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Pseudomonas in a Hospitalized Patients: The Notorious Nosocomial Pathogen

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ABSTRACT

Background: *Pseudomonas* spp. is one of the major threat of nosocomial infections in hospitalized patients due to combination of various virulence factors and weakened host defense. Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care So, it is important to know prevalence of *Pseudomonas* spp.in various clinical infections.

Methods: This hospitalized based prospective study includes 250 *Pseudomonas* isolates subjected to identification by microscopy, culture, speciation and Antibiotic Sensitivity tests with standard guidelines.

Results: In this study out of 2051 clinical samples, the most common was *E. coli* (38.23%), followed by *Klebsiella* (15.94%) and 250 (12.19%) *Pseudomonas* spp. were isolated. Of them,233 were of *P. aeruginosa*,141(56.4%) were from pus. Diabetes mellitus and post operative infections each 33(13.2%) were the most commonly known predisposing factors for patients from whom *Pseudomonas* were isolated, *P. aeruginosa* isolates were most resistant to Piperacillin (78%) while least resistant to Meropenem and Imipenem with resistance of 28% and 22% respectively.

Conclusion: The study underlines the importance of preventing the spread of the resistant bacteria. For this, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented. In the meantime, it is desirable that the antibiotic susceptibility pattern of *Pseudomonas* in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the resistance.

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Introduction

Pseudomonas has become an important cause of gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. *Pseudomonas* infections are complicated and can be life-threatening. *Pseudomonas* infections were described in the literature in the 1800s when physicians began to report a condition causing a blue-green discoloration on bandages and associated with a "peculiar" odour (1).

The pathogenesis of *Pseudomonas* infections is multi-factorial and complex. *Pseudomonas* species are both invasive and toxigenic. The 3 stages, according to Pollack (2000), are bacterial attachment and colonization, local infection, and bloodstream dissemination and systemic disease (2). *Pseudomonas* causes a wide spectrum of diseases; therefore, prognosis is varied. Acute fulminant infections, such as bacteremic pneumonia, sepsis, burn wound infections, and meningitis, are associated with extremely high mortality rates (3).

Although it seldom causes disease in healthy individuals, *Pseudomonas* is a major threat to hospitalized patients, *Pseudomonas* is the second most common organism causing nosocomial infections. *Pseudomonas* are often isolated from ICU infections, respiratory infections, surgical wound infections, diabetic wound infections and various eye and ear infections. The high mortality associated with these infections is due to a combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes, toxins and various other virulence factor. *P. aeruginosa* is the second most common cause of nosocomial pneumonia (17%), third most common cause of urinary tract infection (7%), fourth most common cause of surgical-site infection (8%), and fifth most common isolate (9%) overall from all sites (4). Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care (5). Therefore it is very important to study this microorganism specilly in clinical settings like tertiary care hospitals with antibiotic susceptibility pattern of various *Pseudomonas* isolates.

Materials and Methods

This prospective study was conducted in a Department of Microbiology, Tertiary care center and teaching hospital in city of central India. The study included a total of 2051 samples which were received at Microbiology Laboratory from patients of various clinical conditions admitted to this center during July 2018 to August 2020. Study included various infectious samples like sputum, pus, urine, blood, ear swabs, bronchoalveolar lavage, wound swabs, ET aspirations collected from ICU, OPD and indoor patients irrespective of their diagnosis, age, group, sex socioeconomic status and other socio-demographic factors.

Following inclusion and exclusion criteria was used to select the study subjects. Inclusion criteria: *Pseudomonas* isolates from various infections irrespective to age groups and gender. Exclusion criteria: there was no specific exclusion criterion. All samples were primarily processed by wet mount, Gram stain (Jenson’s modification), and blood agar and Mac Conkey agar cultures.

Characterization

Hanging drop preparation (6) showed actively motile organisms with pus cells whereas gram stain showed pus cells with pink , non sporulated, non-capsulated gram negative bacilli. Part of the sample was subjected for culture on Blood agar, Nutrient agar and MacConkey agar. MacConkey showed spreading, Non-lactose fermenting colony which is oxidase positive. Organisms from colony showed Positive Catalase test (7), Positive

Cytochrome oxidase test (7), Positive Nitrate Reduction test (7), Negative Indole test, Negative Methyl Red test, Positive Citrate Utilization test, Negative Urease Test and Triple Sugar Iron Agar Test showed Alkaline slant, Alkaline butt without H2S without gas that confirms the genus *Pseudomonas*.

