

Prevalence of Pseudomonas Species Isolates from Various Clinical Specimens at Tertiary Care Hospital, Jaipur

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Abstract:

Background: *Pseudomonas aeruginosa* is an important pathogen commonly implicated in serious nosocomial infections such as pneumonia and sepsis. The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide and limiting our therapeutic options. The worldwide emergence of multi-drug resistant bacterial strains in hospitals and community continues to be a problem of due scientific concern, especially infections caused by *Pseudomonas species* and *Pseudomonas aeruginosa* in particular. *P. aeruginosa* is an opportunistic pathogen with inherent resistance to many antibiotics and disinfectants including anti-pseudomonal Penicillins, Ceftazidime, Carbapenems, Aminoglycosides and Ciprofloxacin.

Materials and Methods: This Prospective observational study was conducted in Microbiology Department NIMS medical college Jaipur Rajasthan from July 2018 to December 2018. Clinical specimens were collected from NIMS hospital Jaipur. Clinical isolates were identified by standard and specific microbiological methods. The antibiotic susceptibility pattern was determined by Kirby Bauer Disc diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to determine the results.

Results: During the study period, 80 positive samples of *Pseudomonas species* isolated from various clinical specimens of patients of all ages and both sexes attending outpatients and inpatients at NIMS hospital were processed. Study revealed that most sensitive antibiotic were Polymyxin- B and Colistin (100%) followed by Meropenem (90%) in our study. Norfloxacin appeared as most resistant antibiotic with (80%) rate in Urine sample.

Conclusion: The increase in resistance among *Pseudomonas species* is due to irrational use of antibiotics in present era. This study emphasizes need for a continuous surveillance in hospital to detect the resistant strains. Also more research should be done to find better treatment policies, effective and cheaper alternative antibiotics in developing countries like ours.

Key Word: *Pseudomonas aeruginosa*, multidrug-resistant; antibiotics.

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I. Introduction

In the recent years due to routinely use of antibiotics, NFGNB have emerged as important health care associated pathogens. Non fermenter gram negative bacteria cause various infections such as septicemia, pneumonia, urinary tract infections and surgical site infections¹. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β - lactamases and metallo β - lactamases^[1,2,4,5]. Nonfermenters are being encountered with increasing frequency as agents of opportunistic and very serious infections as well as nosocomial infections. Nonfermenters have a low degree of virulence and most often cause nosocomial infections in patients who are debilitated or immunocompromised^[7,3,6]. *Pseudomonas aeruginosa* is an important pathogen commonly implicated in serious nosocomial infections such as pneumonia and sepsis. The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide and limiting our therapeutic options.^[10] Therefore, the present study was undertaken to find out the antibiotic susceptibility patterns of pathogenic isolates of *Pseudomonas species* from various specimens of hospital acquired infections (HAI).

II. Material And Methods

This Prospective observational study was conducted in Microbiology Department NIMS medical college Jaipur Rajasthan from July 2018 to December 2018. Clinical specimens were collected from NIMS hospital Jaipur.

Study Design: A cross-sectional observation study.

Study Location: This The Present study entitled “DETECTION OF PSEUDOMONAS SPECIES FROM VARIOUS CLINICAL SAMPLE” was carried out at department of microbiology, NIMS medical college jaipur rajasthan.

Study Duration: The study was carried from July 2018 to December 2018

Sample size: 300 patients.

Sample size calculation: During the study period, 300 various clinical samples include urine, body fluids, pus, sputum, swab and other clinical samples were collected and process for isolation and identification of pseudomonas strains by standard method out of which 80 positive Pseudomonas strains were isolated and their antibiotic sensitivity were tested by Kirby Bauer disc diffusion method according to CLSI guidelines (2018). Pseudomonas species ATCC 27853 strains were used as control organism.

Inclusion criteria:

1. Sample was collected from the different OPD, ICU, and Wards in NIMS hospital.
2. Proper collection of the samples
3. Complete identification of the samples.

Exclusion criteria:

1. Any leakage samples or broken container/vials
2. Requested forms are incomplete.
3. If sample information and request form information is mismatch.
4. Repetitive sample from same patient.

Procedure methodology

The organism that grew as non fermenters on MacConkey agar and produced an alkaline reaction in triple sugar iron agar were provisionally considered to be nonfermentive gram negative bacilli and were stocked and stored at -20⁰ C. later on time to time these were revived and identified as per following protocol.

