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ORIGINAL ARTICLE

Antibiotic susceptibility profile of *Pseudomonas species* isolated from clinical specimens to access, watch and reserve drugs across various hospital settings at a tertiary care hospital of central India

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ABSTRACT

Background and Objectives: Over the last decade, hospital-acquired infections, particularly in the critical care setting, have become more common, with Gram-negative bacterial infections having the highest prevalence. This study aims to determine the prevalence and antibiotic susceptibility pattern of *Pseudomonas species* to WHO's, aware class of antibiotics, which are commonly prescribed across various ICU's, medical and surgical wards of our tertiary care teaching hospital.
Materials and Methods: This prospective study conducted from January 2021 to June 2022 at a tertiary care centre of central India identified *Pseudomonas species* from clinical samples using standard procedures and antimicrobial susceptibility testing performed as per Clinical Laboratory Standards Institute (CLSI) guidelines (M100; 32th Edition).
Results: A total of 1490 non duplicate *Pseudomonas species* isolates were grown from 21,019 culture positive clinical samples, of which 1247 were *Pseudomonas aeruginosa.* Out of these 1247 *Pseudomonas aeruginosa* 384 were MDR (30.7%). *Pseudomonas aeruginosa* were most commonly isolated from the pus samples (85%). ICU isolates were significantly more resistant to antibiotics than those from other units. *P. aeruginosa* strains from ICUs showed the highest rates of resistance to ceftazidime (93.9%). Reserve drug colistin showed good susceptibility (98.2%). All the 18 colistin resistant strains were found to be negative for plasmid mediated *mcr*-1,2,3 genes.
Conclusion: The study shall help to generate and disseminate the data so that proper antibiotic policy can be made for judicious use of Access, Watch and Reserve antibiotics and antibiotic de-escalation plan can be put forth.

Keywords: Antimicrobial drug resistance; *Pseudomonas aeruginosa*; Intensive care unit; Multidrug resistant; Colistin; World health organization

INTRODUCTION

Antimicrobial resistance is big threat for public health (1). Non-fermenting Gram-negative pathogen *Pseudomonas aeruginosa* is more common in critically ill patients, particularly in immunocompromised and hospitalized patients, where it contributes 3-15% of blood stream infections with high mortality rates of about 27-48% (2). Patients in intensive care units (ICUs) are more likely to harbour *P. aeruginosa* infections (3).

The World Health Organization (WHO) changed how antibiotics are categorized in its most recent model list of essential medicines. Three categories make up the new model: Key Access Antibiotics (including β-lactam, aminoglycoside and first or sec-

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ond-generation cephalosporin that "should be extensively used antibiotics accessible, cost-effective, and of guaranteed quality; Watch Group antibiotic (includes macrolides, penems, glycopeptides, quinolones, as well as third-generation cephalosporins) only advised for specific indication; and Reserve Group antibiotics (such as fourth-generation and fifth-generation cephalosporins, aztreonam, polymixins) for use when every alternate antibiotic has failed (4).

There are more than 140 species in the genus *Pseudomonas*, the majority of which are saprophytic. More than 25 species are linked with human infections. The majority of human disease-causing Pseudomonads are associated with opportunistic infections. *P. aeruginosa, P. putrefaciens, P. fluorescens, P. putida, P. stutzeri,* and *P. luteola* are a few of them (5).

Pseudomonas aeruginosa is an opportunistic organism that is multi-drug resistant (MDR), causing acute or chronic infection in immunocompromised people with chronic obstructive pulmonary disease, cancer, burn injuries, cystic fibrosis, diseases (COPD), Ventilator-associated pneumonia (VAP) and sepsis (6).

P. aeruginosa is one of the ESKAPE pathogens, along with pathogens like *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Enterobacter.* Carbapenem-resistant *P. aeruginosa* is listed among the "critical" group of pathogens by WHO, which urgently need newer antibiotics (7).

Pseudomonas aeruginosa is responsible for approximately 5-14% of all nosocomial or healthcare-associated infections and 16-40% of cases of ventilator-associated pneumonia (VAP) (8). The Centres for Disease Control and Prevention reported 32,600 cases causing 2,700 deaths due to multidrug-resistant *P. aeruginosa* infection among hospitalized patients in the United States in 2017 (9). MDR *P. aeruginosa* is classified as not being susceptible to at least one antibiotic in at least three antibiotic groups where susceptibility to *P. aeruginosa* is typically anticipated: penicillins, aminoglycosides, carbapenem, fluoroquinolones, and cephalosporins (10). Hence, it is important to generate local susceptibility data for *P. aeruginosa* to AWaRe classification of antibiotics.

