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"Isolation, Identification and Antibiotic susceptibility pattern of Pseudomonas aeroginosa with special reference to Carbapenemase production at a tertiary care hospital in Kanpur"

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INTRODUCTION- *Pseudomonas aeroginosa* is a Gram negative motile bacillus, belonging to the family *Pseudomonaceae.* It is a leading cause of nosocomial infections in especially critically ill and immuno-compromised patients. *P. aeroginosa* is increasingly gaining importance as a multidrug resistant nosocomial pathogen complicating the treatment of inpatients in a hospital, especially in the ICUs

AIM- Isolation, identification and antibiotic susceptibility pattern of *Pseudomonas aeroginosa* with special reference to carbapenem resistance production at a tertiary care hospital.

MATERIAL & METHODS-. The present study was a prospective study conducted at Rama Medical College, Hospital & Research Centre, Kanpur, (U.P.). Total 100 samples were received from admitted patients. The isolated bacteria were identified by colony morphology, gram's stain, microscopy and standard biochemical tests. The *Pseudomonas aeroginosa* isolates were subjected to antibiotic susceptibility test by Kirby Bauer disk diffusion test and Screening for MBL production was done by combined disk synergy test, E-Test & Modified Hodge test.

RESULT- Out of 100 samples, 70 were culture positive and 30 were cultures negative. Out of 70 isolates 21 (30%) were *Pseudomonas aeroginosa species* isolated & 49 (70%) other bacterial species. Out of 21 *Pseudomonas aeroginosa* isolates sensitivity was observe to Polymyxin (100%), Colistin (100%), and Imipenem (95.23%) Meropenem (98.25%) Piperacillin /Tazobactam (93%) by Kirby Bauer disk diffusion test. Out of 21 *Pseudomonas aeroginosa* isolates MBL Screening test was carried out, which 10 (47.61%) were MHT positive 4 (19.04%) were CDST Positive and E – Test was Positive in 2 (9.52%)

CONCLUSION - The study will help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns of *Pseudomonas aeroginosa* species in our region.

KEY WORDS: *Pseudomonas aeroginosa, drug resistance, carbapenem*

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Introduction

Pseudomonas aeroginosa is a Gram negative motile bacillus, belonging to the family *Pseudomonaceae*. It is found in moist environment, disinfectant solutions and water due to its ability to utilize many different organic compounds and survive in nutrient deficient conditions. [1] Its minimal nutritional requirements and tolerance to varied physical conditions allows it to persist on numerous living and non living objects. [2]

It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal.[3]

It is a leading cause of nosocomial infections in especially critically ill and immuno-compromised patients.[4] It is strongly associated with wound infections in burns patients, respiratory infections in cystic fibrosis patients and severe life threatening infections like septicemia in neutropenic patients.[2] *P. aeruginosa* is increasingly gaining importance as a multidrug resistant nosocomial pathogen complicating the treatment of inpatients in a hospital, especially in the ICUs.[5]

The problem of multidrug resistance in gram negative bacilli due to extended spectrum β lactamases and metallo β lactamases (MBLs) is becoming a serious threat to the patients. . In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P. aeruginosa* has been noticed, which is related to high morbidity and mortality. [10]

P. aeruginosa is an opportunistic pathogen with inherent resistance to many antibiotics and disinfectants including anti-pseudomonal Penicillins, Ceftazidime, Carbapenems, Aminoglycosides and Ciprofloxacin [11] Extended-spectrum beta-lactamases (ESBLs) have emerged as an important cause of resistance in Gram-negative bacteria. Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents all over the world intreating Gram positive and Gram negative infections [12].

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Carbapenems are famously stable to AmpC β-lactamases and extended-spectrum-β-lactamases [13]. This group is considered treatment of choice for infections caused by resistant strains of Gram negative bacteria[14].

