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STUDY ON METALLO-BETA LACTAMASE (MBL) PRODUCING PSEUDOMONAS IN CLINICAL ISOLATES

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ABSTRACT

Introduction: Pseudomonas species are responsible for 10% of hospital acquired infection especially in an ICU set up and in burn patients. Metallo-beta lactamase production is the most common mechanism of resistance to carbapenems which is the most commonly used drug to treat *Pseudomonas*. Local prevalence of MBL producing *Pseudomonas* is important information to both microbiologist and clinician to formulate hospital infection control strategy. This cross-sectional descriptive study was conducted in a tertiary care hospital of Bhopal to detect MBL prevalence among clinical isolates of *Pseudomonas* species. **Material & Methods:** 78 Pseudomonas strains isolated from different clinical samples from January 2021 to December 2021. All imipenem resistance strains were checked for MBL production by combined disc test with imipenem, and MBL production was confirmed by MBL E test. **Results:** Among 78 *Pseudomonas* strains 35 were found MBL producing. Polymixin –B and Colistin shows 100% sensitivity against *Pseudomonas* species. Gentamicin shows highly resistant 61.5% against *Pseudomonas*. *Pseudomonas* infection was found comparatively more in female patients i.e. 43 (55.1%) than in male patient 35 (44.8%). **Conclusion:** It is better to prevent MBL *Pseudomonas* than to cure it as most of the antibiotics were found to be ineffective against it. In our study MBL production rate in clinical isolate of *Pseudomonas* was low compared to other studies in India.

KEYWORDS: Pseudomonas, Metallo-beta lactamase production.

INTRODUCTION

Pseudomonas is the gram-negative bacilli that are strict aerobes, motile with one or two flagella, utilise glucose oxidatively, and are oxidase positive. It belongs to Pseudomonadaceae family and in molecular taxonomy to fluorescent group of r RNA group.[1] Another members of this Fluorescent group are Pseudomonas fluorescence and Pseudomonas putida that are rarely involved in clinical diseases in human.[2] With the help of different virulent factors like pyocyanin, exotoxin A, exoenzyme S, protease, phospholipase, rhamnolipids they produce both community acquired infections like otitis externa, keratitis, varicose vein ulcer and hospital acquired infection like Catheter associated urinary tract infection (CAUTI), Ventilator associated pneumonia (VAP), burn infection, bedsore, septicaemia and necrotising pneumonia in cystic fibrosis patients etc.[2] They have highly evolved Quorum sensing mechanism by which they can easily form biofilm and prevent attack of antibiotic. Most important factor that makes it so much dominant in hospital environment that it can resist or even can utilise some disinfectants/ antiseptics like cetrimide for their nutrition,[1] so they easily grow in hospital environment and ICU. They are responsible for 10% of all hospital acquired infection. Aminoglycoside (gentamicin, amikacin, tobramycin), antipseudomonal penicillin (piperacillin, ticarcillin) and cephalosporin like Ceftazidime are used to treat Pseudomonal infection but resistance against these antibiotics are common today. Beta lactamase destroy beta lactam ring of antibiotic and make them ineffective against Pseudomonas.[3] Ccarbapenem is the drug of choice in extended spectrum beta lactamase producing Pseudomonas. This was derived from thienamycin, a naturally derived product of Streptomyces cattleya.[4] Ertopenem, Doripenem, imipenem, Meropenem and Faropenem are example of carbapenem but imipenem and Meropenem are most commonly used carbapenem in India.[4] With the progress of time irrational and inappropriate use of carbapenem led to emergence of carbapenem resistant Pseudomonas- first in Japan in 1991[5] and then in different part of the world. In India first case of MBL

