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ORIGINAL ARTICLE

Detection and prevalence of the bla_{NDM-1} gene in carbapenem-resistant *Klebsiella pneumoniae* bloodstream isolates from a tertiary care institute

Susheem Ramakrishnan[1], Gulnaz Bashir[1*], Nargis Kaur Bali[1], Sabah Rashid[1], Dona Mathew[1], Qounser Nisar[1], Mohd Iqbal Mir[2]

[1]*Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India*
[2]*State-Level VRDL Laboratory, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India*

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ABSTRACT

Background and Objectives: Carbapenem resistance mediated by bla_{NDM-1} in *Klebsiella pneumoniae* has emerged as a major challenge, particularly in intensive care settings with high antibiotic pressure. This compromises therapeutic options and contributes to poor clinical outcomes. The present study aimed to determine the prevalence of bla_{NDM-1} among isolates of *K. pneumoniae* from a tertiary care hospital, evaluate the performance of phenotypic tests against PCR-based detection, assess antimicrobial susceptibility profiles, and analyze clinical outcomes.
Materials and Methods: In this study, over 18 months, 130 non-duplicate *K. pneumoniae* isolates were identified, and antimicrobial susceptibility testing was performed by VITEK-2 Compact and broth microdilution for colistin. Imipenem-resistant isolates were subjected to the Combined disc diffusion test (CDDT) and Double disc synergy test (DDST) for metallo-beta-lactamase (MBL), and conventional PCR targeting bla_{NDM-1}. Demographic data and outcomes were recorded.
Results: Of the 130 isolates, 111 were imipenem-resistant, of which CDDT detected MBLs in 94.6%, and DDST detected MBLs in 76.6%. PCR confirmed bla_{NDM-1} in 77.5% and was more commonly associated with cases of sepsis. bla_{NDM-1} -positive isolates were resistant to β-lactams, fluoroquinolones and aminoglycosides. No isolate was found to be colistin-resistant. 26.7% of the patients with bla_{NDM-1} -positive bacteremia died.
Conclusion: This study highlights the high prevalence of bla_{NDM-1} in *K. pneumoniae* isolates. Among the phenotypic tests, CDDT outperformed DDST and showed the best agreement with PCR, supporting its use as a screening method for MBL, but confirmatory PCR remains essential. The restricted treatment options underscore the need for stringent infection control and robust antimicrobial stewardship to curb transmission and preserve last-line agents.

Keywords: Carbapenemase; Beta-lactamase; New Delhi metallo-beta-lactamase; *Klebsiella pneumoniae*; Polymerase chain reaction; Bloodstream infections; Sepsis

INTRODUCTION

Klebsiella pneumoniae is a major cause of both hospital- and community-acquired infections and is classified by the WHO as a critical-priority pathogen due to its resistance to commonly used antibiotics (1). The carbapenem group of antibiotics are generally considered to be the last resort antibiotic with proven efficacy against life-threatening infections due to multidrug-resistant Gram-negative bacilli

*Corresponding author: Gulnaz Bashir, MD, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India. Tel: +919419081260 Email: gulnaz.bashir@skims.ac.in

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(GNB) (2). However, the rising global prevalence of carbapenem-resistant Enterobacterales (CRE), particularly *K. pneumoniae* harboring the New Delhi metallo-β-lactamase bla_{NDM-1} gene, is a major concern for healthcare providers worldwide. The first documented isolation of an NDM-1-producing *K. pneumoniae* strain was from a Swedish patient with a urinary tract infection who had recently traveled to India (3). In a study published by Kumaraswamy et al. in 2010, the authors reported the existence of NDM-1-producing Enterobacteriaceae isolates from India, Pakistan and the United Kingdom. Their study identified NDM-1-producing *Escherichia coli* and *K. pneumoniae* isolates that were resistant to all antibiotic agents except colistin and tigecycline (4).

