



## **Study of Pseudomonas Species from Clinical Isolates and Antibiogram for Rational Use of Antibiotics**

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### **ABSTRACT**

Antimicrobial resistance is an urgent public health threat, and antibiograms are critical tools for identifying and combating the spread of drug-resistant organisms. Antibiograms summarize data about resistance patterns for selected pathogens. Our aim was to describe and interpret pseudomonas species and antibiograms to guide rational use of antibiotics in hospitals and to monitor the trends of antimicrobial resistance patterns. 172 positive pseudomonas species studied over a one year period from Jan 2022 to Dec 2022. Admitted Patients from 2 tertiary care hospitals were selected as prototypes. Commonly used antibiotics were identified for each species. Further comparisons were done to study changes in Pseudomonas species collected from different sites and their sensitivity patterns. The predominant Pseudomonas species causing infection are Pseudomonas aeruginosa 60%, Pseudomonas putida 20%, Pseudomonas cepacia (Burkholderia cepacia) 15%, Pseudomonas pseudomallei (Burkholderia pseudomallei) 5%. Among all comparative study Pseudomonas pseudomallei is dangerous species mortality rate is high among others. Required long term antibiotics treatment. Among all (90%) isolates showed sensitivity to ceftazidime, (70%) isolates showed sensitivity to levofloxacin, (90%) isolates showed sensitivity to Amikacin, cotrimoxazole and minocycline. (100%) isolates showed sensitivity to meropenem. Pseudomonas pseudomallei ceftazidime resistant strains is an important alarming sign for treating clinician's. It helps medical professionals in a locality to choose appropriate evidence based antibiotics for initiating treatment for emergency cases while waiting for an individual culture and sensitivity report.

**Key words:** - Pseudomonas species, Antibiotic resistance, Antibiograms.

Received 28.05.2023

Revised 20.07.2023

Accepted 11.09.2023

### **INTRODUCTION**

Antibiotics have saved many lives through their successful treatment of infections, and their availability has made many surgeries possible. Antibiotics have also made many surgeries possible because of their availability. In response to selection pressure caused by the misuse of antibiotics, susceptible pathogenic populations are being gradually replaced by increasingly resistant populations. (1).

There are many factors contributing to the rise of antimicrobial resistance (AMR) in India. Due to lack of awareness about the issue of antibiotic resistance and insufficient training on rational antibiotic use. Treatment of infectious diseases becomes a major problem because of the emergence of drug resistant organisms in the hospital and community area. This is especially true in the case of Pseudomonas aeruginosa is an opportunistic pathogen causing a wide variety of infections such as urinary tract infections, respiratory infections, soft tissue infections, dermatitis, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in immune-suppressed patients were severe burns, bed ulcers and in patients suffering from cancer or AIDS. (2).

*B. pseudomallei* previously known as *Pseudomonas Pseudomallei*. *B. pseudomallei* (*Pseudomonas Pseudomallei*) readily grows in commercially available blood culture media. Ashdown's medium (gentamicin containing medium) can be used for selective growth of *B. pseudomallei*. *B. pseudomallei* shows resistance to many antibiotics in clinical use and exhibits a tendency to cause relapse despite successful initial and maintenance therapy. It has the potential of being a biological weapon and causes infections with major problems among individuals with immunosuppression, diabetes, alcoholic and renal failure. Treatment is Intensive Therapy minimum of 10 to 14 days Ceftazidime or Meropenem Or Imipenem. Anyone of the three may be combined with trimethoprim-sulfamethoxazole recommended for neurologic, cutaneous, bone, joint, and prostatic melioidosis (3)

This organic entity is noted for its inborn protection from different antimicrobial specialists and its capacity to secure hereditary determinants for opposition. Since the creature is impervious to a few anti-microbials; carbapenems the last choice for the administration of *P. aeruginosa* diseases (4).

