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# MOLECULAR DETECTION OF THE *BLA*$_{NDM-1}$ GENE AMONG CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIAL ISOLATES FROM INTENSIVE CARE UNIT PATIENTS

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## ABSTRACT

**Background:** Carbapenem-resistant Gram-negative bacteria (CR-GNB) have emerged as a significant healthcare concern, particularly in intensive care units (ICUs), owing to their limited treatment options and association with high morbidity and mortality. The New Delhi metallo-β-lactamase (*Bla*$_{NDM-1}$) gene is one of the most important mechanisms responsible for carbapenem resistance among Gram-negative bacterial pathogens.

**Aim:** To determine the prevalence of the *Bla*$_{NDM-1}$ gene among carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates recovered from ICU patients.

**Materials and Methods:** This hospital-based cross-sectional observational study was conducted over 12 months in the Department of Microbiology of a tertiary care teaching hospital. A total of 911 Gram-negative bacterial isolates obtained from ICU patients were analyzed, among which 290 carbapenem-resistant isolates were included. Identification and antimicrobial susceptibility testing were performed using standard microbiological methods. Molecular detection of the *Bla*$_{NDM-1}$ gene was carried out by polymerase chain reaction (PCR).

**Results:** Among 290 CR-GNB isolates, *Klebsiella pneumoniae* was the predominant organism (126, 44.7%), followed by *Acinetobacter baumannii* (55, 35.3%), *Escherichia coli* (45, 20.5%), and *Pseudomonas aeruginosa* (39, 36.1%). Lower respiratory tract infections accounted for the majority of cases (54.8%), followed by urinary tract infections (17.2%) and pyogenic infections (15.2%). Overall carbapenem resistance among Gram-negative isolates was 31.8%. Resistance to meropenem, imipenem, and ertapenem exceeded 94%, while colistin demonstrated the lowest resistance rate (9.66%). The *Bla*$_{NDM-1}$ gene was detected in 209 out of 290 CR-GNB isolates (72.0%). Highest positivity was observed in *Proteus spp*. (100%) and *Klebsiella pneumoniae* (77.0%). Major risk factors included prior antibiotic exposure (71.3%), prolonged ICU stay >7 days (65.1%), and mechanical ventilation (56.5%).

**Conclusion:** The study demonstrates a high prevalence of NDM-mediated carbapenem resistance among ICU isolates, emphasizing the urgent need for molecular surveillance, antimicrobial stewardship programs, and strict infection control practices essential to limit the spread of these multidrug-resistant pathogens.

**KEYWORDS:** Carbapenem resistance, *Bla*$_{NDM-1}$, ICU, Gram-negative bacteria, Multidrug resistance, PCR.

## INTRODUCTION

Carbapenem-resistant Gram-negative bacteria (CR-GNB) have emerged as one of the most serious threats to global public health, particularly in intensive care unit (ICU) settings, where critically ill patients are highly vulnerable to severe infections. The extensive and often inappropriate use of broad-spectrum antibiotics in healthcare facilities has accelerated the emergence and spread of multidrug-resistant organisms, resulting in limited therapeutic options, increased morbidity and mortality, prolonged hospital stays, and higher healthcare costs.[1] Among the various resistance mechanisms identified in Gram-negative pathogens, the production of carbapenemases is considered the most alarming because of its rapid dissemination and its ability to hydrolyze nearly all β-lactam antibiotics, including carbapenems, which are regarded as last-resort agents for the treatment of severe bacterial infections.[2]

Among carbapenemases, the New Delhi metallo-β-lactamase (NDM) enzyme has attracted worldwide attention because of its high transmissibility and broad-spectrum resistance profile. The NDM gene, commonly designated as *Bla*$_{NDM-1}$, encodes a metallo-β-lactamase capable of inactivating carbapenems and several other β-lactam antibiotics. Since its first identification in 2008 in a clinical isolate of , the NDM gene has spread rapidly across different regions of the world through plasmid-mediated horizontal gene transfer.[3] The mobility of this gene among diverse bacterial species, particularly members of the Enterobacteriaceae family as well as non-fermenting organisms such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* has made its containment extremely challenging.[4]

