Original Article

DOI: 10.7860/JCDR/2021/51650.15629

The Rising Threat of Drug Resistant *Pseudomonas aeruginosa*- A Nightmare for Intensive Care Unit Patients

Microbiology Section

SHIVENDRA DUTT SHUKLA[1], GEETA GUPTA[2], RAZIA KHATOON[3], RITU JAIN[4], ANKITA GUPTA[5]

ABSTRACT

Introduction: Multidrug Resistant (MDR), Extensively Drug Resistant (XDR) and Pan Drug Resistant (PDR) variants manifest a high level of intrinsic resistance to antimicrobial drugs by the help of efflux pump, biofilm formation and aminoglycoside modifying enzymes. The potentiality of *Pseudomonas* spp. to produce variety of drug resistance mechanism has led to evolution of drug resistant phenotypes this poses a challenge for clinicians in the treatment of severe infection among Intensive Care Unit (ICU) patients.

Aim: To determine the phenotypic profiling of β-lactamases and burden of MDR, XDR and PDR *Pseudomonas aeruginosa (P. aeruginosa)* in ICU patients.

Materials and Methods: The present cross-sectional prospective study was carried in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India, after permission from Institutional Ethics Committee (IEC). A total of 115 isolates of *P. aeruginosa* were isolated from 502 human clinical samples from January 2019 to February 2021 and all the clinical samples were non duplicate. Antimicrobial Susceptibility Testing (AST) was performed for all isolates by standard Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA). Phenotypic profiling of Extended Spectrum β-Lactamase (ESBL), Metallo β-Lactamase (MBL) and Ampicillinase C (AmpC) was performed by disc potentiation test; Imipenemase (IMP) - Ethylenediamine Tetraacetic Acid (EDTA) combined disc test and Cefoxitin Cloxacillin Double Disc Synergy Test (CC-DDST), respectively. The obtained results were statistically analysed in numbers and percentages using MS Excel 2013 version.

Results: Out of 502 total human clinical samples, 115 isolates were *P. aeruginosa* giving the prevalence rate of 23%. Among 115 *Pseudomonas* isolates, 60 (52%) were MDR phenotypes, 8 (7%) were XDR phenotypes and there was no PDR phenotypes isolated in present study as all isolates were sensitive to Ticarcillin/Clavulanic acid, Colistin and Polymyxin B. Out of 115 isolates, 59 (51%) were ESBL producers, 26 (23%) were MBL producers, and 6 (5%) were AmpC producers.

Conclusion: Strict antibiotic policies and regular surveillance programme of antimicrobial resistance must be tailored to fend off the emergence of drug resistant *Pseudomonas aeruginosa*.

Keywords: Extended spectrum β-lactamases, Extensively drug, Metallo β-lactamases, Multidrug resistant

INTRODUCTION

About 50 years ago, *P. aeruginosa* was rarely considered as an actual pathogen, but in the 1970s it was documented to be the microorganism which was directly correlated with neutropenic host. In the present scenario, it is amongst the most common pathogen responsible for hospital acquired infection. Respiratory instrument, antiseptics, soaps, sinks, mops and hydrotherapy pools are the variety of sources for this pathogen [1]. *P. aeruginosa* is mainly responsible for nosocomial infection and around 10-20% of nosocomial infection in patients were admitted in the ICUs [2]. This pathogen is categorised into different phenotypic variants which are mainly based on the drug resistance pattern. MDR type is defined as *Pseudomonas* spp. that are resistant to more than one antimicrobial agent in three or more antimicrobial categories. XDR is defined as all those phenotype which shows resistance to more than one antimicrobial agent in all the antimicrobial categories but remains susceptible to only one or two categories. PDR type is defined as those isolates which show resistance to all antimicrobial agents in all antimicrobial class. XDR is a subgroup of MDR and PDR is subgroup of XDR, these categories of drug resistance phenotypes were according to ECDC (European Centre for Disease Prevention and Control) and CDC (Centers for Disease Control and Prevention) [3].

P. aeruginosa is one of the most frequent gram negative non fermentative pathogen in ICU patients causing Urinary Tract Infection (UTI), surgical site infection, and bacteremia but Lower Respiratory Tract Infections (LRTI) is most common and predominating one. MDR, XDR and PDR variants manifest a high level of intrinsic resistance to antimicrobial drugs by the help of efflux pump, biofilm formation, aminoglycoside modifying enzymes and sometimes by mutation in chromosomal gene (ESBL and AmpC hyper expression) [4]. *Pseudomonas* spp. is also able to obtain the resistance by means of horizontal gene transfer mechanism which is responsible for class B carbapenamase (MBL) [5]. Genes responsible for drug resistance are located on integrons which is frequently located in plasmids or transposons and these genes can shift very often and contributes to the dissemination of resistance mechanism around the world [6,7].

