Original Research Paper

Volume - 12 | Issue - 11 | November - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

RESISTANCE PATTERNS OF BETA-LACTAMASES PRODUCING PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT **Background:** Pseudomonas aeruginosa are one among the most versatile, adaptable microorganisms in the environment. They are known to cause diseases in multiple organ systems of humans. Due to their easy acquisition of resistance genes via various modes, multidrug resistance patterns are emerging. Morbidity and mortality especially of immunocompromised and hospitalized individuals are on the rise because of ESBL, MBL and AmpC producing Pseudomonas aeruginosa. **Objective:** To isolate Pseudomonas aeruginosa from various clinical samples and identify production of beta-lactamase enzymes. **Methods:** A prospective study for 6 months was undertaken to identify Pseudomonas aeruginosa from various clinical samples using conventional microbiological techniques. Antimicrobial susceptibility testing was performed according to CLSI guidelines. Further, beta-lactamase enzymes' production was screened and confirmed according to CLSI. **Results:** During our study period, we isolated 189 Pseudomonas aeruginosa from different clinical specimens. Screening for beta-lactamase enzymes revealed 38 (20.1%) probable ESBL, 15 (7.93%) probable MBL and 30 (15.87%) probable AmpC producing Pseudomonas aeruginosa strains. From these, 7 (18.42%) were confirmed as ESBL producers, 6 (40%) as MBL producers and 20 (66.67%) as AmpC producers. Drug resistance to multiple groups of antibiotics was noted in all the beta-lactamase producing strains. **Conclusion:** Routine detection of suspected resistant strains in the diagnostic microbiology laboratory will aid in the early identification of the causative pathogen hence leading to appropriate steps for their treatment. A strict antibiotic policy to curb the misuse of second line antimicrobials will help in cutting down the production of mutants responsible for drug resistance.

KEYWORDS : Pseudomonas aeruginosa, beta-lactamase producers, multidrug resistant, extremely drug resistant, MBL+AmpC producers

INTRODUCTION

Known to be an opportunistic pathogen, Pseudomonas aeruginosa has been increasingly identified to be the etiological cause of serious infections especially in hospitalised patients with weakened immunity.[1] They have been implicated in not only respiratory, urinary and burn infections, but also in systemic diseases. Being able to survive in a wide scope of environmental conditions and a variety of hosts makes this pathogen infectious in the community as well as in hospital settings.[2] This adaptability of Pseudomonas aeruginosa confers intrinsic drug resistance in them and also equips them to easily acquire multiple drug resistance mechanisms from other organisms.[3] A multi drug resistant pseudomonal infection is therefore severely difficult to treat.[4]

Increasing resistance patterns in Pseudomonas aeruginosa to the commonly used antibiotic drug classes such as β-lactams, quinolones and even aminoglycosides and carbapenams have been reported from all over the world.[5] Additionally, chromosomal and plasmid mediated AmpC β-lactamases and six distinct metallo-beta-lactamases (MBLs) have been identified.[6,7] Early detection and correct identification of resistance mechanisms in Pseudomonas aeruginosa ergo become crucial in their treatment especially when they are multidrug or sometimes pandrug resistant. This study has identified the three common resistance mechanisms exhibited by Pseudomonas aeruginosa isolated from various clinical samples in a tertiary care hospital.

MATERIALS AND METHODS

A prospective study was conducted in a tertiary care hospital in Udaipur for a duration of 6 months during which, the Microbiology laboratory received 4795 samples. Conventional plating was performed for all received samples. Large, spreading, irregular, beta-haemolytic colonies on 5% sheep blood agar, non-lactose fermenting colonies on MacConkey agar, oxidase positive, Gram negative, motile bacilli on hanging drop were preliminarily considered as Pseudomonas spp.

Speciation as P. aeruginosa was achieved by checking for green coloured pyocyanin pigment production on nutrient agar, growth at 42°C, a positive citrate test, a positive nitrate test and a positive arginine dihydrolase test. By this, we isolated 189 Pseudomonas aeruginosa from various clinical samples obtained from both IPD and OPD patients.

Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method against a set panel of antibiotics and interpreted according to CLSI 2019 guidelines.[8]

1. CLSI does not provide any method for AmpC beta lactamase detection in PA. Therefore, as for testing Enterobacterales, a zone diameter of cefoxitin < 22mm demonstrated by Pseudomonas aeruginosa isolates were considered as probable AmpC producers. Further confirmation was performed by ceftazidime-imipenem antagonism test. Reductions in inhibition of the zone surrounding the ceftazidime disc placed adjacent to the imipenem disc in a lawn culture of Pseudomonas aeruginosa were confirmed to be AmpC producers.
2. PA isolates showing imipenem zone size < 19mm were considered to be probable MBL producers and confirmed by imipenem+EDTA double disc synergy test as described by Yong et al.[9]
3. For screening of ESBL producers, the same criteria laid down for Enterobacterales was employed, as the principle for Pseudomonas remains the same. Screen positive isolates were those which showed a zone size of < 22mm for ceftazidime and < 27mm for aztreonam. Confirmatory testing was performed by phenotypic confirmatory disc diffusion test using ceftazidime alone and in combination with clavulanic acid. A ≥ 5mm increase in zone size for ceftazidime clavulanate in comparison to ceftazidime alone confirmed ESBL production in the isolates.[10,11]

RESULTS

During the study period, we isolated 1421 Gram negative bacilli of which 189 were confirmed as Pseudomonas aeruginosa. The various samples from which the organism was isolated is elaborated in Table 1.

Table 1: Distribution of samples from which Pseudomonas aeruginosa were isolated.

Samples	Number Of Isolates	Percentage
Respiratory samples (sputum, bronchial aspirate, bronchoalveolar lavage, pleural fluid)	97	51.32%
Pus including swabs	44	23.28%

Urine	38	20.11%
Blood	7	3.8%
Other body fluids	3	1.59%
TOTAL	189	100%

Recovering Pseudomonas aeruginosa from clinical samples was greater among in-patients (68.25%) than in the out patients (31.75%) with a male predominance (78.84%). The distribution of β-lactamase producing Pseudomonas aeruginosa is depicted in Table 2 and the overall antibiotic resistance pattern of Pseudomonas aeruginosa isolates are given in Table 3.

Table 2: Distribution of β-lactamase producing Pseudomonas aeruginosa.

TYPE OF β-LACTAMASE ENZYME	PROBABLE ISOLATES	CONFIRMED ISOLATES
ESBL	38 (20.1%)	7 (3.7%)
MBL	15 (7.93)	6 (3.17%)
AmpC	30 (15.87%)	20 (10.58%)
TOTAL	83 (43.92%)	33 (17.46%)

Table 3: Resistance pattern of isolated Pseudomonas aeruginosa.

Antibiotics	All Isolates (n=189)	All B-lactamase Producers (n=31)	Esbl Producers (n=7)	Mbl Producers (n=6)	Ampc Producers (n=20)
Amikacin	39 (20.63%)	18 (58.06%)	3 (42.86%)	6 (100.00%)	11 (55.00%)
Netilmicin	19 (10.05%)	8 (25.80%)	1 (14.28%)	3 (50.00%)	5 (25%)
Tobramycin	24 (12.70%)	12 (38.70)	1 (14.28%)	4 (66.67%)	9 (45.00%)
Piperacillin-Tazobactam	25 (13.22%)	13 (41.93)	3 (42.86%)	5 (83.33%)	7 (35.00%)
Ceftazidime	38 (20.10%)	19 (16.29)	7 (100.00%)	4 (66.67%)	9 (45.00%)
Cefoperazone-Sulbactam	24 (12.70%)	10 (32.26%)	4 (57.14%)	3 (50.00%)	4 (20.00%)
Cefepime	38 (20.10%)	15 (48.39%)	6 (85.71%)	5 (83.33%)	5 (25.00%)
Ciprofloxacin	68 (35.98%)	23 (74.19%)	5 (71.43%)	5 (83.33%)	14 (70.00%)
Levofloxacin	74 (39.15%)	25 (80.64%)	5 (71.43%)	5 (83.33%)	16 (80.00%)
Aztreonam	32 (16.93%)	13 (41.93)	6 (85.71%)	4 (66.67%)	4 (20.00%)
Imipenem	15 (7.94%)	7 (22.58%)	1 (14.28%)	6 (100.00%)	2 (10.00%)
Meropenem	12 (6.35%)	6 (19.35%)	0 (0.00%)	6 (100.00%)	2 (10.00%)
Polymyxin B	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

