ORIGINAL RESEARCH

Antimicrobial Susceptibility Pattern of Pseudomonas aeruginosa isolates from Different Clinical samples at a Tertiary care hospital

[1]Snehal Patil, [2]Shraddha Chavan, [3]Shailendra Patil, [4]Suvarna Patil

[1]Associate Professor, Department of Microbiology, B.K.L.Walawalkar rural Medical College, Sawarde, Dist. Ratnagiri Maharashtra, India
[2]Pg DMLT, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist. Ratnagiri Maharashtra, India
[3]Professor, Department of Anaesthesia, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist. Ratnagiri Maharashtra, India
[4]Medical Director, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist. Ratnagiri Maharashtra, India

Corresponding author
Snehal Patil
Associate professor, Department of Microbiology, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist; Ratnagiri Maharashtra, India
Email: sausnehal@gmail.com

Received date: 08 October, 2023 Revised date: 13 November, 2023 Acceptance date: 18 December, 2023

ABSTRACT

Background: *Pseudomonas aeruginosa* is a gram negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9-10% of hospital infections. The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is a worldwide concern. **Aims and Objectives:** The study was undertaken to find out antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolated from different clinical samples. **Material and Methods:** 106 samples of *Pseudomonas aeruginosa* isolates were included in this study. Identification was done by standard Microbiological procedures. Antibiotic susceptibility pattern was carried out by Vitek 2 automated system **Results:** Highest percentage of *Pseudomonas aeruginosa* were isolated from pus samples. Amikacin (64.15 %) was found to be more sensitive towards Pseudomonasaeruginosa followed by Cefoperazone/Sulbactum (61.32%), Ciprofloxacin(59.43%) and Meropenem(59.43%). **Conclusions:** *Pseudomonas aeruginosa* is one of the most important bacterial pathogen seriously contributing to the problem of healthcare associated infection. Maximum resistance in Pseudomonas aeruginosa isolates was seen against Ceftazidime,Piperacillin-Tazobactum and Levofloxacin.Hencethere is a need to emphasize the rational use of antimicrobials & strictly adhere to the concept of reserve drugs to minimize the misuse of available antimicrobials.

Keywords: Pseudomonas aeruginosa, nosocomial infection, opportunistic pathogen

INTRODUCTION

Pseudomonas aeruginosa, a virulent microorganism is susceptible to only limited number of antibiotics. It accounts for about 11% of all nosocomial infections and ranks fifth among all the nosocomial pathogens. *Pseudomonas aeruginosa* is increasingly recognized as an emerging opportunistic pathogen ofclinical relevance, often multidrug - resistant organism that commonly inhabits the soil and water of the environment. It is a common cause of nosocomial infections such as burn wound infection, respiratory tract infection, urinary tract infection and acts as an opportunistic pathogen in immune compromised patients. [1,2] P. aeruginosa is one of the most common nosocomial pathogens that contributes significantly to prolonged hospitalization, and increased mortality. [3,4]Numerous different nutrients can be metabolized by pseudomonas. When combined with their capacity to form biofilms, P. aeruginosa thrive in a range of unexpected environments, such as areas wherepharmaceuticals are prepared and antiseptics such quaternary ammonium compounds**.**In spite of advances in medical and surgical care and introduction of wide variety of antimicrobial agents against anti- pseudomonal activities, P. aeruginosa causes life threatening infection and complications. This bacterium frequently causes nosocomial infections that are difficult to treat because of its

inherent resistance and amazing capacity to develop new resistance mechanisms to a variety of antimicrobial drug classes, such as aminoglycosides, fluoroquinolones, and β-lactams.[5] β-lactam resistance in P. aeruginosa is the destruction of the β-lactamase enzyme in antibiotics, changes in the target of antibiotics, decreased intracellular uptake of antibiotics. [6]

The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is a worldwide concern. Unfortunately, P. aeruginosa demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment. [7] Antibiotic resistance is an ever increasing problem for Pseudomonas aeruginosa, which is normally associated with increased morbidity, mortality and costs. Hence the present study was planned to identify and determine the antimicrobial susceptibility pattern of Pseudomonas aeruginosa from different clinical samples.