The clinical samples were processed immediately and cultured as per standard protocol using several of tests:-^{11,12,13}

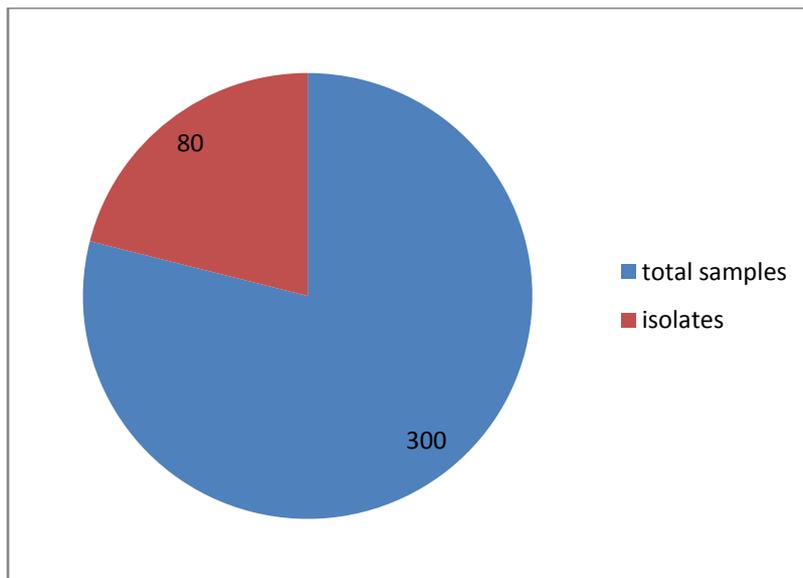
1. Culture characters: Blood agar, McConkey and Nutrient agar.
2. Pigment production : Blood agar and Nutrient agar
3. Morphology : Gram’s stain
4. Motility : Hanging drop preparation
5. Catalase test
6. Oxidase test
7. Indole, Methyl red, Voges Proskauer, Citrate utilization test, Urease test and Triple sugar iron reaction.
8. Oxidation/fermentation (Hugh and Leifson’s media) for glucose, lactose, xylose, mannitol and maltose.

III. Result

The present study was conducted in the department of microbiology, National Institute of Medical Sciences (NIMS) Medical College and tertiary care Hospital Jaipur, Rajasthan from July 2018 to December 2018 to detect Pseudomonas species from various clinical samples.

Table 3: Incidence of Pseudomonas species isolates from Clinical Samples

No. of sample tested	300
No. of isolates of Pseudomonas spices	80
Incidence	26.66%



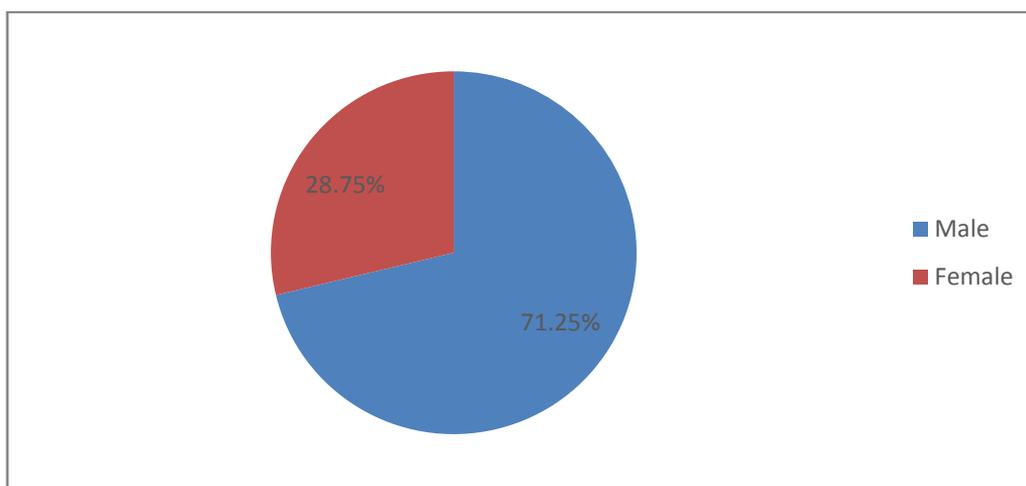
As the shown in above **TABLE 3** and **GRAPH 1**: Incidence of Pseudomonas species isolates 80 (26.66%) out of 300 from various clinical samples.