DISCUSSION

Pseudomonas aeruginosa is amongst the most diverse, ecologically significant bacteria of the Pseudomonas genera. The significant properties of the pathogen include its widespread distribution in nature, exhibition of multiple virulence factors and demonstrations of resistance to a wide range of antimicrobials.[5] Resistance acquisition is quite easy for Pseudomonas aeruginosa and are commonly observed as production of β-lactamase enzymes such as AmpC, extended-spectrum and metallo- β-lactamases.[12] Multiple β-lactamase producing Pseudomonas aeruginosa can lead to disastrous therapeutic failure and hence poses a significant clinical challenge if they remain undetected. Therefore, the present study was undertaken to identify ESBL, MBL and AmpC producing Pseudomonas aeruginosa from various clinical samples and map their antibiogram.[4,13]

Being ubiquitous, Pseudomonas aeruginosa frequently inhabits the hospital environment causing nosocomial infections in hospitalised and immunocompromised patients increasing their prevalence in healthcare institutes. Prevalence of Pseudomonas aeruginosa infections in India has been estimated to vary from a minimum of 10.5% up to 30%.[14] We isolated 13.30% of Pseudomonas aeruginosa from a total of 1421 Gram negative bacteria from different clinical samples tested during our study period. Among them, 3.7% were confirmed ESBL producers, 3.17% were MBL producers and 10.58% was AmpC β-lactamase producing Pseudomonas aeruginosa.

The most common method of ESBL production in Pseudomonas aeruginosa is by hydrolysis of the beta-lactam ring of beta-lactam antibiotics commonly demonstrated by members of Entero-bacteriaceae to exhibit resistance. This type of enzyme production in Pseudomonas aeruginosa isolates varies from 12.78% as evidenced by Choudhary V et al.[3] to 20.27% as captured by Aggarwal R et al.[1] and even as dangerously high as 57.76% as documented by Easwaran S et al.[6] The large variation in ESBL producing isolates of Pseudomonas aeruginosa is directly proportional to the varied antibiotic prescription all over the world although the extremely high frequency of horizontal gene transfer is one of the modes.[6] Obviously, the isolates were resistant to cephalosporin ceftazidime along with >85% resistance to cefepime and aztreonam. Our study suggests less action of fluoroquinolones in ESBL producing Pseudomonas aeruginosa. However, carbapenems, higher aminoglycosides and polymyxins can be used to manage infections caused due to such bugs.

A study conducted in Shimla, Himachal Pradesh[5] used two methods (combined disc test and E-test) to confirm MBL production and arrived at 15.56% isolates. Another study from Kota, Rajasthan[15] also used two methods (combined disc test and double disc test) for confirming their imipenem resistant Pseudomonas aeruginosa isolates as MBL producers and recorded 34.16%. All of our studies have observed that MBL producing Pseudomonas aeruginosa are resistant to not only carbapenems but also cephalosporins, fluroquinolones and aminoglycosides, rendering them possible to treat with only polymyxins which are potentially toxic to human life. Despite this drawback, sporadic resistance against colistin has made clinicians lose patients to MBL producing multidrug resistant PA infections. Such mechanisms may also be a result of loss of the Opr D porin making the pathogen impermeable to carbapenems or an upregulation of an efflux pump system responsible for pumping out the carbapenems out of the bacterial system.[3] The confirmation of these mechanisms however, could not be performed in our laboratory.

More than a decade ago, Upadhyay S et al.[16] from Varanasi demonstrated AmpC production in Pseudomonas aeruginosa isolates (59.4%) as recommended by CLSI. On similar lines, we also have performed AmpC testing. Considerable resistance was observed towards aminoglycosides and fluoroquinolones leaving cephalosporins and carbapenems to treat AmpC producing Pseudomonas aeruginosa infections.

Overall, our Pseudomonas aeruginosa isolates showed a low level of individual beta-lactamase production with just 1.05% MBL+AmpC production. Co-production of a varying combination of beta-lactamases was noted by authors Choudhary V et al.,[3] Upadhyay S et al.,[16] Ilyas M et al.,[17] Pramodhini S et al.[18] and Tankhiwale S et al.[19]

Multidrug and extreme drug resistance is a problem of the new age particularly stemming from misuse of antibiotics. The dissemination of these resistance patterns has been documented to occur via plasmids, transposons and chromosomes carrying the particular genes. Such genetic transfers convert simple pathogens to life threatening super bugs. Therefore, as clinical microbiologists and infection preventionists, we are direly required to promote dedicated and continuous monitoring of antibiotic resistance patterns alongside the identification of pathogens causing them; follow stringent infection prevention practices in the community and especially in the healthcare set ups; and abide by strict antibiotic policing to save our precious, limited antimicrobial repertoire.