Biofilm is described as "a structural community of bacterial cells bounded in self-founded polymetric matrix adherent to biotic or abiotic surface". Any surface either biotic or abiotic is appropriate for bacterial colonisation and biofilm formation. Phenotypes that are biofilm producers are more drug resistant than biofilm non producers. The ability of microorganism to produce biofilm could be a constructive strategy to intensify its survival and existence under suppressed condition like antibiotic therapy or host invasion [8,9]. The potentiality of *Pseudomonas* spp.to produce variety of drug resistance mechanism has led to evolution of drug resistant phenotypes. This poses as a challenge for our clinician for the treatment of such kind of severe infection. This type of situation draws attention for the detection of phenotypes those are producing different kind of mechanism for the drug resistance to avoid treatment failure and hospital acquired infection [10].

The aim of the present study was to determine the drug resistance pattern in association with phenotypic profiling of β-lactamases and burden of MDR, XDR, and PDR *P. aeruginosa* among ICU patients at a tertiary care hospital of Ghaziabad, Uttar Pradesh, India.

MATERIALS AND METHODS

The present cross-sectional prospective study was carried in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. The study was carried from January 2019 to February 2021. Permission from IEC was taken before carrying out the study (Reference No: SU/2021/092 [3]). Written informed consent was taken from all participants of the study. The obtained results were statistically analysed in numbers and percentages using MS Excel 2013 version.

Sample size calculation: The sample size was done by using the formula $n=z^2pq/e^2$ where 'p' is the prevalence, q=1-p, 'e' is the precision of the estimate. If the values are normally distributed, then 95% of the values will fall within two standard deviations of the mean and the value of 'z' corresponding to 1.96. The prevalence of MDR *P. aeruginosa* was taken to be 50% based on the study conducted by Gill JS et al., [2]. So according to calculation a total of 502 patients samples those are admitted in the ICU were enrolled in the study.

Inclusion criteria: All the ICU samples including samples obtained after an invasive procedure and from the indwelling catheters were incorporated in the study.

Exclusion criteria: Present study did not include samples from the paediatric ICU, thereby resulting in the exclusion of patients below 10 years of age. Patient with evidence of septicemia and known diagnosis of *P. aeruginosa* infection were also excluded from the study.

Sample collection and processing: All the suitable clinical samples that fulfilled the determined inclusion criterion were procured individually. Different clinical samples like Endotracheal (ET) aspirate, Blood, Pus, and Urine were collected with aseptic precaution in sterile universal container and were directly sent to the Microbiology laboratory as early as possible, samples were kept in refrigerator at 2-8°C temperature in case of inevitable situation. The entire clinical sample received in microbiology laboratory was tested for the isolation, identification and AST. A total of 115 isolates of *P. aeruginosa* were isolated from 502 human clinical samples in the course of two year and all the clinical samples were non duplicate. These *Pseudomonas* isolates were identified by conventional methods as per standard microbiology laboratory protocol and finally identified by observing the culture characteristic on routine laboratory culture media viz., blood agar and MacConkey agar plates. Bacterial colonies on MacConkey agar plates showed non lactose fermenting pale colour colonies and were oxidase test positive. Whereas on nutrient agar, the bacterial colonies were pigmented, non pigmented and oxidase positive. Species level identification was performed with the help of manual biochemical test methods and finally pure isolates of *P. aeruginosa* was used for further investigation. Standard operating procedure for the isolation and identification of bacteria were followed [11].

AST: AST was performed for all clinical isolates by standard Kirby-Bauer disc diffusion method on Mueller Hinton agar (Hi-media labs, Mumbai, India). *P. aeruginosa* control strain ATCC (American Type Culture Collection) 27853 were used during the study. Zone of inhibition was interpreted according to Clinical and Laboratory Standard Institute (CLSI) guidelines [12].