Pseudomonas isolates, confirmed by above standard microbiological tests were further speciated as per the following scheme of identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing performed on *Pseudomonas* spp. by modified Kirby Bauer method using Mueller Hinton agar (Hi Media) as per the standard laboratory procedures. Interpretation as sensitive, intermediate or resistant was done with reference to standard CLSI guidelines.

Statistical analyses

The interpretation and analysis of the data were done by using Microsoft Excel and analyzed with the help of SPSS 20.0 version. The quantitative data were expressed as numbers and percentages in tabular form and figures.

Results

During the study period, an extensive analysis of 2051 clinical specimens revealed Escherichia coli as the dominant isolate 784(38.23%), while Coagulase-Negative *Staphylococcus* (CONS) emerged as the least frequent 48(2.34%). Notably, 19 samples (0.93%) showed no microbial growth.
Predisposing Factors for *Pseudomonas* Infections: The study identified significant clinical drivers, including: Postoperative complications and diabetes mellitus 33 cases each (13.2%) as key risk factors. Burn injuries 25(10%), trauma, and fetal diseases 16 each (6.4%), Tuberculosis 12(4.8%) and respiratory infections 11(4.4%) also played notable roles. Interestingly, 35.6% of patients (89 cases) exhibited no discernible predisposing conditions, highlighting the opportunistic nature of these infections.

These isolates were predominantly obtained from inpatients 184(74%), underscoring their critical association with hospitalized cases, while a smaller proportion 66(26%) were from outpatients.

Among *Pseudomonas* isolates (n=250), the majority were retrieved from pus samples 141 (56.4%), followed by blood 55 (22%), respiratory specimens 28(11.2%), urine 20(8%) and miscellaneous sources 6(2.4%).

P. aeruginosa was found as the principal species (233 cases) which showed a preference for pus/wound/ear swabs 133 (62.33%), followed by blood 52(22.31%), respiratory specimens 26(11.15%), urine 16 (6.87%), and other samples 6(2.58%). *P. putida* represented 9 isolates, primarily from pus 4(44.44%) and urine 3(33.33%), with lesser contributions from blood and respiratory sources 1 each (11.11%). *P. fluorescens* was the next speies with 3 isolates, predominantly from pus 2(66.66%) and respiratory specimens 1(33.33%). *P. stutzeri* was isolated in 5 cases, equally distributed between pus 2(40%) and blood 2(40%), with a minor presence in urine 1(20%).

Antibiotic resistance investigations highlighted the fact that *P. aeruginosa* shows an alarming resistance to Piperacillin (78%) and Ciprofloxacin (70%), with notable tolerance to Meropenem (28%) and Imipenem (22%), offering critical therapeutic insights. In comparison, *P. putida* exhibited high resistance to Piperacillin (78%) and Ciprofloxacin (67%), but demonstrated the least resistance to Imipenem (11%). Also, *P. fluorescens* maintained consistent resistance (67%) to multiple antibiotics but showed no resistance to Imipenem and only 33% resistance to Meropenem.

Table 1. Various biochemicals tests for speciation of genus *Pseudomonas*.

Species of *Pseudomonas*	Pyo-Cyanin	Fluorecein	Arginine hydrolysis	Gelatin liquefaction	Nitrate reduction	Denitri - fication	OF Glucose	OF Mannitol	Growth at 42 °C
P. aeroginosa	+	+	+	+	+	v	+	+	+
P. fluorescens	-	+	+	+	V	-	+	+	-
P. putida	-	+	+	-	-	-	+	V	-
P. stutzeri	-	-	-		+	+	+	V	V
P. mendocina	-	-	+		+	+	+	-	V
P. alacaligenes	-	-	-		V	-	-	-	-
P. pseudo-alcaligenes	-	-	V		+	-	-	-	-

Table 2. Distribution of the microorganisms isolated from clinical specimens.

