



ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM VARIOUS CLINICAL SAMPLES (SPUTUM AND PUS)

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Abstract

A significant proportion of nosocomial and many infections are contributed by the *Pseudomonas spp.* major changes were observed from time to time in *Pseudomonas sp.* prevalence and multidrug-resistant (MDR) with enormous morbidity and mortality. The spread of antimicrobial resistance has now become a worldwide issue as a consequence of the haphazard use of antimicrobials. Place and duration: This study was done in the Department of Microbiology, Novus Path Labs, India and Nepal and its associates Hospitals. *Pseudomonas sp.* was isolated from different clinical samples like sputum, pus. Identification of *Pseudomonas sp.* was done by biochemical tests. Antibiotic susceptibility test (AST) was done by Kirby-Bauer disc diffusion method. Data Analysis was done by percentage method. Extended-spectrum β -lactamase (ESBLs) production was detected by the combined disc diffusion test. Sensitivity was seen in colistin and polymyxin B-100%, meropenem-86.58% and piperacillin-tazobactam- 82.92%. Resistance was seen in co-trimoxazole-80.48%, cefixime-82.92%, cefoperazone/sulbactam-59.75% and ciprofloxacin-39.02%.

Keywords: Antibiotic susceptibility test (AST), Kirby-Bauer disc diffusion method, *Pseudomonas aeruginosa*, Multiple drug resistance (MDR), Extended-spectrum β -lactamase (ESBL)

INTRODUCTION

Pseudomonas is an ultimate example of the opportunistic nosocomial pathogen, which in immune-compromised patients causes a broad range of infections and contributes to severe morbidity. The mortality due to nosocomial pseudomonal pneumonia is around 70 percent, despite therapy. Sadly, *P. aeruginosa* displays resistance to several antibiotics, thus endangering the option of effective therapy. In isolates that are multidrug tolerant, the relative contributions to phenotypic multidrug resistance from various molecular pathways are not well-known. [6] While several surveillance studies have been performed over the years to control the resistance of *Pseudomonas sp.* to different antimicrobial agents, studies identifying trends in concurrent resistance to various antibiotics over time are rare.[14] One of the most serious complications associated with *Pseudomonas* is antibiotic resistance. Due to their ability to be naturally resistant to several pathogens, a limited number of antibiotics are successful. Most gram-negative bacteria (including *Pseudomonas*) are responsible for the production of ESBL, which shows resistance too many antibiotics of new generations. [13] Therefore, we study the pattern of antibiotic resistance of *Pseudomonas sp.*, as it is the first study in the Novus Path Labs, India and Nepal and its associates Hospitals region of Uttarakhand, India.

MATERIALS AND METHOD

The present study was done at the Novus Path Labs, India and Nepal and its associates Hospitals region of Uttarakhand. In this study total of 20 strains of *Pseudomonas sp.* were isolated from different clinical samples (Sputum, pus samples) in the hospital. Identification of *Pseudomonas sp.* was done by biochemical tests. Antibiotic susceptibility test (AST) method was done by Kirby-Bauer disc diffusion method as per CLSI (clinical and laboratory standards institute) criteria.[18] All the analysis was performed using the simple percentage method.

Identification of *Pseudomonas spp.*

All clinical samples collected were inoculated aseptically on MacConkey agar and Blood agar media plates in case of sputum and pus samples while an additional chocolate agar plate was used in case of sputum samples which were incubated at 37°C for 24 Hrs. pus samples were inoculated by using a 4mm inoculating loop. In the case of the pus sample, 10⁵CFU/ml of growth was considered for significant bacteria. [10] Further *Pseudomonas sp.* was identified by cultural characteristics, morphological characteristics. Presumptive *Acinetobacter spp.* isolates was subjected to identification as per procedure described by N. Ullah, *et. al.*, 2019.

Identification of *Pseudomonas spp.* was done by biochemical tests

Biochemical tests:- *Pseudomonas sp.* indicated Oxidase positive reaction. In mannitol test showed strong motility. The color of citrate converted to blue from green indicated a positive reaction. The urease tube remains unchanged indicates a negative reaction. [12] Triple sugar iron (TSI) indicated alkaline slant /no change in the butt. Species identification of *Pseudomonas* was performed by the Oxidative-Fermentative (O-

F) test for Glucose, sucrose, lactose, and mannitol; Liquefaction of gelatin, by beta hemolysis on a blood agar plate and test for nitrate reduction. The urease hydrolysis is also used for its identification. Other tests include decarboxylation of Arginine, Lysin, and Ornithine. The growth at 35° C and at 42° C for time duration of 18-24 hours on two tubes of trypticase soy agar (TSA). [20]