producing Pseudomonas was reported in 2002.[6] Mechanism of carbapenem resistance are mainly three types, first due to increase expression of porin in cell wall, second due to increase activity of efflux pump and third- production of metallo-beta lactamase. Metallo-beta lactamase production is the most common mechanism of carbapenem resistance.[7] Metallo-beta lactamase is a zinc dependent enzyme belonging to Ambler class B that can hydrolyse all beta lactam antibiotics including carbapenem8. Ambler class A, C, D. beta lactamases use serine as active site so they can be easily degraded by beta lactamase inhibitor like clavulanic acid or sulbactam8. But metallo-beta lactamase cannot be inhibited by clavulanic acid or sulbactum so MBL producing Pseudomonas is now emerging as a nightmare for treating physician. Besides that, resistance determinant of MBL is located in highly mobile genetic element allowing easy dissemination from patient to patient or even from patient to health care providers.[8] So prevention is the always better option than treatment of MBL Pseudomonas infection. Clinician in every hospital should know the local prevalence of MBL producing Pseudomonas to formulate proper antibiotic policy and hospital infection control strategy to prevent outbreak of this dangerous superbug. Keeping it in mind we have conducted a research to find out MBL positivity rate in clinical isolates of *Pseudomonas* in a tertiary care hospital of Bhopal, India.

MATERIAL AND METHODS

This is a descriptive cross-sectional study conducted in the Department of Microbiology, L.N Medical College Bhopal M.P over a period of 1 year January 2021 to December 2021. Depending upon site of infection samples were collected like urine, pus, sputum, BAL, ear swab etc in sterile container. Sample Processing All samples were inoculated immediately into Blood agar and MacConkey agar media and incubated for 18-24 hrs. at 37°C in incubator. Next day growth was observed, and gram stain was performed. Identification and antibiogram of oxidase positive growth was done in fully manual method.[16] Carbapenem resistance was suspected when either imipenem was resistant (mic>8 µl/ml).MBL production was tested in all carbapenem resistant Pseudomonas species by Combined disc test with imipenem.[17]

Combined disc test

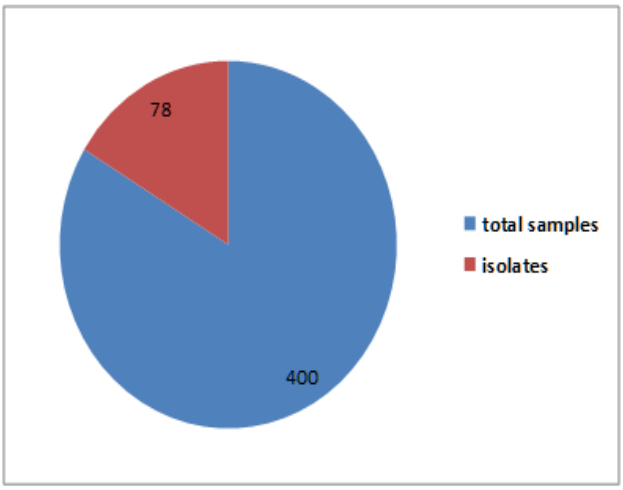
Two 10 µg IPM disks were put on the MHA plate seeded with the test organism. 10 µL of EDTA solution (750 µg) was added to one of them. The plate was incubated for 16-18 hrs at 35°C. If the increase in inhibition zone with the IPM + EDTA disk was >7 mm than the IPM disk alone, it was suspected as MBL positive.[9]

RESULTS

The present study was conducted in the department of microbiology, L N Medical College and tertiary care Hospital Bhopal Madhya Pradesh from January 2021 to December 2021 to detect *Pseudomonas* species from various clinical samples.

Table 1: Incidence of pseudomonas species isolates from clinical samples.

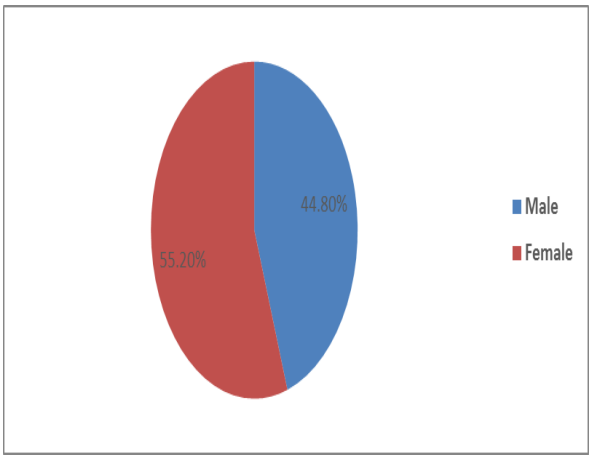
No. of sample tested	400
No. of isolates of Pseudomonas spices	78
Incidence	19.5%



As the shown in above **TABLE 1** and **GRAPH 1:** Incidence of Pseudomonas species isolates 78 (19.5%) out of 400 from various clinical samples**.**

Table 2: Distribution of Pseudomonas species according to gender (n=78).