During the study period, 80 positive samples of Pseudomonas species isolated from various clinical specimens of patients of all ages and both sexes attending outpatients and inpatients at NIMS hospital were processed.

Distribution Pseudomonas species according to Age and Sex;

TABLE 4: Distribution of Pseudomonas species according to gender

Sex	Number	%
Male	57	71.25
Female	23	28.75
Total	80	100

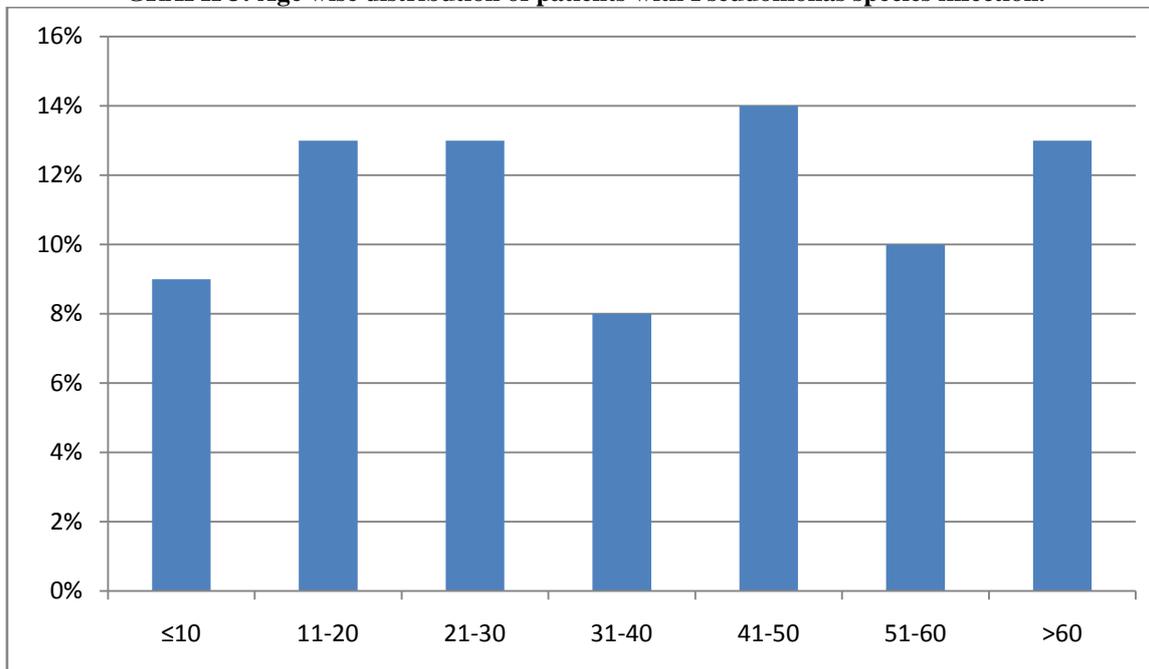


As shown in **TABLE 4** and **GRAPH 2** Pseudomonas infection was found comparatively more in male patients i.e. 57 (71.25%) than in female patient 23 (28.75%). The male to female ratio is 2.4:1

TABLE 5: Age wise distribution of patients with Pseudomonas species infection

Age (in yrs)	Total cases (n=80)	Percentage (%)
≤10	9	11.25
11_20	13	16.25
21_30	13	16.25
31_40	8	10
41_50	14	17.5
51_60	10	12.5
>60	13	16.25
Total	80	100

GRAPH 3: Age wise distribution of patients with Pseudomonas species infection.



