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PRODUCTION OF METALLO BETA LACTAMASE IN CLINICAL ISOLATES OF PSEUDOMONAS AERUGENOSA IN TERITARY CARE HOSPITAL JAMNAGAR"

Microbiology

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ABSTRACT

Introduction: The present study was 250 clinical isolates of Pseudomonas Aerugenosa from various clinical samples were included in this study. For further processing we used carbapenem (Imipenem) Resistant strains and placed them for production of Metallo beta lactamase by Antimicrobial disc diffusion method was performed according to CLSI guideline. All Carbapenem resistant strain placed for various phenotypic methods to detect MBL production. The phenotypic methods used were Combine Disc Diffusion test and Epsilometer test. Epsilometer test gave better results then other test. **Materials and Methods** Disc diffusion method were used and Epsilometer test . **Results:** Total 250 clinical isolates of Pseudomonas Aerugenosa out of which 119 were MBL producing in which 45.3% were males and 54.6% were females. Out of 119 MBL producers, highest isolates, 34.4% were from pus samples, Followed by blood 26%, urine 19.3%, endotracheal tube aspirates and sputum 8.4%, Miscellaneous 3.3%. Out of 119 MBL producing Pseudomonas aeruginosa isolated, in which 38 (31%) were from Surgery, followed by medicine 28 (23.5%), PICU 13 (10.9), TBCD 12 (10%) ICU 9 (7.5%), Pediatrics 08 (6.7%), Ortho 6(5%) ENT 5 (4.2%),. **Discussion and Conclusion:** 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 antibiotic susceptibility pattern. Out of 250 isolates of Pseudomonas aeruginosa screened, 119 were MBL producers.

KEYWORDS

INTRODUCTION

Pseudomonas Aerugenosa is a large group of aerobic, non sporing, gram negative bacilli, motile which are motile by polar flagella. They are ubiquitous, typically saprophytic, mostly found in soil, water, and other moist environment. Pseudomonas Aerugenosa producing metallo beta lactamase was first reported from Japan in 1991 and since then has been reported from many parts including Asia, Europe, Australia.Metallo beta lactamases belongs to ambler class B and have ability to hydrolyze a wide variety of beta lactam agents. The gene responsible for the production of MBLs are part of integrons and are carried on transferable plasmids. In 2017 Government of India came into notice that Pseudomonas Aerugenosa is an important pathogen in containment of antimicrobial resistance. Multidrug resistance Pseudomonas aerugenosa are increasing threats that contribute to high mortality. The pathogenesis of pseudomonas aerugenosa depends on its ability to make biofilms, these are micro aggregates of bacterial colony which surround device and cover them and also mucosal surfaces. Recently there is no standardised methods for detection of MBLs production has been proposed and despite PCR is highly accurate and reliable.The aim of this study to detect MBL producing Pseudomonas aerugenosa in different clinical isolates from different wards and to determine their antibiogram and to guide physician and surgeons prescribing proper antibiotics and controlling hospital infection.

MATERIALS AND METHODS

This prospective study was done from January year 2020 to December 2020 in the Department of Microbiology Shri at a Tertiary Care Hospital Jamnagar with obtaining approval from the Institute Ethics Committee.

Study Design and Sample:

A total 250 samples collected from which Pseudomonas aerugenosa isolated from various clinical samples received in Microbiology Laboratory for bacterial culture and sensitivity. Samples included sputum, urine, wound swabs, bronchoalveolar lavage, endotracheal aspirate, central line, pus, blood, and indwelling catheters.

Inclusion Criteria:

Pseudomonas Aerugenosa from various clinical samples were included in the study.

Exclusion Criteria:

All the clinical isolates other then Pseudomonas Aerugenosa were excluded from the study and repeat isolates from the same patients.

Isolation of Pseudomonas aerugenosa is done by various conventional methods like colony morphology, pigmentation, on nutrient agar, Mac conkey agar, microscopy gram stain smear

4. Ceftazidime - EDTA Combined disk synergy test (CDST- CAZ)[3] examination, oxidase test, suger fermentation, and biochemical reaction. Different phenotypic methods done for detection MBL production.

