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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES FROM VARIOUS CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL, JAIPUR RAJASTHAN.

Clinical Microbiology

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ABSTRACT

Background: Pseudomonas aeruginosa is one of the most common pathogens isolated from a variety of clinical samples. Furthermore, Pseudomonas aeruginosa is also one of the common pathogens responsible for generalized sepsis and subsequent mortality. Thus, it becomes critical to keep track of the prevailing drug sensitivity pattern of Pseudomonas species. **Objective:** This study aims to determine the antibiotic susceptibility pattern of Pseudomonas aeruginosa isolates in patients admitted to different clinical wards. **Material and Methods:** This was a single centre, laboratory-based, cross-sectional study conducted over a period of 14 months (May 2021 to June 2022) at the Department of Microbiology, SMS Medical College, Jaipur. The isolates were identified by standard protocols using biochemical tests. A total of 132 clinical samples in which the causative organism was Pseudomonas aeruginosa was tested for drug sensitivity and resistance pattern using as per Clinical and Laboratory Standards Institute (CLSI) guidelines using Kirby-Bauer's disc diffusion method. **Results:** The mean and median age of the participants was 31.8 years and 28 years, respectively, ranging from a minimum of 8 days to a maximum of 80 years. All the 132 (100.0%) isolates were sensitive to Colistin, followed by Piperacillin & Tazobactam (87.8%), Aztreonam (75%), Amikacin (75%), and Imipenem (74.2%). The isolates of Pseudomonas aeruginosa were least sensitive antibiotics to cefoperazone + sulbactam (65.2%), ciprofloxacin (65.9%), and tobramycin (67.4%). **Conclusion:** Pseudomonas aeruginosa is one of the commonly isolated opportunistic pathogen and it is becoming more resistant to commonly used antibiotics. Carbapenems and aminoglycosides were the two classes of drugs that showed highest activity against Pseudomonas aeruginosa.

KEYWORDS

Pseudomonas aeruginosa, antibiotics, drugs, resistance, sensitivity

INTRODUCTION-

Pseudomonas aeruginosa is a gram-negative bacterium that is known for its clinical importance and is a leading cause of hospital-acquired infections[1]. This bacterium is highly adaptable and can infect various body sites, making it a significant concern in clinical settings[2]. *Pseudomonas aeruginosa* commonly affects individuals with weakened immune systems, such as hospitalized patients, individuals with chronic illnesses, and those with compromised immune function[3]. It opportunistically causes infections, particularly in wounds, burns, surgical sites, urinary tract, respiratory tract, and bloodstream[4]. *Pseudomonas aeruginosa* is adept at forming biofilms, which are complex communities of bacteria encased in a protective matrix. Biofilms contribute to the bacterium's persistence and resistance to antibiotics, making infections difficult to eradicate[5,6]. Biofilm-associated infections are often seen in patients with chronic wounds, cystic fibrosis, and medical devices such as catheters and ventilators. *Pseudomonas aeruginosa* possesses an array of virulence factors that contribute to its pathogenicity. These factors include toxins, enzymes, flagella for motility, pili for attachment, and various secreted factors[4]. They enable the bacterium to evade the host immune response and cause damage to host tissues. *Pseudomonas aeruginosa* is a leading cause of healthcare-associated infections, including ventilator-associated pneumonia (VAP), urinary tract infections (UTIs), surgical site infections, and bloodstream infections[1]. These infections can prolong hospital stays, increase healthcare costs, and pose significant risks to patient outcomes.

Pseudomonas aeruginosa has shown a remarkable ability to develop resistance to multiple antibiotics[7]. This bacterium possesses various intrinsic mechanisms of resistance and can acquire additional resistance through genetic mutations and the transfer of resistant genes[4]. This makes treating *Pseudomonas* infections challenging and underscores the need for appropriate antibiotic stewardship. Its ability to cause opportunistic infections, develop antibiotic resistance, form biofilms, and produce virulence factors contributes to its clinical significance[3]. Efforts to prevent and control *Pseudomonas* infections, along with prudent antibiotic use, are crucial in managing these infections and improving patient outcomes.