Phenotypic Profiling of β-lactamases: (MBL/ESBL/AmpC detection):

Ordinary steps before performing phenotypic methods

1. 4-5 bacterial colonies were touched with a straight wire and transferred to nutrient broth and the turbidity matched with 0.5 McFarland standards.
2. Excess liquid was removed by squeezing the swab against the inner side of the suspension tube.
3. Lawn culture was made on Muller Hinton Agar (MHA) plate with a sterile cotton swab.
4. Inoculum was allowed to dry for 15 minutes before placing the antibiotic disc.

Phenotypic detection of ESBL: Bacterial inoculum was prepared and lawn culture was made on MHA plates, after drying for 15 minutes disc of ceftazidime and ceftazidime+clavulanic acid (disk potentiation test) were incorporated on MHA plates after overnight incubation at 37°C plates were interpreted as ESBL positive if the zone size was ≥5 mm for ceftazidime+clavulanic in comparison to zone size of ceftazidime alone [12].

Phenotypic detection of MBL: The IMP-EDTA combined disc test: Bacterial inoculum was prepared and lawn culture was made on MHA plates, after 15 minutes of drying the two imipenem disc one with 10 µL of EDTA (750 µg) and the other disc without EDTA were placed on MHA culture plate 30 mm apart and incubated overnight at 37°C, a ≥7mm increase in the zone size in IMP+EDTA disc was considered as MBL positive strain [13].

Phenotypic detection of AmpC β-lactamase:

Cefoxitin Cloxacillin Double Disc synergy Test (CC-DDST)

The principle of this method is based on inhibitory effect of cloxacillin on AmpC production. Bacterial inoculum was prepared and lawn culture was made on MHA plates, after 15 minutes of drying two antibiotic discs one of cefoxitin (30 µg) and other disc of cefoxitin (30 µg)/cloxacillin (230 µg) were placed on MHA culture plates 24 mm apart with centers and incubated overnight at 37°C. A difference of ≥4 mm in the inhibition zone of cefoxitin/cloxacillin and cefoxitin disc was considered as AmpC producers [14].

Inhibitor-based method: Microbial inoculum of isolates were prepared in normal saline and turbidity was maintained with 0.5 McFarland standard and finally inoculated evenly on MHA plates then two cefoxitin disc (30 µg) with and without boronic acid (400 µg) were placed on dry MHA plate 30 mm apart. After overnight incubation at 37°C aerobically, a zone size of 5 mm or more around the disc of cefoxitin+boronic acid compared to the cefoxitin disc alone was considered as AmpC positive isolates [15].

STATISTICAL ANALYSIS

The data included demographic information i.e., age, sex and period of ICU stay. Also, bacterial culture and their drug resistance pattern and β-lactamase profiling was examined. MS Excel 2013 version was used to analysed the data.

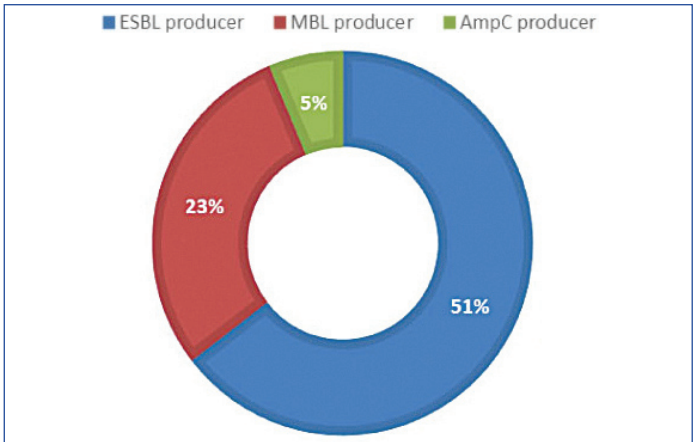
RESULTS

Out of 502 total clinical samples, 115 isolates were *P. aeruginosa* giving the prevalence rate of 23%. MDR and XDR phenotypes of *P. aeruginosa* were commonly isolated from ET aspirates followed by urine, pus and blood [Table/Fig-1]. Out of 115 isolates, 59 (51%) were ESBL producers, 26 (23%) were MBL producers, and 6 (5%) were AmpC producers as shown in [Table/Fig-2]. Rest 24 isolates were not producing any type of β-lactamase enzymes.