Sex	Number (%)
Male	35(44.8)
Female	43(55.2)
Total	78(100)



As shown in **TABLE 2** and **GRAPH 2** Pseudomonas infection was found comparatively more in female patients i.e. 43 (55.1%) than in male patient 35 (44.8%). The female to male ratio is 2.4:1

Table 3: Antibiogram of pseudomonas species.

Sr. No.	Antibiotic	Sensitive (%)	Resistant (%)
1	Levofloxacin	53 (67.9)	25 (32.1)
4	Piperacillin-Tazobactum	59 (75.6)	19 (24.3)
5	Ceftazidime	51 (65.3)	27 (34.6)
6	Amikacin	53 (67.9)	25 (32.1)
7	Gentamicin	30 (38.5)	48 (61.5)
9	Ciprofloxacin	43 (55.2)	35 (44.8)
10	Imipenem	43 (55.2)	35 (44.8)
11	Meropenem	40 (51.2)	38 (48.7)
12	Polymyxin- B	78 (100)	0 (0)
13	Colistin	78 (100)	0 (0)

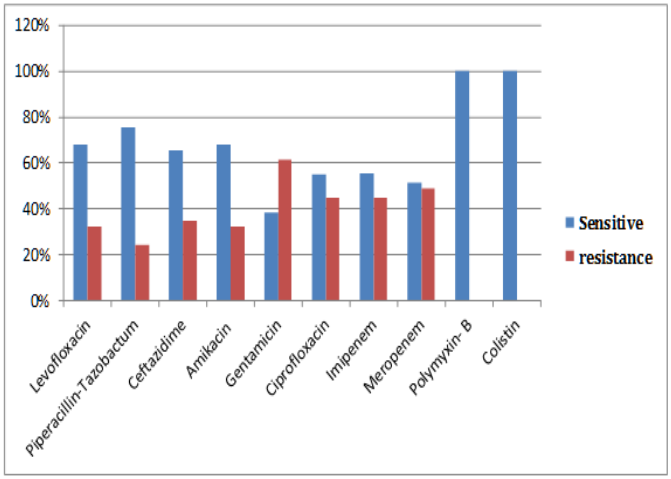
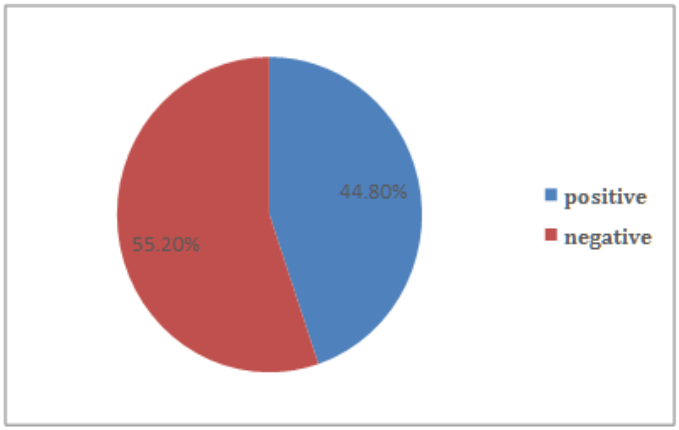


TABLE 3: and **GRAPH 3** revealed that most sensitive antibiotic were Polymyxin- B and Colistin (100%) followed by Piperacillin-Tazobactum (75%) in our study. Gentamicin appeared as most resistant antibiotic with (61.5%) rate in Clinical samples.