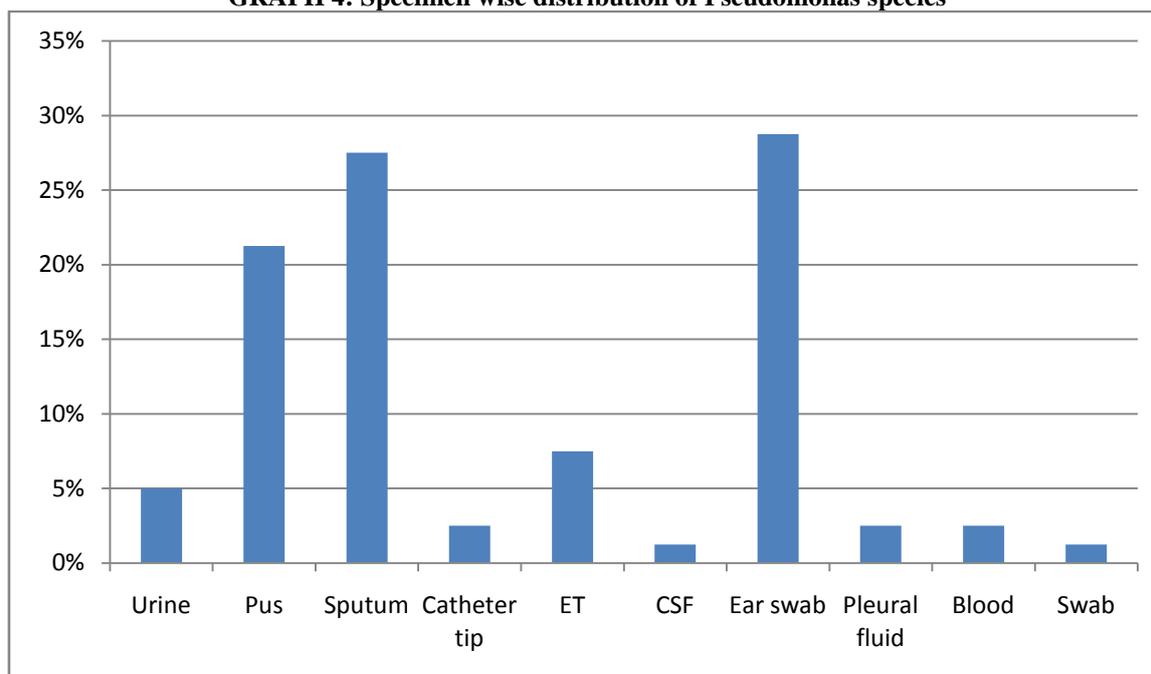
As shown in **TABLE 5** and **GRAPH 3** maximum pseudomonas infections were found in the age group between 41-50 yrs i.e. 17.5% while minimum from 31-40 yrs i.e. 8%

Sample distribution:

TABLE 6: Specimen wise distribution of Pseudomonas species

Clinical Specimen	No. of samples (n=80)	Percentage (%)
Urine	4	5
Pus	17	21.25
Sputum	22	27.5
Endotracheal secretion	6	7.5
CSF	1	1.25
Ear Swab	23	28.75
Pleural Fluid	2	2.5
Blood	2	2.5
Catheter Tip	2	2.5
Swab	1	1.25

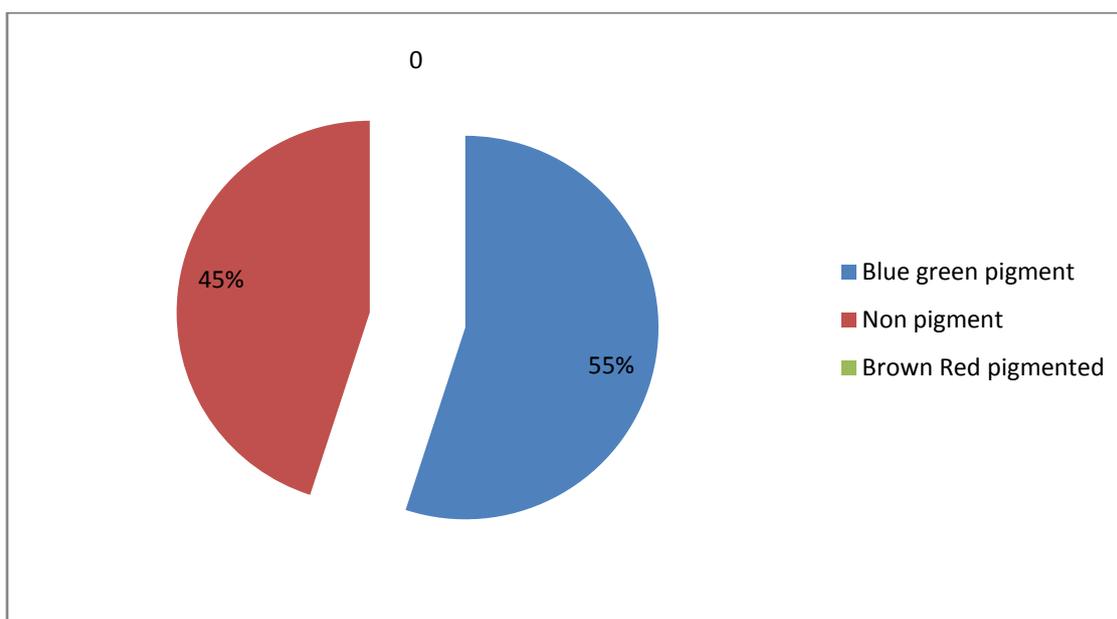
GRAPH 4: Specimen wise distribution of *Pseudomonas* species