The Carbapenems available for use in India are Imipenem and Meropenem. [21] However Carbapenem resistance has been observed frequently in Pseudomonas aeruginosa which is due to decreased outer membrane permeability, increase efflux system, alteration of penicillin binding protein and Carbepenem hydrolysing enzyme – carbepenamase.[22] They have potent hydrolyzing activity not only against carbepanamase but also against other β lactamase antibiotics.[23] These MBL producing P. aeruginosa strains have been reported to be important cause of nosocomial infection associated with clonal spread. [24] Unfortunately, resistance to carbapenem in Enterobacteriaceae is difficult to detect by routine disc diffusion method used by many microbiology laboratories[14].

The aim of the present study is to isolate, identify and see the antibiotic susceptibility pattern of Pseudomonas aeroginosa with special reference to carbapenemase production at a tertiary care hospital in Kanpur.

MATERIAL AND METHODS

Study setting & Design:- This Prospective study was conducted in the Department of Microbiology, Rama Medical College Hospital & Research Centre Kanpur. Sample from OPD and inpatients admitted to the IPD ward were collected.

Study period:

This study was conducted from January 2019 to December 2019.

Size of the study sample:

100 samples were collected.

Inclusion criteria:

All clinical samples (Pus, Urine, Blood, Fluids, Respiratory specimens) were taken.

Exclusion Criteria :Patients on antibiotics for the last two weeks.

Statistical analysis:

A suitable statistical test was carried out according to study.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Ethical consideration :

Ethical clearance was taken from the Institutional Ethical Committee of Rama Medical College Hospital and Research Centre.

Sample collection & processing:

All clinical samples (Pus, Urine, Blood, Fluids, Respiratory specimens) were taken by standard methods. Gram stain was performed before culture, and then sample was inoculated on Blood Agar, MacConkey's Agar & CLED agar in case of urine, incubated for 18-24 hours then on next day growth was observed. After identification of morphology by Gram stain, biochemical tests were performed, to identify the isolates. Antibiotics Susceptibility Test was performed by using Kirby Bauer disc diffusion method following CLSI guideline 2018.

GRAM STAIN :- [124]

The suspected colonies were stained using gram stain method and their shape, color,and arrangement were observed under light microscope.

BIOCHEMICAL TESTS - Used For Identification of *Pseudomonas aeruginosa* :-

All bacteriological isolates were examined and confirmed by biochemical tests as per the standard operative procedure.

Test includes:- O/F Test, Oxidase test, Catalase test, Nitrate reduction, Glucose, Lactose, Mannitol, Sucrose, Citrate, Indole, Urease,. H_2S test

HANGING DROP METHOD [125] – For motility.

CETRAMIDE AGAR – For pigment, the slant was inoculated with a portion of isolated colony and incubated at 37˚C overnight and was observed for color change from green to blue Tubes with negative result were further incubated for 7 days.

ANTIMICROBIAL SUSCEPTIBILITY TEST [126]

AST was performed by Kirby –Bauer disc diffusion method.

Kirby –Bauer disc diffusion method-

Antibiotic susceptibility pattern was done on Mueller Hinton Agar by Kirby- Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute(CLSI) [125]

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

- After drying the surface of agar plate for 3-5 minutes the antibiotic discs were applied using either sterile forceps or multidisc dispenser.
- For each strain a bacterial suspension adjusted to 0.5 McFarland was used.
- Discs were placed closer than 20 mm (center to center) on the MHA plate.
- Maximum up to 6 disc were applied on a 100 mm plate.
- The plates were then incubated at 37ºC for 18-24 hours. The zones of complete growth of inhibition around each of the disc were measured by using a ruler.
- The interpretation of zone size into sensitive , intermediate or resistance was based on the standard zone size interpretant chart as per CLSI guidelines.
- The control strains used were *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853