AIMS & OBJECTIVE

1. To identify *Pseudomonas aeruginosa* isolates from different clinical samples using standard Microbiological procedures
2. To carry out antimicrobial susceptibility testing by using Vitek 2 compact automated system as per CLSI guidelines

MATERIAL AND METHODS

A total 106 samples of *Pseudomonas aeruginosa* isolates from different clinical specimen were studied and identified by standard microbiological procedures from September 2018 – September 2021 in Microbiology Department of B.K.L. Walawalkar Rural Medical College,

Nature of Sample: Blood, Sputum, Urine/Foley's Tip, Pus, ET Culture, Wound Swab, Tissue, Ear Swab, Wound Aspirate, Ascetic Fluid, Synovial Fluid, Peritoneal Fluid, Pleural Fluid. Samples were collected by using standard Microbiological procedures with sterile septic techniques. All these samples were transported to the microbiology department for further studies.

Inclusion criteria

Pseudomonas aeruginosa isolated from Pus, Urine, Sputum, Blood, Wound Swab, ET Culture, Synovial Fluid, Pleural fluid, Peritoneal Fluid, Ascetic Fluid, Ear Swab, and Foley's Tip and repeated isolation of the same strain of *Pseudomonas aeruginosa* from clinically significant patient were included in the study.

Exclusion criteria

Patient with colonization of *Pseudomonas aeruginosa* with no apparent clinical infection and organism isolated from collected specimens other than Pseudomonas aeruginosa were excluded.

All the samples were inoculated on Blood gar, MacConkey agar and Chocolate agar. Bacterial species was identified by using Gram stain morphology, Motility, oxidase test and Vitek 2 compact automated system.

Samples were processed in biosafety cabinet.18-24 hr. pure culture *of Pseudomonas aeruginosa* was used for Preparation of Bacterial suspension. A sterile swab is used to transfer a sufficient number ofcolonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (0.45% NaCl) in aclear plastic test tube.Vortexit for homogenous suspension. Putthetube in Densi CHEK plus then rotate 3600rpm after that read density, acceptable range0.50-0.63for bacteria. ID (Gram negative) and AST pair setup Tube fixed pipetting (red for gram -). 145µl of suspension was used for *Pseudomonas aeruginosa*. Add the above volume to 3ml of saline Vitek Tube Compact Cassette. The test suspension tube is placed into the"cassette", with Reagent card. Sealed test kits were moved into the reader/incubator automatically.Test kitsincubate and analyze automatically.

The reagent cards are available for the identification GN ID Card Gram-negative fermenting and non-fermenting bacilli. (GNIDcard).

Quality control: The Vitek 2 compact machine was validated using the standard strain as per the manufacturer's instructions. *Pseudomonas aeruginosa* 27853 was used. During the study period, the control strain was checked at regular intervals.

Antimicrobial susceptibility testing was done for gram negative (Oxidase positive) organismwith AST (406) card. Antimicrobial susceptibility testing was done by using a panel of antibiotics by Vitek 2 automated system. [8]

Vitek 2 automated system gives minimum inhibitory concentration (MIC) values for each antibiotics in the panel. MIC values were interpreted as per CLSI guidelines. [9]

OBSERVATIONS AND RESULTS

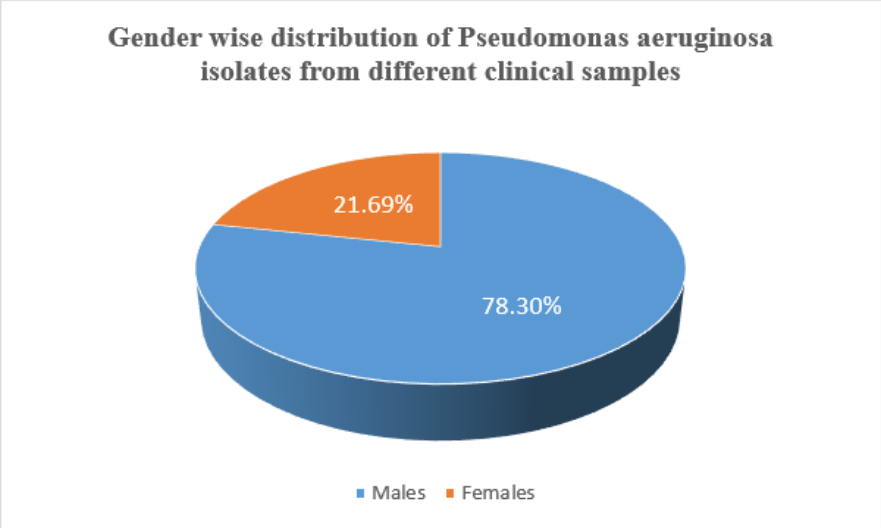
The present study included 106*Pseudomonas aeruginosa* isolates from clinical specimens obtained from patients with pyogenic wound infection, Respiratory tractinfection, Urinary tractinfection, Ear infection and processed by using standard Microbiological procedures.