Patients admitted to ICUs are especially susceptible to infections caused by NDM-producing organisms because of prolonged hospitalization, invasive procedures, mechanical ventilation, urinary catheterization, immunosuppression, and

extensive exposure to antimicrobial agents.[5] These infections commonly present as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections, and sepsis. Management of such infections is particularly difficult because NDM-producing bacteria frequently exhibit resistance not only to carbapenems but also to aminoglycosides, fluoroquinolones, and cephalosporins, leaving only a few therapeutic options such as colistin, tigecycline, and combination antimicrobial therapy.[6]

The rapid dissemination of the NDM gene has become a major concern in developing countries, including India, where irrational antibiotic use, overcrowded healthcare facilities, inadequate infection control practices, and poor antimicrobial stewardship significantly contribute to the spread of resistant organisms.[7] Several studies from tertiary care hospitals in India have reported an increasing prevalence of NDM-positive Gram-negative isolates in ICU settings, highlighting the urgent need for prompt detection and effective infection prevention strategies.[8] Although conventional phenotypic methods are useful for screening carbapenem resistance, molecular techniques such as polymerase chain reaction (PCR) provide more accurate and definitive detection of the *Bla*$_{\text{NDM-1}}$ gene and play a vital role in epidemiological surveillance.[9]

Molecular detection of the *Bla*$_{\text{NDM-1}}$gene is therefore essential for assessing the burden of carbapenem resistance, monitoring the dissemination of resistant strains, guiding antimicrobial therapy, and implementing effective infection control measures in ICUs. s[10] Early identification of NDM-producing pathogens can assist clinicians in selecting appropriate treatment regimens and help healthcare institutions prevent outbreaks of multidrug-resistant infections.

The present study was undertaken to determine the prevalence of the *Bla*$_{\text{NDM-1}}$gene among carbapenem-resistant Gram-negative bacterial isolates obtained from ICU patients. The objectives of the study were to isolate and identify Gram-negative bacterial pathogens, assess their carbapenem resistance patterns, and detect the presence of the *Bla*$_{\text{NDM-1}}$ gene using polymerase chain reaction (PCR) techniques.

## MATERIALS AND METHODS

This hospital-based cross-sectional observational study was conducted in the Department of Microbiology, MGM Medical College and Hospital, from January 2023 to December 2023. Ethical approval for the study was obtained from the Institutional Ethics Committee**.** A total of 290 non-duplicate carbapenem-resistant Gram-negative bacterial (CR-GNB) isolates obtained from ICU patients admitted to the Surgical Intensive Care Unit (SICU), Medical Intensive Care Unit (MICU), and Emergency Medical Services ICU (EMS-ICU) with suspected bacterial infections were included in the study.

Clinical specimens, including urine, blood, respiratory secretions, wound swabs, catheter tips, and other relevant samples from patients diagnosed with catheter-associated urinary tract infection (CAUTI), bloodstream infection (BSI), catheter-related bloodstream infection (CRBSI), surgical site infection (SSI), and ventilator-associated pneumonia (VAP), were collected and processed using standard microbiological techniques.[11,12]

Isolation and identification of bacterial pathogens were performed using conventional culture methods and standard biochemical tests. Antimicrobial susceptibility testing was carried out by the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[13]Carbapenem-resistant isolates were identified phenotypically and subsequently subjected to molecular analysis. Carbapenem resistance was genotypically verified by detecting the *Bla*$_{\text{NDM-1}}$ gene using polymerase chain reaction (PCR). Genomic DNA extracted with the HiPurA Bacterial Genomic DNA Purification Kit (MB505) and amplification was performed with NDM-specific primers (Forward:5′-GGTTTGGCGATCTGGTTTTC-3′;

Reverse: 5′ CGGAATGGCTCATCACGATC-3′) at a concentration of 10 pmol each, generating a PCR product of 621 bp.[14, 15]

**Statistical Analysis:** Data were entered into Microsoft Excel and analyzed using SPSS software version 27.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Associations between categorical variables were assessed using the Chi-square test or Fisher's exact test, as appropriate. A p-value of less than 0.05 was considered statistically significant.

