ORIGINAL RESEARCH

Prevalence of NDM – 1 in the Clinical Isolates of Pseudomonas aeruginosa in a tertiary Hospital in Central India

Amrita[1], Ramanath Karicheri[2*], Dhananjay Kumar Pandey[3]

[1]PhD Scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University Indore M.P, India.
[*2]Professor, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University Indore, M.P, India.
[3]Associate Professor, Department of Pharmacology, Government Medical College, Azamgarh, U.P, India.

Corresponding Author
Ramanath Karicheri
Professor, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University Indore, M.P, India
Email: ramanath.karicheri@gmail.com

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ABSTRACT

Background: The present study was undertaken to detect the prevalence of the blaNDM-1 metallo beta lactamases (MBLs) in the isolates of Pseudomonas aeruginosa, which were recovered from various clinical samples from hospitalized patients in a tertiary care centre in Indore, India.**Methods:**A total of 200 isolates of P. aeruginosa which were obtained from various clinical samples were subjected to antibiotic susceptibility testing by the disc-diffusion method and MIC detection by the Etest method against imipenem, meropenem, piperacillin, tobramycin, ceftazidime, tigecycline and colistin. The presence of blaNDM-1 was detected by PCR.**Result:**In the present study, four isolates of P. aeruginosa, which carried the blaNDM-1 gene, were resistant to imipenem and meropenem. These blaNDM-1 carrying isolates remained susceptible to colistin.**Conclusion:**Emergence of the P. aeruginosa carrying NDM-1gene, which exhibited resistance to imipenem and meropenem, is a threat in the treatment of Pseudomonas infectionss.

Key words:P. aeruginosa, Multidrug resistant, Carbapenems, blaNDM-1.

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a prominent gram-negative organism that is responsible for inducing nosocomial infections, such as those affecting the urinary tract, bloodstream, and ventilator-associated pneumonia. Resistance to carbapenems has been frequently identified in P. aeruginosa. The resistance observed towards carbapenems can be attributed to several factors. Firstly, the loss of OprD porin results in a reduction in outer membrane permeability. Secondly, the up-regulation of an active efflux pump system across the cytoplasmic membrane enhances the resistance. Lastly, modifications are made to the penicillin binding proteins and carbapenem hydrolyzing enzymes[1]. In recent times, P. aeruginosa has developed acquired metallo-beta lactamases (MBL) as a resistance mechanism. This is due to the MBLs' ability to hydrolyze all beta-lactams, including carbapenems, in vitro[2], except for monobactams. The proliferation of multidrug-resistant Pseudomonas aeruginosa (MDRPA) strains is a matter of concern due to the severe scarcity of effective antimicrobial alternatives. MDRPA infection risk factors include protracted hospitalization, antimicrobial therapy exposure, and immunocompromised states[3].Pseudomonas aeruginosa is prevalent in hospital settings, where it has the propensity to persist on both living and non-living objects in close proximity to patients. The presence of an MBL-positive isolate within a hospital environment presents an amplified therapeutic challenge.[4] It is acknowledged as a significant risk to infection control in hospitals, particularly when the isolates possess resistance to multiple drugs. The simple dissemination of these genes to other bacteria is due to the fact that they are transported on highly mobile genetic elements, leaving little room for effective antimicrobial agents. The objective of this research work was to ascertain the prevalence of blaNDM-1 in

P. aeruginosa among patients admitted to our tertiary care hospital and to monitor the clinical outcome subsequent to treatment for these patients.

MATERIALS AND METHODS

Bacterial Isolates

From January 2020 to December 2022, this study was conducted on a total of two hundred consecutive non duplicate isolates of *P. aeruginosa* which were isolated from different clinical specimens such as urine, pus, blood and body fluids from the patients who were admitted to a 1000 bedded tertiary care hospital in Indore, India. All the specimens were collected by using strict aseptic precautions and they were immediately processed without any delay. The bacterial identification was performed by routine conventional microbial culture and biochemical tests using standard recommended techniques[5].

Screening for the Carbapenemase Production

By disc diffusion, all the *P. aeruginosa* isolates with reduced susceptibilities to meropenem and imipenem (the diameters of the zones of inhibition were ≤ 15mm) were screened for the production of carbapenemase. The phenotypic detection of the carbapenemase production was performed by the modified Hodge test by using a meropenem disc (10 µg) as per the CLSI guidelines[6]. The screening for the metallo-beta-lactamase production was performed by the combined – disc test, by using two imipenem discs (10 µg), one containing 10 µl of 0.1 M (292 µg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO), which were placed 25 mm apart on a Mueller Hinton agar plate. An increase in the zone diameter of >4 mm around the imipenem-EDTA disc as compared to that around the imipenem disc alone was considered as positive for the metallo-β-lactamase production. The MBL production of the isolates was detected by the MBL (IP/IPI) E-test method (AB Biodisk, Solna, Sweden) as per the manufacturer's instructions.