Antimicrobial susceptibility Testing:

- In the present study antibiotic susceptibility testing were carried out against following antibiotics.Piperacillin, ciprofloxacin, ceftizoxime, ofloxacin, gentamicin, amikacin.
- All the antibiotic discs were procured commercially from pathoteq Biological Laboratories.
- The diameter of the zone of inhibition measured according to CLSI guideline.
- Antibiotic susceptibility pattern were for (in percentage) piperacillin 25.6, ciprofloxacin 20, gatifloxacin 10, ceftizoxime 8, amikacin 12, ofloxacin 12, gentamicin 12.4, imipenem 100.

1. Imipenem (IMP) -EDTA combined disc test

This test was first described by Yong et al. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two 10 µg imipenem discs 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 discs were compared after 16 to 18 hours of incubation in air at 35^{0}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. Imipenem –EDTA double disc synergy test (DDST)

As described by Lee et al, test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An imipenem (10 µg) disc was placed 20 mm centre to centre from a blank disc containing 10µL of 0.5M EDTA (750µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition in the area on the far side of the drug was interpreted as a positive result.

3. Ceftazidime - EDTA Double disc synergy test (DDST-CAZ) [8]

Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An ceftazidime (30 µg) disc was placed 20 mm centre to centre from a blank disc containing 10µL of 0.5M

EDTA (750µg). Enhancement of the zone of inhibition in the area between ceftazidime and the EDTA disc in comparison with the zone of inhibition in the area on the far side of the drug was interpreted as a positive result.

Two 30 µg ceftazidime discs 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 ceftazidime and ceftazidime - EDTA discs were compared after 16 to 18 hours of incubation in air at 35^{0}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.

5. **5)E-STRIP –** Enz Mic ™ (Himedia) this strip contain two portion ESBL + /ESBL these part contain Ceftazidime,Cefotaxime ,EDTA+Clavulanic acid and Ceftazidime,Cefotaxime & EDTA respectively .

Interpretation Criteria

Report	Formula	Interpretative Criteria
MBL Positive Strain	ESBL/ESBL += >8	When the ratio of the value obtained for ESBL the value obtained for ESBL+ is more then 8 or No zone obtained for ESBL and zone obtained in ESBL +

RESULTS:

In this study phenotypic characterization, antibiotic susceptibility of Pseudomonas aerugenosa with special reference to metallo beta lactamase detection was conducted in the Department of Microbiology, Shri M.P. Shah Govt Medical College Jamnagar.

A total of 250 Pseudomonas Aerugenosa isolated from various clinical samples were studied. The results of the study are as follows.

Organism	Total number of imipenem resistant isolates	MBL production		Non - MBL production	
		No. of isolates	Percentage (%)	No. of isolates	Percentage (%)
P. aeruginosa	250	119	47.6	131	52.4

Age-wise Distribution

Age (years)	Number of cases	Percentage (%)
< 10	21	17.6
11-20	16	13.4
21-30	09	7.5
31-40	15	12.6
41-50	16	13.4
51-60	22	18.4
61-70	16	13.4
>70	04	3.3
Total	119	100

Out of the 119 MBL producing P.aeruginosa isolates studied, maximum no. of cases (18.4%) were in the 51-60 age group years, followed by <10 (17.6%) and the least number of cases (3.3%) were in the age group >70 years.

Table – 12: Sex-wise distribution

Sex	No of cases	Percentage (%)
Male	54	45.3
Female	65	54.6
Total	119	100

Among 119 isolates of MBL Producing Pseudomonas Aerugenosa 45.3% (54 cases) were male and 54.6 (65 cases) were females.

Ward Wise Distribution

Wards	No.of cases	Percentage%
Surgical ward	38	31
Medical ward	28	23.5
ICU	09	7.5
PICU	13	10.9
TBCD Ward	12	10
ENT Ward	05	4.2
Ortho Ward	06	5.0
Pediatrics	08	6.7
TOTAL	119	100

Out of 119 MBL producing pseudomonas aeruginosa highest number of samples 31% were from Surgical wards followed by 23.5% from Medical wards (Medicine) and remaining 45.5% were from , TBCD, pediatrics, ENT,ortho,ICU ,and PICU.