## RESULT

**Out of the 911 Gram-negative isolates recovered, 290 (31.8%) were carbapenem resistant.**

**Table 1. Prevalence of CR-GNBs in Various Infections**

| Type of Infection | *Klebsiella pneumoniae* | *Acinetobacter baumannii* | *E. coli* | *Pseudomonas aeruginosa* | *Enterobacter spp.* | *Proteus spp.* | *Citrobacter spp.* | Total |
|---|---|---|---|---|---|---|---|---|
| Bacteraemia | 12 | 11 | 2 | 3 | 3 | 1 | 1 | 33 |
| Lower Respiratory Tract Infection (LRTI) | 85 | 30 | 15 | 21 | 5 | 1 | 2 | 159 |
| Pyogenic Infection | 14 | 8 | 13 | 3 | 5 | 1 | 0 | 44 |
| Urinary Tract Infection (UTI) | 13 | 6 | 14 | 11 | 1 | 2 | 3 | 50 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gastrointestinal Infections | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 4 |
| **Total** | **126** | **55** | **45** | **39** | **14** | **5** | **6** | **290** |

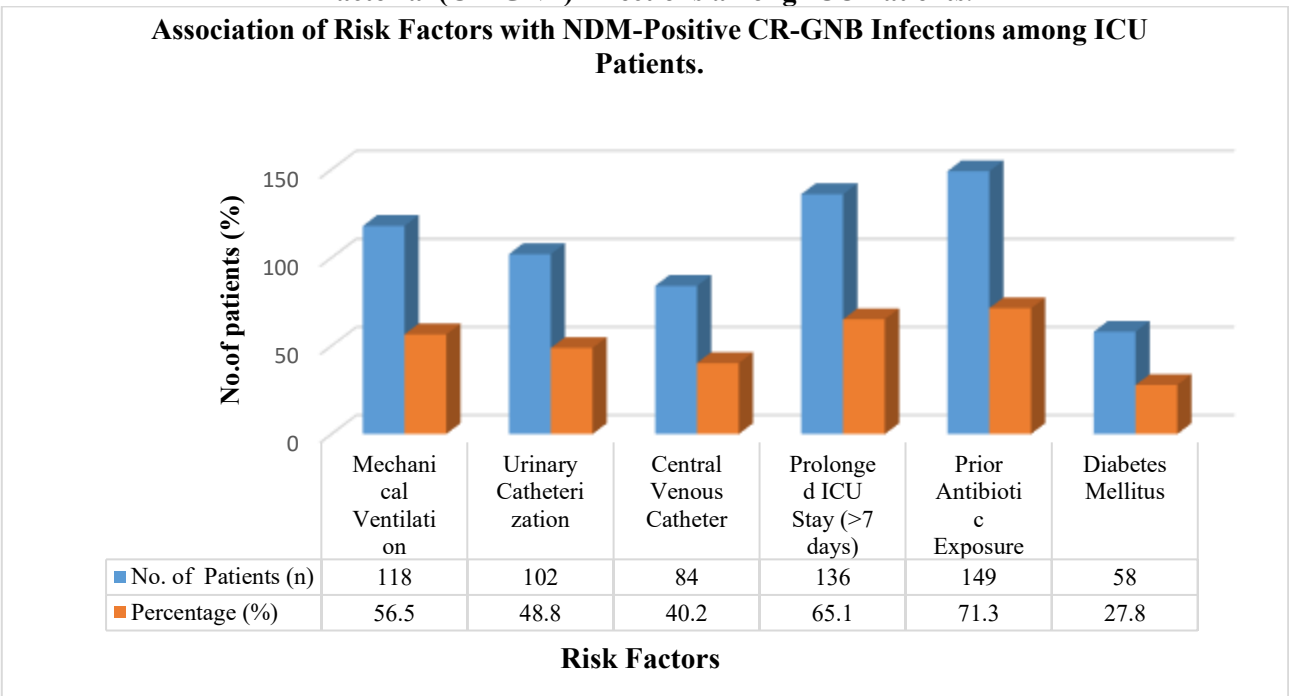
**Table 2. Prevalence of Carbapenem Resistance among GNBs and *Bla*$_{NDM-1}$Gene Detection among CR-GNB from ICU Patients.**

| Organism | Total no.of GNBs (n) | Carbapenem Resistance | | Detection of *Bla*$_{NDM-1}$ | |
|---|---|---|---|---|---|
| | | Total no.of CR-GNB Isolates (n) | Carbapenem-Resistance (%) | *Bla*$_{NDM-1}$ Positive in CR-GNB isolates (n) | *Bla*$_{NDM-1}$ Positive percentage (%) |
| ***Klebsiella pneumoniae*** | 282 | 126 | 44.7 | 97 | 77.0 |
| ***Acinetobacter baumannii*** | 156 | 55 | 35.3 | 39 | 70.9 |
| ***Escherichia coli*** | 220 | 45 | 20.5 | 32 | 71.1 |
| ***Pseudomonas aeruginosa*** | 108 | 39 | 36.1 | 25 | 64.1 |
| ***Enterobacter spp.*** | 81 | 14 | 17.3 | 7 | 50.0 |
| ***Proteus spp.*** | 26 | 5 | 19.2 | 5 | 100.0 |
| ***Citrobacter spp.*** | 38 | 6 | 15.8 | 4 | 66.7 |
| **Total** | **911** | **290** | **31.8** | **209** | **72.0** |