Antibiotics For *Pseudomonas aeruginosa*: Piperacilin (100µg), Piperacillin/ Tazobactam (100/10µg), Ticarcillin/clavulanic Acid(75/10), Ceftolozane – Tazobactam (30/10µg). Cteftazidime(30µg), Cefepime(30µg), Aztreonam(30µg), Doripenem(10µg), Imipenem(10µg), Meropenem(10µg), Colistin(10µg), PolymyxinB(300µg), Gentamicin(10µg), Tobramycin(10µg), Amikacin(30µg), Netilmycin(30µg), Ciprofloxacin(5µg), Levofloxacin(5µg), Norfloxacin (10µg), Lomefloxacin (10µg), Ofloxacin(5µg), Gatifloxacin (5µg),

Intrinsically Resistance Antibiotics For *Pseudomonas aeruginosa*[125]

Amoxyclav(30µg), Ampicillin/Sulbactum(10/10µg), . Amoxicillin – Clavulanate, Cefotaxime (30µg), Ceftriaxone (30µg), Ertapenem (10µg), Tetracycline (10µg), Tigecycline (15µg), Trimethoprim (5µg), Trimethoprim Sulfamethoxazole (5µg), Chloramphenicol (10µg).

Antibiotics For Other Gram Negative :

Co-trimoxazole(10µg), Cefotaxime(30µg), Nalidixic Acid (30µg), Cefoperazone/ sulbactam(75/10µg),

Antibiotics For Gram Positive :

Amoxyclav(30µg), Erythromycin(5µg), Clindamycin(2µg), Tetracycline(10µg), Linezolid (10µg), Vancomycin(5µg), Teicoplanin(30µg), Co-Trimoxazole(25µg), Cefoxitin(30µg),Oxacillin(1µg)

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Detect Carbapenemase Resistant Gram negative bacilli [123]

Screening for MBL production was done in Imipenem resistant isolates by the following:

1. Combined Disk Synergy Test (CDST)- The IMP-EDTA combined disk test was performed as described by . Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two 10 µg imipenem disks were placed on the plate, and appropriate amounts of 10 µL of EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive.

2. E-test -

The E-Test MBL strip containing a double sided seven-dilution range of IPM (4 to 256 µg/mL) and IPM (1 to 64 µg/mL) in combination with a fixed concentration of EDTA has been reported to be the most sensitive format for MBL detection. The E-test was done according to manufacturer's instructions. MIC ratio of IP (Imipenem)/IPI (Imipenem-EDTA) of >8 or >3 log 2 dilutions indicates MBL production.

3. Modified Hodge Test (MHT):

In MHT, a lawn culture of 1:10 dilution of 0.5 McFarland's standard *E.coli* ATCC 25922 broth was done on a Mueller Hinton agar plate. A 10µg Imipenem disk was placed in the centre of plate and 10µl of 50mM zinc sulphate solution was added to Imipenem disk. then Imipenem resistant *P.aeruginosa* were streaked from edge of the disk to the periphery of the plate in 4 different directions. After overnight incubation, the plates were observed for presence of a cloverleaf shaped zone of inhibition and the plates with such zones were interpreted as positive Modified Hodge test.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