Antibiotic Susceptibility Pattern Of The Isolates

Antibiotics	No. of isolates (sensitive)	Percentage (%)
Piperacillin	64	25.6
Ciprofloxacin	50	20
Gatifloxacin	25	10
Ceftizoxime	20	8
Amikacin	30	12
Ofloxacin	30	12
Gentamicin	31	12.4
Imipenem	250	100

Distribution of MBL Producers In Different Clinical Samples

Specimens	No. of cases	MBL POSITIVE	Percentage (%)
Pus	87	41	34.4
Blood	56	31	26
Urine	64	23	19.3
Sputum	16	10	8.4
Miscellaneous	07	04	3.3
Endotracheal aspirate	20	10	8.4
Total	250	119	100

Out of 250 isolates 119 (47.6%) were MBL producers. In which 34.4% from PUS, Followed by blood 26%, urine 19.3%, endotracheal tube aspirate and , sputum 8.4 %, Miscellaneous 3.3%.

Detection MBL by Various Methods

Test	No of MBL producing isolates	Percentage positivity (%)
Combined disc test IMP	31	26
Combined disc test CZA	30	25.2
Double disc synergy IMP and CZA	24	20.1
E - Strip	34	28.5
TOTAL	119	100

Detection of Metallo beta lactamase were by E-Strip 28.5%,followed by combined disc synergy test (IMP,IMP+EDTA) 26%, combined disc synergy test (CZA,CZA+EDTA)25.2% , double disc synergy test (IMP,EDTA,CZA)20.1%.

DISCUSSION

Antibiotic when first introduced was considered as a magic bullet.[16] Unfortunately the genes expressing resistance to antimicrobials have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration.

Pseudomonas aerugenosa is known to cause a variety of infections like urinary tract infections, wound infections, septicemia and device infections is now emerging as MBL producer and is spreading it in horizontal way to members of Enterobacteriaceae.[65]

REFERENCES

1. Patil SS, Munjappa B,Gadgil SA,Gadve A. Prevalence of asymptomatic hepatitis B virus and hepatitis C virus infections in patients with maintenance hemodialysis of a tertiary care hospital in western Maharashtra. Indian Journal of Microbiology Research. 2018; 5(3):382-386.
2. Bhaumik P, Debnath K. Prevalence of Hepatitis B and C among Hemodialysis Patients of Tripura, India. Euroasian Journal of Hepato-Gastroenterology. 2012;2(1):10-13.
3. Carolyn Chi, Patel P, Pilishvili T, Moore M, Murphy T, Strikas R. Guidelines for vaccinating Kidney Dialysis Patients with Chronic Kidney diseases summarized from Recommendations of the Advisory Committee on Immunization Practices (ACIP). 2012.https://www.google.com/search?client=firefox-bd&q=Guidelines+for+vaccinating+Kidney+Dialysis+Patients+ International Journal of Health and Clinical Research, 2021;4(11):240-242 e-ISSN: 2590-3241, p-ISSN: 2590-325X Ingin et al International Journal of Health and Clinical Research, 2021; 4(11):240-242 www.ijhcr.com 242 with+Chronic+Kidney+diseases+summarized+from+Recommendations+of+the+Advisory+Committee+on+Immunization+Pra ctices+%28ACIP%29.+December+2012
4. Kokane H, Panchawalwar V. Seroprevalence of Hepatitis B in maintenance dialysis patients and associated risk factors in tertiary care institute. Med Pulse International Journal of Medicine. 2018; 6(1):43-47.
5. Kapse, Joshi, Verma. Prevalence of HIV, HBV, and HCV Markers in Multi-transfused Patients. International Journal of Scientific Study, 2017; 5(7):1
6. Kalantari H, Ebadi S, Yaran M, Maracy MR, Shahshahan Z. Prevalence and risk factors of hepatitis B and C viruses among hemodialysis patients in Isfahan, Iran. Adv Biomed Res. 2014; 3:73.