As shown in the **TABLE 6** and **GRAPH 4** *Pseudomonas* species isolated maximum from Ear swab i.e. 23 (28.75%) followed from Sputum 22 (27.5%), Urine 4 (5%), Pus 17 (21.25%), Catheter tip 2 (2.5%), ET 6 (7.5%), CSF 1 (1.25%), Blood 2 (2.5%), Pleural fluid 2 (2.5%), Swab 1 (1.25%).

TABLE 7: Percentage wise Distribution of Pigment producing *Pseudomonas* species

Pigment	No.	%
Blue- Green	44	55
Non-Pigmented	36	45
Brown Red pigmented	0	0
Total	80	100



As shown in **TABLE 7** and **GRAPH 5** out of 80 isolate of *Pseudomonas* species, 44 (55%) isolates producing Blue- Green pigment (Pyocyanin), 36 (45%) was Non-pigmented, 0 (0%) isolates producing Brown Red pigment (Pyomelanin).

TABLE 8: Antibiogram of *Pseudomonas* species

Sr. No.	Antibiotic	Sensitive	Percentage (%) Sensitive	Resistant	Percentage (%) Resistant
1	Norfloxacin	1	20	4	80
2	Ticarcillin	47	58.75	33	41.25
3	Piperacillin	59	73.75	21	26.25
4	Piperacillin-Tazobactam	67	83.75	13	16.25
5	Ceftazidime	58	72.5	22	27.5
6	Amikacin	51	63.75	29	36.25
7	Gentamicin	54	67.5	26	32.5
8	Tobramycin	64	80	16	20
9	Ciprofloxacin	60	75	20	25
10	Imipenem	53	66.25	27	33.75
11	Meropenem	72	90	8	10
12	Polymyxin- B	80	100	0	0
13	Colistin	80	100	0	0
14	Nitrofurantoin	2	40	3	60