Table 1: Distribution of Pseudomonas aeruginosa isolates from differentclinicalsamples

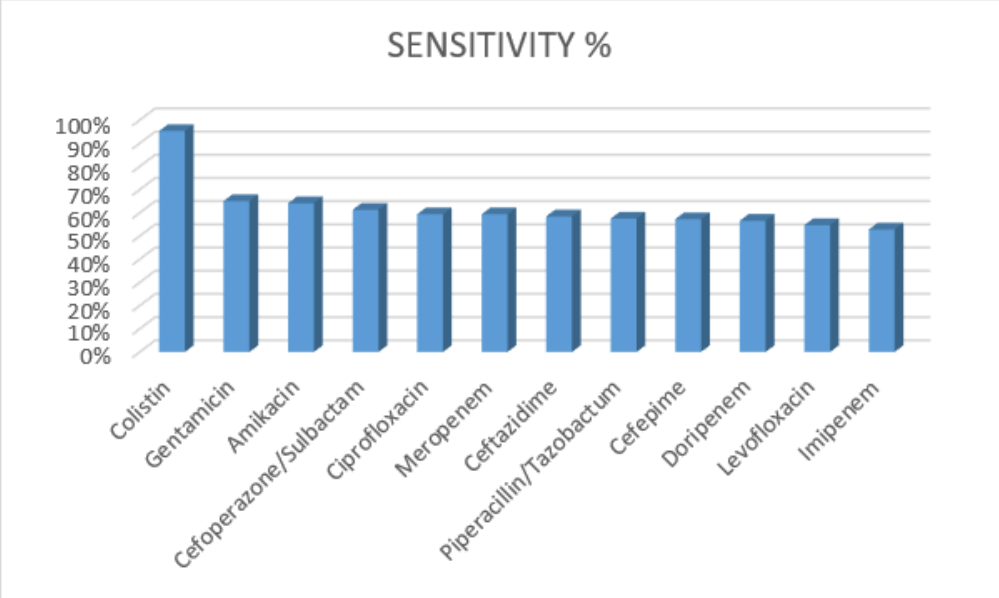
Sr.No	SAMPLES	FREQUENCY	PRECENTAGE
1	Pus	28	26.41%
2	Urine	26	24.52%
3	Sputum	12	11.3%
4	WoundSwab	9	8.49%
5	Tissue	7	6.60%

6	Blood	6	5.66%
7	Foley'sTip	3	2.83%
8	EarSwab	3	2.83%
9	AsciticFluid	3	2.83%
10	PleuralFluid	2	1.88%
11	SynovialFluid	2	1.88%
12	ETCulture	2	1.88%
13	WoundAspirate	1	0.94%
14	BronchoalveolarLavage	1	0.94%
15	PeritonealFluid	1	0.94%
	Total	106	100%

Among 106*Pseudomonas aeruginosa* isolates, frequency of Pus sample was found to be 28(26.41%), Followed by Urine 26(24.52%), Sputum 12(11.3%), Wound Swab 9(8.49%), Tissue 7(6.60%), Blood 6(5.66%), Tip 3(2.83%), Ear Swab 3(2.83%), Ascitic Fluid 3(2.83%), Pleural Fluid 2(1.88%), Synovial Fluid 2(1.88%), Wound Aspirate 2(1.88%),ET Culture2(1.88%), Broncho alveolar Lavage1(0.94%) and Peritoneal Fluid 1 (0.94%).



Graph 1: Gender wise distribution of Pseudomonas aeruginosa isolates from different clinical samples



Graph2: Anti microbial susceptibility pattern of Pseudomonas aeruginosa isolates from different clinical samples

Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates from different clinical samples towards Colistin was (95.28%) followed by Gentamycin (65.09%), Amikacin(64.15. %), Cefoperazone and Sulbactum (61.32%)Ciprofloxacin (59.43%), Meropenem (59.43%), Ceftazidime (58.49%), Piperacillin and Tazobactum (57.54%), Cefepime (57.28%), Doripenem (56.60%), Levofloxacin (54.71%), Imipenem (52.83%) and Ticarcillin and Clavulanic acid (49.05%)

DISCUSSION

Pseudomonas aeruginosa can be commonly encountered in the clinical specimens and considered as the normal bacterial flora of the pharynx, mucous membranes and skin. Whenthis organism is isolated from the clinical samples, efforts should be made to substantiate the clinical relevance in a particular patient.