**Table 3. Antibiotic Resistance Pattern among CR-GNB Isolates**

| Antibiotic | Resistant Isolates (n) | Percentage (%) |
|---|---|---|
| Meropenem | 290 | 100 |
| Imipenem | 287 | 98.97 |
| Ertapenem | 273 | 94.14 |
| Ceftazidime | 252 | 86.9 |
| Ciprofloxacin | 231 | 79.66 |
| Gentamicin | 214 | 73.79 |
| Piperacillin–Tazobactam | 205 | 70.69 |

**Figure 1. Association of Risk Factors with NDM-Positive Carbapenem-Resistant Gram-Negative Bacterial (CR-GNB) Infections among ICU Patients.**



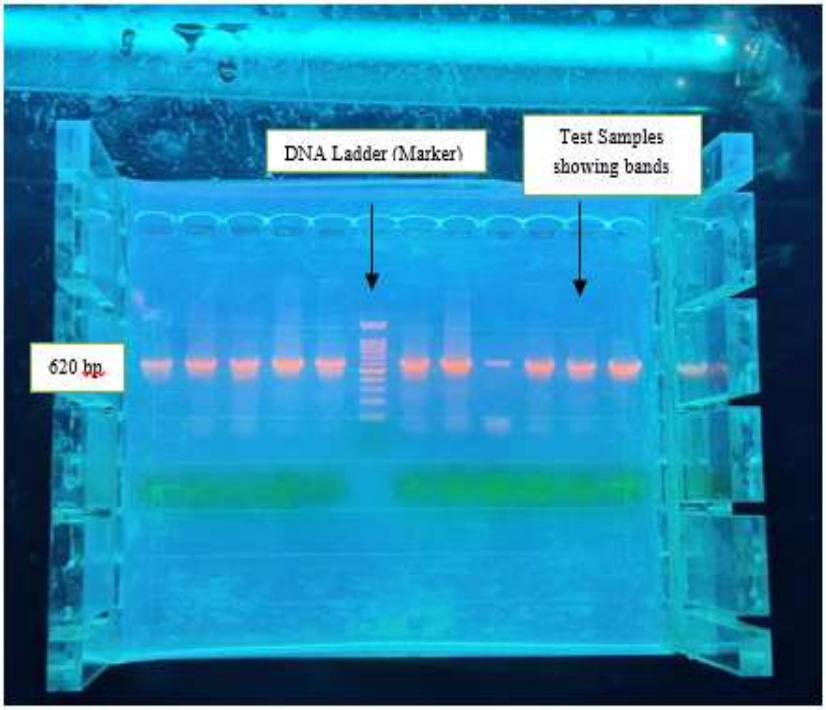



**Figure 2. PCR detection of *Bla*$_{NDM-1}$ gene by Agarose gel electrophoresis**

Among the 290 CR-GNB isolates, Lower Respiratory Tract Infections (LRTIs) were the most common source of infection, accounting for 159 (54.8%) isolates, followed by Urinary Tract Infections (UTIs) with 50 (17.2%) isolates and Pyogenic Infections with 44 (15.2%) isolates. Bacteraemia contributed 33 (11.4%) isolates, while Gastrointestinal Infections accounted for only 4 (1.4%) isolates. *Klebsiella pneumoniae* was the predominant pathogen (126 isolates, 43.4%), followed by *Acinetobacter baumannii* (55, 19.0%), *E. coli* (45, 15.5%), and *Pseudomonas aeruginosa* (39, 13.4%).

Among 911 Gram-negative bacterial isolates recovered from ICU patients, 290 (31.8%) were carbapenem-resistant. The highest resistance rate was observed in *Klebsiella pneumoniae* (44.7%), followed by *Pseudomonas aeruginosa* (36.1%) and *Acinetobacter baumannii* (35.3%). Lower resistance rates were noted among *E. coli* (20.5%), *Proteus spp.* (19.2%), *Enterobacter spp*. (17.3%), and *Citrobacter spp*. (15.8%). The distribution of carbapenem resistance among organisms was statistically significant (p=0.001).