Sample type	MDR *Pseudomonas aeruginosa* (%)	XDR *Pseudomonas aeruginosa* (%)
ET aspirate	26 (43.34)	03 (37.5)
Pus	12 (20)	01 (12.5)
Urine	18 (30)	02 (25)
Blood	2 (3.33)	01 (12.5)
BAL fluid	2 (3.33)	01 (12.5)
Total	60	08

[Table/Fig-1]: Sample wise distribution of MDR and XDR *Pseudomonas aeruginosa* isolates.
ET: Endotracheal; BAL: Bronchoalveolar lavage

Demographic distribution: Out of the total 60 MDR phenotypes, 41 were isolated from male patients and 19 were isolated from female patients and out of total eight XDR phenotypes, five were isolated from male patients and three were isolated from female



[Table/Fig-2]: Prevalence rate of ESBL, MBL and AmpC production by *P. aeruginosa*.

patients. The frequency of MDR was highest in patients between 31-50 years age group and a higher predominance of both MDR and XDR in males was observed as shown in [Table/Fig-3].

S. No.	Age group (in years)	MDR		XDR	
		Males (41)	Females (19)	Males (5)	Females (3)
1	11-20	02 (4.9%)	02 (10.5%)	--	--
2	21-30	04 (9.8%)	02 (10.5%)	--	--
3	31-40	16 (39%)	04 (21.1%)	01 (20%)	--
4	41-50	14 (34%)	06 (31.6%)	02 (40%)	01 (33.3%)
5	51-60	02 (4.9%)	02 (10.5%)	01 (20%)	01 (33.3%)
6	>60	03 (7.4%)	03 (15.8%)	01 (20%)	01 (33.3%)

[Table/Fig-3]: Age and sex distribution of MDR and XDR *Pseudomonas aeruginosa*.

Drug resistance pattern of MDR/XDR *P. aeruginosa*: The highest resistance for MDR was found to be for cetazidime followed by gentamicin, cefepime, ciprofloxacin, amikacin, aztreonam, piperacillin, ticarcillin/clavulanic acid piperacillin-tazobactam and least resistance was found to be meropenem and imipenem. In the present study, higher resistance for XDR *Pseudomonas aeruginosa* was found to be for ceftazidime followed by gentamicin, amikacin, piperacillin-tazobactam, and ticarcillin/clavulanic acid and these phenotypes were completely resistant for the drugs like cefipime, aztreonam, imipenem, meropenem and piperacillin. [Table/Fig-4] shows the resistance pattern of MDR, XDR *P. aeruginosa* against various anti-pseudomonal drugs.

Drug	MDR *P. aeruginosa* N (%)	XDR *P. aeruginosa* N (%)
Colistin (10 µg)	Nil	2 (25%)
Amikacin (30 µg)	46 (76%)	5 (62.5%)
Piperacillin-Tazobactam (100 µg/10 µg)	23 (38%)	4 (50%)
Piperacillin (100 µg)	36 (60%)	8 (100%)
Gentamicin (10 µg)	51 (85%)	6 (75%)
Meropenem (10 µg)	10 (16%)	8 (100%)
Imipenem (10 µg)	11 (18%)	8 (100%)
Ciprofloxacin (5 µg)	48 (80%)	6 (75%)
Ticarcillin/clavulanic acid (75 µg/10 µg)	29 (48%)	4 (50%)
Aztreonam (30 µg)	46 (76%)	8 (100%)
Cefepime (30 µg)	48 (80%)	8 (100%)
Ceftazidime (30 µg)	52 (86%)	7 (87.5%)
Polymyxin B (300 Units)	Nil	2 (25%)

[Table/Fig-4]: The resistance pattern of MDR, XDR *Pseudomonas aeruginosa* against various anti-pseudomonal drugs.

Prevalence of MDR/XDR *Pseudomonas aeruginosa*: A total of 115 *Pseudomonas* isolates were processed, out of which 60 (52%) were MDR phenotypes, 8 (7%) were XDR phenotypes and 47 (41%) were Non Drug Resistant *Pseudomonas aeruginosa* (NDRPA) and there was no PDR phenotypes isolated in present study as all the phenotypes were sensitive to colistin and polymyxin B. The most important risk factors associated with *P. aeruginosa* infections in ICU patients as reported by this study was mechanical ventilation followed by endotracheal intubation. Prolonged ICU stay was also a crucial point related to infections in ICU patients as shown in [Table/Fig-5] and at the last underlying conditions like hypertension and Chronic Obstructive Pulmonary Disease (COPD) was highly associated with infections in ICU patients.

