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EMERGING RESISTANCE TO CARBAPENEM AND ANTIMICROBIAL RESISTANCE IN PSEUDOMONAS AERUGINOSA ISOLATED FROM VARIOUS CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL, UDAIPUR, RAJASTHAN.

Medical Microbiology

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ABSTRACT

Introduction: Pseudomonas aeruginosa represents a great threat to public health worldwide, due to its high ability to acquire resistance to different antibiotic classes. Carbapenem are effective against multidrug resistant (MDR) Pseudomonas aeruginosa, but their widespread use has resulted in the emergence of carbapenem-resistant strains, which is considered a major global concern. This study aimed to determine the MBL producing Pseudomonas aeruginosa and its antimicrobial resistance pattern isolated from different clinical samples. **Material And Methods:** This prospective study was started from May 2020 to December 2022 in Department of Microbiology in Pacific Medical College and Hospital Udaipur, Rajasthan. It was done on various clinical samples i.e Urine, Endotracheal, Tracheostomy, Blood, Pus, Sputum, and CSF were collected from IPD and OPD patients. The collected samples were inoculated onto Nutrient agar, Blood agar and MacConkey agar plates. All plates were incubated aerobically at 37°C and observed for microbial growth at 24 and 48 hours. Identifications were done on the basis of phenotypically and biochemical characterizations Antimicrobial susceptibility testing Antimicrobial susceptibility testing will be done on Mueller Hinton agar (MHA) according to CLSI 2022 guidelines for Kirby Bauer disc diffusion test. After 24 hours of incubation zone of inhibition were measured. **Result And Conclusion:** In our research study we have analyzed 1000 various clinical samples i.e. Urine, ET, TT Blood, Pus, Sputum, and CSF and isolate 400 samples of Pseudomonas species. Out of 400 Pseudomonas species 300 were identified as Pseudomonas aeruginosa. The prevalence of Pseudomonas aeruginosa was 30 percent Out of 300 Pseudomonas aeruginosa 110 were isolated from Urine samples, 25 were isolated from ET samples, 18 were isolated from TT samples, 45 were isolated from Blood samples, 58 were isolated from Pus samples, 40 Pseudomonas aeruginosa were isolated from Sputum samples and 4 were isolated from CSF samples. Study clearly indicated that maximum number of MBL producing Pseudomonas aeruginosa was isolated from Urine sample i.e (37.5%) followed by Pus (25%), ET (15%), TT (10%), Blood (7.5%) and Sputum (5%). While Pseudomonas aeruginosa isolated from CSF sample shown zero MBL producer.

KEYWORDS

Metalloid-β-lactamases, Pseudomonas aeruginosa, prevalence, antimicrobial resistance.

INTRODUCTION

Statement of problem Antibiotic resistance in bacterial populations is a widespread and developing clinical problem that has been identified as a public health threat. As a result, area-specific monitoring studies are needed to profile distinct bacteria responsible for specific diseases, as well as their resistance patterns, in order to create data that will aid clinicians in selecting the appropriate empirical treatment. Importance of the study Antibiotic resistance in bacterial populations is a widespread and developing clinical problem that has been identified as a public health threat [1]. As a result, area-specific monitoring studies are needed to profile distinct bacteria responsible for specific diseases, as well as their resistance patterns, in order to create data that will aid clinicians in selecting the appropriate empirical treatment. Increased sickness, death, and medical expense are caused by Pseudomonas aeruginosa high level of inherent resistance to several structurally unrelated antimicrobial drugs [2]. One of the main factors influencing the formation and spread of multidrug resistance in India is the virtually non-existence of antibiotic policies and guidelines in our nation to assist doctors in making sensible decisions regarding antibiotic therapy [3]. In order to create strategies to manage infections caused by these bacteria in patients admitted, particularly in intensive care units, regular monitoring and documenting of resistance is necessary. Pseudomonas species is a type of opportunistic nosocomial pathogen that causes an extensive range of infection in immunocompromised patients and causes significant morbidity. Despite treatment, the death rate from nosocomial pseudomonal pneumonia is over 70%. Unfortunately, P. aeruginosa is resistant to a variety of antibiotics, making it difficult to choose the best treatment [4]. As a result, the current study was conducted to establish the antibiotic susceptibility patterns of metallo-beta-lactamase producing P. aeruginosa as a means of generating local data for planning and advocacy with respect to empiric therapy, antibiotic stewardship, and infection control from a variety of hospital acquired infections specimens (HAI) Non sporing non fermenters GNB are a type of aerobic bacteria that don't use carbohydrates for energy or break them down instead of fermenting or oxidising them [5]. They can also be found on the mucous membranes of people and animals as normal flora. More than 120 species of the genus Pseudomonas are harmful to plants, animals, and people and are common in moist settings including water and soil ecosystem[6]. Due to the synthesis of pigments including pyoverdine, a brilliant yellow-green pigment, and pyocanin, a blue-green pigment, Pseudomonas species are readily identifiable on agar. P. aeruginosa is the Pseudomonas species that is most frequently linked to infecting humans, despite being a naturally occurring organism in the environment [7]. The bacterium is classified as an opportunistic pathogen that mostly affects immunocompromised people and causes nosocomial infections. Only when there is a possibility of transmission to a compromised patient, such as when nurses' hands or respirators are involved in the transfer, is it important to isolate Pseudomonas aeruginosa from healthy carriers or ambient areas [8]. In addition to the genera Rugamonas, Mesophilobacter, Azotobacter, Cellvibrio, and Azomonas, Pseudomonas is a member of the bacterial family Pseudomonadaceae [9]. These bacteria live in both soil and water. Gram-negative, nonsporulating, strict aerobic bacteria are referred to as Pseudomonads. They're nonacid fast rods with oxidase positive, catalase positive, and diameters of 0.5–1 µm and 1.5–5 µm. These bacteria have polar flagella and are motile. Most organisms die in acidic environments (pH 5.5 or lower) [10].