All CR-GNB isolates demonstrated resistance to meropenem (100%). Resistance was also very high against imipenem (98.97%) and ertapenem (94.14%). High resistance rates were observed for ceftazidime (86.9%), ciprofloxacin (79.66%), gentamicin (73.79%), and piperacillin–tazobactam (70.69%). Colistin retained the highest activity, with resistance observed in only 28 (9.66%) isolates. The overall resistance pattern was statistically significant (p<0.001).

Out of the 290 carbapenem-resistant Gram-negative bacterial isolates, 209 isolates (72.0%) were positive for the *Bla*$_{NDM-1}$ gene by molecular detection methods. The highest prevalence of *Bla*$_{NDM-1}$ positivity was observed in *Proteus* spp., where all 5 isolates (100%) carried the gene. *Klebsiella pneumoniae* demonstrated 97 positive isolates out of 126 CR isolates (77.0%), followed by *Escherichia coli* with 32 out of 45 isolates (71.1%) and *Acinetobacter baumannii* with 39 out of 55 isolates (70.9%). *Pseudomonas aeruginosa* showed 64.1% positivity, while *Enterobacter spp.* had the lowest positivity rate at 50.0%. The distribution of the *Bla*$_{NDM-1}$gene among different organisms was statistically significant (p=0.004).

Prior antibiotic exposure was the most frequent risk factor, observed in 149 (71.3%) patients, followed by prolonged ICU stay greater than 7 days in 136 (65.1%) patients. Mechanical ventilation was present in 118 (56.5%) patients, urinary catheterization in 102 (48.8%), and central venous catheterization in 84 (40.2%). Diabetes mellitus was noted in 58 (27.8%) patients. The association between these risk factors and NDM-positive CR-GNB infection was statistically significant (p=0.009).

## DISCUSSION

The present study evaluated the prevalence of carbapenem-resistant Gram-negative bacteria (CR-GNB) among ICU patients, with particular emphasis on the molecular detection of the *Bla*$_{NDM-1}$ gene. The study also assessed associated risk factors and antimicrobial resistance profiles of the isolates. The high prevalence of multidrug-resistant organisms and the detection of *Bla*$_{NDM-1}$ among CR-GNB isolates indicate the growing role of NDM-1 in mediating carbapenem resistance in intensive care settings. These findings highlight the importance of molecular surveillance, infection control strategies, and judicious antimicrobial use to prevent the emergence and spread of NDM-producing pathogens.

In the current study, Lower Respiratory Tract Infections (LRTIs) constituted the most common source of CR-GNB isolates (54.8%), followed by Urinary Tract Infections (17.2%) and Pyogenic Infections (15.2%). The predominance of LRTIs may be attributed to the frequent use of mechanical ventilation and prolonged hospitalization among critically ill

patients. Similar observations were reported by Nair et al. [16], who found ventilator-associated pneumonia to be the leading infection caused by carbapenem-resistant organisms in ICU settings. Likewise, Gupta et al. [17] documented respiratory tract infections as the most frequent presentation among patients infected with carbapenem-resistant Enterobacteraceae. The high prevalence of respiratory infections in the present study reflects the vulnerability of ICU patients to nosocomial pulmonary infections.

Among the isolated pathogens, *Klebsiella pneumoniae* was the predominant organism (43.4%), followed by *Acinetobacter baumannii* (19.0%), *Escherichia coli* (15.5%), and *Pseudomonas aeruginosa* (13.4%). Similar findings were reported by Paul et al. [18], who identified *Klebsiella pneumoniae* as the principal carbapenem-resistant pathogen in tertiary care hospitals. A multicenter Indian study by Khurana et al. [19] also demonstrated that Klebsiella species accounted for the majority of carbapenem-resistant isolates. The predominance of *Klebsiella pneumoniae* is particularly concerning because of its ability to acquire and disseminate multiple resistance determinants, including NDM enzymes.

The present study demonstrated an overall carbapenem resistance prevalence of 31.8% among Gram-negative bacterial isolates. *Klebsiella pneumoniae* showed the highest resistance rate (44.7%), followed by *Pseudomonas aeruginosa* (36.1%) and *Acinetobacter baumannii* (35.3%). Comparable resistance rates were reported by Wattal et al. [20], who documented increasing carbapenem resistance among Enterobacterales and non-fermenters in Indian tertiary care hospitals. Similarly, Veeraraghavan et al. [21] observed a rising prevalence of carbapenem- resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii* across intensive care units in India. The increasing burden of carbapenem resistance highlights the urgent need for continuous surveillance and molecular monitoring.