S. No.	Risk factors	No. and percentage of positive cases of *Pseudomonas aeruginosa* (N=115)
1	Tracheostomy (ET Tube)	78 (68%)
2	Foley catheter	64 (56%)
3	Intravenous catheter	12 (10%)
4	Dialysis	NA
5	Mechanical ventilation	89 (77%)
6	Previous surgery	59 (51%)
7	**ICU stay**	
	<7 days	24 (21%)
	7-15 days	38 (33%)
	>15 days	53 (46%)
8	**Underlying conditions***	
	COPD	54 (47%)
	Malignancy	NA
	Diabetes	16 (14%)
	Hypertension	77 (67%)

[Table/Fig-5]: Number and percentage of *P. aeruginosa* with relevance to risk factors in ICU patients.
*Multiple conditions

DISCUSSION

Emergence of MDR, XDR and PDR phenotypes in *P. aeruginosa* has become a serious threat in recent years and the treatment of these phenotypes is very challenging task for the clinicians. Prevalence of MDR and XDR documented in last ten years from India is shown in [Table/Fig-6] [1,2,4,16-27]. Different types of molecular mechanism are responsible for resistance against these antibiotics, production of variety of β-lactamases, integration of *bla* genes to the integrons and due to incompetency of porin genes to intensify

S. No.	Study	Year	Place	Prevalence	
				MDRPA	XDRPA
1	Nagaveni S et al., [16]	2011	Karnataka	80%	---
2	Kalaivani R et al., [17]	2013	Puducherry	33%	----
3	Shrivastava G et al., [1]	2014	Madhya Pradesh	24.7%	11.6%
4	Biswal I et al., [18]	2014	Delhi	36.2%	-----
5	Senthamarai S et al., [19]	2014	Tamilnadu	41.3%	-----
6	Dash M et al., [20]	2014	Odisha	84.7%	35.7%
7	Pramodhini S, et al., [21]	2016	Puducherry	26%	----
8	Gill JS et al., [2]	2016	Pune	50%	2.3%
9	Basak S et al., [22]	2016	Wardha (Maharashtra)	37.1%	13.8%
10	Gupta R et al., [4]	2016	Aligarh	80.1%	----
11	Singh NP et al., [23]	2017	Delhi	15.2%	----
12	Yadav S et al., [24]	2018	Kanpur	50%	----
13	Pattnaik D et al., [25]	2019	Odisha	59%	18%
14	Mehta I et al., [26]	2019	Gujarat	29.2%	----
15	Sarkar S et al., [27]	2020	Kolkata	51.7%	-----
16	Present study	2021	Ghaziabad	52%	7%

[Table/Fig-6]: Prevalence of MDR and XDRPA as documented in last ten years from India [1,2,4,16-27].

their expression level or target site modification [28]. The prevalence rate of *Pseudomonas aeruginosa* in present study was 23% which was higher to 14.7% rate reported by Gill JS et al., [2]. However, Gupta R et al., obtained the prevalence rate of 28%, while lower prevalence rate of 2.76% was reported by Senthamarai S et al., in Tamilnadu [4,19]. Prevalence rate of MDR and XDR *P. aeruginosa* in present study was 52% and 7%, respectively. However, Gill JS et al., obtained the prevalence rate of 50% and 2.3%; Saderi H and Owlia P reported a frequency of 54.5% and 33% for MDR and XDR *P. aeruginosa*, respectively in Iran while Mirzaei B et al., in Tehran found the prevalence rate of 16.5% and 15.53% for MDRPA and XDR *P. aeruginosa*, respectively [2,29,30]. The only relief in present study was 0% PDR *P. aeruginosa* phenotypes, thereby showing the effectiveness of certain antibiotics for this pathogen while Shrivastava G et al., reported PDR *P. aeruginosa* phenotype with 6.06% prevalence rate, similar result of 4% PDR *P. aeruginosa* was described by Jayakumar S and Appalaraju B [1,31].

In the present study, MDR and XDR *P. aeruginosa* phenotypes were most frequently isolated from lower respiratory tract followed by urine, pus and blood samples. Similar results were also reported by Gupta R et al., [4]. However, Gill JS et al., reported urine and wound samples considered for the majority of the positive isolates [2]. Our results were also agreed with Prakash V et al., [32].

In the present study, it was found that male patients (68%) were predominant than female patients (32%) in case of MDR *P. aeruginosa* and in case of XDR *P. aeruginosa* phenotypes, the prevalence in male (63%) was more than female (38%). These results were in agreement with Mirzaei B et al., [30].