In our study total 106 isolates of *Pseudomonas aeruginosa* were included during period from September 2018-September 2021. Samples such as Pus, Blood, urine, sputum, wound aspirate, Body fluid, Broncho alveolar lavage were included in the study. Total 106 clinical isolates of Pseudomonas aeruginosa were processed by standard microbiological procedures.

Among 106 Pseudomonas aeruginosa isolates, frequency of Pus sample was found to be 28(26.41%) followed by sputum 12(11.3%), urine26(24.52%), ET1(0.94%), Foley's Tip 3(2.83%), Ear swab 3(2.83%), Blood 6(5.66%), Foley's Tip 3(2.83%),EarSwab3(2.83%),Asceticswab3(2.83%), Pleural Fluid 2 (1.88%), Synovial Fluid 2 (1.88%), ET Culture 2 (1.88%), PeritonealFluid 1 (0.94), and Broncho alveolar lavage 1(0.94%), Wound swab 9(8.49%), Wound Aspirate1(0.94%).[Table1]

This study correlates with study done by Gyawali R, et. al [10] in which *Pseudomonas aeruginosa* were contributed from pus swab samples, (28.6%) from urine samples, (14.3%)from sputum samples, (10.1%)from tips,and(3.6%) from the fluid samples.

Out of 106 *Pseudomonas aeruginosa* isolates 78.30 % were from male patients and21.69% were female patients. [Table No.2] This finding correlates with the study done by Siddique.M, et.al.[13] in which 75.36% were from males and 24.64% were from females.

Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates from different clinical samples towards Gentamycin (65.09%) followed by Amikacin(64.15. %), Cefoperazone and Sulbactum (61.32%)Ciprofloxacin (59.43%), Meropenem (59.43%),Ceftazidime(58.49%), Piperacillin and Tazobactum (57.54%), [Table no.3]

Our study is in agreement withstudy done by Gyawali. R. et. Al [10] in which *Pseudomonas aeruginosa* were susceptible to Gentamycin (67.9 %), Meropenem (67.9 %)and Ciprofloxacin(64.3%).

The study does not correlate with study done by Srividya Y.*et al.* [11] in which Amikacin was (42.85 %), Piperacillin/ Tazobactum (42.85%) and Ciprofloxacin (25.0%).

In our study Maximum resistance in *Pseudomonas aeruginosa* isolates was seen towards Ceftazidime (41.51%), Piperacillin/Tazobactum (42.46%), Cefepime (42.72%), Doripenem (43.40%), Levofloxacin (45.29%), Imipenem (47.17%), and Ticarcillin/Clavulanic Acid(50.95%).

The study does not correlate with the study done by Tiwari N.t al. [12] in which 60% of the isolates were resistant to Ceftazidime followed by Cefepime (52%), Levofloxacin (49%), Ticarcillin/clavulanic acid (49%), Meropenem & Gentamycin (44%) and Ciprofloxacin (43%).

The study correlates with study done by, Pokharel. K, et.al. [13] in which they found that resistance towards Ceftazidime was 57.21% and 56.52% towards Piperacillin-tazobactum in P*seudomonas aeruginosa* isolates.

The Vitek 2 compact system identifies Non fermenting Gram negative bacilli along with their antibiotic susceptibility pattern with in a time period of 8 to 16hrs as per Simgamsetty S.*et al.* [14]

In this study occurrence of *Pseudomonas aeruginosa* isolates from Pyogenic wound infection were(43.39%), urinary tract infection(28.15%), respiratory tract infections were(15.09%), Ear infections(2.83%)and in Septicemia patients(5.60%).

The study is in agreement with study done by Siddiqaua M. *et al.* [15] in which they detectedthe maximum no of *Pseudomonas aeruginosa* isolates which was 64.49% from wound12.31% from urine samples in contrary Fatima A, *et al.*[16] found that isolates of P. aeruginosa were 60% from respiratory tract infection followed by UTI (13.3%),wound infections, (12.2%) and septicemia patients (10%) .

CONCLUSION

Among 106*Pseudomonas aeruginosa* isolates maximum number of isolates were fromPus sample. Maximum resistance in *Pseudomonas aeruginosa* isolates was seen against Ceftazidime, Piperacillin-Tazobactum, Levofloxacinand Imipenem.

In our study *Pseudomonas aeruginosa*s trains were predominantly isolated from Pyogenic wound infection were (43.39%), and urinary tract infection (28.15%), respiratory tract infections were(15.09%).

