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RESEARCH ARTICLE

PFGE GENOTYPING OF METALLO BETA LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA

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Abstract

Purpose: Indiscriminate use of antibiotics since a long time has led to the emergence of organisms resistant to many antibiotics. This study was carried out for genotyping of metallo beta lactamase (MBL) producing *P.aeruginosa*using pulsed field gel electrophoresis (PFGE) and compare the PFGE pattern with the antibiotic susceptibility pattern obtained.

Methods: In this study the MBL producing *P.aeruginosa* isolates were subjected to antibiotic sensitivity test against 9 antibiotics and further subjected to PFGE to determine their genetic pattern.

Results: All the isolates were resistant to meropenem(10µg) and 88% were resistant to imipenem(10 µg). Isolates showed maximum sensitivity to amikacin (30 µg) and ciprofloxacin (5µg).Identical PFGE pattern was observed in 23 out of 25 isolates and 2 isolates were found to have completely different pattern.

Conclusion: Our findings show a dominant genomic pattern of the MBL isolates although the phenotypic antibiotic resistance test showed no conclusive phenotypic pattern.

Introduction:-

Microorganisms resistant to multiple antibiotics are termed as multidrug resistant (MDR) or more recently as superbugs. Bacteria have developed several mechanisms through which they can resist the action of antibiotics(Mahtab et al., 2021). The most common mode is enzymatic inactivation of the antibiotic for example production of beta-lactamses (enzymes produced by organisms that cleave beta lactam antibiotics. Emergence and dissemination of beta lactamases and Carbapenemases has posed a global public health risk(Tooke et al., 2019). Similarly, Methicillin resistant *Staphylococcus aureus*(MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) are prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide. Vancomycin-resistant enterococci (VRE) clones occurring in different hospitals, countries, and regions have been demonstrated in several places(Breves et al., 2015). Whenunderstanding disease mechanisms, organism's virulence and host predisposition must be considered. Hospitals are exposed to a greater danger of diseases outbreak that are caused by bacteria. In addition, there are people who are immunocompromised, in such cases and also in older people; diseases are easily spread(Collins AS 2008). In suspicious cases of an outbreak, determining the exact time by which spread occurs is important because the source of the outbreak is always avery crucial information. Typing of these drug resistant strains will enable in identifying prevalent genotypes, which will help in empirical treatment(Chambers & Deleo, 2010).Further, the causative organisms associated with nosocomial outbreaks are frequently found to be clonally

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related, implying a common origin. Molecular typing of MDR organisms provides data regarding source and rate of dissemination of resistance in populations. They provide an important tool for evaluation and development of preventive measures for dissemination and treatment(Vivas et al., 2019).

Materials and Methods:-

Strains of Metallo β lactamase producing *Pseudomonas aeruginosa* isolated from various clinical specimens were included in the study. These strains were established to be metallo beta lactamase producers in a previous work carried out in our laboratory(Pal & Joshi, 2016). Further antibiotic sensitivity test and PFGE analysis was carried in this study.

Antibiotic Sensitivity Test:

The antibiotic susceptibility testing was performed as per the Clinical and Laboratory Standards Institute (CLSI) recommendations by Kirby–Bauer method. MBL-producing *P. aeruginosa* culture suspension was prepared by adjusting the culture density to 0.5 McFarland standard. Mueller Hinton Agar was used for growing the lawn of the culture by swabbing onto the agar plate. Different antibiotic discs were then placed equidistant and the plate and incubated at 37^0C for 18 hrs. .Antibiotics used were Imipenem (10µg), Meropenem (10µg), Tobramycin(30 µg), Piperacillin (100 µg), Ceftazidime (30 µg), Amikacin (30 µg), Gentamicin (10 µg), Levofloxacin (5 µg), Ciprofloxacin(5 µg). .

PFGE Analysis:

These strains were then subjected to PFGE using Bio-Rad CHEF-DR III Pulsed Field Electrophoresis System.

Plug preparation:

The test strains were cultured on nutrient agar plate overnight at 37°C and a loopful of bacteria was suspended in 1 ml saline to achieve McFarland 4 turbidity. Cell suspension was treated with 100µl of 37%-41% Formaldehyde and incubated for 1 hour at 4°C. After incubation the cells were washed 3 times with normal saline solution.Low melting temperature agarose2% (w/v) was prepared in distilled water. An equal volume (100 µl each) of cell suspension and agarose was added to the tubes and gently mixed by pipetting 2 - 3 times. This mixture (100µl)was immediately dispensed into plug molds and incubated at 4°C for 10 min.

Lysis of the cells in agarose plugs:

The plugs were removed from the molds and transferred to the cell lysis solution containing 0.5 mM EDTA, 1% sarcosyl, proteinase K (2 mg/ml). Cell lysis was done at 55°C in shaking water bath overnight. Next day, plugs were washed three times with sterile ultrapure water and then three times with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA), pH 7.6.