In present study results, the highest resistance for MDR *P. aeruginosa* was found to be for ceftazidime followed by gentamicin, cefepime, ciprofloxacin, amikacin, aztreonam, piperacillin, ticarcillin/clavulanic acid piperacillin-tazobactam and least resistance was found to be meropenem and imipenem, similar results were also obtained from study conducted by Biswal I et al., in burn patients [18]. Present study results were also agreed with Gupta R et al., and Nasser M et al., who also reported similar results of resistance pattern of MDRPA [4,33].

For MDR *P. aeruginosa* isolates, the drug of choice is carbapenems but increasing resistance towards carbapenems is now a serious threat. In the present study, the resistance pattern for Imipenem and Meropenem was lowest as 18% and 16%, respectively. However, Bhatt P et al., reported the resistance pattern of 61% and 54%, respectively for MDR *P. aeruginosa* isolates [34]. In the present study, drug resistance patterns revealed that >50% isolates were resistant to fluroquinolones, gentamicin, cephalosporin's and aminoglycosides. The treatment and management options for such type of bacterial strains are limited which may result in treatment failures and thereby causing significant morbidity and mortality. The good efficacy of the carbapenems as it is an effective antibiotic in the management of nosocomial infections and it is found to be the precious weapon against MDR *P. aeruginosa* infections. In the current study, MDR *P. aeruginosa* isolates showed the lowest resistance to carbapenems, whereas piperacillin alone showed a resistance rate of 60% whereas β-lactam/β-lactamase inhibitor piperacillin/Tazobactam showed a lower resistance of 38% only, indicating that β-lactamase inhibitor markedly increases the spectrum of activity of β-lactams, which makes the combination drug the preferred choice against *P. aeruginosa* infections. In present study, higher resistance for XDR *Pseudomonas aeruginosa* was found to be for ceftazidime followed by gentamicin, amikacin, piperacillin-tazobactam and ticarcillin/clavulanic acid. These phenotypes were completely resistant for the drugs like cefepime, aztreonam imipenem, meropenem and Piperacillin. Similar results were also reported by Shrivastava G et al., [1]. However; Woradet S et al., from Thailand reported that among ICU patients XDR *Pseudomonas aeruginosa* isolates were least resistant to ciprofloxacin, carbapenem and 3rd generation cephalosporins [35]. β-lactamase producing non fermenter gram negative bacilli have emerged as a serious threat in hospitalised patients. They create a serious problem to the topical β-lactam therapy as well as other antimicrobial agents. The various β-lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposons. As various phenotypic mechanisms of resistance like AmpC and MBL were detected in the present study which is very effective in degrading the anti-pseudomonal agents these days. In the present study, authors found that the overall prevalence of ESBL, MBL and AmpC was 59 (51%), 26 (23%) and 6 (5%), respectively which agreed with Shrivastava G et al., [1]. However, Sarkar S et al., reported the overall prevalence of ESBL, MBL and AmpC was 36.8%, 12.9% and 12.4%, respectively. Another study by Umadevi S et al., reported the highest prevalence of MBL (65.7%) followed by ESBL (19.4%) and AmpC (16.4%) among the β-lactamases in her study [27,36]. However, Gupta R et al., reported the highest prevalence of AmpC (42.8%) among the other β-lactamases in their study [4]. Production of multiple β-lactamases by *P. aeruginosa* is therapeutic challenge and there is a need for urgent jurisdiction to control the spread of such type of resistant strains. Management and treatment of infections caused by *Pseudomonas* spp. is less complicated than drug resistant ones. The problem of bacterial drug resistance to commonly used antibiotics is very frequent globally as drug resistance is a greater problem in developing countries especially due to easy availability of antibiotics over the counter. To the best of our knowledge, this was the first study that includes information regarding drug resistance pattern in MDR and XDR phenotypes of *P. aeruginosa* among ICU patients. Also, the β-lactamase profiling has been included in the present study which is very crucial factors to be detected early for the better treatment of such infections. The susceptibility pattern of *P. aeruginosa* varies from one region to another with the prevalence of different drug resistant genes [37].

Limitation(s)

Molecular characterisation of ESBL, AmpC and MBL production could not be studied due to limited resources. This study was limited to patients admitted in ICU of a single hospital. Therefore, the results may not be applicable to other geographical locations. Also, the resistance pattern against all the available anti-pseudomonal drugs were not checked.