Nonetheless, protection from the current enemy of pseudomonas anti-infection agents has been expanding because of the securing of at least one components, for example, creation of  $\beta$ -lactamases and depression of external film penetrability, procurement of plasmid-interceded obstruction qualities and aminoglycoside adjusting catalysts, and mutational change of antimicrobial targets, for example, topoisomerases or ribosomal proteins (fluoroquinolones and aminoglycosides opposition), hyper production of an inducible AmpC  $\beta$ -lactamase (Penicillin's and Cephalosporin's), and constitutive articulation of efflux siphon (beta-lactams, fluoroquinolones and aminoglycosides opposition) (5).

These systems have led to the development of MDR and PDR strains. Beta-lactam anti-toxins are the most often recommended antimicrobial specialists in treating both gram positive and gram negative diseases. Creation of beta lactamases is the most widely recognized system of bacterial obstruction and is plasmid interceded and likewise, Expanded range beta-lactamases (ESBL) delivering organic entities display co-protection from numerous different classes of anti-infection agents bringing about the restriction of restorative choices. The most well-known imported  $\beta$ -lactamases are penicillinases having a place with the sub-atomic class A serine  $\beta$  lactamases (TEM, SHV, CTX-M families) and class D (OXA-type); butPSE, CARB, PER, VEB, GES, and IBC families are less regularly revealed.

### **Survey of the Past Work**

Phytophthora infestans is an unavoidable living thing found in countless normal settings, where it causes defilements in plants, animals, and individuals. It has a spot with the assortment Pseudomonas. An amount of 202 creature types have been recognized; due to the existence structure's ability to make due with unimportant dietary necessities and to get through numerous natural conditions, it has obtained the ability to endure in both neighborhood center settings (6, 7).

Pseudomonas aeruginosa is one of the Pseudomonas creature classes that is constantly associated with human illnesses. It can moreover be isolated from clinical equipment, sanitizers, cleaning agents, sinks, mops, prescriptions, and physiotherapy and hydrotherapy pools in facility settings, notwithstanding different things. This living thing can in like manner be found in neighborhood like pools, whirlpools, warm tubs, contact point of convergence plan, home humidifiers, soil, rhizosphere, and vegetables, as well as in the environment (8).

P. aeruginosa conveys less multiresistance plasmids, develops less security from cephalosporin and less inalienable impediment than K. pneumonia, Enterobacter spp and Stenotrophomonas maltophilia separately. However P. aeruginosa is unique on account of the traits of both inalienable and obtained assurance from different immunizing agents poisons (9).

Bacterial separates with non-vulnerability to somewhere near one expert in no less than three antimicrobial classes are named as Multidrug safe (MDR). Broadly drug safe (XDR) is portrayed as non-lack of protection to something like one expert in everything with the exception of two or less antimicrobial groupings (i.e., bacterial separates stay powerless against several antimicrobial classes). Container drug safe (PDR) is described as non-frailty to all experts in each and every antimicrobial classification.(6,9) There are various parts of resistance in P. aeruginosa including making of serum poison hydrolyzing impetuses, target site changes, efflux siphon, and reduced permeability. Occasion of no less than one instrument prompts compromising the choice of convincing treatment.

P. aeruginosa interfaces with the host through a protein, needle-like appendage known as the sort III release system (T3SS). It is a huge hurtfulness determinant and is connected with extreme prominent defilements and requires pilin-mediated bacterial epithelial contact. After contact, this structure is sanctioned and dials back signal transduction; achieving cell destruction or adjustments in have safe responses. This system uses complex release/development equipment to inject a lot of factors, called effectors proteins, directly into the cytoplasm of eukaryotic cells to stifle cell capacity for bacterial perseverance. It contains 3 sections: release gadget, development/zeroing in on contraption, and radiated harms (effector proteins) and related chaperones (10).