Pseudomonas aeruginosa infections are becoming more common in hospitals and it has been identified as a major nosocomial pathogen, especially in patients with impaired immune systems[8,9]. Drug-resistant bacteria have emerged as a result of the concurrent widespread use of antimicrobial drugs and bacterial resistance mechanisms that have evolved over time[2]. Due to the emergence of resistance, the effectiveness of many antibiotics in the treatment of infections has become fairly restricted, and the threat posed by antimicrobial-resistant organisms is growing and intensifying[10,11]. In critical care units (ICUs), *P. aeruginosa* was the second most frequent cause of pneumonia, the fourth most frequent source of urinary tract infection, and the sixth most frequent blood stream isolate. In the hospital setting, numerous possible infection reservoirs have been discovered, including endoscopes, cleaning supplies, disinfectants, sinks, produce, flowers, and physiotherapy pools[12,13]. The selection of effective treatments is greatly hampered by the rising prevalence of healthcare-associated infections (HAIs) caused by Multi-Drug Resistance *P. aeruginosa* strains, which is linked to significant morbidity and mortality[1,10]. *P. aeruginosa* infections are hard to treat and frequently need combined medication. In *P. aeruginosa*, multidrug resistance is defined as resistance to at least three of the antibiotic classes' penicillin/cephalosporin/monobactam, carbapenem, aminoglycoside, and fluoroquinolone[1]. This study aims to determine the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates in patients admitted to different clinical wards.

MATERIAL AND METHODS :

Study Design: This was a single-centre, laboratory-based, cross-sectional, observational study. **Study Duration:** 14 months (May 2021 to June 2022). **Study Settings:** The present study was conducted at the Department of Microbiology, SMS, Medical College, Jaipur. The data collection for the present study was started after ethical approval from the Institutes' Ethical Committee. **Sample Size:** sample size was calculated at 95% confidence level, α error of 0.05 expecting 17.2% resistance among 2nd generation cephalosporins. **Selection of Clinical Samples for Study:** Non-lactose fermenting gram-negative bacilli (NFGNB) isolated from various clinical specimens like pus, urine, sputum, blood, throat swab, tracheal swab, ear swab, cerebrospinal fluid (CSF), pleural fluid, corneal swab were included in the study. **Inclusion Criteria**: Specimens showing non-lactose fermenting, oxidase-positive growth. **Exclusion Criteria**: Specimens showing non-lactose fermenting, oxidase negative growth.

Specimen collection, isolation, and identification

Three to five millilitres of clinical samples were collected through a sampling port into clean and dry containers using 24 gauge needle and syringe after cleaning the sampling port area with 70 % alcohol [12]. The specimens were transported immediately to the lab and inoculated on MacConkey and blood agar, and incubated aerobically at 37 °C for 24 h. Non-lactose fermenting pale colonies from MacConkey and large flat dark greenish colonies from blood agar (after sub-culturing on nutrient agar) were tested for conventional biochemical tests: oxidase test, catalase, citrate utilization, and oxidative fermentation.

The isolates were further sub-cultured on to two nutrient agar plates and incubated separately (at 37 °C for pigment production and growth at 42 °C). *Pseudomonas aeruginosa* ATCC 27853 was used as a quality control strain.

Identification of Bacteria: The isolates were identified based on-
i. Colony morphology on MacConkey and Blood agar,
ii. Gram's staining
iii. Oxidase test
iv. Triple sugar iron test
v. Pyocyanin pigment production test (choloform test)
vi. Growth at 42 degree Celsius
v **Susceptibility tests:** Antimicrobial susceptibility tests were done by the Kirby-Bauer disk diffusion method as per the recommendations of CLSI against a panel of antipseudomonal antimicrobials of standard strengths[14]. After incubation, inhibition zones were recorded as the diameter of the clear zones around the disc and interpreted according to the performance standard for the antimicrobial disk susceptibility test (CLSI 2021) .
o MDR- Non susceptible to ≥ 1 agent in ≥3 antimicrobial categories.
o XDR-Non susceptible to ≥1 agent in all but < 2 categories.
o PDR- Non-susceptible to all antimicrobial agents listed[15].