7. Malhotra R, Soin D, Grover P, Galhotra S, Khutan H, Kaur N. Hepatitis B virus and hepatitis C virus co-infection in hemodialysis patients: A retrospective study from a tertiary care hospital of North India. J Nat SciBiol Med. 2016;7(1):72
8. Ibrahim N MR, Sidiq Mohammed Saleem Z, R Hussein N. The Prevalence of HIV, HCV, and HBV Among Hemodialysis Patients Attending Duhok Hemodialysis Center, Int J Infect. 2018; 5(1):e63246.
9. Kosaraju K, Faujdar SS, Singh A, Prabhu R. "Hepatitis Viruses in Heamodialysis Patients: An Added Insult to Injury?" Hepatitis Research and Treatment.2013:1-4
10. Prakash S,Jain A,Sankhwar SN,Usman K,Prasad N,Saha D,Singh KP,Jain P, Singh DD. Prevalence of hepatitis B & C viruses among patients on hemodialysis in Lucknow, Uttar Pradesh. Clinical Epidemiology and Global Health. 2014; 2(1):19-23.
11. Güvenir M, Guler E, Oygar D, Behlul A,Suer K. Evaluating the Prevalence of HBV, HCV, and HIV in Hemodialysis Patients in North Cyprus. Hepat Mon. 2019; 19(1):e84699.
12. Kansay S, Sekhon J, Rana S. Seroprevalence of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus among hemodialysis patients in a Tertiary Care Teaching Hospital in a developing country. Indian J Sex Transm Dis. 2019; 40:120-5.
13. Khullar S, Singh PR, Khatri PK, Kumar MV. Seroprevalence of hepatitis B virus and hepatitis C virus infection in haemodialysis patients at tertiary care hospital in Western Rajasthan, India. Journal of the academy of clinical microbiologist. 2020; 22(1):23-27.
14. Saha D, Agarwal SK. Hepatitis and HIV infection during haemodialysis. J Indian Med Assoc. 2001;99(4):194-9, 203, 213.
15. Datta S, Chatterjee S, Veer V, Chakravarty R. Molecular biology of the hepatitis B virus for clinicians. J Clin Exp Hepatol 2012; 2:353-65.
16. Kramvis A, Kew M, François G. Hepatitis B virus genotypes. Vaccine 2005; 23:2409-23.
17. Sudan SS, Sharma RK. Prevalence of Hepatitis B and C infection on maintenance haemodialysis. Bombay Hosp J 2013; 45. Available from: http://www.bhj.org.in/journal/2003_4502_april/prevalence_301.htm. [Last accessed on 2016 Mar].
18. Reddy GA, Dakshinamurthy KV, Neelaprasad P, Gangadhar T, Lakshmi V. Prevalence of HBV and HCV dual infection in patients on haemodialysis. Indian J Med Microbial 2005; 23:41-3.
19. Chandra M, Khaja MN, Hussain MM, Poduri CD, Farees N, Habeeb MA, et al. Prevalence of hepatitis B and hepatitis C viral infections in Indian patients with chronic renal failure. Intervirology 2004; 47:374-6.
20. Moloughney BW. Transmission and post exposure management of blood borne virus infections in the health care setting: Where are we now? CMAJ 2001; 165:445-51
21. Salama G, Rostaing L, Sandres K, Izopet J. Hepatitis C virus infection in French hemodialysis units: A multicenter study. JMed Virol 2000; 61:44-51.
22. Hinrichsen H, Leimenstoll G, Stegen G, Schrader H, Fölsch UR, Schmidt WE, et al. Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: A multicentre study in 2796 patients. Gut 2002; 51:429-33.
23. Kelley VA, Everett Kitchens J, Brannon LE, Connor K, Martinez EJ, Pearson TC, et al. Lack of seronegative hepatitis C virus infections in patients with chronic renal failure. Transplantation 2002; 74:1473-5.
24. Wreghitt TG. Blood borne virus infections in dialysis units – A review. Rev Med Virol 1999; 9:101-9.