Restriction Digestion of Plugs:

The restriction enzymeBcuI (SpeI) (10 U/µL)was used for digestion. A single plug was placed in a sterile 1.5 ml micro centrifugetube. The plug was incubated for three hours with the appropriate restriction enzyme (30 units per plug) at 37°C for 6 hours.

Loading the Samples:

The DNA sample plug were placed on a smooth clean surface, and cut to size using a razor blade. Agarose plugs were placed in the sample wells using a spatula and were gently pressed down to the bottoms of the wells. Each sample well was then filled with Low Melt Preparative Grade Agarose (at an agarose concentration equal to that of the gel) and allowed to solidify at room temperature for 10–15 minutes. An appropriate size standard was also loaded.

Electrophoresis:

The gel from the casting tray was transferred to electrophoresis chamber and covered with 1,900 ml of 0.5 X TBE pH 8.0 with 50uM of thiourea.DNA restriction fragments were separated by PFGE using a CHEF DR III system (Bio-Rad, USA) at 14 °C, 6 V/cm, for 18hrs, with a time switch of 5–30 seconds.

Staining the Gel: After the run, the gel was stained with 10µl Ethidium bromide (10mg/ml) in 0.5X TBE buffer for 20 minutes. The gel was visualized in the gel doc system.

Interpretation PFGE banding patterns was done according to the criteria of Tenover et al(Tenover et al., 1995). Isolates were considered genetically indistinguishable or identical, if their restriction patterns showedthe same

number of bands with the same identical apparent size, these were designated as genomic pattern A. Isolates were considered to be unrelated if their PFGE patterns showed differences in seven or more bands.

Results:-

Table 1:- The rate of antibiotic resistance determined by AST.

Sr.no	Antibiotic	Resistant no. (%)	Intermediate no. (%)	Sensitive no. (%)
1.	Imipenem (IMP) (10µg)	22 (88)	0.00	3 (12)
2.	Meropenem(MEM) (10µg)	25 (100)	0.00	0.00
3.	Tobramycin (TOB) (30µg)	12 (48)	3 (12)	10 (40)
4.	Piperacillin (PIP) (100µg)	10 (40)	5 (20)	10 (40)
5.	Ceftazidime (CAZ) (30µg)	16 (64)	7 (28)	8 (32)
6.	Amikacin (AMK) (30µg)	7 (28)	6 (24)	12 (48)
7.	Gentamicin (GEN) (10µg)	17 (68)	0.00	8 (32)
8.	Levofloxacin (LVX) (5µg)	10 (40)	5 (20)	10 (40)
9.	Ciprofloxacin (CIP) (5µg)	09 (36)	5 (20)	11(44)

By AST, maximum isolates were found to be sensitive to Amikacin (5µg)and Ciprofloxacin (5 µg). Besides Imipenem and Meropenem maximum resistance was found for the drugs like Gentamicin (10µg) and Ceftazidime (30µg). (Table 1)

PFGE pattern analysis:

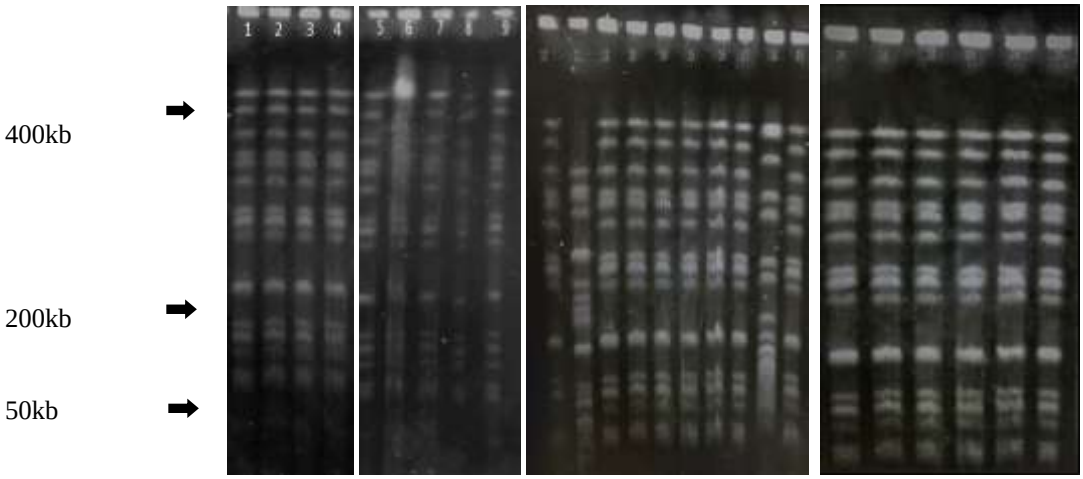

Figure 1:- PFGE pattern for MBL producing *Pseudomonas aeruginosa*.