T2ss facilitates the extracellular centering of a sweeping scope of something like 12 unmistakable fetoproteins, including toxins and subsidiary impetuses. This structure is coordinated by larger part distinguishing and expects a critical part in the overall hurtfulness of this bacterium. Two pragmatic sort II systems have been depicted, for instance, xcp and hx.c (5).

Changes in characteristics, called xcp, happened in the periplasmic accumulation of released proteins like elastase (LasB), lipase (LipA), acid neutralizer phosphatase (PhoA), or phospholipase C (PlcH). The xcp characteristics encode proteins which resemble Pull proteins, involving Klebsiella species. Outflow structures are proportioned across Gram-negative infinitesimal life forms and T2SS stays unequivocal and heterologous release. The Xcp structure is encoded in a gathering containing 11 characteristics, xcpP-Z; xcpA (twelfth quality) generally called PilD which is found elsewhere on the chromosome drew in with type IV concealment (3,7).

## Identification of Research Gap

A sharp microorganism is connected with continuous illnesses like *P. aeruginosa*. It is one of the most broadly perceived explanations behind center and neighborhood pollution in the US. Unsafe *P. aeruginosa* is occasionally destructive, and the advancement of multidrug safe limits perplexes the endeavor of treating the tainted patients essentially further. *P. aeruginosa*'s trademark and acquired insurance from an enormous number of hostile to microbials that are essentially immaterial is a result of various changes, including dynamic efflux structures, lessened cell wall permeability, plasmid getting, enunciation of various proteins, and the improvement of biofilms. Hostility to microbial maltreatment has achieved a diminishing in weak microorganisms while simultaneously extending safe masses. Hostile to disease use should be bound to a base since hostile to contamination block is in many cases associated with a lessening in real wellbeing.

Hyper transformation, compensatory changes, and cross co-decision are massively critical components in the assurance and dauntlessness of microorganisms that are particularly unsafe and safe. Pathogenesis is depicted by the formation of danger factors that are both extracellular and cell-related. Larger part recognizing, which is a cell thickness subordinate framework, is responsible for the statement of various destructiveness factors. These additional danger factors, which are encoded by exo characteristics, consolidate elastase, lipase, protease, and different cytotoxins, among others. It is understood that elastase and dissolvable protease are good for debasing a broad assortment of tissue parts, including proteinaceous parts of connective tissue and the cell surface receptors on neutrophils, among others. Despite how the bacterium is helpless to counter agents poisons in vitro, this living being is moreover known for the advancement of biofilms, which makes it hard to kill with antimicrobial treatment accepting it is accessible. Strength to various enemies of contamination classes is made by the minuscule living beings in their biofilms.

This study thinks about the sub-atomic attributes of opposition and harmfulness determinants of *P.aeruginosa*. As phenotypic recognition of ESBLs in no fermenters is confounded, ESBLs might be undervalued and underreported in these strains. The finding of the ongoing review shows the wide appropriation of ESBL variations and conjunction of MBL encoding qualities in certain strains. More prominent hereditary variety of MDR confines were seen by ERIC PCR investigation. Till date, aminoglycosides altering catalysts and transformations in QRDR qualities are believed to be answerable for significant level aminoglycoside opposition and fluoroquinolone obstruction separately.

Yet, ongoing reports recommend other procured opposition systems like 16S RMTases and PMQR determinants. Nonetheless, there are very few reports from India. The ongoing review reports the presence of 16S RMTases and qnr B quality which are prevalently found in Enterobacteriaceae and this recommends the level quality exchange may happen between the individuals from Enterobacteriaceae family and *P.aeruginosa*. This concentrate additionally shows the commonness of AME qualities. *P.aeruginosa* is known for its inborn obstruction towards numerous antimicrobials and this is predominantly because of the efflux component. There isn't a lot of information accessible in India in regards to the commonness of this component.