Correct and rapid identification and antimicrobial susceptibility testing of nonfermenting gram negative bacilli by Vitek 2 is very important because it will help in early initiation of appropriate antimicrobial therapy and proper management of patients. Antibiotic resistance in *Pseudomonas aeruginosa* are emerging as a critical human health issue. There is an urgent need to resolve the issue by taking some preventive measures.Combined efforts of healthcare professionals and researchers are required to educatepeopleabouttheproperuseof antibioticsand other infectioncontrolmeasures.To stop the spread of antibiotic resistance, stringent infection control

procedures and ongoing surveillance are crucial.

REFERENCES

1. Riou M, Carbonnelle S, Avrain L, Mesaros N, Pirnay JP, BilocqF. In vivo development of antimicrobial resistance in Pseudomonas aeruginosa strains isolated from the lower respiratory tract of intensive care unit patients with nosocomial pneumonia and receiving antipseudomonal therapy. Int J Antimicrob Agents 2010; 36: 513-522.
2. Brown SP, Cornforth DM, Mideo N. Evolution of virulence in opportunistic pathogens: generalism, plasticity and control. Trends Microbiol 2012; 20: 336-342.
3. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, *et al.* Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34(2):344–53.
4. Sonmezer MC, Ertem G, Erdinc FS, Kaya Kilic E, Tulek N, Adiloglu A, *et al.* Evaluation of risk factors for antibiotic resistance in patients with nosocomial infections caused by Pseudomonas aeruginosa. Can J Infect Dis Med Microbiol 2016; 2016:1321487.
5. Strateva T & Yordanov D. Pseudomonas aeruginosa – a phenomenon of bacterial resistance. J of Medical Microbiology: 2009; 58: 1133- 48.
6. Lin SP, Liu MF, Lin CF, Shi ZY. Phenotypic detection and polymerase chain reaction screening of extended-spectrum b-lactamases produced by Pseudomonas aeruginosa isolates. Journal of Microbiology, Immunology, and Infection. 2012; 45: 200-207
7. Obritsch MD, Fish DN, Mc Laren R, Jung R. National surveillance of anti-microbial resistance in pseudomonas aeuroginosa obtained from intensive care unit patients from 1983-2002. Antimicrobs agents chem; 2004; 48: 4606-10
8. Ling TK, Tam PC, Liu ZK, Cheng AF. Evaluation of VITEK 2 rapid identification and susceptibility testing system against gram-negative clinical isolates. J Clin Microbiol. 2001;39(8):2964-6.
9. Weinstein MP. Performance standards for antimicrobial susceptibility testing. Clin Lab. 28th ed S.l. 2018:M100-
10. Rabin Gyawali, Ram Bahadur Khadaka, Basudha Shrestha, Sarita Manandhar. Antimicrobial Susceptibility Patterns of Pseudomonas species isolated fromvarious clinical samples at a tertiary are hospital. Journal of institute of science andtechnology(Oct2020);25(2):49-54.
11. Srividya Yeruva and Vasantha Kabra Identification and Antimicrobial Susceptibility testing of Non Fermenting Gram Negative Bacteria by Vitek 2 in a Teaching Hospital in Mahbubnaga Int.J. Curr.Microbiol. App.Sci (2018) 7(10): 234-240
12. Tiwari N., Rajdev, S. and Mullan, S. (2017) Resistance Trends among *Pseudomonas aeruginosa* Isolates in a Tertiary Care Centre in South Gujarat. *Advances in Microbiology*, **7**, 188-194.
13. Pokarel K, Dr. Bhatt C P. Prevalence of Pseudomonas aeruginosa and its AntibioticSensitivityPatternjournalofNepalHealthResearchCouncilApril(2019);17(1):109-113
14. Susmitha Simgamsetty, Padmaja Yarlagadda, Bindu Madhav Yenigalla and Ramesh Babu Myneni. 2016. Ease with Vitek 2 systems, Biomerieux in identification of non- lactose fermenting bacteria including their antibiotic drug susceptibility: our experience. Int J Res Med Sci., 4(3): 813-817
15. Siddhiqua M, Ferdousi R S. Antibiotic Resistance pattern in Pseudomonas aeruginosaisolatedFromPrivatemedicalcollegehospital. KYAMCJournal,April(2018);9(1):16-19.
16. Fatima A. Alhussaina,, Nagarajkumar Yenugadhati , Fahad A. Al Eidana,, Sameera Al Johani , Motasim Badri ,Risk factors, antimicrobial susceptibility pattern and patient outcomes of Pseudomonas aeruginosa infection: A matched case-control study: Journal of Infection and Public Health 14 (2021) 152–157.