Procedure:
i. About 4-5 colonies of the test strain were touched with a straight wire and transferred to 1mL of normal saline and turbidity matched to 0.5 McFarland standards using this as inoculum.
ii. A sterile swab was dipped in the inoculum; excess was expressed by rotating the swab against the inner side of the tube.
iii. Lawn culture was made on Muller Hinton Agar (MHA) plate by swabbing the entire surface & repeating it twice, rotating the plate 60 degrees each time.
iv. Plate was allowed to dry for 15 minutes before placing the antibiotic disc.
v. Antimicrobial susceptibility testing was done with the antimicrobial agents given in table below. *P. aeruginosa* American Type Culture Collection (ATCC) 27853 was used as control and results interpreted as per Clinical Laboratory Standards Institute (CLSI) 2021 criteria[14]

RESULTS:
During the period of data collection a total of one hundred thirty-two isolates of *Pseudomonas aeruginosa* were identified from different clinical samples like pus, blood, sputum, throat swab, ear swab, cerebrospinal fluid (CSF), urine, pleural fluid, corneal swab etc. received in Department of Microbiology, SMS Medical College, Jaipur, (Rajasthan).

Out of the total 132 participants included in the study, 30% were in the age group of 16-30 years; 20% each in 1-15 and 51-70 years of age. The mean and median age of the participants was 31.8 years and 28 years, respectively, ranging from a minimum of 8 days to a maximum of 80 years. Of the total 132 participants, 75% were males and 25% were females (Table 1).

Table 1: Age and Gender (n=132)

Age Group (Years)	Gender					
	Female		Male		Total	
	n	%	N	%	n	%
<1	4	12.1	2	2.0	6	4.6
1-15	8	24.2	19	19.2	27	20.5
16-30	7	21.2	33	33.3	40	30.3
31 -50	6	18.2	20	20.2	26	19.70
51-70	7	21.2	20	20.2	27	20.5
>70	1	3.0	5	5.1	6	6.1
Total	33	25.0	99	75.0	132	100.0

Of the total 132 specimens tested for culture & sensitivity, 31% of requests were received from ICU, 30% from surgical ward and 23.5% from medical ward. Furthermore, more than one-third (34.1%) of samples were isolated from pus samples, 23.5% isolated were recovered from urine, and 15.2% from throat swabs, and 6.1% were isolated from CSF (Table 2).

Table 2: Specimen for culture and sensitivity testing (n=132)

	Freq.	Percent
ASCITIC FLUID	1	0.76
BLOOD	4	3.03
CSF	8	6.06
EAR SWAB	1	0.76
OTHERS	12	9.09
PLEURAL FLUID	4	3.03
PUS	45	34.09
SPUTUM	6	4.55
THROAT SWAB	20	15.15
URINE	31	23.48
Total	132	100.00

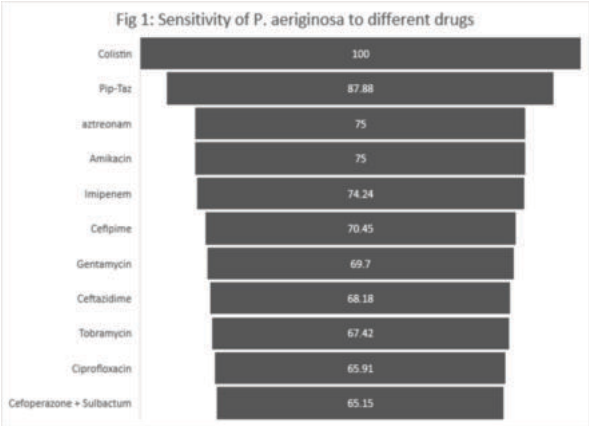

Figure 1 shows the results of the antibiotic sensitivity tests conducted on the 132 isolates of *Pseudomonas aeruginosa*. All the 132 (100.0%) isolates were sensitive to Colistin, followed by Piperacillin & Tazobactam (87.8%), Aztreonam (75%), Amikacin (75%), and Imipenem (74.2%). The isolates of *Pseudomonas aeruginosa* were least sensitive antibiotics to cefoperazone + sulbactam (65.2%), ciprofloxacin (65.9%), and tobramycin (67.4%).