Table 4 and Graph 4: Results of CD test of all 74 P. aeruginosa isolates using IPM (10 µg) for MβL detection. (n= 78).

Sample	CDT using IPM	
Total isolates (%)	Positive No. (%)	Negative No. (%)
78 (100)	35 (44.8)	43 (55.2)



As shown in **TABLE 7** and **GRAPH 7**: Out of 78 Pseudomonas isolates 35 isolates were found to be Metallo beta lactamase production by using disk diffusion test.

DISCUSSION

The name *Pseudomonad* is used to denote Gram-negative, non-sporulating bacteria that are purely aerobic.[11] *P. aeruginosa* is a prevalent pathogenic agent in neonatal critical care units due to its widespread distribution, strong preference for wet environments, and capacity to thrive in a variety of settings. Predisposing factors with considerable morbidity and fatality rates in P. aeruginosa septicaemia are invasive diagnostic and therapeutic procedures, and indiscriminate antibiotic usage.[12]

Pseudomonas aeruginosa is a bacterium that has been linked to major nosocomial infections such pneumonia and sepsis. Multidrug-resistant P. aeruginosa strains are becoming more common over the world, restricting our therapeutic options.[15] From January to December 2021, the current investigation was conducted at the Department of Microbiology, L N Medical College and Hospital, Bhopal (Madhya Pradesh). The goal of this study was to identify antibiotic susceptibility patterns in 78 positive *Pseudomonas* species strains obtained from clinical specimens of patients at various Burhanpur hospitals. *Pseudomonas species* were found in 55.2 percent of female patients compared to 44.8 percent of male patients. Somayeh Safarirad [14] had a similar observation (2021). Females accounted for 54.1 percent of the total, while males accounted for 45.9%.The increase rate of isolation of Pseudomonas speices in Female as compared to Male patients is due to anatomical differences to hormones changes and stages of women's reproductive life cycle, contaminated environment, daily activites and poor personal hygiene.

In the present study, out of 78 Pseudomonas species isolated from various clinical samples, Maximum number isolated from from Urine i.e. 26 (33.3%) followed from Pus 19 (24.3%), Ear swab 11 (14.1%), Sputum 10(12.8%), Catheter tip 5 (6.4%), ET 3 (3.8%), CSF 2 (2.5%), stool 2 (2.5%).

Based on Disk Diffusion Results 35(44.8%) out of 78 isolates were Imipenem resistant *Pseudomonas aeruginosa*. Imipenem resistant isolates were collected from 15 (42.8%) male and 20(57.1%) female. Similar observation was made by Somayeh Safarirad (2021).[11] i.e. female was 54.1% as compared to male 45.9%. The antibiotic susceptibility of 78 isolated strains of *Pseudomonas* species shows 100% susceptibility to Polymyxin- B and Colistin followed by Piperacillin-Tazobactum (75%) in our study. Study by Zahra Norouzi Bazgir et al[12].

CONCLUSION

400 separate non-repetitive clinical samples, including ear swabs, catheter tips, urine, and sputum, were collected under aseptic conditions from diverse IPD and OPD, yielding a total of 78 strains of Pseudomonas species.

- 19.5 percent of clinical sample isolates contained *Pseudomonas species*.
- Out of 78 Pseudomonas species, females showed a higher proportion of isolation (55.1%) than males did (44.8 percent).
- The age range between 31-40 years and 41-50 yrs found to have the most pseudomonas infections i.e.23% and 22% while minimum from less than 10 yrs age with 11-20 yrs i.e. 10.2%
- Pseudomonas speices were isolated maximum from Urine 26 (33.3%) followed from Pus 19 (24.3%), Ear swab 11 (14.1%), Sputum 10(12.8%), Catheter

tip 5 (6.4%), ET 3 (3.8%), CSF 2 (2.5%), stool 2 (2.5%).

- In our investigation, 78 isolated strains of the pseudomonas bacterium were tested for antibiotic susceptibility shows 100% susceptibility to Polymyxin- B and Colistin followed by piperacillin-tazobactum (65%). followed by Imepenem (44.8%), Ciprofloxacin (44.8%), Ceftazidime (34.6%), Amikacin (32.1%).
- Based on disc diffusion findings, 35 (44.8%) out of 78 isolates were *Pseudomonas* aeruginosa imipenem resistant.