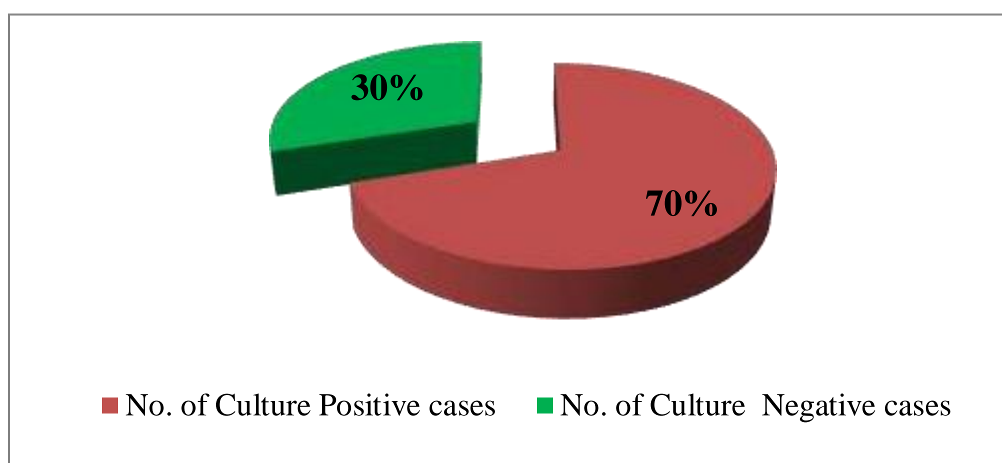
RESULTS

Table No.1: Distribution of culture positive and culture negative cases.

No. of Culture Positive cases	No. of Culture Negative cases	TOTAL
70	**30**	**100**

In the present study, out of 100 patients, 70 were culture positive and 30 were cultures negative.

Graph No.1: Distribution of culture positive and culture negative cases.



TableNo.2: Total Organism isolates.

Organism	No = 70
Escherichia Coli	20 (28.58%)
Staphylococcus aureus	05 (7.14%)
Acinetobacter spp	07 (10%)
Enterococcus faecalis	06 (8.58%)
Coagulase Negative Staphylococcus	03 (4.28%)
Klebsiella spp.	8 (11.42%)
Pseudomonas aurginosa.	**21 (30%)**
Total	**70 (100%)**

Out of 70 culture positive cases, **21 (30%)** were ***Pseudomonas aurginosa*** and **49 (70%)** other bacterial species.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Table No.3: Types of Clinical sample with *Pseudomonas aurginosa*.isolates.

Clinical sample	*Pseudomonas aurginosa.* N=21
Pus	8 (38.09%)
Urine	1 (4.76%)
Blood	4 (19.04%)
C.S.F.	2 (9.52%)
E.T. tube	4 (19.04%)
Ascitic fluid	1 (4.76%)
Folish Tip	1 (4.76%)
TOTAL	**21 (100%)**

Table No. 4: Age & Gender wise distribution of Male and Female *Pseudomonas aurginosa*.

AGE	MALE (N=15)	FEMALE (N=6)
10-20	4 (26.67%)	0 (0%)
21-30	5 (33.34%)	1 (16.67%)
31-40	1 (6.67%)	3 (50%)
41-50	4 (26.67%)	1 (16.67%)
51-60	1 (6.67%)	1 (16.67%)
TOTAL	**15 (100%)**	**6 (100%)**

Table No.5: Sensitivity pattern of Pseudomonas aurginosa.

Antibiotics	*Pseudomonas aurginosa* . (N=21)
Ampcillin	10 (47.61)
Piperacillin Tazobactem	19 (90.47)

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Ciprofloxacin	10 (47.61%)
Ofloxacin	5 (23.80%)
Cotrimoxazole	5 (23.80%)
Amikacin	14 (66.66%)
Gentamicin	15 (71.42%)
Netilmicin	15 (71.42%)
Cefotaxime	4 (19.04%)
Cefatazidime	6 (28.57%)
Cefepime	16 (76.19%)
Cefoperazone/sulbactum	6 (28. 57%)
Aztreonam	16 (76.19%)
Imipenem	20 (95.23%)
Meropenem	21(100%)
colisitin	21 (100%)
Polymyxin-B	21 (100%)

Table No.6. MBL Screening Test

Isolates	**No. of strains isolated**	**Strain Resistance To Imipenem (%)**	**MBL Screeening Positive**
Pseudomonas aurginosa	**21**	**11 (52.38%)**	**10 (47.61%)**

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

Out of 21 isolates of Pseudomonas aeroginosa, 11 (52.38%) isolates are resistance to Imipenem MBL Screening was observed in 10 (47.61%)

Table No.7. Detection of MBL By Different Methods.

METHOD		MHT	CDST	E- TEST
MBL Isolation Detection	No.	10	4	2
	%	(47.61%)	(19.04%)	(9.52%)

FIGURE 27 : CDST

DISCUSSION

In this chapter we have discussed all the result obtained in present study the result obtained in present study co-related with other similar studies.