Organism	**Pus (pus wound swab ear swab)**	**Respiratory specimen**	**Urine**	**Blood**	**Others***	**Total**
E. coli	196(30.91)	113(22.03)	309(55.98)	106(48.18)	60(45.45)	784(38.23)
Klebsiella spp.	125(19.72)	67(13.06)	101(18.3)	7(3.18)	27(20.45)	327(15.94)
Staphylococcus spp.	61(9.62)	163(31.77)	22(3.99)	31(14.09)	5(3.79)	282(13.75)
Pseudomonas spp.	141(22.24)	28(5.46)	20(3.62)	55(25.0)	6(4.55)	250(12.19)
Acinetobacter spp	13(2.05)	69(12.50)	2(0.39)	7(3.18)	11(8.33)	102(4.97)
Candida	32(5.05)	44(8.58)	8(1.45)	4(1.82)	9(6.82)	97(4.73)
Proteus spp.	15(2.72)	19(3.7)	29(4.57)	9(4.09)	6(4.55)	78(3.8)
Enterobacter spp.	3(0.54)	1(0.16)	56(10.92)	1(0.45)	3(2.27)	64(3.12)
CONS	*36(5.68)*	1(0.76)	6(1.17)	5(0.91)	0(0)	48(2.34)
No growth	0(0)	15(2.92)	0(0)	0(0)	4(3.03)	19(0.93)
Total	622	520	553	225	131	2051

* Sputum, ET Aspirate, pleural fluid

**Corneal scrapping, intracatheter tip, drain fluids

Table 3. The predisposing factors contributing to *Pseudomonas* infections.

Predisposing factor	No of patients	Percentage
Un-known predisposing factors	**89**	**35.6**
DM	33	13.20
Post-operative	33	13.20
Burn	25	10
Fetal diseases	16	6.40
Trauma	16	6.40
Tuberculosis	12	4.80
Respiratory tract infections	11	4.4
Human immunodeficiency	7	2.8
Catheterization	5	2
Cardiac diseases	3	1.2

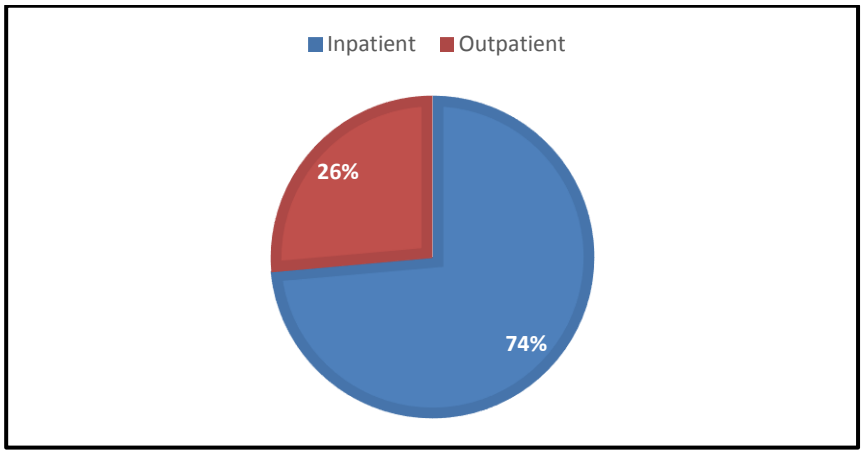

Fig 1. Inpatient-Outpatient wise distribution of cases showing *Pseudomonas* isolates.

Table 4. *Pseudomonas* isolates from various clinical specimens.

Specimens (n=250)	No of patients	Percentage
Pus (pus wound swab ear swab)	141	56.40
Respiratory specimen (sputum, ET Aspirate, pleural fluid)	28	11.20
Urine	20	8.00
Blood	55	22.00
Others (corneal scrapping, intracathetertip, drain fluids)	6	2.40
Total	250	100.00

