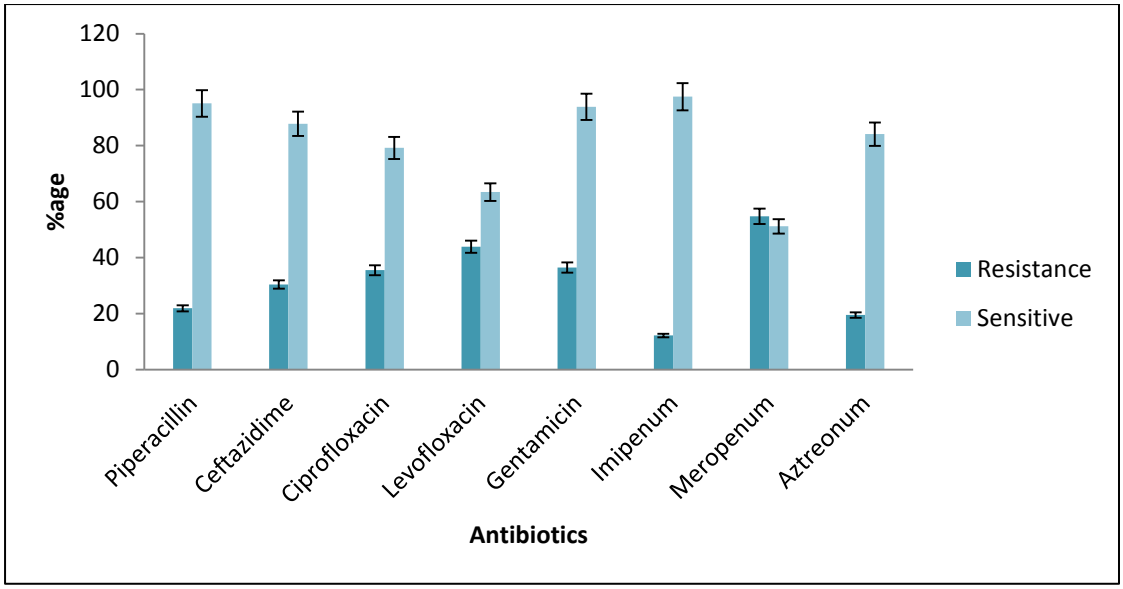

Figure 1: Percentage of antimicrobial resistance of *P. aeruginosa*isolated from non-critical areas.

Table 5: Antimicrobial sensitivity profile of isolated *P. aeruginosa* from Critical areas (N=28)

Antibiotics	Resistant N (%)	Sensitive N (%)
Piperacillin	28 (100 %)	0 (0 %)
Ceftazidime	28 (100 %)	0 (0 %)
Ciprofloxacin	14 (50 %)	6 (21.4 %)
Levofloxacin	11 (39.2 %)	8 (28.5 %)
Gentamicin	28 (100 %)	0 (0 %)
Imipenum	17 (60.7 %)	4 (14.2 %)
Meropenum	28 (100 %)	0 (0 %)
Aztreonum	17 (60.7 %)	5 (17.8 %)

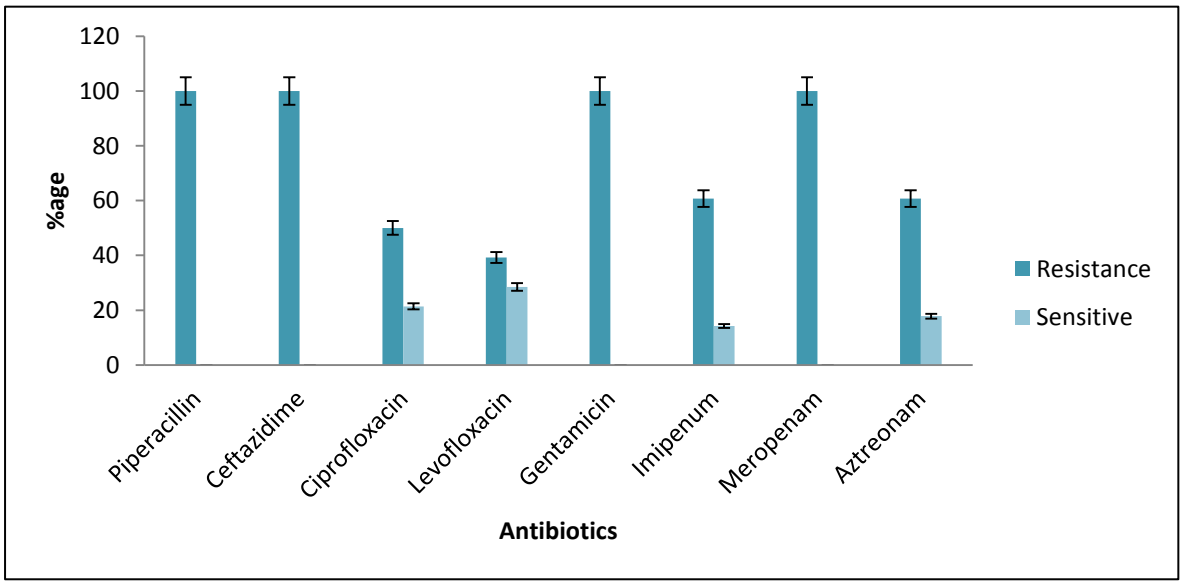

Figure 2: Percentage of antimicrobial resistance of *P. aeruginosa*isolated from critical areas.