CONCLUSION

Although the study period was limited and mechanisms of beta lactamase production was identified by only phenotypic methods, it underscores the need to identify the presence of ESBL, MBL and AmpC-producing Pseudomonas aeruginosa causing difficult to treat infections. The availability of a precise antibiotic susceptibility pattern of the isolate coupled with the type of resistance mechanisms identified will help made an informed decision about the antibiotics to be chosen and implemented for their treatment.

REFERENCES

1. Aggarwal R, Choudhary U, Bala K. Detection of extended-spectrum β-lactamase in Pseudomonas aeruginosa. Indian J Pathol Microbiol. 2008;51:224-4.
2. Toval F, Guzmán-Marte A, Madriz V, Somogyi T, Rodríguez C, García F. Predominance of carbapenem-resistant Pseudomonas aeruginosa isolates carrying blaIMP and blaVIM metallo-β-lactamases in a major hospital in Costa Rica. J Med Microbiol. 2015;64(1):37-43.
3. Choudhary V, Pal N, Hooja S. Phenotypic detection of ESBL, AmpC and MBL β-lactamases among Clinical Isolates of Pseudomonas aeruginosa in a tertiary care

Hospital of North India. Int J Curr Med Pharma Res. 2018;04(12):3902-3906.
4. Kumar V, Sen MR, Nigam C, Galhot R, Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing Pseudomonas aeruginosa in burn patients: A prospective study. Indian J Crit Car Med. 2012;1(3):136.
5. Minhas N, Sharma PC. Phenotypic detection of metallo and AmpC β-lactamases producing strains of P. aeruginosa in the state of Himachal Pradesh (India). Al Ameen J Med Sci. 2015;8(4):259-65.
6. Easwaran S, Yerat RC, Ramaswamy R. A study on detection of extended-spectrum beta-lactamases (ESBL) and comparison of various phenotypic methods of AmpC detection in Pseudomonas aeruginosa from various clinical isolates in a tertiary care teaching hospital. Muller J Med Sci Res. 2016;7:35-9.
7. Salimi F, Eftekhar F. Coexistence of AmpC and extended-spectrum β-lactamases in metallo-β-lactamase producing Pseudomonas aeruginosa burn isolates in Tehran. Jundishapur J Microbiol. 2013;6(8):e7178.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI guideline M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
9. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798-801.
10. Nithyalakshmi J, Vidhyarani R, Mohanakrishnan K, Sumathi G. ESBL producing Pseudomonas aeruginosa in clinical specimens: Is it a scary nightmare or a paper tiger? Indian J Microbiol Res. 2016;3(3):287-291.
11. Das S, Basak S. ESBL producing Pseudomonas aeruginosa: A threat to Patient Care. Int J Health Sci Res 2017;7(4):132-137.
12. Khan JA, Iqbal Z, Rahman SU, Farzama K, Khan A. Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. Pak J Pharma Sci. 2008; 21: 311-5.
13. Rafiee R, Eftekhar F, Tabatabaei SA, Tehrani DM. Prevalence of Extended-Spectrum and Metallo β-Lactamase Production in AmpC β-Lactamase Producing Pseudomonas aeruginosa Isolates From Burns. Jundishapur J Microbiol. 2014;7(9):e16436.
14. Saxena S, Banerjee G, Garga R, Singh MA, Verma SK, Kushwaha RA. ESBL, MBL and AmpC β-lactamases produced by superbugs: An emerging threat to clinical therapeutics. Int J Pharm Sci. 2015;7:353-6.
15. Chand AE, Chauhan PS, Sharma S, Afridi D. Prevalence of Metallo-beta-lactamase Production in Imipenem-resistant Pseudomonas in Tertiary Care Centre at Kota Region. Int J Sci Stud. 2016;4(3):87-91.
16. Upadhyay S, Sen MR, Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries 2010;4(4):239-242
17. Ilyas M, Khurram M, Ahmad S, Ahmad I. Frequency, susceptibility and co-existence of MBL, ESBL & AmpC positive Pseudomonas aeruginosa in Tertiary Care Hospitals of Peshawar, KPK, Pakistan. J Pure App Microbiol 2015;9(2):981-8
18. Pramodhini S, Umadevi S, Seetha K. Prevalence of antimicrobial resistance in clinical isolates of Pseudomonaas aeruginosa in a tertiary care hospital, Puducherry, India. Int J Curr Microbiol App Sci 2015;4:718-26
19. Tankhiwale S. Beta-lactamases in P. aeruginosa: A threat to clinical therapeutics. Curr Pediatr Res 2016;20(1&2):253-257