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A Study of Metallobetalactamase Producing Pseudomonas Aeruginosa in Patients Attending D.Y.Patil Hospital and Research Centre, Kolhapur.

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Abstract: *The aim was to study the susceptibility pattern of Pseudomonas aeruginosa in hospitalized patients so as to enable the clinician to select appropriate regimen at lower rate of side effects of side effects of antibiotics & decreased health care cost &to control the spread of drug resistance. Pseudomonas aeruginosa is one of the most common pathogen causing nosocomial infections.Acquired drug resistance is frequent in nosocomial isolates of Pseudomonas aeruginosa. Acquired Metallobetalactamase (MBL) has recently emerged as one of the most worrisome resistance mechanism. The study was conducted at Department of Microbiology, D. Y. Patil Hospital & Research Centre during the period from January 2012 to December2012.The percentage of MBL Producer in Pseudomonas aeruginosa isolates in the present study by Double Disc Synergy Test (DDST) & Modified EDTA Sunergy Test (MEDST) were 23%. We have observed that both the methods showed 100% correlation.*

Keywords: *Pseudomonas aeruginosa, Metallobetalactamase*

I. Introduction:

Pseudomonas aeruginosa, an opportunistic and worrisome nosocomial pathogen, is a gram negative, aerobic rod, belonging to bacterial family Pseudomonadaceae. It is the pseudomonas[5] most frequently recovered from the clinical specimens. It is reported to be a leading cause of nosocomial infections, including pneumonia, UTI, Burn infection, meningitis and bacteremia. Its ability to survive on inert materials, live on minimal nutritional requirement, with its tolerance to a wide variety of physical conditions and antiseptics; has contributed enormously to its ecological success and its role as an effective opportunistic pathogen.[1]

Infections caused by *Pseudomonas aeruginosa* are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs. Its general resistance is due to a combination of factors: 1) It is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall. 2) It can become resistant through mutation in chromosomal genes which regulate resistance genes. 3) It can acquire additional resistance genes from other organisms via plasmids. It produces enzymes namely 6- lactamases, which are responsible for wide spread β lactam resistance. These β-lactamases hydrolyze the amide bond of the four-membered characteristic β-lactam ring, thus rendering the antimicrobial ineffective.[2]

Based on molecular studies, two types of carbapenem - hydrolyzing enzymes have been described: 1) Serine enzymes (possessing a serine moiety at the active site) and 2) Metallobetalactamases

Characteristics of Metallo-β-lactamase(MBL):

Metallobetalactamase requires zinc for their catalytic activity. Their activity is inhibited by metal chelators such as EDTA and Thiol compounds. Metallobetalactamase hydrolyzes all β lactam antibiotics including carbapenems, with the exception of aztreonam (monobactam). MBL producing strains are not susceptible to serine β lactamase inhibitors(eg-clavulanate).[3]

II. Materials and Methods:

A total of 100 *Pseudomonas aeruginosa* isolated from various clinical specimens over a period of one year from January 2012 to December 2012 in the Department of Microbiology, D.Y. Patil Medical College and Research Institute, Kolhapur were selected for the study. These isolates were subjected to antibiotic susceptibility testing using Kirby- Bauer disc diffusion technique, and screened for MBL production

a) imipenem-EDTA Double Disc Diffusion Synergy Method (Imp-EDTA DDDS):

The suspected strains were inoculated into sterile peptone broth and the turbidity adusted to 0.5Mc Farland. Dried Muller Hinton agar plate was inoculated with a sterile cotton swab dipped in the above broth. After drying two 10µg imipenem disc was applied firmly on the the surface of agar, at a distance of 20 mm

centre to centre on the plate. To one of the Imipenem discs 10µl of 0.5M EDTA was added and the plates were incubated for 16 to 18 hrs or overnight.

Interpretation : The zone diameters of the two imipenem discs were compared. Difference in the inhibition zones between the two discs by ≥7mm was considered as positive result.

Control Strains: Pseudomonas aeruginosa ATCC 27853 was used as a negative control for the test.

b) Imipenem –EDTA Combined disc method:

The suspected strains were inoculated into sterile peptone broth and the turbidity adjusted to 0.5Mc Farland. Dried Muller Hinton agar plate was inoculated with a sterile cotton swab dipped in the above broth, after drying a 10µg imipenem disc was applied firmly on the surface of the agar. Another blank filter paper disc (Hi media) was placed at a distance of 10mm (edge to edge).To the blank disc 10µl of a 0.5M EDTA a solution was added. Plates were incubated at 37℃ for 16 to 18 hrs or overnight.