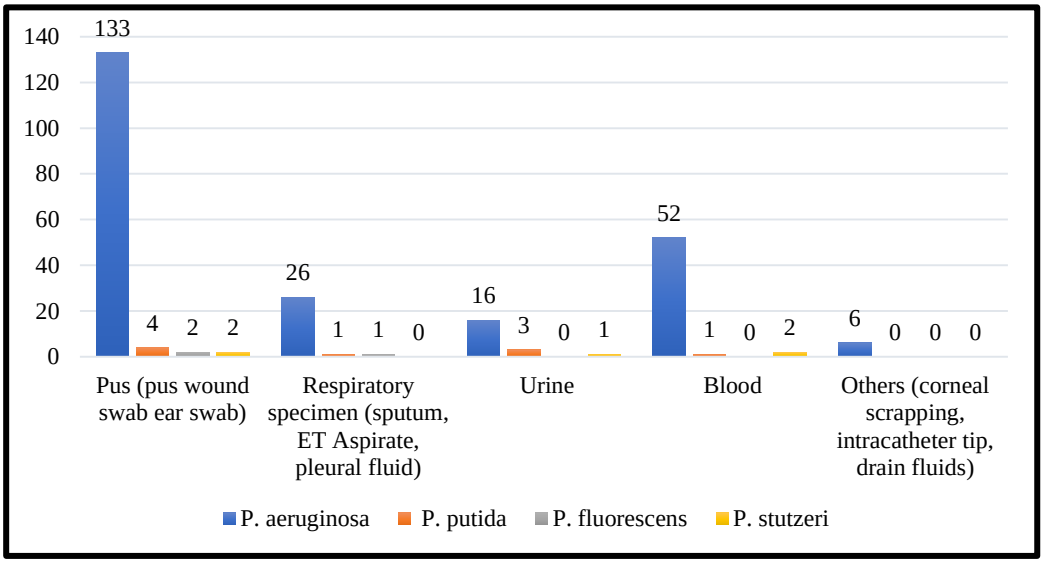

Fig 2. Various species of *Pseudomonas* isolated from different clinical specimens [n=250].

Table 5. Antibiotic susceptibility pattern of the *P. aeruginosa* isolates.

Antibiotic	Sensitive	Percentage	Resistant	Percentage
Piperacillin	51	22	182	**78**
Ciprofloxacin	70	30	163	70
Gentamicin	75	32	158	68
Amikacin	103	44	130	56
Tobramycin	82	35	151	65
Cotrimoxazole	89	38	144	62

Cefepime	70	30	163	70
Ceftazidime	77	33	156	70
Meropenem	168	72	65	28
Imipenem	182	78	51	**22**

Table 6. Antibiotic susceptibility pattern of other species of *Pseudomonas* isolates.

Antimicrobial	***P. putida* (n=9)**		***P. stutzeri* (n=5)**		***P. fluorescens* (n=3)**	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Piperacillin	7 (78 %)	2 (22 %)	4 (80%)	1 (20%)	2 (67%)	1 (33 %)
Ciprofloxacin	6 (67 %)	3 (33 %)	3 (60%)	2 (40%)	2 (67%)	1 (33 %)
Gentamicin	5(55 %)	4 (44 %)	3 (60%)	2 (40%)	2 (67%)	1 (33 %)
Amikacin	4 (46 %)	5(55 %)	2 (40%)	3 (60%)	1 (33%)	2 (67%)
Tobramycin	5(55 %)	4 (44 %)	3 (60%)	2 (40%)	2 (67%)	1 (33%)
Cotrimoxazole	5(55%)	4 (44 %)	3 (60%)	2 (40%)	2 (67%)	1 (33 %)
Cefepime	6 (67 %)	3 (33 %)	2 (40%)	3 (60%)	1 (33%)	2 (67%)
Ceftazidime	5(55 %)	4 (44%)	3 (60%)	2 (40%)	2 (67%)	1 (33%)
Meropenem	3 (33 %)	6 (67%)	0(0%)	5 (100%)	1 (33%)	2 (67%)
Imipenem	1 (11 %)	8 (89%)	0(0%)	5 (100%)	0(0%)	3 (100%)

Table 7. Resistance pattern of the *P. aeruginosa* isolates in various studies.

Antibiotic	**Rajat R *et al* 2012 (27)**	**Sailaja BSG, Prasad PD 2019 (8)**	**Juyal D *et al* . 2013 (9)**	**Kaur A *et al* . 2018 (28)**	**Present study**
Piperacillin	50 %	72 %	47.87 %	64.6 %	78 %
Ciprofloxacin	49 %	58 %	75.53 %	42.8 %	70 %
Gentamicin	63%	78 %	51.06 %	44.3 %	68 %
Amikacin	-	78 %	27.66 %	39.1 %	56 %

Tobramycin	68 %	-	-	-	65 %
Cotrimoxazole	-	79 %	91.49 %	-	62 %
Cefepime	30 %	50 %	-	57.8 %	70 %
Ceftazidime	43 %	50 %	71.28 %	60.9 %	67 %
Meropenem	39 %	100 %	-	35.5 %	28%
Imipenem	14%	100%	28.72%	28.6%	22%

In addition, *P. stutzeri* recorded the highest Piperacillin resistance (80%), with moderate resistance to other agents. Importantly, no resistance was observed for Meropenem and Imipenem.