Aims And Objective

- Isolation and identification of Pseudomonas species
- To find out prevalence of Pseudomonas aeruginosa from various clinical samples.
- To study antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from various clinical samples for detection of antimicrobial resistance strain.

MATERIAL AND METHODS

This prospective study was started from May 2020 to December 2022 in department of Microbiology in Pacific Medical College and Hospital Udaipur, Rajasthan. It was done on various clinical samples i.e Urine, Endotracheal, Tracheostomy, Blood, Pus, Sputum, and CSF were collected from IPD and OPD patients. The collected samples were inoculated onto Nutrient agar, Blood agar and MacConkey agar plates.

Statistical Analysis: Chi – Square analysis of study as per following formula

$\chi^2 = \sum(O_i - E_i)^2/E_i$

Where
- O_i = observed value (actual value)
- E_i = expected value.

The Chi-Square test gives a P-value to help you know the correlation if any

For statistical analysis we have considered the following parameters
1. Anova
2. Chi-Square Test

Source	Degrees of Freedom	Mean Square	F statistic	p-value
Groups (between groups)	k – 1	MSG = SSG / (k - 1)	F = MSG / MSE	P (x > F)
Error (within groups)	n – k	MSE = SSE / (n - k)		
Total	n – 1	Sample Variance = SS (total) / (n - 1)		

Chi-Square Test

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

All plates were incubated aerobically at 37°C and observed for microbial growth at 24 and 48 hours. Identifications were done on the basis of phenotypically and biochemical characterizations Antimicrobial susceptibility testing Antimicrobial susceptibility testing will be done on Mueller Hinton agar (MHA) according to CLSI 2022 guidelines for Kirby Bauer disc diffusion test. After 24 hours of incubation zone of inhibition were measured [11].

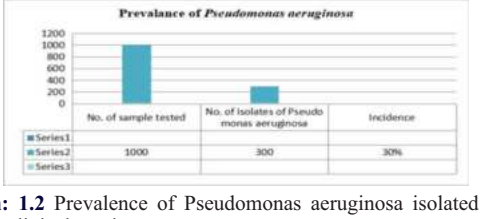
Two-Three colonies of specific bacteria were collected by loop from fresh sub cultured media and inoculated into 0.9% saline solution and mixed well by vortex mixture. The turbidity of the saline solution compared with standard McFarland's solution. A cotton swab was dipped into the turbid saline solution and made lawn on Mueller Hinton agar (MHA) [12]. Specific antibiotic disks were placed on the inoculated MHA media through forceps and disks were slightly pressed on the agar to place it well. After 18-20 hours of incubation either different clear zones or no clear zone appeared on inoculated MHA according to the susceptibility and resistance to specific antibiotics around antibiotic disks. Clear zone indicates susceptibility of bacteria to the specific antibiotic and no clear zone indicates resistance to the antibiotic [13].

Results And Observations

In our research study we have analyzed 1000 various clinical samples i.e. Urine, ET, TT Blood, Pus, Sputum, and CSF and isolate 400 samples of pseudomonas species. Out of 400 Pseudomonas species 300 were identified as Pseudomonas aeruginosa the basis of morphological and biochemical estimation. These 300 samples of Pseudomonas aeruginosa were further processed to study the antimicrobial resistance patterns and detect metallo-beta-lactamase.

Table: 1.1 Prevalence Of Pseudomonas Aeruginosa Isolate From Various Clinical Samples.

No. of sample tested	No. of isolates of Pseudomonas aeruginosa	Prevalence
1000	300	30%

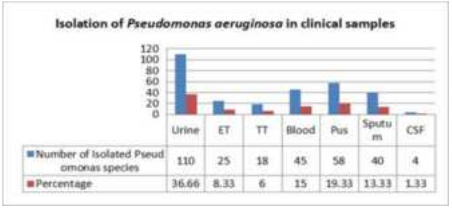


Graph: 1.2 Prevalence of Pseudomonas aeruginosa isolated from various clinical specimen.