The study can be strengthened further by associating the site of infection and clinical condition of the patient with the isolate's resistance characteristics.

MATERIALS AND METHODS

This prospective, study was conducted in the department of microbiology of a tertiary care teaching centre of central India for a duration of 18 months. Ethical approval for the study was obtained from the institutional ethics committee (27088/MC/IEC/2021).

The varied specimens collected from patients were processed for isolation and identification of *Pseudomonas species.* All the specimens were inoculated on blood agar, MacConkey agar, and incubated at 37°C in ambient air at the aerobic section of bacteriology laboratory. All the isolates obtained from non repetitive clinical samples were included for analysis except those grown from faecal specimens and rectal swabs. Identification of the *Pseudomonas species* was done by Gram staining, observing colony morphology, pigment production and conventional biochemical methods viz. Hugh leifson oxidative / fermentative test, oxidase test, motility testing. Phenotypic characterization of various *Pseudomonas species* was done using the scheme given in (Table 1).

The antimicrobial susceptibility of bacterial isolates was determined by the Kirby-Bauer disk diffusion method, and minimal inhibitory concentration (MIC) of colistin was determined by the broth microdilution method (gold standard). Interpretation of antibiotic susceptibility testing was done as per the Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (M100; 32th Edition) (12, 13). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the controls for disc diffusion testing. *P. aeruginosa* ATCC 27853 (MIC: 1-4 µg/mL) and in-house *P. aeruginosa* (MIC >16 µg/mL) was used as negative and positive control respectively for colistin broth microdilution.

Among colistin resistant *Pseudomonas species*, the most prevalent plasmid mediated colistin resistance genes *mcr*-1,*mcr*-2, and *mcr*-3 were traced by PCR. DNA extraction, primer sequence selection and PCR reaction were performed as per Soni et al. (14). The Gel Doc system was used to visualize the DNA bands following electrophoresis. By comparing the relative mobility of the PCR products to that of 100-bp molecular markers, the PCR product sizes were calculated.

Details of patients such as the demographic parameters, primary clinical diagnosis, location of stay in the hospital, duration of hospital stay, were noted in a structured proforma received in clinical microbiology department (Table 2).

Table 1. Phenotypic Characterization of *Pseudomonas species* (11).

Test	*P. aeruginosa*	*P. fluorescens*	*P. putida*	*P. stutzeri*	*P. luteola*	*P. oryzihabitans*
Oxidase	+	+	+	+	-	-
Growth						
Cetrimide	+	+	+	-	-	-
at 42C	+	-	-	+	+	-
Nitrate reduction	+	-	-	+	+	-
Gelatin hydrolysis	+	+	-	-	+	-
Esculin hydrolysis	-	-	-	-	+	-

RESULTS

A total of 1490 non duplicate *Pseudomonas species* were isolated from 21,019 culture positive clinical samples, of which 1247 were *Pseudomonas aeruginosa*. Out of these 1247 *P. aeruginosa*, 384 (30.7%) were multidrug resistant (MDR). Most of the *Pseudomonas species* isolated were from ICU patients (42.4%) followed by medical and surgical wards (41%), and then OPD patients (define OPD for the first time) (16.5%). Majority of isolates were from the age group of 46-65 years (49%), and the least were from infants (7%) (Table 2).

A total of 1490 non duplicate *Pseudomonas species P. aeruginosa* (83.2%), *P. fluorescens* (12%), *P, putida* (1.8%), *P. luteola* (1.5%), *P. stutzeri* (0.8%) were isolated.

Majority of *P. aeruginosa* were isolated from pus specimen (655) followed by respiratory samples (200), blood cultures (150), sterile fluid (142) and urine (100) (Table 3).

P. aeruginosa isolated from OPD showed good susceptibility to colistin (99.6%), followed by meropenem (79.2%), cefepime (73.1%), amikacin (65%), gentamicin (61.7%), piperacillin-tazobactam (59.7%), imipenem (56.5%), ciprofloxacin (51.5%), and ceftazidime (35.7%) (Table 4).

18 out of 1490 (1.2%) of *Pseudomonas species* were resistant to colistin which is agent of last resort drug. All the 18 colistin resistant strains were screened for plasmid mediated *mcr*1,2,3 genes and were found to be negative (Table 5).

DISCUSSION

Healthcare-associated infections constitute a potentially controllable source of morbidity and mortality, despite dramatic improvements in the critical care setting during the past ten years.

ICU patients continue to experience much more ICU-acquired infections than do non-ICU patients (15).