This comprehensive study reveals a striking diversity in Pseudomonas isolates and their antimicrobial resistance profiles. The findings underscore the critical need for precise and dynamic therapeutic strategies to combat these versatile pathogens, especially in hospital settings.

Discussion

Pseudomonas infections are emerged as an important pathogen and responsible for the nosocomial and various other infections. It is one of the important causes of morbidity among hospital patients. The pre-eminent of *Pseudomonas* infections due to its resistance to common antibiotics and antiseptics, and its ability to establish itself widely in hospitals. As Pseudomonas causes serious infections, and is one of the leading causes of hospital acquired infections, several studies were carried out to detect antibiotic sensitivity pattern for the various drugs available. Such study helps clinicians for the better management of patients.

In present study the isolation rate of *Pseudomonas* was 12.19% (Table 2) and comparable with other studies. In our study, out of 2051 clinical isolates, the most common was *E. coli* (38.23%) followed by *Klebsiella* spp. (15.94%), *Pseudomonas* spp. (12.19%), *Staphylococcus* spp. (13.75%). Less common isolates found were *Acinetobacter* spp. (4.97%), *Candida* (4.73%), *Proteus* spp. (3.8%), *Enterobacter* spp. (3.12%) and CONS (2.34%). In the study by Sailaja and Prasad (8) (2019) out of 302 isolated samples, *Klebsiella* species (30.4%), *Staphylococcus aureus* (24.83%), *Escherichia coli* (20.8%), *Pseudomonas* spp. (16.5%) were the prevalent pathogens followed by *Streptococcus pneumoniae* (5.3%), *Proteus* species (1.6%) and *Enterococcus* spp. (0.3%). Thus, the prevalence of *Pseudomonas* spp. was more as compared to present study 12.19%. In the study by Juyal et al. (2013) (9) among 2585 clinical samples, non-fermenters like *Staphylococcus aureus* was most common followed by *E. coli*, *K. pneumoniae* and *Citrobacter* species. The proportion of the *Pseudomonas* isolates in the study was 4.83% as compared to present study 12.19%.

In the study, diabetes mellitus and postoperative infections each 33(13.2%) were the most commonly known predisposing factors for patients from whom *Pseudomonas* were isolated (Table 3). Also, there were 89(35.6%) patients with no known predisposing factors were noted.

Out of the 250 cases studied, (Fig 1) 184 (73.6%) were inpatients and 66 (26.4%) were outpatients. Most of the non fermenters exist as environmental commensals in the hospital. Among them, Pseudomonas species is responsible for substantial proportion of nosocomial infections in modern era. That might be the reason for the more proportion of IPD cases in our study.

In present study (Table 4) the maximum clinical isolates of (56.4%) were from pus, (22%) from blood, (11.2%) from respiratory specimen, (8%) from urine and 6 (2.4%) from another specimen. *Pseudomonas* species can cause infections in

almost all parts of the body, with the most common being skin, soft tissues, lungs, and wounds. These findings align with studies by Jamshaid A. K. et al.(10), Shenoy et al.(11) (2002), Arshi et al.(12) (2007), Murase et al. (13), Stark and Maki (14)(1984), Juyal et al.(9) (2013), Gad (15)(2007), Malini et al. (16)(2009), and Henwood et al.(17) However, they differ from the study by Ergin et al. (18) (1999), which reported more respiratory isolates 37% compared to our 11.2%. The percentage of blood samples from which *Pseudomonas* samples were isolated was 55 (22%) in our study which was far more as compared to studies from Juyal et al (9) (2013) 5.7% and Malini et al (16) (2009) with 5.6% blood isolates.

This variation in the composition of isolates in various studies by different clinical workers might be due to different hospital settings, associated clinical conditions, different population groups by ethnicity, religion climates, habits, socioeconomic status in a diverse country like India.

In the present study (Fig 2), 233 (93.2%) samples of *P. aeruginosa*, 9(3.6%) isolated samples of *P. putida*,5 (2%) samples of *P. stutzeri* and rest 3 (1.2%) samples of *P. fluorescens* were found in total 250 samples. The proportion of *P. aeruginosa* observed by Yan et al (19) in (92.3%) samples, by Patel et al (20)in 3300(99.1%) samples and by Erginn et al (16) in 9298 (91.67%) samples comparable to our study but was disproportionate as compared to the study by Gad G et al 15733 (75.8%), Sidhu S et al (21) 301 (77%) and Juyal et al (9)3290 (77%).