Table 3: Comparative Sensitivity pattern to selected antibiotics among Pseudomonas aeruginosa isolates in different studies

	Present Study	Anil C.	Sharma J.	Tadvi J.	K Sukesh	Babu S.	Das S	Bindu D.
Colistin	100.0	-	95	-	-	-	-	100
Pip-Taz	87.88	-	64	98	-	76.4	64	84
aztreonam	75.0	-	47	-	-	-	-	-
Amikacin	75.00	82	-	56	-	-	67	79
Imipenem	74.24	100	70	-	-	81.3	71	-
Cefipime	70.45	-	45	-	-	-	-	76
Gentamycin	69.7	-	55	54	37	-	-	73
Ceftazidime	68.18	-	44	82	53	-	-	70
Tobramycin	67.42	-	-	-	32	-	60	84
Ciprofloxacin	65.91	72	-	-	51	-	-	73
Cefoperazone + Sulbactam	65.15	65	56	-	-	-	-	-

DISCUSSION-
The development of a rational antibiotic prescription plan is largely dependent on the detection and isolation of common causative pathogens for a variety of infections as well as identifying their sensitivity to various commonly prescribed antibiotics. Appropriate antibiotic prescription is critical for preventing the further emergence and spread of antibiotic resistance. *Pseudomonas aeruginosa* is one of the most prevalent gram-negative microorganisms isolated from hospitalised patients' clinical samples. *Pseudomonas* are not only linked with substantial morbidity and death, but also represent a significant economic burden due to their high treatment costs and lengthier hospital stays. In this regard, we studied the pattern of infection and drug sensitivity of *Pseudomonas aeruginosa* isolated from all the samples tested in the department of microbiology during the period of study. During the period of study, *Pseudomonas* was isolated from a total of 132 samples sent for microbiological testing.

There was a remarkable diversity in the age group of the population-ranging from a neonate of 8 days to elderly patients aged 80 years. These findings imply that *Pseudomonas* can infect patients of all age groups. However, most of the participants were in the age group of 16-30 years of age followed by 1-15 years, and 51-70 years. The mean and median age of participants from which *Pseudomonas* was isolated was 31.8 years and 28 years. Similar to our findings, Anil C. et al., (2013) reported that most of patients having *P. aeruginosa* infection were in the age group 21-40 years (60, 41.40%), followed by patients of > 60 years of age (45, 31.00%)[16]. K SUKESH.et al., (2017) reported that most of patients having *P. aeruginosa* infection were in the age group between31 to 45 years[17]). Golia S. et al.,(2016) reported that most of patients having *P. aeruginosa* infection were in the age group 41-60 years (40, 33.3%), followed by patients of > 60 years of age (35, 29.16%)[18]). Das S. et al., (2020) found that out of 1000 clinical samples 102 strains of *P. aeruginosa* were isolated. Most of the patient was from age group of 20-40 years (53 in number) and then of older age group >60 years (23 patients)[19].

Of the total 132 participants 75% were males and 25% females. K Anil C. et al., (2013) reported that out of these 145 strains of *P. aeruginosa*, 80 (55.17%) were from females and 65 (44.83%) were from males[16]. Golia S. et al., (2016) reported that out of the 120 specimens of *P. aeruginosa*, 80 (66.6%) were from males and 40 (33.3%) were from females(18). Das S. et al., (2020) reported that out of the total 102 strains of *P. aeruginosa,* 44% were male and 58% were female([19]).