CONCLUSION(S)

Strict antibiotic policies and regular surveillance programme of antimicrobial resistance should be tailored to fend off the emergence of drug resistant *P. aeruginosa*. Colistin and Polymyxin B still shows high sensitivity against MDR *P. aeruginosa* and XDR *P. aeruginosa* phenotypes. Early detection of β-lactamases should be performed regularly for all clinical isolates of *Pseudomonas aeruginosa* to guide antibiotic selection and for the better management of serious infection in ICU patients.

REFERENCES

[1] Shrivastava G, Bhatambare GS, Patel KB. Evaluation of prevalence and antibiogram of multidrug resistant, extensively drug resistant and pan drug resistant *Pseudomonas aeruginosa* in patients visiting a tertiary care hospital in Central India. CHRISMED J Health Res. 2014;1:145-49.
[2] Gill JS, Arora S, Khanna SP, Kumar KH. Prevalence of multidrug-resistant, extensively drug-resistant, and pan drug-resistant *Pseudomonas aeruginosa* from a tertiary level intensive care unit. J Glob Infect Dis. 2016;8(4):155-59.
[3] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.
[4] Gupta R, Malik A, Rizvi M, Ahmed SM. Incidence of multidrug-resistant *Pseudomonas* spp. in ICU patients with special reference to ESBL, AMPC, MBL and biofilm production. J Glob Infect Dis. 2016;8(1):25-31.
[5] Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? Clin Infect Dis. 2002;34(5):634-40.