## MATERIAL AND METHODS

### Sample Collection

A total number of 2854 patient's culture samples collected from the 2 tertiary care Hospital Goa, over a one year period from Jan 2022 to Dec 2022. From those 172 patients samples were positive for pseudomonas species studied. Admitted Patients from 2 tertiary care hospitals were selected as prototypes. Commonly used antibiotics were identified for each species. Further comparisons were done to study change in Pseudomonas species collected from different sites and their sensitivity patterns.

### Isolation and Identification

For Urine, Tissue a loop full of samples streak on 5% sheep blood agar (BA) and Macconkey agar (Mac) with selective supplements (HiMedia Laboratories, Mumbai, India) and incubated at 37°C for 24hrs. For Blood culture (8-10ml) withdrawn from suspected patients were inoculated directly into BD BACTEC PLUS-Aerobic/F Medium vials (30 mL) and processed using BACTEC 9050/Fx40 automated blood culture system. Bacterial growth in the culture bottle was streaked on previously discussed Blood and Macconkey agar medium and incubated at 37°C for 24hrs. Then proceed for Gram stain and After Gram stain make a suspension 0.5 McFarland standard as per CLSI Guidelines. The bacterial isolates were identified to the species level by the VITEK2 system (BioMérieux, Lyon, France). The antimicrobial susceptibility (MIC) values derived from the VITEK 2 compact system according to clinical and laboratory standards (CLSI and EUCAST) guidelines.

### Statistical Method

The resistance of Pseudomonas spp. to individual antimicrobials was presented in absolute numbers and

percentages .In MsExcel using percentage formula check the resistance antimicrobial pattern of Pseudomonas species by Chi -square test

.Chi-square test gives P- value ( 0.05) null hypothesis. It helps to decide if to hold onto or reject the hypothesis.

$$X^2 = \sum \frac{(\text{Observed value} - \text{Expected value})^2}{\text{Expected value}}$$

## RESULT AND DISCUSSION

The predominant Pseudomonas species causing infection are Pseudomonas aeruginosa 60%, Pseudomonas putida 20%, *Pseudomonas cepacia* (*Burkholderia cepacia*) 15%, *Pseudomonas pseudomallei* (*Burkholderia pseudomallei*) 5% Fig(1)

.Among all comparative studies *Pseudomonas pseudomallei* (*Burkholderia pseudomallei*) is a dangerous species and mortality rate is high among others. Required long term antibiotics treatment. In Fig (2) Antimicrobial Resistance pattern for Pseudomonas species is shown in %.

As per Table no. 5:- Study of six antibiotics (p Value) Pseudomonas species for Tissue specimen the drug of choice will be meropenem, amikacin, Ceftazidime ,Minocycline, Cotrimoxazole statistically proven. For all Pseudomonas species for Urine and Blood specimens the drug of choice will be meropenem, amikacin, Minocycline, Cotrimoxazole statistically proven.

**Table 1: Pseudomonas species identified in clinical isolates.**

S. No	Total no. Pseudomonas aeruginosa	Total no. Pseudomonas putida	Total no. Pseudomonas cepacia (Burkholderia)	Total no. Pseudomonas pseudomallei (Burkholderia)	Summation	Specimen
1	11	4	13	3	31	Blood
2	48	15	4	0	67	Urine
3	52	18	3	1	74	Tissue, Burn, Wound
TOTAL	111	37	20	4	172	

Eg:-  $111 \times 31 / 172 = 20.00$

$\{11 - 20.00\}^2 / 20.00 = - 0.9$

**Table 2: Resistance pattern for Pseudomonas species in %.**

Organisms	Specimen	%	Chi Square	Summation
<i>P.aeruginosa</i>	BLOOD	20±1	4.05	11
	URINE	14.41±1	78.29	48
	TISSUE	8.60±1	5.046	52
<i>P.putida</i>	BLOOD	20±1	12.8	4
	URINE	14.41±1	0.024	15
	TISSUE	8.60±1	10.27	18
<i>P.cepacia</i>	BLOOD	20±1	2.45	13
	URINE	14.41±1	7.52	4
	TISSUE	8.60±1	3.64	3
<i>P.pseudomallei</i>	BLOOD	20±1	14.45	3
	URINE	14.41±1	14.41	0
	TISSUE	8.60±1	6.71	1