Of the total 132 specimens from which *Pseudomonas aeruginosa* was isolated: most common specimen was that of the pus (34%) followed by the 23.5% urine and 15% were throat swab. Other bodily fluids constituted less than 10% of samples individually. The source of the specimen depends upon the underlying pathological condition. In the present study, most common source of pus was infected wounds (both trauma & surgery) and pus originating from the ear. Qayoom S. et al., (2019) identified that of the 71 specimens isolated from patients; most of the *Pseudomonas* aeruginosa were isolated from 29(40.84%) from pus from in-patient department. Samad A et al., (2017) reported that most *Pseudomonas aeruginosa* were isolated from tracheal aspirate (23.3%) followed by pus/wound swab (22.7%), urine (20.9%), blood(12.5%), bronchoalveolar lavage (11.8%), CSF(2.8%), pleural fluid(0.4%),and sputum(0.2%)[20]. Das S. et al.,(2020) reported that out of the 102 strains of *P. aeruginosa* isolated: most samples were isolated from urine (64%) and Ascitic fluid (33.3%) respectively[19]). Wajid M. et al.,(2021) of the 142 isolates of *Pseudomonas aeruginosa:* most of the specimens were isolated from pus & body fluids (51.4%), followed by respiratory samples (36.6%) & urine (7.04%)[21].

Of the total 132 specimens tested for culture & sensitivity 96.2% were received from inpatients ward (31% requests received from ICU, 30% from surgical ward and 23.5% from medical ward) and only 3.8% were received from outpatient's department. Qayoom S. et al., (2019) reported that among a total of 71 isolates of *Pseudomonas aeruginosa,* 29(40.84%) were cultured from the samples received from in-patient department[22]. Das S. et al.,(2020) reported that out of the 102 sample of *P. aeruginosa;* most samples originated from the specimen sent from the inpatients department in comparison to outpatients department[19]. Dash M. et al., (2022) reported that out of 327 isolates, 229 isolates (70%) were detected from in-patients admitted in the hospital and rest 98 isolates (30%) were isolated from out-patients (community-acquired infection)[23].

Among the aminoglycosides, the *P. aeruginosa* were most sensitive to amikacin (75%) followed by gentamycin (69.7%) and least sensitive to Tobramycin (67.4%). Among the cephalosporins group of antibiotics-isolated specimens of *P. aeruginosa* most sensitive to (70.45%) to Cefepime, followed by ceftazidime (68.2%), and least sensitive to Cefoperazone + Sulbactam (65.15%). Among the total 132 specimens with *Pseudomonas aeruginosa* subjected to drug sensitivity test with various antibiotics: 87.9% were sensitive to Pip-Taz, 74% sensitive to Imipenem, 75% were sensitive to Aztreonam, 100% sensitive to Colistin and 65.9% sensitive to Ciprofloxacin.

Collectively, in the present study, the isolated specimen of *Pseudomonas aeruginosa*, were most sensitive to Colistin (100%), followed by Pip-TAZ (87.88%), and were least sensitive to Cefoperazone + Sulbactam (65.15%) and Ciprofloxacin (65.9%) (Table 3). Anil C. et al.,(2013) reported that the resistance pattern of the *P. aeruginosa* to various antibiotics tested was in the order: chloramphenicol (72.41%) > ceftriaxone (68.96%) > piperacillin (55.17%) > co-trimoxazole (51.72%), cefoperazone+ Sulbactam (34.48%) > ciprofloxacin (27.59%) > amikacin (17.25%) > imipenem (0.0%)[16]. Bindu D. et al., (2022) reported that the antibiotic resistance rate of the isolates were 29.7% to ceftazidime, 16.2% to Piperacillin-tazobactam, 27 % to gentamicin and ciprofloxacin, 16.2% to tobramycin and imipenem, 24.3% to meropenem, 27% to ciprofloxacin, 13.5% to aztreonam, 21.6% to amikacin, 24.3% to cefepime and levofloxacin, 21.6 to tigecycline. All strains were sensitive to colistin.