Interpretation: The presence of even a small zone of synergistic inhibition was interpreted as positive.

Control Strains: *Pseudomonas aeruginosa* ATCC 27853 was used as a negative control.

III. Observations and Results:

Out of the 100 *Pseudomonas aeruginosa* isolated,72% were from admitted patients and 28% were from outpatients .Most of these isolates were from pus and wound swabs 50%. Antimicrobial susceptibility testing showed maximum resistance to cephalosporins.76% of the isolates were sensitive to imipenem. Out of the 100 Pseudomonas isolates 23% were MBL producers.

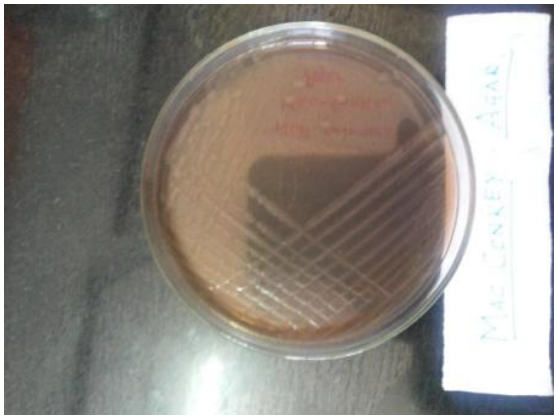

Fig. 1: Showing Growth of *Pseudomonas aeruginosa* on MacConkey Agar (NLF colonies)

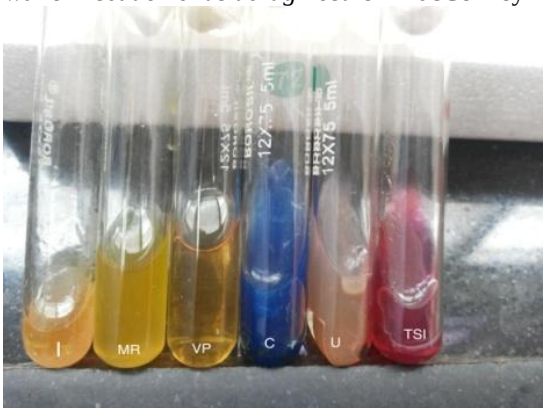

Fig. 2: Showing Biochemical Reactions

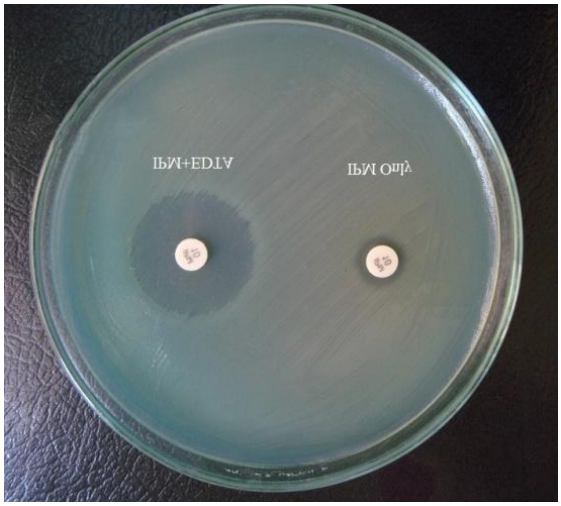

Fig.3: Showing Imipenen EDTA combined disc method.

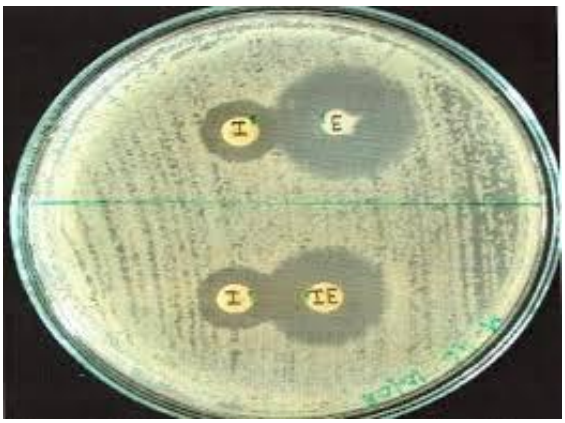

Fig. 4: Showing Imipenen EDTA Double disc diffusion Synergy method.