Table 2:- Antibiotic resistance phenotype and PFGE pattern.

Isolate	Resistance phenotype	PFGE Pattern
1	IMP,MEM,PIP	A
2	IMP,MEM,PIP	A
3	IMP,MEM,TOB,PIP	A
4	IMP,MEM,TOB,PIP	A

5	MEM,TOB,PIP,GEN	A
6	MEM,GEN, LVX,CIP	A
7	IMP,MEM,CAZ,GEN,CIP	A
8	IMP,MEM,PIP,CAZ,GEN,CIP	A
9	IMP,MEM,TOB,PIP,CAZ,GEN,LVX,CIP	A
10	MEM,GEN,CAZ	A
11	IMP,MEM,TOB,CAZ,AMK,GEN,LVX	B
12	IMP,MEM,TOB,PIP,CAZ,GEN,LVX,CIP	A
13	IMP,MEM,CAZ,GEN	A
14	IMP,MEM,TOB,CAZ,GEN	A
15	IMP,MEM,AMK,GEN	A
16	IMP,MEM,AMK,CAZ,GEN	A
17	IMP,MEM,PIP,LVX	A
18	IMP,MEM	C
19	IMP,MEM,TOB,CAZ,AMK,GEN,LVX,CIP	A
20	IMP,MEM,TOB,CAZ,AMK,GEN,LVX,CIP	A
21	IMP,MEM,TOB,CAZ,AMK,GEN,LVX	A
22	IMP,MEM,CAZ,GEN	A
23	IMP,MEM,TOB,PIP,CAZ,GEN,LVX,CIP	A
24	IMP,MEM,TOB,PIP,CAZ,GEN,LVX,CIP	A
25	IMP,MEM,PIP,CAZ,AMK	A

A total of 25 isolates were subjected to DNA macro-restriction analysis by Pulsed-field Gel Electrophoresis. Analysis of PFGE profiles was done by comparing the pattern of bandsobserved on visual inspection of EtBr stained-gels. A total of three patterns were found among the isolates. All 23 isolates were found to be completely indistinguishable or identical and were marked as type A. Whereas, the other two patterns found in isolates 11 and 18 were found to be completely unrelated as their PFGE patterns showed differences in seven and more of their bands. All the isolates were found to be resistant to Meropenem (10 µg)whereas 3 isolates belonging to pattern A were found to be sensitive to Imipenem (10 µg). Besides this isolate 11 (pattern B) was found to be resistant to all the antibiotics in the pannel.Isolate 18 (pattern C)was resistant to Meropenam (10 µg),Imipenem (10 µg), Tobramycin (30µg),Piperacillin (100µg),Gentamicin (10µg)and intermediate resistance was seen for Ceftazidime (30 µg) and was sensitive to Amikacin (30 µg),Levofloxacin (5 µg), Ciprofloxacin (5 µg).

Discussion and Conclusion:-

Prevalence of carbapenem resistant *P. aeruginosa* is high among nosocomial infections. PFGE can be considered as gold standard for genotyping of strains(Mulliqi, 2018) .This technique shows results with highly discriminatory PFGE patterns and correlation among the strains.Although a single technique cannot be relied upon completely(Harbottle et al., 2006). This study was carried out to determine correlation between phenotypic resistance pattern and genotyping by PFGE.

Various antibiotic patterns are reported in studies conducted for MBL producing *P. aeruginosa*(Kali et al., 2013), (Saderi et al., 2010),(Parkins et al., 2006).Our study showed the most effective antibiotics for infections caused by MBL *P. aeruginosa* are amikacin and ciprofloxacin. In a study by Ranjan et al MBL-producing *P.aeruginosa* showed 100 % resistance to ciprofloxacin. However, resistance to meropenem 100% and imipenem 93.10% was comparable with our study(Ranjan et al., 2014).Tsakris et al reported 100% resistance to ciprofloxacin and amikacin (Tsakris et al., 2011).The isolate 11 showed resistance to all antibiotics also the pattern obtained was different from other isolates of pattern A and isolate 18 (pattern C) which showed sensitivity to two or more antibiotics. Thus, in conjunction with the study of Selim et al, we also report that the antibiotic susceptibility pattern cannot be considered basis of genetic relatedness or difference in strains. Although both different genotype pattern showed unique antibiotic susceptibility but this difference in antibiotic resistance phenotype may not confirm clonal distinction.

The PFGE pattern revealed a significanthomogeneity among these*P. aeruginosa* isolates (Fig. 1), which suggested that these *P. aeruginosa* isolates were mostly originating from common origin.12 distinct clonal patterns were

obtained in study by Akya et al and inter- and intra-hospital dissemination of resistant clones was concluded(Akya et al., 2015).