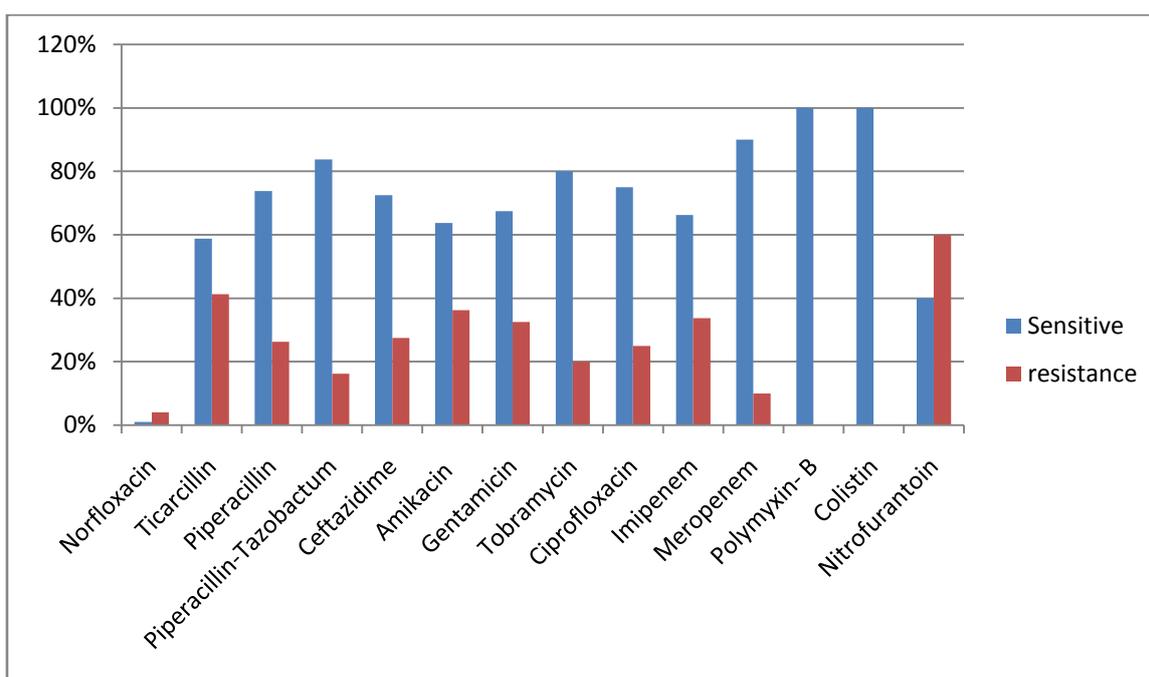


TABLE 8:and **GRAPH 6** revealed that most sensitive antibiotic were Polymyxin- B and Colistin (100%) followed by Meropenem (90%) in our study. Norfloxacin appeared as most resistant antibiotic with (80%) rate in Urine sample.

IV. Discussion

The term Pseudomonad is used to describe strictly aerobic Gram-negative, nonsporulating bacteria.^[8] Due to ubiquitous nature of *P. aeruginosa*, its high affinity to moist environments, and ability to survive at various conditions, it remains as a common pathogenic agent in neonatal intensive care units. Immunodeficiency status of newborn, invasive characteristics of diagnostic and therapeutic procedures and indiscriminating use of antimicrobials are predisposing factors with significant morbidity and mortality rates in *P. aeruginosa* septicaemia.^[9]

Pseudomonas aeruginosa is an important pathogen commonly implicated in serious nosocomial infections such as pneumonia and sepsis. The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide and limiting our therapeutic options.^[10]

The present study was conducted in the Department of Microbiology, NIMS Medical College and Hospital, Jaipur (Rajasthan) from July 2018 to December 2018. This study was undertaken to determine antimicrobial susceptibility pattern in 80 positive strains of *Pseudomonas* species isolated from various clinical specimens of the patients of NIMS hospital.

In the present study, *Pseudomonas* species was common in male patients i.e. 71.25% as compare to female patients i.e. 28.75% almost similar observation was made by Gagan et al(2016).^[15] i.e. male was 70% as compare to female 30%. Arora D et al (2010)¹², Flegoo et al(2014)¹³ and Anurave K et al (2013).The increase rate of isolation of *Pseudomonas* spices in Male as compare to female patients is due to more exposing in contaminated environment, daily activites most common is accidental injuries among men.

In the present study out of 80 *Pseudomonas* species strains most of patients maximum *pseudomonas* infections were found in the age group between 41-50 yrs i.e. 17.5% while minimum from 31-40 yrs i.e. 8% comparable to Prachee Singh Anuradha Tolpadi ^[16] Maximum number of *Pseudomonas aeruginosa* were isolated from the age group of above 60 years (40.50%), followed by 41 to 60 years (25%).

In the present study, out of 80 *Pseudomonas* species isolated from various clinical samples, Maximum number isolated from Ear swab 23 (28.75%) followed by Sputum 22 (27.5%), Pus 17 (21.25%) and CSF 1(1.25%). Observation are compared with Kumar et al. ^[17] (2013) Bacteria isolated from Ear swab samples pure cultures were *Pseudomonas* spp (38/90) in 42.22%, *Pseudomonas* spp was the most predominant organism causing CSOM in this region and this is in agreement with many previous investigators.

In the present study, out of 80 *Pseudomonas* species isolated from various clinical samples, Maximum number isolated from Ear swab 23 (28.75%) followed by Sputum 22 (27.5%), Pus 17 (21.25%) and CSF 1(1.25%). According to study Piyush Tripathi et al.^[18] (2011) out of 298 patient sputum samples 102 (34.23%) patient samples were isolated with *pseudomonas* strains.

According to our study the antibiotic susceptibility of 80 isolated strains of *Pseudomonas* species shows more effective antibiotic were Polymyxin- B and Colistin (100%), Meropenem (90%) and piperacillin Tazobactam (83.75%) was second most effective drug of choice for *Pseudomonas* infections followed by Gentamicin (67.5%) and Amikacin (63.75%). Nitrofurantoin is drug of choice for *Pseudomonas* in Urine infection.