Antibiotic susceptibility test

Antibiotic susceptibility test (AST) method was done by Kirby-Bauer disc diffusion method on *Pseudomonas spp.* following the guidelines of the Clinical Laboratory Standards Institute 2015. First, the inoculums were prepared for AST with the help of nutrient broth by taking 5/6 colony of *Pseudomonas spp.* that matched to 0.5 McFarland standard (1.5×10^8 CFU/ml) within 15 minutes, a sterile cotton swab was dipped into the inoculums suspension and pressed inside the wall of the tube above the fluid level, and inoculate over the dried surface of Mueller Hinton Agar (MHA) plate. After 3-5 minutes antibiotic discs were applied and gently pressed down to ensure complete contact with agar. [8] The antibiotic disc used for AST in present study were purchased from Hi-media laboratories; co-trimoxazole (25µg), ciprofloxacin (5µg), ceftazidime (30µg), cefixime(30µg), gentamicin (10µg), amikacin(30µg), meropenem(10µg), cefoperazone/sulbactam (75/3030µg), piperacillin -tazobactam(100/10µg), colistin(10µg), polymyxin-B(300unit), piperacillin (100µg), tobramycin(10µg), cefotaxime(30µg) and amoxicillin/clavulanic acid (30µg). [3] Zone of inhibition was measured in mm was concluded with interpretative criteria CLSI 2007 and isolates were sensitive, intermediate, and resistant. *Pseudomonas aeruginosa* ATCC-27853 was used as a control strain. [19]

RESULTS

The total number of isolates of *Pseudomonas spp.* collected was 20. Gender wise distribution of isolates were male and female patients, as shown in table 1.1. The sample distribution for collected isolated was Pus 54.87%, Sputum 6%, as shown in table 1.1. The highest number of isolates of *Pseudomonas sp.* were collected from patients at age interval between 31 to 45 years. In every age group isolates collected from male patients was greater than female patients. Morphology of *Pseudomonas sp.* is in gram-negative rods belongs to the family of Enterobacteriaceae. Sensitivity was seen 100% (n=82) in colistin and polymyxin-B, 86.58% (n=71) in meropenum, 82.92% (n=68) in piperacillin -tazobactam, 68.29%(N=56) in tobramycin, 67.07% (n=55) in gentamicin, 64.63%(n=53) in amikacin, 58.58%(n=48) in piperacillin, 56.09%(n=46) in ceftazidime and 51.21%(n=42) in ciprofloxacin. Resistance was seen 80.48% (N=66) in co-trimoxazole, 82.92% (n=68) in cefixime, 59.75% (n=49) in cefoperazone/sulbactam, 39.02%(n=32) in ciprofloxacin and 36.58%(n=30) in piperacilin. The maximum number of MDR strains were isolated from the pus samples (n=18) as compared to other samples. MDR strains were seen higher in males than females in the case of pus samples, whereas females were seen in greater numbers than males in pus samples. The total number of ESBL producing strains of *Pseudomonas sp.* in clinical samples was 30.48%(n=25). The distribution of ESBL strains of *Pseudomonas sp.* in clinical samples was pus 60%(n=25), sputum 36%(n=9).

Table: 1.1 Sample collections**1.2 *Acinetobacter* species from different Clinical Samples**

S. No.	Clinical Samples	No. of Samples	Positive for <i>Acinetobacter</i> spp.
			Number
1.	Sputum	10	6
2.	Pus	10	5
	Total Sample Collected	20	11

S. No.	Clinical Samples	Male	Female	No. of Samples
1.	Sputum	7	3	10
2.	Pus	4	6	10
	Total Sample Collected	10	10	20

Table- 1.3 Culture Positivity of Study Population

Culture	Frequency
No growth	6
Growth	5
Total	11

Statistical analysis was done by descriptive statistics using simple ratio and percentages method. Microsoft office 2007 was used to generate Tables.

CONCLUSION

At the end of our study, we concluded that the most effective treatment for treating infections related to *Pseudomonas* spp. in the Uttarakhand region is colistin, polymyxin-B, meropenem, and piperacillin-tazobactam. Maximum isolates of *Pseudomonas* spp. obtained from hospitalized patients indicated nosocomial infections. It is very necessary to conduct routine surveillance for resistance strains at regular intervals of time to protect many lives from harmful MDR strains. Our study is an attempt to regulate the use of antibiotics that are irrelevant and wasteful. It is also a public health concern to take adequate measures, maintain cleanliness and hygiene to reduce the spread of nosocomial infections. To minimize the spread of

hospital-acquired infections, it is the primary responsibility of all health care staff and all clinicians, and all patients along with their relatives to facilitate adequate hand washing with soap or sanitization of all areas. We can protect many lives from life-threatening MDR strains in this way and also aim to keep our society healthy.

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