**Table no.3: Resistance Pattern for Pseudomonas Species in %.**

Organisms	Specimen	Meropenem (%)	Amikacin (%)	Ceftazidime (%)	Levofloxacin (%)	Cotrimoxazole (%)	Minocycline (%)
<b>P.aeruginosa</b>	TISSUE	100±1	95.24±1	95.24±1	79.85±1	79.85±1	83.69±1
	URINE	100±1	94.76±1	92.67±1	82.26±1	71.84±1	73.92±1
	BLOOD	100±1	100±1	83.00±1	73.74±1	55.35±1	82.83±1
<b>P.putida</b>	TISSUE	100±1	76.00±1	100±1	76.00±1	76.00±1	76.00±1
	URINE	100±1	87.67±1	87.67±1	81.00±1	81.00±1	87.67±1
	BLOOD	100±1	100±1	78.78±1	89.89±1	89.89±1	84.34±1
<b>P.capacia</b>	TISSUE	100±1	100±1	100±1	67.67±1	67.67±1	100±1
	URINE	100±1	76.00±1	76.00±1	76.00±1	100±1	100±1
	BLOOD	100±1	93.3i±1	77.93±1	62.54±1	70.24±1	77.93±1
<b>P.pseudo mallei</b>	TISSUE	100±1	100±1	67.67±1	67.67±1	100±1	100±1
	URINE	100±1	100±1	51.00±1	51.00±1	100±1	100±1
	BLOOD	100±1	100±1	51.00±1	51.00±1	100±1	100±1

**Table 4: Study of Six Antibiotics Chi Square Test**

Organisms	Specimen	Meropenem Chi Square	Amikacin Chi Square	Ceftazidime Chi Square	Levofloxacin Chi Square	Cotrimoxazole Chi Square	Minocycline Chi Square
<b>P.aeruginosa</b>	TISSUE	83.53	78.81	78.81	62.49	62.49	67.37
	URINE	73.25	68.13	66.09	55.96	45.91	47.90
	BLOOD	64.0	64.0	47.81	39.16	22.57	47.65
<b>P.putida</b>	TISSUE	83.53	59.77	85.53	59.77	59.77	59.77
	URINE	73.25	61.21	61.21	54.74	54.74	61.21
	BLOOD	64.0	64.0	43.85	52.79	52.79	49.08
<b>P.capacia</b>	TISSUE	83.53	83.53	83.53	51.56	51.56	83.53
	URINE	73.25	49.91	49.91	49.91	73.25	73.25
	BLOOD	64.0	57.59	43.06	28.93	35.93	43.06
<b>P.pseudo mallei</b>	TISSUE	83.53	83.53	51.56	51.56	83.53	83.53
	URINE	73.25	73.25	18.84	18.84	73.25	73.25
	BLOOD	64.0	64.0	18.84	18.84	64.0	64.0