Analysis of antimicrobial susceptibility patterns revealed universal resistance to meropenem (100%) and extremely high resistance to imipenem (98.97%) and ertapenem (94.14%). Resistance to ceftazidime, ciprofloxacin, gentamicin, and piperacillin–tazobactam was also notably high. However, colistin retained significant activity, with resistance observed in only 9.66% of isolates. Similar findings were reported by Doi [22], who observed limited therapeutic options against carbapenem-resistant organisms, with polymyxins remaining among the few effective agents. The comparatively lower resistance to colistin in the present study suggests its continued utility as a last-resort treatment option, although emerging resistance remains a significant concern.

Molecular detection revealed that 72.0% of CR-GNB isolates carried the $Bla_{\text{NDM-1}}$gene, highlighting the widespread dissemination of NDM-mediated resistance in ICU settings. Similar prevalence rates were reported by Bora et al., who detected the $Bla_{\text{NDM-1}}$gene in approximately 70% of carbapenem-resistant isolates from intensive care units.[23] The highest positivity observed in *Klebsiella pneumoniae* (77.0%) is consistent with the findings of Kaur et al., who identified *Klebsiella pneumoniae* as the predominant reservoir of NDM genes in hospitalized patients.[24] The detection of the gene in multiple bacterial species in the present study supports the plasmid-mediated horizontal transfer of resistance determinants among Gram-negative organisms. The 100% positivity observed in *Proteus spp.,* although based on a smaller sample size, indicates the potential for rapid dissemination of NDM genes even in less frequently isolated pathogens. These findings emphasize the need for molecular surveillance and strict infection control strategies in ICUs.

Assessment of risk factors revealed that prior antibiotic exposure (71.3%) and prolonged ICU stay exceeding seven days (65.1%) were the most common factors associated with NDM-positive CR-GNB infections. Similar observations were made by Tumbarello et al. [25], who reported previous broad-spectrum antibiotic use as an independent predictor of carbapenem-resistant infections. Likewise, Papadimitriou-Olivgeris et al. [26] demonstrated that prolonged ICU hospitalization significantly increased the likelihood of acquiring carbapenem-resistant Gram-negative organisms. These findings underscore the role of antibiotic selection pressure and extended healthcare exposure in the emergence of resistant pathogens.

Mechanical ventilation and urinary catheterization were also frequently associated with NDM-positive infections in the present study. This may be explained by the increased risk of biofilm formation on invasive devices, facilitating bacterial colonization and infection. Comparable observations were documented in previous ICU-based studies where invasive procedures significantly increased the risk of multidrug-resistant infections. [27,28] The significant association between these risk factors and NDM-positive isolates highlights the importance of antimicrobial stewardship programs, strict aseptic measures, and early infection control interventions in reducing the burden of resistant pathogens.

Similar results were reported by Falagas et al. [29], who identified invasive medical devices as major risk factors for healthcare-associated multidrug-resistant infections. The disruption of natural host barriers and formation of biofilms on medical devices facilitate bacterial colonization and persistence, contributing substantially to ICU-acquired infections.

Overall, the findings of this study are consistent with national and international reports demonstrating the increasing prevalence of carbapenem-resistant Gram-negative pathogens in ICUs. The predominance of *Klebsiella pneumoniae*, the significant role of invasive procedures and prior antibiotic exposure, and the high level of resistance to commonly used antimicrobials emphasize the necessity for stringent infection-control practices, antimicrobial stewardship programs, and routine surveillance of resistance mechanisms in critical care settings.

## CONCLUSION

The present study highlights the significant burden of carbapenem-resistant Gram-negative bacteria (CR-GNB) and the widespread presence of the $Bla_{\text{NDM-1}}$gene among ICU isolate with *Klebsiella pneumoniae* emerging as the predominant pathogen. Lower respiratory tract infections were the most common clinical presentation, followed by urinary tract and pyogenic infections. A high prevalence of carbapenem resistance was observed among major Gram-negative pathogens, particularly *Klebsiella pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. The antimicrobial susceptibility profile demonstrated extensive resistance to carbapenems and several commonly used antibiotics, leaving limited therapeutic options. Colistin retained comparatively better activity against CR-GNB isolates, although emerging

resistance remains a concern. Molecular analysis revealed a significant burden of NDM-mediated resistance, emphasizing the rapid dissemination of resistant determinants in critical care settings. Important risk factors associated with NDM-positive infections included prolonged ICU stay, invasive procedures, and prior antibiotic exposure. These infections were associated with considerable morbidity and mortality among ICU patients. These findings emphasize early molecular detection, strict infection control measures, rational antibiotic use, and effective antimicrobial stewardship programs to limit the spread of NDM-producing pathogen and improve patient outcomes in intensive care settings.