Out of 233 samples of *P. aeruginosa*, there were 133 (62.33%) samples from pus/wound swab/ ear swab which was very comparable to Patel et al (20) (61.7%) was higher than Agarwal et al (22) 2008 (39.2%), Variya et al (23) (22.1%), Attal R et al (24) (28.6%), Javiya et al (25) (26.7%) 306 and lower than Juyal et al (9) 2013 (77%), 290 Rashid M et al (26) (66.5%).

P. aeruginosa (Table 5) isolates were most resistant to Piperacillin (78 %) followed by Ciprofloxacin (70 %), Cefepime (70%), Gentamicin (68%), Ceftazidime (67%), Tobramycin (65%), Cotrimoxazole (62%) and Amikacin (56%). P. aeruginosa isolates were least resistant to Meropenem and Imipenem with resistance of 28% and 22% respectively.

In a study Cho C.H. and Lee SB (2018) (29) P. putida resistance of Tobramycin (0%), Ceftazidime (12.5%), Ciprofloxacin (12.5%). Imipenem (18.7%,), Piperacillin (25%), and Ticarcillin (100%) was found. *P. fluorescens* isolates were most resistant to Piperacillin, Ciprofloxacin, Gentamicin, Tobramycin, Cotrimoxazole, Ceftazidime (67% each) followed by Amikacin, Cefepime, Meropenem (33%). and were least resistant to Imipenem with no resistance. In a study conducted in India by Trivedi (2015) (30) all the *P. fluorescens* isolates were 100% susceptible to Ceftazidime, Piperacillin/Tazobactam, Gentamicin, Tobramycin and Colistin. In a study by Juyal D et al (2013) (9) the resistance for various antimicrobials was Amikacin (28.57%), Imipenem (21.43%), Gentamicin (53.57%), Cefepime (53.57%), Piperacillin (53.57%), Ceftazidime (60.71%), Ciprofloxacin (67.86%), Cotrimoxazole (82.14%). 80% of *P. stutzeri* isolates were resistant to Piperacillin highest of all antimicrobials followed by Ciprofloxacin, Gentamicin, Tobramycin, Cotrimoxazole, Ceftazidime (60% each), Amikacin, Cefepime (40% each) and they were not at all resistant to Meropenem and Imipenem.

Previous study conducted by Bisharat et al . (2012)(31) showed *Pseudomonas stutzeri* susceptibility to Gentamicin (99%), Ofloxacin (99%), Amikacin (98%), Imipenem (98%), Ciprofloxacin (97%), Meropenem (97%), Ceftazidime (95%), Piperacillin (93%),Polymixin-B (92%), Trimethoprim-sulfamethoxazole (91%), Piperacillin/tazobactam (91%), Cefepime (71%), Ceftriaxone (60%), Amoxicillin-Clavulanic acid (50%), Cefotaxime

(50%), Nitrofurantoin (27%), Cefuroxime (14%) and Cefoxitin (12.5%).

Extensive resistance to antimicrobials is challenging and threats to the management of infections. This is due injudicious use, no fixed antibiotic policy, easily over the counter availability of antimicrobials, extensive use of broad spectrum antibiotics etc. This should always be considered in case of *Pseudomonas* infections as it is very common in patients of DM, burns, nosocomial infections, immunocompromised patients. The high incidence of resistance due to multiple mechanism in *Pseudomonas* is alarming and requires urgent action from both therapeutic and infection control perspective.

Study indicated that growth of *Pseudomonas* cannot be overlooked and should be confronted with high index of suspicion. Precise identification of these bacteria upto genus and species level, imperative clinico-microbiological correlation and careful antibiotic prescription shall go a long way in improving clinical outcomes of patients.

Conclusion

This study underlines the importance of preventing the spread of the resistant bacteria. For this, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented. In the meantime, it is desirable that the antibiotic susceptibility pattern of *Pseudomonas* in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the resistance.

The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community epidemiologist to promote greater understanding of this problem. Frequent hand washing to prevent spread of organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

Also, this study gives an alarming sign towards high prevalence of *Pseudomonas* needs to be prevent its spread. For this, it is critically important to have equipment decontamination, strict protocols for hand washing, hospital infection prevention training to the staff and strict antibiotic policies need to be implemented.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare that they have no conflict of interest.

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