Sarwat T. et al., (2015) reported that the nosocomial isolates showed more resistance to antibiotics like ceftazidime (60%), tobramycin (46.5%), ticarcillin (58.46%), ticarcillin/clavulanic acid (38.46%) and piperacillin/tazobactam (26.15%) as compared to the community isolates which showed resistance of 32%, 0%, 40%, 24% and 8% respectively[24]. Sharma J. et al., (2015) reported that most of isolates were found to be highly sensitive to Colistin (95.4%), Polymixin B (95%), Levoflox (83.3%), Imipenem (70%), Netilmycin (66%) and Piperacillin+ Tazobactam (64.5%). However, they showed resistance towards Ofloxacin (65%), Piperacillin (64%), Ceftazidime (56.3%), Cefoprazone (58%), Cefepime (55%), Aztreonam (53%), Cefoperazone + sulbactam (46%) and Gentamicin (45%)[25]). Tadvi J. et al.,(2015) reported that majority of *Pseudomonas* isolates were susceptible to Piperacillin Tazobactam (98%), Meropenem (93.33%) and Levofloxacin (92.66%) and followed by Ceftazidime (82%), Cefoperazone (81.33%), Piperacillin (80.66%), Amikacin (56%), Gentamicin (54.66%)[26].

Golia S. et al.,(2016) reported that among 112 isolated pathogens 35(31.25%) showed resistance to aminoglycosides, 30(26.78%) to cephotaxime[18]. Resistance rates to Piperacilin/ tazobactam, ceftazidime, ofloxacin varied from 10-15(8.92% to 13.39%). 10/112(8.92%) isolates were multi-drug resistant. K Sukesh et al.,(2017) reported that maximum isolates of *Pseudomonas aeruginosa* isolated from various samples are resistant to tobramycin (68%) followed by gentamicin (63%), piperacillin (50%), ciprofloxacin 49%) and ceftazidime (43%)[17].

Kaliyamoorthi D. et al., (2018) reported that among Cephalosporins highest resistance was observed among 1st generation cefazolin (83.9%) followed by 2nd generation Cefotaxime (17.2%) and Ceftazidime (23.7%) the least was seen among 4th generation Cefepime which showed 6.5% resistance[27]. All isolates were sensitive to Imipenem. Babu S. et al.,(2018) found that most number of isolates being sensitive to Imipenem (81.3%), Piperacillin+ Tazobactam (76.4%), and Piperacillin (67.6%) Das S. et al., (2020) found that isolates of *P. aeruginosa* showed good sensitivity against antibiotics like Amikacin (67.7%), Imipenem (71%), Piperacillin-Tazobactam (64%), Tobramycin (60%)[28]). Wajid M. et al.,(2021) observed that the highest resistance among *Pseudomonas aeruginosa* isolates were noted for levofloxacin (47.3%), ciprofloxacin(45.7%), ceftazidime(38.7%)(21). Dash M. et al .,(2022) observed that most of *P. aeruginosa* were highly resistant to ceftazidime 77.7%, cefepime 64.8%, piperacillin 45%, ciprofloxacin 38.9%, levofloxacin 36.1%, gentamicin 37.3% and amikacin 30%. *P. aeruginosa* was least resistant to imipenem 6.4%, followed by meropenem 8% and piperacillin/tazobactam 11.3%[23]).

CONCLUSION-

The results of this study showed very clearly that some *P. aeruginosa* isolates were resistant to multiple antipseudomonal drugs. Colistin was the only antibiotic that was effective against all isolates of *P. aeruginosa*. To stop the *P. aeruginosa* from becoming resistant to multiple drugs, everyone must come up with ways to limit antibiotic use. An answer can be found if an infection control committee keeps working to help people understand the problem better. Encourage people to wash their hands well so that germs don't spread. Monitoring how well antimicrobials work on a regular basis is important for local, regional, and national isolates. This would help and direct the doctors in giving the right mixtures of anti-microbials to limit and stop the spread of *P. aeruginosa* strains that are resistant to multiple drugs.

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