In our research study, out of 300 Pseudomonas aeruginosa 110 were isolated from Urine samples, 25 were isolated from ET samples, 18 were isolated from TT samples, 45 were isolated from Blood samples, 58 were isolated from Pus samples, 40 Pseudomonas aeruginosa were isolated from Sputum samples and 4 were isolated from CSF samples (table: 2). These isolated Pseudomonas aeruginosa were purified for further investigations.

Table 5.2: Number Of Isolated Pseudomonas Aeruginosa From Different Types Of Clinical Samples

S. No.	Type of Clinical Sample	Number of Isolated Pseudomonas aeruginosa	Percentage
1	Urine	110	36.66
2	ET	25	8.33
3	TT	18	6
4	Blood	45	15
5	Pus	58	19.33
6	Sputum	40	13.33
7	CSF	4	1.33



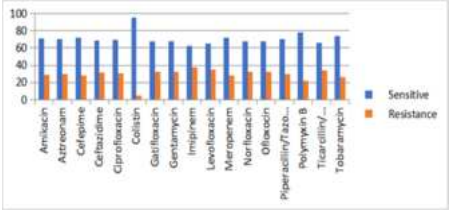
Graph 1.2: Graphical representation of isolated Pseudomonas aeruginosa from different clinical samples

Table 1.2 and Graph 1.2 showed that maximum number of Pseudomonas aeruginosa was isolated from urine i.e. 36.66%, followed by pus 19.33%, blood 15%, sputum 13.33%, ET 8.33% and TT 6%, while the minimum number was isolated from CSF i.e. 1.33%. Our study clearly showed that the prevalence of Pseudomonas aeruginosa was higher in urine samples compared to the rest of the clinical samples.

Antimicrobial Susceptibility Pattern Of Pseudomonas Aeruginosa Isolated From Various Clinical Samples

All strains of Pseudomonas aeruginosa were subjected to antibiotic sensitivity tests by the disc diffusion method, these were examined for zones of inhibition around each disc following standard antibiotic susceptibility chart of the CLSI guideline 2022 [14].

Table 1.4: Antibiogram of Pseudomonas aeruginosa (N=300) from various clinical sample



Antibiogram of Pseudomonas aeruginosa clearly revealed that Colistin shown maximum sensitivity i.e (95%) followed by Polymixin B (78.33%), Tobramycin (73.33%), Cefepime and Meropenem (71.66%). Antibiotic Amikacin have shown sensitivity i.e (71.33) respectively. Piperacillin/Tazobactam and Aztreonam have shown similar sensitivity i.e 70% against Pseudomonas aeruginosa infection. Ciprofloxacin, Ceftazidim and Gentamycin have shown 69.66%, 68.33% and 68% sensitivity. Antibiotic Norfloxacin and Gatifloxacin have shown similar sensitivity i.e 67.66% followed by Oflaxacin, Ticarcillin/Clavulanicacid and Levofloxacin i.e 67.33%, 66% and 65% respectively. Our study clearly indicates that Imipenem have shown minimum sensitivity i.e 62.66%.

Statistical Observations:

Table 5.23: ANNOVA table: Antibiogram of Pseudomonas aeruginosa (N=300) from various clinical sample

Source	DF	Sum of Square (SS)	Mean Square (MS)	F Statistic (df1,df2)	P-value
Factor A - rows (A)	16	0.0001941	0.00001213	1.181e-7 (16,16)	1
Factor B - columns (B)	1	14715.1685	14715.1685	143.2778 (1,16)	0.00065
Error	16	1643.2605	102.7038		
Total	33	16358.4291	495.71		

DISCUSSION AND CONCLUSION

In our study antibiotic Amikacin have shown sensitivity (71.33%) respectively. Among the aminoglycosides, resistance to Amikacin was seen in 28.67% of the isolates in our study, while lower rate of resistance were reported from Pakistan, 6.73% by Nadeem, et al. [15] and 8% by Nadeem and Qasmi, et al. [16], a resistance of 21% was reported by Farida, et al. (2010) [21], Jamshaid, et al. (2008) reported 24% resistance, 25% in Nagaveni, et al. also reported similar finding in 2010 [17].

Relative findings also reported from various studies which showed higher rates of resistance i.e. 42.8% by Murugan, et al. [18,19], 50% by Viren, et al. study [20] and 73% in Tehran by Horeih Saderi, et al. [21], 74% in Behera, et al. study [22,23]. In 2012 higher rate of resistance has been reported by Madhu Sharma, et al. i.e. 91.2% [24], 96.6% by Awari, et al. and 97% by Bhale Rao, et al. [25,26].

Clinical isolates that have acquired carbapenemases are a serious problem as carbapenems are one of the last lines of defence against Pseudomonas aeruginosa. The importance of β-lactams in treating Pseudomonas aeruginosa infections, and the widespread occurrence of antibiotic-resistant bacteria, has led to a large amount of research into the mechanism of β-lactam action and bacterial resistance.

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