A total of 1490 *Pseudomonas species* were isolated from 21,019 culture-positive clinical samples, accounting for an isolation rate of 7% which is similar to a study conducted by Maharjan et al. (6.48%) (16). Out of 1490 *Pseudomonas species* isolated 418 (28%) were multidrug resistant.

The demographic data from our study showed patients most commonly affected by *Pseudomonas species* infections were males between in the age group of 46-65 years which is similar to finding by Gupta et al. who reported patients >50 years had higher risk for acquiring infections (17). *Pseudomonas species* in this study were predominantly from ICU (42.4%), followed by medical and surgical wards (41%) and then OPD (16.5%), this outcome was consistent with the findings of Stéphan et al. that older patients had a higher incidence of infection in the ICU than younger patients as aging leads to variable decline of physiologic functions and differential changes to organ systems (18).

In our study higher rate of *Pseudomonas* infection were seen in burn patient (10%) in concordance with study by Nikokar et al. (19). Demonstrating a higher frequency of *Pseudomonas species* among the patients with burn infections (47%).

Pseudomonas aeruginosa was the most common among species isolated. In this study, most of the *P. aeruginosa* were isolated from pus samples (85%) followed by respiratory samples (90.9%), blood cultures (73.1%), urine (84.7%) and various body fluids, viz. cerebrospinal fluid and pleural fluid (79.7%). This is in accordance with a study conducted by Maharjan et al. (15). where most of the *P. aeruginosa* isolated were from pus sample (19.1%).

Pseudomonas aeruginosa isolated from OPD

Table 2. Demographic and clinical details of the patients

Parameter	% distribution (number of cases) Total number of cases=1490
Age	0-1 years (5%)
	1-18 years (16%)
	18-45 years (20%)
	46-65 years(49%)
	>65 years (10%)
Gender	
Male	887 (59.5%)
Female	603 (40.4%)
Primary diagnosis	
Acute exacerbation of chronic obstructive pulmonary disease(COPD)	69 (4.6%)
Pneumonia	45 (3%)
Pulmonary Koch's	20 (1.3%)
Idiopathic pulmonary fibrosis	29 (1.9%)
Lung abscess	26 (1.7%)
Chronic bronchopulmonary aspergillosis	36 (2.4%)
Ruptured amoebic liver abscess	25 (1.6%)
Cirrhosis	25 (1.6%)
Hospital-acquired pneumonia	61 (4%)
Diabetes	20 (1.3%)
Renal disease	43 (2.8%)
Hypertension	10 (0.67%)
Acute suppurative otitis media	70 (4.6%)
Chronic suppurative otitis media	140 (9.3%)
Cystic fibrosis	40 (2.6%)
Burn	159 (10.6%)
Post operative LSCS	120 (8%)
Meningitis	63 (4.2%)
Urinary tract infection	75(5%)
Peritonitis	64 (4.2%)
Location of patient in hospital	
OPD	246 (16.5%)
Wards	
Medical	250 (16.7%)
Surgical	362 (24.2%)
ICU	
Medical	289 (19.3%)
Surgical	343 (23%)
Mean duration of stay in the hospital (among admitted patients)	6.2 days

showed good susceptibility to colistin (99.6%), followed by meropenem (79.2%), cefepime (73.1%), amikacin (65%), gentamicin (61.7%), piperacillin-tazobactam (59.7%), imipenem (56.5%), ciprofloxacin (51.5%), and ceftazidime (35.7%). The widespread use of third-generation cephalosporins in hospital settings may be to blame for the high level of resistance in *Pseudomonas* infections.

ICU isolates were more resistant to antibiotics than those from other units, suggesting apparent misuse/overuse of antimicrobials in critically ill patients being referred to tertiary care teaching hospitals. ICU

Table 3. Distribution of *Pseudomonas species* across all clinical samples

S No	Isolates	Type of sample					Total
		Blood	Pus	Respiratory Sample	Sterile fluids	Urine	
		N (%)					
1	*Pseudomonas aeruginosa*	150 (73.1%)	655 (85.1%)	200 (90.9%)	142 (79.7%)	100 (84.7%)	1247 (83.2%)
2	*Pseudomonas fluorescens*	27 (13.17%)	93 (12%)	15 (6.8%)	30 (16.8%)	15 (12.7%)	180 (12%)
3	*Pseudomonas Luteola*	6 (2.9%)	12 (1.56%)	1 (0.4%)	2 (1.1%)	1 (0.8%)	22 (1.5%)
4	*Pseudomonas Putida*	15 (7.3%)	6 (0.78%)	2 (0.9%)	3 (1.6%)	2 (1.6%)	28 (1.8%)
5	*Pseudomonas stutzeri*	7 (3.4%)	3 (0.39%)	2 (0.9%)	1 (0.5%)	0 (0%)	13 (0.8%)
	Total	205 (100)	769 (100)	220 (100)	178 (100)	118 (100)	1490 (100)

Table 4. Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* across various hospital units (% susceptibility) (as per CLSI M100, 32 Edition) (12).