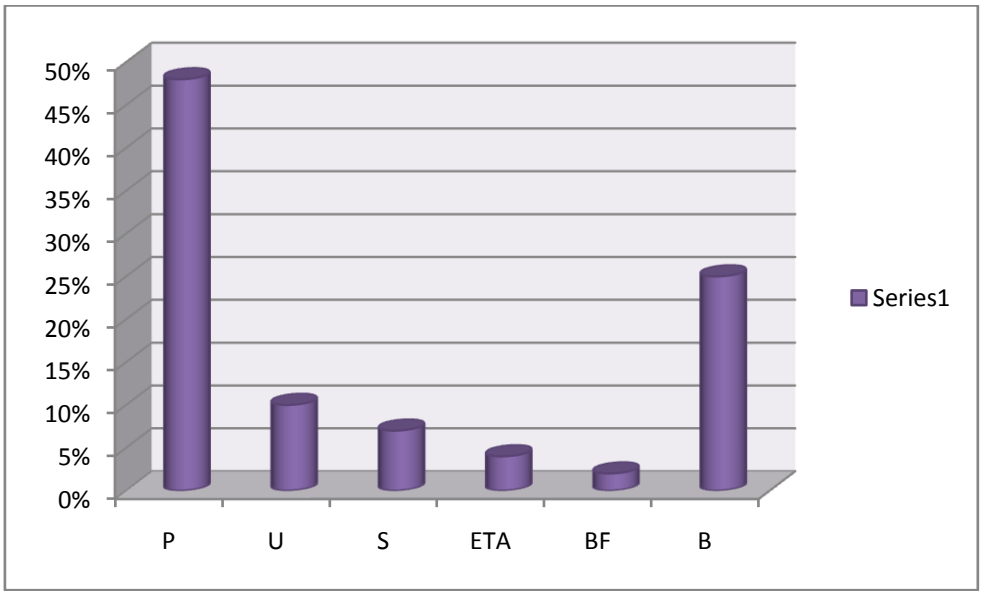

Fig. 5: Bar-diagram Showing Sample wise distribution of *P. aeruginosa* isolates

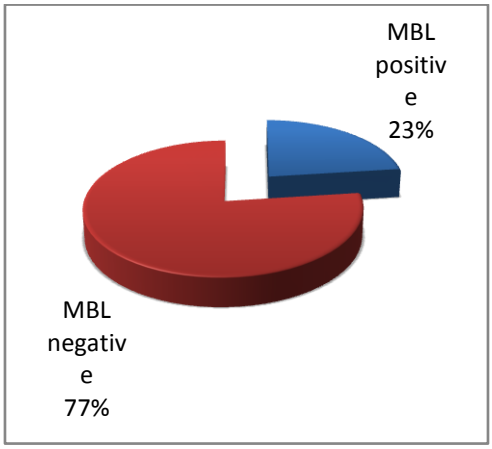

Fig. 6: Showing Percentage of MBL producers

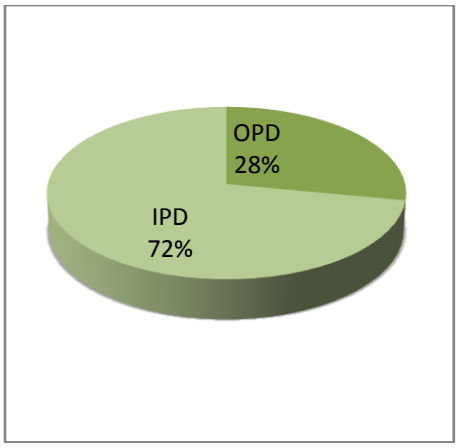

Fig. 7: Showing Inpatient and outpatient wise distribution

IV. Discussion:

The overall prevalence of MBL producers are found to vary greatly in different geographical areas and in different institutes.In the present study,an attempt has been made to know the prevalence of MBL producing Pseudomonas aeruginosa isolated from various clinical samples and their antibioyic susceptibility pattern.Out of 100 isolates of Pseudomonas aeruginosa scrrened,23%were MBL producers.The results were compared with other studies and disussed as follows:

Study series	year	Pus (%)	Blood (%)	Urine (%)	Sputum (%)	Endotracheal aspirate (%)	Body fluids (%)
Shenoy S et al [1]	2002	42.33	18.9	34.9	-	20	-
Rashid A et al[4]	2007	65.6	-	29.25	1.02	1.7	-
Aggarwal R et al[2]	2008	39.29	18.9	14.19	18.9	-	15.38
Varaiya et al[6]	2008	22.17	4.16	21.6	-	24.5	-
Attal RO et al[7]	2010	28.6	12.1	22.9	25	5	6.4
Deeba B et al[8]	2011	53	20.8	12.4	3.5	1.8	6.4
Present study	2013	50	26	11	7	4	2

Table 1: Distribution of *pseudomonas aeruginosa* in various clinical samples

Study series	Year	Inpatients (%)	Outpatients (%)
Shampa Anupurba et al[3]	2006	73.43	26.57
Rashid A et al[4]	2007	70	30
Prashant D P et al[8]	2011	84.93	15.07
Deeba B et al[9]	2011	95.5	4.5
Present study	2013	72	28

Table 2: Inpatient- outpatient wise distribution in various studies

Study series	Year	Percentage of MBL producers (%)
Sederi H et al[5]	2008	39.06
Varaiya et al[6]	2008	20.8
Attal RO et al[7]	2010	11.4
Deeba B et al[8]	2011	11.66
Prashant D P et al[8]	2011	7.8
John S et al [10]	2011	27.7
Present study	2013	23

Table 3: Percentage of MBL producers in various studies

V. Conclusion:

Detection of mettalobetalactamase production is of paramount importance both in hospital and community isolates. This is because these strains are probably more prevalent but not routinely screened in most of the labs. These enzymes constitute a serious threat to the use of currently available antibiotics mainly cephalosporins. Institutional outbreaks are increasing because of the selective pressure due to the indiscriminate use of extended spectrum cephalosporins and carbapenems and lapses in effective control measures. So vigilance and timely recognition of infection with resistant bacteria and appropriate antibiotic therapy, is the only answer to the current multidrug resistant bacterial population.

Acknowledgement:

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References:

[1]. Shenoy S, Baliga S, et al. Antibiotic sensitivity Of Pseudomonas aeruginosa strains isolated from various clinical specimens. Ind J Med Sci 2002; 56: 427-30.
[2]. Aggarwal R., Chaudary U. Extended spectrum β-lactamases (ESBL) – an emerging threat to clinical therapeutics. Indian J. Med Microbiol 2004; 22 (2): 75-80.
[3]. Anupurba S, Bhattacharjee A, Garg A, Ranjan sen M. Antimicrobial susceptibility of Pseudomonas aeruginosa isolated from wound infections. Indian J Dermatol 2006; 51(4): 286-288.
[4]. Rashid A, Chowdhury A, et al. Infections by Pseudomonas aeruginosa and antibiotic resistance pattern of the isolates from Dhaka Medical College hospital Bangladesh J Med 2007; 1(2): 48-51.
[5]. Saderi H, Karimi Z, Owlia P et al. Phenotypic detection of Metallo-beta-lactamase producing Pseudomonas aeruginosa strains isolated from burned patients. Iranian J of Pathology 2008; 3(1): 20-24.
[6]. Varaiya A, Kulkarni N, Bhalekar P et al. Incidence of metallo-beta-lactamase producing Pseudomonas aeruginosa in ICU patients.Ind J Of Med Res 2008; 127: 398-402.
[7]. Attal RO, Basak S et al. Metallo beta lactamase producing Pseudomonas Aeruginosa. An emerging threat to clinicians. Journal of Clinical and Diagnostic Res 2010; (4): 2691-2696.
[8]. Prashant DP, Basavaraj VP. ESBL and MBL mediated resistance in Pseudomonas aeruginosa: An emerging Threat to Clinical Therapeutics. Journal of Clin and Diagnostic Research 2011; 5(8): 1552-1554.
[9]. Deeba Bashir, Manzoor Ahmad T, Bashir Ahmed F, Gulnaz B et al. Detection of Metallo -beta-lactamase producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. African J Of Microbiol Res 2011; 5(2): 164 -172.
[10]. John S, Balaguruthan R. Metallo-beta-lactamase producing Pseudomonas aeruginosa and Acinetobacter baumanni. Indian Journal of Medical Microbiology 2011; 29 (3): 302-304.