The rapid spread of NDM-1-producing *K. pneumoniae* is due to horizontal transfer of epidemic broad host-range plasmids carrying the bla_{NDM} gene (5). To date, 67 variants of NDM-type carbapenemases (NDM-1 to NDM-67) have been identified, with NDM-1 being the most prevalent worldwide and NDM-1, 4, 5, 6 and 7 being the most prevalent in India (5). NDM-1-producing *K. pneumoniae* has been mainly associated with UTI, peritonitis, septicaemia, pulmonary infections, skin and soft tissue infections and device-associated infections (6). The detection of NDM-producing GNB can be done by both phenotypic and genotypic methods. The phenotypic methods include the combined disc diffusion test (CDDT), the double disc synergy test (DDST) and the gradient diffusion strips (Epsilometer-test). Studies evaluating these phenotypic methods in comparison to molecular tests have shown high sensitivities and specificities ranging from 90-100%. Colorimetric assays, such as the Carba NP test, can also be used to detect NDM production. For confirmatory and epidemiological purposes, molecular and genomic methods are increasingly employed. These include nucleic acid amplification tests (NAATs) like PCR and LAMP, cartridge-based systems (e.g., the Xpert Carba-R assay), and advanced techniques such as multilocus sequence typing (MLST), microarray analysis, and whole-genome sequencing (WGS) (6).

Although data on the prevalence of bla_{NDM-1} in *K. pneumoniae* are widely available, they are scarce for the Union Territory of Kashmir. This study aimed to detect the bla_{NDM-1} gene by conventional PCR in nosocomial *K. pneumoniae* isolates from blood samples. Additionally, it sought to delineate the antimicrobial susceptibility profiles of these isolates, identify the patient subset with the highest burden of bla_{NDM-1}-producing *K. pneumoniae* infection, and analyze their associated clinical outcomes.

MATERIALS AND METHODS

Study design. This prospective hospital-based study was carried out in the Department of Microbiology of our Institute for a period of 18 months from 1st March 2023 to 31st August 2024. Blood samples received in the department from all areas of the hospital were processed on the BACT/ALERT®3D system (BioMérieux, France) for the recovery of bacterial pathogens. The samples positive for the growth of *K. pneumoniae* isolates were included in the present study. Only the first sample from each patient was considered. A pre-designed data collection form was used to fill in relevant demographic and clinical details of the patients, including provisional/definitive diagnosis, antibiotic intake/usage, number of days of hospital admission and any co-morbid conditions. In addition, the clinical outcome in terms of discharge with recovery or death was also noted.

Antimicrobial susceptibility testing (AST). AST for the *K. pneumoniae* isolates was performed using the VITEK-2 system, while for colistin susceptibility testing, the microbroth dilution method was used. Results were interpreted as specified by the Clinical and Laboratory Standards Institute (CLSI M100 32nd ed.) (7). *Escherichia coli* (ATCC 25922) was used as a quality control strain for AST. *K. pneumoniae* isolates resistant to either meropenem or imipenem were screened for MBL production by CDDT and DDST as described by Yong et al. and Lee et al. (8, 9).

CDDT and DDST. For performing CDDT and DDST, 0.5M EDTA was prepared, pH was adjusted to 8.0 and autoclaved. Then, 0.5 McFarland suspensions of the isolates were inoculated as a lawn onto Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd, Nashik, India). For CDDT, two discs of imipenem were placed 20mm apart, and 10µl 0.5M EDTA was added to one of the imipenem discs and incubated at 35°C for 16-18 hours. An increase in the zone of inhibition around the imipenem-EDTA disc of ≥7mm than that of the imipenem disc alone was considered as MBL positive (8). For DDST, an imipenem disc was placed

20mm apart from a blank disc containing 750 µg of EDTA (10 µl of 0.5M EDTA) onto the Mueller-Hinton agar with the test organism inoculated as a lawn and incubated at 35°C for 16-18 hours. An enhancement of the zone of inhibition in the area between the imipenem and EDTA discs was considered as DDST positive (9).

DNA extraction. DNA extraction was done by the boiling centrifugation method according to the Centers for Disease Control and Prevention (CDC) for all the carbapenem-resistant isolates of *K. pneumoniae* and control strains (10). A clinical isolate of *K. pneumoniae* harbouring the bla_{NDM-1} gene identified by PCR and gene sequencing, obtained from the Tata Medical Center, Kolkata, was included as a positive control to check primer specificity and reaction conditions due to the unavailability of a bla_{NDM-1}-positive standard strain.