[6] Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: The quiet before the storm? Clin Microbiol Rev. 2005;18(2):306-25.
[7] Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterisation of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother. 2000;44(4):891-97.
[8] Gurung J, Khyriem AB, Banik A, Lyngdoh WV, Choudhury B, Bhattacharyya P. Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. Indian J Crit Care Med. 2013;17(4):214-18.
[9] Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian J Med Microbiol. 2008;26(4):333-37.
[10] Obritsch MD, Fish DN, MacLaren R, Jung R. National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob Agents Chemother. 2004;48(12):4606-10.
[11] Collee JG, Mackie TJ, McCartney JE. Mackie & Mccartney Practical Medical Microbiology. New York: Churchill Livingstone, 1996. Print.14th edition.
[12] Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
[13] Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol. 2003;41(10):4623-29.
[14] Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M. Practical approach for reliable detection of AmpC beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol. 2011;49(8):2798-803.
[15] Thomson KS. Controversies about extended-spectrum and AmpC beta-lactamases. Emerg Infect Dis. 2001;7(2):333-36.
[16] Nagaveni S, Rajeshwari H, Oli AK, Patil SA, Chandrakanth RK. Widespread emergence of multidrug resistant *Pseudomonas aeruginosa* isolated from CSF Samples. Indian J Microbiol. 2011;51(1):02-07.
[17] Kalaivani R, Shashikala P, Sheela Devi C, Prashanth K, Saranathan R. Phenotypic assays for detection of ESBL and MBL producers among the clinical isolates of multidrug resistant *Pseudomonas aeruginosa* from a tertiary care hospital. Int J Cur Res Rev. 2013;05(17):28-35.
[18] Biswal I, Arora BS, Kasana D, Neetushree. Incidence of multidrug resistant *Pseudomonas aeruginosa* isolated from burn patients and environment of teaching institution. J Clin Diagn Res. 2014;8(5):DC26-29.
[19] Senthamarai S, Reddy SK, Sivasankari S, Anitha C, Somasunder V, Kumudhavthi Ms, et al. Resistance pattern of *Pseudomonas aeruginosa* in a tertiary care hospital of Kanchipuram, Tamilnadu, India. J Clin Diagn Res. 2014;8(5):DC30-32.
[20] Dash M, Padhi S, Narasimham MV, Pattnaik S. Antimicrobial resistance pattern of *Pseudomonas aeruginosa* isolated from various clinical samples in a tertiary care hospital, South Odisha, India. Saudi J Health Sci. 2014;3:15-19.
[21] Pramodhini S, Umadevi S, Seetha KS. Detection of virulence determinants and its association with drug resistance in clinical isolates of *Pseudomonas aeruginosa*. Int J Res Med Sci. 2016;4:3917-23.
[22] Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: A study. J Pathog. 2016;2016:4065603.
[23] Singh NP, Rani M, Gupta K, Sagar T, Kaur IR. Changing trends in antimicrobial susceptibility pattern of bacterial isolates in a burn unit. Burns. 2017;43(5):1083-87.
[24] Yadav S, Sujatha R, Deepak S. Emergence of multidrug resistant *Pseudomonas aeruginosa* in a tertiary care center. Int J Health Sci Res. 2018;8(8):52-55.
[25] Pattnaik D, Panda SS, Singh N, Sahoo S, Mohapatra I, Jena J. Multidrug resistant, extensively drug resistant and pan drug resistant gram negative bacteria at a tertiary care centre in Bhubaneswar. Int J Community Med Public Health. 2019;6:567-72.
[26] Mehta I, Surani S, Mehta M, Summaiya M. Prevalence of multidrug resistant *Pseudomonas aeruginosa* infection in burn patients at a tertiary care centre. Saudi J Pathol Microbiol. 2019;4(3):210-14.
[27] Sarkar S, Dutta S, Namhata A, Banerjee C, Sengupta M, Sengupta M. Beta-lactamase profile and biofilm production of *Pseudomonas aeruginosa* isolated from a tertiary care hospital in Kolkata, India. J Clin Diagn Res. 2020;14(10):DC22-27.
[28] Pfeifer Y, Cullik A, Witte W. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. Int J Med Microbiol. 2010;300(6):371-79.
[29] Saderi H, Owlia P. Detection of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) *P. aeruginosa* isolated from patients in Tehran, Iran. Iran J Pathol. 2015;10(4):265-71.
[30] Mirzaei B, Bazgir ZN, Goli HR, Iranpour F, Mohammadi F, Babaei R. Prevalence of Multidrug Resistant (MDR) and Extensively Drug-Resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC Res Notes. 2020;13(1):380.
[31] Jayakumar S, Appalaraju B. Prevalence of multi and pan drug resistant *Pseudomonas aeruginosa* with respect to ESBL and MBL in a tertiary care hospital. Indian J Pathol Microbiol. 2007;50(4):922-25.
[32] Prakash V, Mishra PP, Premi H K, Walia A, Dhawan S, Kumar A. Increasing incidence of multidrug resistant *Pseudomonas aeruginosa* in inpatients of a tertiary care hospital. Int J Res Med Sci. 2014;2(4):1302-06.
[33] Nasser M. Phenotypic Demonstration of ß-lactamase (ESßLs, MßLs, and Amp-C) among MDR *Pseudomonas aeruginosa* isolates obtained from burn wound infected in Yemen. J Appl Biol Biotech. 2019;7(06):31-34.
[34] Bhatt P, Rathi KR, Hazra S, Sharma A, Shete V. Prevalence of multidrug resistant *Pseudomonas aeruginosa* infection in burn patients at a tertiary care centre. Indian J Burns. 2015;23:56-59.
[35] Woradet S, Chaimay B, Putthanachot N, Sangsa N, Sirisa P. Antimicrobial agents administration among patients with extensively drug-resistant *Pseudomonas aeruginosa* infection in intensive care unit in tertiary care: A hospital-based study, India. J Clin Diagn Res. 2020;14(7):DC01-04.
[36] Umadevi S, Joseph NM, Kumari K, Easow JM, Kumar S, Stephen S, et al. Detection of extended spectrum beta lactamases, ampc beta lactamases and metallobetalactamases in clinical isolates of ceftazidime resistant *Pseudomonas aeruginosa*. Braz J Microbiol. 2011;42(4):1284-88.
[37] Pragasam AK, Veeraraghavan B, Nalini E, Anandan S, Kaye KS. An update on antimicrobial resistance and the role of newer antimicrobial agents for *Pseudomonas aeruginosa*. Indian J Med Microbiol. 2018;36:303-16.

PARTICULARS OF CONTRIBUTORS:
1. PhD Scholar, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.
2. Professor, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.
3. Professor, Department of Microbiology, Hind Institute of Medical Sciences, Sitapur, Uttar Pradesh, India.
4. Assistant Professor, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.
5. Assistant Professor, Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shivendra Dutt Shukla,
PhD Scholar, Department of Microbiology, Santosh Medical College and Hospital,
Ghaziabad-201009, Uttar Pradesh, India.
E-mail: shivendrafshukla@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 31, 2021
- Manual Googling: Oct 12, 2021
- iThenticate Software: Oct 14, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 29, 2021**
Date of Peer Review: **Sep 10, 2021**
Date of Acceptance: **Oct 15, 2021**
Date of Publishing: **Nov 01, 2021**