TABLE 9: Antibiotic resistance in *Pseudomonas aeruginosa* isolated from patients from 2012 to 2014^[19] Comparable to our study 2018

Year	2012		2013		2014		2018	
	Sensitivity percentage	Resistance percentage						
Colistin	100	0	100	0	94.7	5.3	100	0
Meropenem	100	0	70.9	29.1	59.9	40.1	90	10
Imipenem	100	0	73.9	26.1	72.2	27.8	66.25	33.75
Amikacin	0	100	8.7	91.3	8.7	91.3	63.75	36.25
Gentamicin	60	40	34.8	65.2	44.4	55.6	67.5	32.5
Ceftazidime	60	40	43.5	56.5	35.5	46.7	72.5	27.5
Ciprofloxacin	20	80	21.7	78.3	25.5	74.5	75	25

According to our study the antibiotic susceptibility of 80 isolated strains of *Pseudomonas* species shows Norfloxacin appeared as most resistant antibiotic with (80%) rate in Urine sample. followed by Ticarcillin (41.25%), Amikacin (36.25%), Imepenem (33.75%), Gentamicin (32.5), Ciprofloxacin (25%), Ceftazidime (27.5%) compare with a study by Laxmi et al, resistance to gentamycin, ciprofloxacin and ceftazidime was 18%, 25% and 42% respectively, which was comparatively less in Gentamicin and high in Ceftazidime as compared to the present study.

As this study concluded that due to the rational use of antibiotics in present era it is difficult to control the multiple drug resistance. More and more studies should be undertaken in future to monitor the emergence of resistance to these antibiotics. Also they should be focused on better treatment policies, effective and cheaper alternative antibiotics in developing countries like ours. Also clinicians should be also aware of their use of these drugs in order to preserve this useful antibiotic and prolong its therapeutic usefulness.

V. Conclusion

The present study was conducted for “PREVALENCE OF PSEUDOMONAS SPECIES ISOLATES FROM VARIOUS CLINICAL SPECIMENS AT TERTIARY CARE HOSPITAL, Jaipur”. The present study was carried out on 80 strains of *Pseudomonas* species isolated from 300 various non repetitive clinical samples. The isolates were further tested for isolation of *Pseudomonas* species and their antibiogram susceptibility pattern as per CLSI guidelines 2018.¹⁴

A total 80 strains of *Pseudomonas* species were collected from 300 various non repetitive various clinical samples Pus, Urine, Sputum, Ear swab, Catheter tip etc. and were obtained under aseptic condition from different IPD and OPD.

- Incidence of *Pseudomonas* species isolates from clinical samples was 26.66%.
- Out of 80 *Pseudomonas* species maximum percentage of isolation was seen in male (71.25%) than in female (28.75%). The male to female ratio was 2.4:1

- Maximum pseudomonas infections were found in the age group between 41-50 yrs i.e. 17.5% while minimum from 31-40 yrs i.e. 8%
- Pseudomonas species isolated maximum from Ear swab i.e. 23 (28.75%) followed from Sputum 22 (27.5%) , Urine 4 (5%), Pus 17 (21.25%), Catheter tip 2 (2.5%), ET 6 (7.5%), CSF 1 (1.25%), Blood 2 (2.5%), Pleural fluid 2 (2.5%), Swab 1 (1.25%).
- All Pseudomonas species were most sensitive to Polymyxin- B and Colistin (100%) followed by Meropenem (90%) in our study.
- Highest resistance in Pseudomonas species was towards Ticarcillin (41.25%), Amikacin (36.25%), Imepenem (33.75%) and Gentamicin (32.5%).
- Norfloxacin appeared as most resistant antibiotic with (80%) rate in Urine sample.

Finally, the result of our study indicated that Polymyxin-B and Colistin is the only antimicrobial agent which showed (100%) sensitive followed by Meropenem (90%) may use as the drugs of choice for treating the Pseudomonas species infections.

The increase in resistance among Pseudomonas species is due to rational use of antibiotics in present era. This study emphasizes need for a continuous surveillance in hospital to detect the resistant strains.

This will help in making of conventional antibiograms.

Based on that, patient can be given appropriate treatment and will prevent further development of drug resistance. Also more research should be done to find better treatment policies, effective and cheaper alternative antibiotics in developing countries like ours. The finding of the studies should be shared with hospital infection control committee to help in the formulation of infection control policies. So that primary care givers can use antibiotic rationally.

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