The current era's irrational usage of antibiotics has resulted in a surge of resistance among *Pseudomonas* species. This study emphasises the importance of ongoing hospital surveillance to find resistant pathogens. This will aid in the creation of traditional antibiogram. As a result, the patient can receive proper treatment, preventing the development of drug resistance in the future. In developing nations like ours, to provide better treatment programmes, additional research should be conducted, as well as effective and less expensive alternative antibiotics. To assist in the creation of infection control policy, the research's findings should be shared with the hospital's infection control committee.

REFERENCES

1. Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al., editors. In: Koneman's Color Atlas and textbook of Diagnostic Microbiology. USA: Lippincott Williams and Wilkins Company; Nonfermenting Gram negative bacilli, 2006; 6: 305–91.
2. Steinberg JP, Rio DC. Gram negative and Gram variable bacilli In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious diseases. Philadelphia, USA: Elsevier Publication, 2005; 6(2): 2751–68.
3. Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: Geographic patterns, Epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999) Clin Infect Dis, 2001; 32: 104–13.
4. Bergogne-Berezin E, Towner KJ. Acinetobacter spp. as Nosocomial Pathogens: Microbiological, Clinical and Epidemiological Features. Clin Microbiol Rev, 1996; 9(2): 148-65.
5. Mehta M, Punia JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isolated from various clinical specimens- a retrospective study. Indian J Med Microbiol, 2001; 19(4): 232.
6. Nagoba BS, Deshmukh SR, Ulka G Gude et al. Invitro susceptibility of Pseudomonas aeruginosa to different antibiotics. Indian J Med Microbiol, 1997; 15: 185-186.
7. Mishra B, Bhujwala RA, Shrinivas. Non fermenters in human infections. Indian J Med Res, 1986; 83: 561-6.
8. Rubin SJ, Granato PA and Wasilaskas BL. Glucose nonfermenting gram negative bacteria. In: Lennette EH, Balwos A, Hausler Jr. WJ and Shadomy HJ, editors. Manual of clinical Microbiology 4th edition, washington, D.C: American society for Microbiology, 1985; 330-49.
9. Gardner, B Griffin, Swartz, Kunz. Nonfermentative gram negative bacilli of nosocomial interest. American J Med, 1970; 48: 735-749.
10. Koneman E, Allen S, Janda W, Schreckenberger P, Winn W. Colour Atlas and textbook of Diagnostic Microbiology. Lippincott –Raven Publishers, Philadelphia, 2006; 6.
11. Bergey's Manual of Systematic Bacteriology, 2001.
12. Moniri R, Mosayebi Z, Movahedian AH Increasing trend of antimicrobial drug-resistance in Pseudomonas aeruginosa causing septicemia. Iranian J Publ Health, 2006; 35.
13. Mansoor T, Musani MA, Khalid G, Kamal M Pseudomonas aeruginosa in chronic suppurative otitis media: sensitivity spectrum against various antibiotics in Karachi. J Ayub Med Coll Abbottabad, 2009; 21: 120-123.
14. Tripathi P, Banerjee G, Saxena S, Gupta MK, Ramteke PW Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection. Afr J Microbiol Res, 2011; 5: 2955-2959.
15. Syrmis MW, O'Carroll MR, Sloots TP, Coulter C, Wainwright CE, et al. Rapid genotyping of Pseudomonas aeruginosa isolates harboured by adult and paediatric patients with cystic fibrosis using repetitive-element-based PCR assays. J Med Microbiol, 2004; 53: 1089-1096.
16. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother, 2010; 54: 1160-1164.
17. Dundar and Otkun and Metin Otkun *In-Vitro* Efficacy of Synergistic Antibiotic Combinations in Multidrug Resistant *Pseudomonas Aeruginosa* Strains. Yonsei Med J., 2010; 51(1): 111-116.