TABLE NO -1: Comparison of Age Wise Distribution With Other Studies

Serial no.	Study	Years	Result
1	AI-Ibran E[127] et. al.	2014	28% cases were in the age group of 41-60
2	Tadvi J[128] et. al.	2016	72.72% cases were in the age group of 16-36
3	Sapna G[129] et. al.	2017	40% cases were in the age group of 13-30
4	**Present Study**	**2019**	**44% cases were in the age group of 21-40**

In the present study 44% cases were in the age group of 21-40. These results were in accordance with Sapna G. et. al. and Tadvi J et. al. and was in contrast with AI- Ibran et al .

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

TABLE 2: Comparison Of Gender Wise Distribution With Other Studies

Serial no.	Study	Years	Male	Female	Total
1	AI-Ibran E [127] et. al.	2014	26(47.27%)	29(52.72%)	55
2	Sapna G [129] et. al	2017	44(44%)	56(56%)	100
3	. Pragathi E[130] et. al.	2017	136(23.85%)	434(76.14%)	570
4	**Present Study**	**2019**	**44 (62.85%)**	**26 (37.14%)**	**100**

Male (62.85%) were more affected than Female (37.14%) in the present study. the findings accordance with Pragathi E. et al. and was in contrast with AI-Ibran E et. al In India higher incidence of clinical injuries .

TABLE 3: Organisms Isolated In Different Studies

Serial no.	Study	Years	Result
1	Upadhaya S et. al .[133]	2018	*Pseudomonas* followed by *Staph* and *CoNS*
2	Atoyebi OA et. al. [132]	2017	*Pseudomonas* followed by *Staph* and *Klebsiella spp*
3	**Present Study**	**2019**	***E.coli ,Enterobacter, Pseudomonas* followed by *Klebsiella spp* and *Staph***

In the present study most common isolate was *Pseudomonas aurginosa*. The result was in accordance with the study conducted by Atoyebi OA et. al. and was in contrast with Upadhaya S et. al.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

TABLE 23: MBL Detection In Other Studies.

Serial no.	Study	Years	Results
1	Franklin et. al. [137]	2010	83%
2	Behera B et. al.[138]	2012	26.9%
3	Mendiratta DK et .al. [139]	2013	19.05%
4	Mehul S C.[140] et . al.	2014	12.7%
5	Ami Varaiya[141]. et. al.	2017	5%
6	**Present study**	**2019**	**(30%)**

In present study, Out of 21 isolates of *Pseudomonas aeruginosa*, 11(52.38%) were resistance to imipenem . 10(47.61%) isolates were found to be MBL producers.Of 21 isolates of MBL were isolated from Clinical patients,. The prevalence of detect Metallo- beta – lactamases producing *Pseudomonas aeruginosa* in our Study (30%) was in higher than Ami Varaiya et. al. (5%) and less than Franklin et. al.(83%).

CONCLUSION

The present study has given us the knowledge regarding incidence of bacterial colonization of burn wounds in our hospital. It was seen that Gram negative organisms were more prevalent. *Pseudomonas aeroginosa* was the most common microorganism followed by *Klebsiella spp*, *Escherichia Coli*, *Acinetobacter spp* and *Enterococcus Faecalis.* The antibiotic susceptibility testing showed that overall Piperacillin/tazobactam was the most effective drug. In addition, Imipenem was the most effective drug for Gram negative.

In the present study the Prevalence of MBL *P. aeruginosa* in clinical patients were found to be high . Piperacillin/tazobactam seems to be effective option for the treatment of *P. aeruginosa* infections. Colistin and Polymyxin B should be reserved for MBL cases of *Pseudomonas aeruginosa* in clinical patients. Hence proper infection control practices and Antibiotic Policies should be implemented to control the spread of these MBL strains.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 09, 2023

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