PCR detection of bla_{NDM-1} gene. Carbapenem-resistant *K. pneumoniae* were screened for the presence of bla_{NDM-1} carbapenemase genes by targeting 237 bp fragments as described by Solanki et al. (11). Primers obtained from Integrated DNA Technologies Inc. consisted of forward primer 5' GCATAAGTCGCAATCCCCG 3' and reverse primer 5' CTTCCTATCTCGACATGCCG 3'. DNA amplification for the bla_{NDM-1} gene fragments for each isolate was done in a reaction mixture of 25 µl containing 5 µl of DNA, 0.25 µl of 10 pmol of each primer (forward and reverse), 0.25 µl of 10 mM dNTPs, 0.25 µl (1.25U) Taq DNA polymerase and 2.5µl of 10x Taq buffer. All PCR reagents were obtained from Sigma-Aldrich. Amplification was performed on an Applied Biosystems 7500 thermal cycler using the following program: initial denaturation at 94°C for 3 minutes; 30 cycles of denaturation at 94°C for 1 minute, annealing at 53.5°C for 1 minute, and extension at 72°C for 1.5 minutes; and a final extension at 72°C for 5 minutes. Each run included positive and negative (sterile deionized water) controls. PCR amplicons of the bla_{NDM-1} gene (expected size: 237 bp) were analyzed by 1.5% agarose gel electrophoresis and visualized under ultraviolet light using a gel documentation system. A 100 bp DNA ladder (Thermo Fisher Scientific) was included for size comparison.

Statistical analysis. Descriptive and inferential statistical analyses were carried out in the present study. All the data was analyzed using SPSS v 22.0 and R environment v 3.2.2. Chi-square and Fisher's exact test were used for categorical variables, and $P<0.05$ was taken as significant. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using PCR as the reference method. The agreement between phenotypic tests and PCR was assessed.

Ethical clearance. The ethical clearance for the study was given by the Institute's Ethical Clearance Committee bearing no: SIMS 131/IEC-SKIMS/2023-28; protocol no: 042/2023.

RESULTS

A total of 130 non-duplicate *K. pneumoniae* isolates recovered from the blood samples of patients admitted to or attending the OPD of our Institute during the study period were included in this study. Of the 130 isolates, 111 (85.4%) were resistant to imipenem, and 19 (14.6%) were sensitive to it. Out of the 111 imipenem-resistant isolates, 105 (94.6%) were metallo-beta-lactamase (MBL) positive and 6 (5.4%) MBL negative by CDDT, showing >5mm zone enhancement around imipenem plus EDTA disc as compared to the imipenem disc alone. DDST detected MBL production in 85 (76.6%) isolates, whereas 26 (23.4%) isolates were MBL negative by this method. All the isolates found to be MBL producers by DDST were positive for MBL production by CDDT also (Fig. 1). PCR confirmed the presence of the bla_{NDM-1} gene in 86/111(77.5%) imipenem-resistant *K. pneumoniae* isolates, whereas 25/111 (22.5%) were bla_{NDM-1}-negative (Fig. 2).

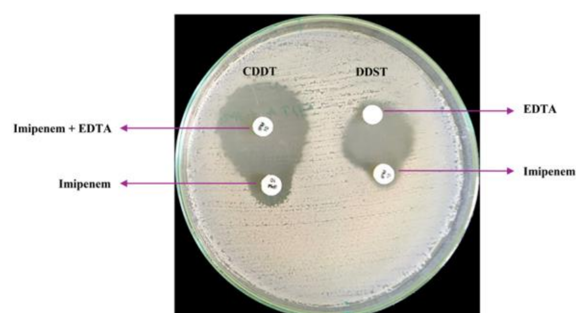

Fig. 1. Phenotypic tests (CDDT & DDST) for MBL screening.

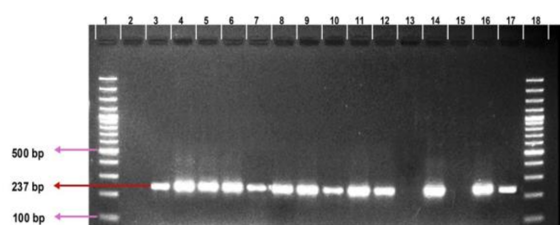

Fig. 2. Electrophoretic analysis of bla_{NDM-1} PCR amplification in *K. pneumoniae* isolates. Lanes: 1 & 18, 100 bp DNA ladder; 2, negative control (no template); 3, positive control; 4–12, 14, 16–17, bla_{NDM-1} -positive isolates (237 bp); 13 & 15, bla_{NDM-1} -negative isolates.