AWaRe Classification	Antimicrobial agents (mcg)	Outpatient department	Intensive Care unit	Wards
Access	Amikacin (30)	160 (65%)	396 (62.6%)	394 (64.3%)
	Gentamicin (10)	152 (61.7%)	383 (60.6%)	375 (61.2%)
Watch	Ceftazidime (30)	88 (35.7%)	39 (6.1%)	79 (12.9%)
	Ciprofloxacin (5)	127 (51.6%)	156 (24.6%)	279 (45.5%)
	Imipenem (10)	139 (56.5%)	352 (55.6%)	354 (57.8%)
	Meropenem (10)	195 (79.2%)	412 (65.1%)	447 (73%)
	Piperacillin/ tazobactam (100/10)	147 (59.7%)	307 (48.5%)	333 (54%)
Reserve	Cefepime (30)	180 (73.1%)	282 (44.6%)	419 (68.4%)

Table 5. Colistin MIC (by broth microdilution method) of various *Pseudomonas species*

Pseudomonas species	Total number of isolates	Colistin MIC ≤2 (Intermediate)	Colistin MIC ≥4 (Resistant)	*mcr*-1,2,3 among Colistin resistant (MIC≥4)
Pseudomonas aeruginosa	1247	1230	17	Negative
Pseudomonas fluorescens	180	179	1	Negative
Pseudomonas luteola	22	22	0	Negative
Pseudomonas putida	28	28	0	Negative
Pseudomonas stutzeri	13	13	0	Negative
Total	1490	1472	18	

strains of *P. aeruginosa* presented the highest resistance rates to ceftazidime (93.9%) followed by ciprofloxacin (75.4%). Reserve drug colistin used as a last resort drug to treat severe infections caused by multidrug resistant *Pseudomonas* showed good susceptibility (98.2%).

A study conducted by Feretzakis et al. reports the highest resistance rates to gentamicin (57.97%) and cefepime (56.67%), followed by fluoroquinolones (55.11%) and carbapenems (55.02%), while a sensitivity rate of 97.41% was reported to colistin among ICU strains of *P. aeruginosa* (20). The findings of the study have important implications for public health, clinical practitioners and policy makers in the area by providing salient groups of antibiotics which can be used for patient management.

The plasmid mediated colistin resistance genes (*mcr-1, mcr-2, mcr-3*) among non fermenters have not been reported till date from any centre of India and were not seen in any of our colistin resistant isolates. This may be explained by the suspected presence of other members of the *mcr* family (*mcr-4* to *mcr-10*) or alternative colistin resistance mechanisms, such as chromosomal mediated or LPS modification, may be the cause of colistin resistance in our strains (21).

The drug resistance data presented here is limited

to a single tertiary care set up, further larger prospective studies looking for syndromic drug resistance patterns are warranted. We could evaluate antibiotic susceptibility for *P. aeruginosa* for which disc diffusion interpretive criteria were available in CLSI. Antibiotic susceptibility testing of the rest of *Pseudomonas species* (*P. fluorescens, P. putida, P. stutzeri,* and *P. luteola*) could not be done, but colistin broth microdilution was done for all *Pseudomonas* spp., and interpretive criteria were used in accordance with clinical efficacy trials of *Pseudomonas aeruginosa*. Correlating patient outcome with the implementation of a final antimicrobial treatment would further strengthen the data for patient welfare. Additionally, other molecular analysis for the colistin resistance's root cause may have been carried out.

CONCLUSION

In the intensive care unit, increased resistance to antimicrobials has been observed among *Pseudomonas aeruginosa*. The emergence of resistance during treatment in *P. aeruginosa* is alarming and this limits treatment options. It is substantially due to the incomplete infection clearing that results in poor clinical outcomes. One main approach to be addressed is the use of combination testing. The rationale for the theory of combination therapy is that it minimises emergence of resistance during treatment and brings about synergy, where appropriate inhibitory concentrations (MIC) can be achieved by using two antibiotics that have distinct ranges of activity.

The purpose of this study is to generate and disseminate the data so that attention of policy makers can be drawn to judicious use of Access, Watch and Reserve antibiotics and appropriate antibiotic policy can be framed along with antibiotic de-escalation plan can be drafted for each health care setup. The findings of the study addressed vital antimicrobial resistance concerns and lead to further research on mechanism and pattern of drug resistance.

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