**REFERENCES**


1. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: World Health Organization; 2014.
2. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791-1798.
3. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, *Bla*$_{NDM-1}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046-5054.
4. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:249856.
5. Vincent JL. Nosocomial infections in adult intensive-care units. Lancet. 2003;361(9374):2068-2077.
6. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010;36(Suppl 3).
7. 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(9):597-602.
8. Rahman M, Prasad KN, Pathak A, Mishra P, Yadav A, Gupta A. Prevalence and molecular characterization of New Delhi metallo-β-lactamases NDM-1, NDM-5, and NDM-7 in multidrug-resistant Enterobacteriaceae from India. Int J Antimicrob Agents. 2018;51(2):252-256.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 34th ed. Wayne (PA): CLSI; 2024.
10. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis. 2017;215(Suppl 1).
11. Mackie and McCartney Practical Medical Microbiology, 14$^{th}$ ed.
12. Koneman's colour atlas and textbook of diagnostic microbiology. 6$^{th}$ edn. Lippincott` Williams and Wilkins, Philadelphia 2006.
13. Wayne P.A., CLSI 2016, Clinical and Laboratory standard institute (CLSI), Performance standards for Antimicrobial Susceptibility Testing, 22$^{nd}$ Informational supplement.
14. 13.HipurA Bacterial Genomic DNA Purification Kit(MB505).Himedia Laboratories. Pvt.Ltd
15. Nordmann P, Naas T, Poirel L (2011) Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791-8.
16. Nair PK, Vaz MS, Bairy I, Rao SP. Clinical and microbiological profile of ventilator-associated pneumonia in intensive care units. J Clin Diagn Res. 2016;10(6):DC05–DC09.
17. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis. 2011;53(1):60–67.
18. Paul D, Dhar D, Maurya AP, Mishra S, Sharma GD, Chakravarty A. Occurrence of *Bla*$_{NDM-1}$ and other resistance genes among carbapenem-resistant *Klebsiella pneumoniae* isolates. Indian J Med Microbiol. 2017;35(4):555–558.
19. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial Gram-negative pathogens. Indian J Med Res. 2017;145(5):670–675.
20. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug-resistant organisms in a tertiary care hospital in India. J Assoc Physicians India. 2010;58(Suppl):32–36.
21. Veeraraghavan B, Walia K. Antimicrobial susceptibility profile and resistance mechanisms of global priority pathogens in India. Indian J Med Res. 2019;149(2):87–96.
22. Doi Y. Treatment options for carbapenem-resistant Gram-negative bacterial infections. Clin Infect Dis. 2019;69(Suppl 7):S565–S575.
23. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of *Bla*$_{NDM-1}$ gene in carbapenem-resistant *Klebsiella pneumoniae* from a tertiary care centre in India. Indian J Med Microbiol. 2014;32(1):34-8.
24. Kaur A, Singh S, Kaur A. Prevalence of NDM metallo-beta-lactamase in clinical isolates of *Klebsiella pneumoniae* in tertiary care hospitals. Int J Appl Basic Med Res. 2018;8(2):111-5.
25. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing organisms. Clin Infect Dis. 2012;55(7):943–950.
26. Papadimitriou-Olivgeris M, Marangos M, Fligou F, Christofidou M, Sklavou C, Vamvakopoulou S, et al. Risk factors for KPC-producing *Klebsiella pneumoniae* acquisition. J Hosp Infect. 2012;80(1):11–17.
27. Joseph NM, Sistla S, Dutta TK, Badhe AS, Rasitha D, Parija SC. Ventilator-associated pneumonia in a tertiary care hospital in India: incidence and risk factors. J Infect Dev Ctries. 2009;3(10):771-7.
28. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem-resistant *Klebsiella pneumoniae* isolated from bloodstream infection: Indian experience. Pathog Glob Health. 2017;111(5):240.