In conclusion, our study identifies the similar genomic pattern of isolates which can be crucial in determination of outbreak and critical in better planning of therapy to control spread in hospital settings.

References:-

1. Akya, A., Salimi, A., Nomanpour, B., & Ahmadi, K. (2015). Prevalence and Clonal Dissemination of Metallo-Beta-Lactamase-Producing *Pseudomonas aeruginosa* in Kermanshah. 8(7), 0–4. https://doi.org/10.5812/jjm.20980v2
2. Breves, A., Miranda, C. A. C., Flores, C., Filippis, I. De, & Clementino, M. M. (2015). Methicillin- and vancomycin-resistant Staphylococcus aureus in health care workers and medical devices. June, 143–152.
3. Chambers, H. F., & Deleo, F. R. (2010). NIH Public Access. 7(9), 629–641. https://doi.org/10.1038/nrmicro2200.Waves
4. Collins AS. 2008 Apr Preventing Health Care–Associated Infections. In: Hughes RG, editor. Patient Safety and Quality: An Evidence-Based Handbook for Nurses. Rockville (MD): Agency for Healthcare Research and Quality (US). Chapter 41.
5. Harbottle, H., White, D. G., Mcdermott, P. F., Walker, R. D., & Zhao, S. (2006). Comparison of Multilocus Sequence Typing , Pulsed-Field Gel Electrophoresis , and Antimicrobial Susceptibility Typing for Characterization of Salmonella enterica Serotype Newport Isolates. 44(7), 2449–2457. https://doi.org/10.1128/JCM.00019-06
6. Tsakris, A., Poulou, A., Kristo, I., Pittaras, T., Spanakis, N., Pournaras, S., & Markou, F. (2011). Large Dissemination of VIM-2 – Metallo- -Lactamase – Producing Pseudomonas aeruginosa Strains Causing Health Care-Associated. 47(11), 3524–3529. https://doi.org/10.1128/JCM.01099-09
7. Kali, A., Srirangaraj, S., Kumar, S., Divya, H. A., Kalyani, A., & Kali, A. (2013). Detection of metallo-beta-lactamase producing Pseudomonas aeruginosa in intensive care units. 4, 686–693.
8. Mahtab, T., Jyoti, A., Khusro, A., Redwan, B. M., Zidan, M., Mitra, S., Bin, T., Dhama, K., Hossain, K., Gajdács, M., Umar, M., Sahibzada, K., Hossain, J., & Koirala, N. (2021). Journal of Infection and Public Health Antibiotic resistance in microbes : History , mechanisms , therapeutic strategies and future prospects. 14, 1750–1766. https://doi.org/10.1016/j.jiph.2021.10.020
9. Mulliqi, G. (2018). Molecular epidemiology of Pseudomonas aeruginosa in University Clinical Center of Kosovo. 2039–2046.
10. Pal, R., & Joshi, M. R. R. (2016). Research article detection of metallo β lactamase in lactose non-fermenting fermenting gram negative organisms comparison of different methods organisms-comparison.
11. Parkins, M. D., Pitout, J. D. D., Church, D. L., Conly, J. M., & Laupland, K. B. (2006). Treatment of infections caused by metallo-Î2-lactamase-producing Pseudomonas aeruginosa in the Calgary Health Region. Clinical Microbiology and Infection, 13(2), 199–202. https://doi.org/10.1111/j.1469-0691.2006.01591.x
12. Ranjan, S., Gs, B., & Babu, P. R. S. (2014). Comparison of Epidemiological and Antibiotic Susceptibility Pattern of Metallo Beta Lactamase-Positive and Metallo Beta Lactamase-Negative Strains of Pseudomonas Aeruginosa. 2, 109–113. https://doi.org/10.4103/0974-2727.141509
13. Saderi, H., Lotfalipour, H., Owlia, P., & Salimi, H. (2010). Detection of Metallo- β -Lactamase Producing Pseudomonas aeruginosa Isolated From Burn Patients in Tehran , Iran. 609–612. https://doi.org/10.1309/LMQJF9J3T2OAACDJ
14. Seventer, J. M. Van, & Health, P. (2020).
15. Tenover, F. C., Arbeit, R. D., Goering, R. V, Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. (1995). GUEST COMMENTARY Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis : Criteria for Bacterial Strain Typing. 33(9), 2233–2239.
16. Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H. A., Takebayashi, Y., & Spencer, J. (2019). β -Lactamases and β -Lactamase Inhibitors in the 21st Century. Journal of Molecular Biology, 431(18), 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002
17. Vivas, R., Andre, A., Barbosa, T., Dolabela, S. S., & Jain, S. (2019). Multidrug-Resistant Bacteria An Overview. 25(6), 890–908. https://doi.org/10.1089/mdr.2018.0319.