**Table no. 5:- Study of Six Antibiotics p Value.**

Organism	Specimen	Meropenem Chi Square	Amikacin Chi Square	Ceftazidime Chi Square	Levofloxa- cinn Chi Square	Cotrimo- xazole Chi Square	Minocyc- linene Chi Square
<b>P. aeruginosa</b>	<b>TISSUE</b>	<b>83.53</b> 0.0078vs	78.81 0.0193ss	78.81 0.0193ss	62.49 0.2275ns	62.49 0.2275ns	67.37 0.2275-
	<b>URINE</b>	<b>73.25</b> 0.0505nqs	68.13 0.1100nss	66.09 0.1454nss	55.96 0.1454nss	45.91 0.8038nss	47.90 0.7403nss
	<b>BLOOD</b>	<b>64.0</b> 0.1899nss	64.0 0.1899nss	47.81 0.7433nss	39.16 0.9474nss	22.57 1.0000nss	47.65 0.7487nss
<b>P.putida</b>	<b>TISSUE</b>	<b>83.53</b> 0.0078vss	59.77 0.3066nss	85.53 0.0078vss	59.77 0.3066nss	59.77 0.3066nss	59.77 0.3066nss
	<b>URINE</b>	<b>73.25</b> 0.0505nqss	61.21 0.2630nss	61.21 0.2630nss	54.74 0.4845nss	54.74 0.4845nss	61.21 0.2630nss
	<b>BLOOD</b>	<b>64.0</b> 0.1899nss	64.0 0.1899nss	43.85 0.8600nss	52.79 0.5595nss	52.79 0.5595nss	49.08 0.6991nss
<b>P.capacia</b>	<b>TISSUE</b>	<b>83.53</b> 0.0078vs	83.53 0.0078vss	83.53 0.0078vss	51.56 0.6069nss	51.56 0.6069nss	83.53 0.0078vss
	<b>URINE</b>	<b>73.25</b> 0.0505nqss	49.91 0.6690nss	49.91 0.6690nss	49.91 0.6690nss	73.25 0.0505nqss	73.25 0.0505nqss
	<b>BLOOD</b>	<b>64.0</b> 0.1899nss	57.59 0.3796nss	43.06 0.8787nss	28.93 0.9985nss	35.93 0.9783nss	43.06 0.8787nss
<b>P.pseudo mallei</b>	<b>TISSUE</b>	<b>83.53</b> 0.0078vs	<b>83.53</b> 0.0078vss	51.56 0.6069nss	51.56 0.6069nss	<b>83.53</b> 0.0078vss	<b>83.53</b> 0.0078vss
	<b>URINE</b>	<b>73.25</b> 0.0505nqss	73.25 0.0505nqss	18.84 1.0000nss	18.84 1.0000nss	73.25 0.0505snqss	73.25 0.0505nqss
	<b>BLOOD</b>	<b>64.0</b> 0.1899nss	64.0 0.1899nss	18.84 1.0000nss	18.84 1.0000nss	64.0 0.1899nss	64.0 0.1899nss

83.25:- The two-tailed P value equals 0.0078. (20 %)

By conventional criteria, this difference is considered to be very statistically significant.

78.81:- The two-tailed P value equals 0.0193. ( 1 % ).

By conventional criteria, this difference is considered to be statistically significant. 73.25:- The two-tailed P value equals 0.0505. (7.2 %)

By conventional criteria, this difference is considered to be not quite statistically significant.

:- The two-tailed P value equals 0.1899(76.36%)

By conventional criteria, this difference is considered to be not statistically significant.

## CONCLUSION

- For all Pseudomonas species for Tissue specimen the drug of choice will be meropenem, amikacin, Ceftazidime, Minocycline, Cotrimoxazole statistically proven.
- For all Pseudomonas species for Urine and Blood specimens the drug of choice will be meropenem, amikacin, Minocycline, Cotrimoxazole statistically proven.
- *Pseudomonas pseudomallei* (*Burkholderia pseudomallei*) ceftazidime resistant strains is an important alarming sign for treating clinicians and microbiologists.
- Early clinical suspicion, along with appropriate culture processes (MIC) and awareness among health care providers is needed for effective control of infection.
- It helps Medical professionals in a locality to choose appropriate evidence based antibiotics. And also helps in initiating treatment for emergency cases while waiting for a culture and sensitivity report.

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#### CITATION OF THIS ARTICLE

Shradha R. Gaonkar and Alka Sagar. Study of *Pseudomonas* Species from Clinical Isolates and Antibigram for Rational Use of Antibiotics. *Bull. Env. Pharmacol. Life Sci., Spl Issue [2]: 2023: 146-152.*