In critical units, all *P. aeruginosa* isolates were reported to be multi drug resistant. Multi drug resistant (MDR) means that microorganisms are resistant to penicillin, cephalosporins, quinolones and aminoglycosides (Dash et al., 2014). The antimicrobial sensitivity profile of this MDR, *P. aeruginosa*is is represented in Table 5. This isolated *P. aeruginosa*from critical areas was most resistant to piperacillin 100 %, ceftazidime 100 %, gentamicin 100 %, and meropenem 100 % as shown in Figure 2.

DISCUSSION

*P. aeruginosa*is a ubiquitous Gram-negative rod which is linked to numerous infections for instance pneumonia, bacteremia, urinary tract, skin infection, etc. *P. aeruginosa*particularly affects immune-compromised patients (Juan et al., 2010). This species is considered as a major opportunistic human pathogen, which is accountable for universal nosocomial diseases with escalating medical along with veterinary consequences (P.D et al., 2009). Due to the emergence of MDR clinical isolates, *P. aeruginosa*is considered a global health problem (M.E et al., 2015). This scenario leaves the clinicians with few therapeutic antibacterial drugs for the cure of contagious diseases (Farooq et al., 2019).

In the current research, a 29.49 % frequency of *P. aeruginosa*was observed, which was like findings of India as 32.1 % (Rajat et al., 2012). On contrary, the low prevalence of 2.1 % was observed by a previous study done in Nigeria (OKon et al., 2009). The varied prevalence of *P. aeruginosa*may be due to the different places and the way of receiving clinical samples for examination, studied population, geographical locations, and types of hospitals. In our study, higher isolation of *P. aeruginosa*was observed in pus samples (30 %) from non-critical areas. These results were supported by previous studies where pus/wounds specimens were the frequent cause (Saeed et al., 2018).Most of the patients have complications on wound locations and are very effortless intentions for nosocomial pathogens. Thus, this is the basic fact to justify the existence of the maximum number of isolates in pus samples. Poor hygiene and inadequate antiseptic measures in the wards may be contributed to acquiring the resistant strains (Farooq et al., 2019).

In our observation, the highest distribution of *P. aeruginosa*was found in the surgery ward (46.2 %) among non-critical areas. These results have coincided with the previous study where 29.6 % of *P. aeruginosa*was isolated from post-operative patients (Ranjan et al., 2010). On the other hand, Kumari has recorded a lower isolation rate of *P. aeruginosa*from the surgical ward (Kumari et al., 2019). According to research, the highest isolates of *P. aeruginosa*as of MICU among critical areas, whereas lower isolation 42.9 % was found by Saeed (Saeed et al., 2018).

According to antimicrobial susceptibility profile of *P. aeruginosa*from non-critical units, it showed resistance to meropenem (54.8 %) followed by levofloxacin (43.9 %) and ciprofloxacin (35.3 %). Bayani reported the same results as in our study (Bayani et al., 2013; Rytekar et al., 2017). Minimum resistance was found against imipenem (12.19 %). This result relates with the investigation made by Rakhee (Rakhee et al., 2014). However, isolates of *P. aeruginosa* from critical areas were opposed to various groups of antibiotics. Currently, available drugs in our research for MDR *P. aeruginosa*contain Fluoroquinolones (ciprofloxacin, levofloxacin), Cephalosporins (ceftazidime), Aminoglycosides (gentamicin), Anti-pseudomonalpenicillins (piperacillin), Monobactum (aztreonam), and Carbapenems (Imepenum, meropenem). Like other studies, high resistance was

demonstrated in our research against all beta-lactam antibiotics (Savas et al., 2005).

Concerning antibiotic sensitivity, the maximum resistance was found against piperacillin (100 %), ceftazidime (100 %), gentamicin (100 %), and meropenem (100 %) while these isolates expressed the highest susceptibility to levofloxacin (28.5 %). However, the highest resistance rate against piperacillin, ceftazidime, and meropenem was reported in previous studies respectively (Asghar and Faidah, 2009; Al-agamy et al., 2011; Asghar, 2012). Previous studies reported the highest resistance against piperacillin (100 %), gentamicin (98 %) (Ameen et al., 2015). Thus, the differences in the resistance rate may be due to prescribing activities of all hospitals as well as the accumulative stress of particular antimicrobial agents (Sarwat et al., 2015).

From the above findings, we have analyzed that the resistance rate is still higher in critically ill patients than the non-critical patients. This study emphasizes the imperative require for balanced apply of antibiotics and severe constancy to thought of reserve prescription, to reduce the misuse of all currently accessible antimicrobials (Javiya et al., 2008).

CONCLUSION

The present study uncovered the resurgence of *P. aeruginosa*in contrast to different antibiotics. It plays a great role in apprehending the manifestation of multi drug resistant isolates in intensive care units been escalating at a frightening speed. Therefore, consistent surveillance and appropriate measures are required to reduce HAIs and antimicrobial resistance. Regular monitoring of *Pseudomonas* sources in different wards and proper management of wards disinfection and instrumental sterilization along with hand hygiene is mandatory to reduce the Hospital acquired infections and resistance.

AUTHOR'S CONTRIBUTION

Romah Ishfaq and Hubza Ruatt Khan wrote the first draft of the manuscript. Mehvish Javeed and Muhammad Ikrama Tanveer analyzed the data carefully while Asma Ashraf modified the final draft. All authors accept responsibility for the remarks made in the published paper.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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