Molecular Detection of The MBL Genes

DNA was extracted by using the spin column method (QIAGEN; GmbH, Hilden, Germany) as per the manufacturer's instructions. A multiplex PCR assay was performed to detect five families with the acquired MBL genes (blaIMP, blaVIM, blaSPM, blaGIM, blaSIM) in a single reaction. Multiplex PCR for the blaOXA-23 and the blaOXA-24 genes and simple PCR for blaKPC were carried out on the isolates by using the Gene Amp 9700 PCR System (Applied Biosystems, Singapore). The primers and the cycling conditions for the PCR were as has been described earlier[7-9]. PCR for the detection of blaNDM-1 was carried out by using primers, as has been reported previously[10]. Briefly, the program consisted of an initial denaturation step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30s, primer annealing at 49°C for 30s, and extension at 72°C for 30s; followed by a final extension for 10 min at 72°C, which resulted in a 813 bp product

RESULT

Out of the 200 clinical isolates of P. aeruginosa, 40 were found to be carbapenem resistant. The screening for MBL production was carried out on these 40 isolates, and 20 of them found to be positive. Among these 20 isolates, sixteen were found to be positive for blaVIM and four were found to be positive for blaNDM-1.

DISCUSSION

The emergence of NDM-1 was initially identified in a Klebsiella pneumoniae isolate from a Swedish patient of Indian descent in 2008, as a result of the prevalent use of carbapenems; it has since been linked to an increasing number of infections in patients from around the globe. In a southern Indian study[11], Nagarajan et al. documented a significant prevalence of blaNDM-1 in carbapenem-resistant K. pneumoniae (75% of the isolates) and E. coli (66%). A comparable investigation conducted at a tertiary care hospital situated in the northeastern part of India revealed that every carbapenem-resistant strain of K. pneumoniae isolate contained the blaNDM-1 gene[12]. Additionally, we have documented the initial occurrence of the blaNDM-1 gene being identified in a Raoultella ornitholytica clinical isolate[13]. Prior research has identified that the majority of the NDM-1-encoding genes were situated on plasmids, with those investigations primarily concentrating on Enterobacteriaceae[14]. The current investigation verified that the blaNDM-1 genes of all four P. aeruginosa isolates that tested positive for NDM were situated on plasmids measuring 50 kb in length. Due to the fact that all four blaNDM-1-carrying P. aeruginosa isolates were obtained from cases of hospital-acquired infections, this could lead to an unprecedented spread of multidrug-resistant pathogens that induce untreatable infections in the hospital environment.

CONCLUSION

P. aeruginosa, a pathogen notorious for antibiotic resistance in hospital-acquired infections, causes soft tissue infections, including surgical site infections, respiratory tract infections in ventilator-dependent patients, and severe infections in burn cases and patients with catheterized systems, respectively. Carbapenems are the antibiotics that are most commonly employed in the management of these infections. In order to overcome resistance to carbapenems, more toxic drugs such as Colistin must be administered to the patients. Particularly when the isolates are multidrug resistant, accurate identification and reporting of NDM-1-producing P. aeruginosa will assist in infection control management by preventing its spread.

REFERENCES

1. Laupland KB, Parkins MD, Church DL , Gregson DB, Louie TJ, Conly JM, et al. Population-based

epidemiological study of infections caused by carbapenem-resistant Pseudomonas aeruginosa in the Calgary Health Region: Importance of metallo-beta lactamase (MBL)-producing strains. J Infect Dis. 2005; 192:1606–12.
2. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta lactamase and its correlation with molecular structure. Antimicrob. Agents Chemother. 1995; 39:1211–33.
3. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial Infections Due to Multidrug-Resistant Pseudomonas aeruginosa: Epidemiology and Treatment Options. Pharmacotherapy. 2005 Oct; 25(10):1353-64.
4. Gray HK, Beaird OE, Smith EA, Schaenman JM, Yang S. Domestically Acquired NDM-1–Producing Pseudomonas aeruginosa, Southern California, USA, 2023. Emerg Infect Dis. 2023;29(11):2382-2385. https://doi.org/10.3201/eid2911.230646.
5. Collee JG, Miles RS, Wan B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A editor. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed.. Edinburgh: Churchill Livingstone; 1996; 131–150.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: twenty second Informational Supplement M100-S22. CLSI, Wayne, PA, USA, 2012.
7. Woodford N, Tierno PM , Young K, Tysall L, Palepou MF, Ward E, et al. Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. Antimicrob. Agents Chemother. 2004; 48: 4793–9.
8. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother. 2007; 59:321-2.
9. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006;27:351-3.
10. Hornsey M, Phee L, Wareham DW. A Novel variant, NDM-5, of the New Delhi metallo-beta-lactamase in a Multidrug-Resistant Escherichia coli ST648 Isolate Recovered from a patient in the United Kingdom. Antimicrob. Agents Chemother. 2011; 55:5952-4.
11. Nagaraj S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. Indian J Med Microbiol. 2012;30:93-5.
12. Bora A, Ahmed G. Detection of NDM-1 in clinical isolates of K. pneumoniae from Northeast India. J Clin Diagn Res. 2012;6: 794- 800.
13. Khajuria A, Praharaj AK, Grover N, Kumar M. First Report of blaNDM-1 in Raoultella ornithinolytica. Antimicrob. Agents Chemother. 2013; 57: 1092-1093.
14. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010; 10: 597–602