Additionally, 19 CDDT-positive and 9 DDST-positive isolates were bla_{NDM-1} -negative by PCR, indicating the presence of other MBLs or false-positive phenotypic results. Using PCR for bla_{NDM-1} as the reference standard, CDDT demonstrated a sensitivity of 100.0% (95% CI: 95.8-100.0) and a specificity of 24.0% (95% CI: 9.4-45.1), with a PPV of 81.9% and NPV of 100.0%. DDST showed a sensitivity of 88.4% (95% CI: 79.7-94.3) and specificity of 64.0% (95% CI: 42.5-82.0), with a PPV of 89.4% and NPV of 61.5%. Cohen's Kappa confirms DDST shows moderate agreement, while CDDT only shows fair agreement with PCR (Table 1).

Slightly more bla_{NDM-1} -positive isolates were recovered from male patients (n=49, 57%) than from female patients (n=37, 43%). Most positive isolates came from patients under 10 years of age (n=34, 39.5%), followed by those aged 50-59 years (n=16, 18.6%). The highest number of bla_{NDM-1} -positive *K. pneumoniae* isolates was recovered from the neonatal intensive care unit (NICU) (n=25, 29.1%), followed by the neurosurgery ICU and paediatric ICU, each with nine isolates (6.9%). Maximum recovery of bla_{NDM-1} -positive *K. pneumoniae* isolates was from patients with sepsis (n=27, 31.4%), followed by those with CNS disorders (n=15, 17.4%), respiratory tract disorders (n=14, 16.3), neoplastic disorders (n=11, 12.8%), trauma (n=6, 7%), pyrexia of unknown origin (n=4, 4.6%) and miscellaneous (n=9, 10.5%). Prior intake of ceftriaxone, piperacillin-tazobactam, colistin, vancomycin and carbapenems was seen more often in patients from whom bla_{NDM-1} -positive strains were recovered. Seventy patients (81.4%) from whom bla_{NDM-1} -positive *K. pneumoniae* were recovered had a hospital stay of >1 week, whereas 16 patients (18.6%) had a hospital stay of <1 week. The majority of these patients (n=63, 73.3%) recovered fully, whereas 23 (26.7%) expired (Table 2).

All the bla_{NDM-1} harbouring isolates were resistant to amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, cefuroxime, piperacillin-tazobactam, imipenem and meropenem, whereas nine isolates were moderately sensitive to colistin, three isolates were moderately sensitive to levofloxacin, two to amikacin and gentamicin each and one was moderately sensitive to ciprofloxacin. None of the isolates were resistant to colistin. It was observed that 67 (77.9%) of the bla_{NDM-1} -producing *K. pneumoniae* strains had the MIC for imipenem ≥ 16 μg/mL while 54 (62.8%) had the MIC for meropenem ≥ 16 μg/mL (Table 3).

Table 1. Comparison of phenotypic tests (CDDT and DDST) with PCR for detecting the bla_{NDM-1} gene in *K. pneumoniae* isolates.

Phenotypic Test		PCR for the bla_{NDM-1} gene		Sensitivity (95% CI) (%)	Specificity (95%CI) (%)	PPV (%)	NPV (%)	Kappa
		Positive (n=86)	Negative (n=25)					
CDDT	Positive	86	19	100.0	24.0	81.9	100.0	0.33*
	Negative	0	6	(95.8-100)	(9.4-45.1)			
DDST	Positive	76	9	88.4	64.0	89.4	61.5	0.52**
	Negative	10	16	(79.7-94.3)	(42.5-82.0)			

P-value <0.001 (Highly significant).

Abbreviations: CDDT-Combined Disk Diffusion Test; DDST-Double Disk Synergy Test; PCR-Polymerase Chain Reaction; PPV-Positive Predictive Value; NPV-Negative Predictive Value; Kappa: *Fair agreement, ** Moderate agreement

Footnote: CDDT and DDST detect all the metallo-beta-lactamases, whereas the PCR in this study was specific to the bla_{NDM-1} gene.

Table 2. Demographic and clinical details of patients from whom *bla*$_{NDM-1}$ -positive and negative *K. pneumoniae* were recovered.

Variable	*bla*$_{NDM-1}$-positive n=86 (%)	*bla*$_{NDM-1}$-negative n=25 (%)	P-value
Gender			
Male	49 (57)	16 (64)	0.689
Female	37 (43)	9 (36)	
Age group			
0-19	38	5 (20)	0.004
20-39	13	8	
40-59	21	6 (24)	
>60	14	6 (24)	
Location			
Neonatal ICU	25 (29.1)	2 (8)	<0.001
Pediatric ICU	9 (10.5)	0	<0.001
Neurosurgical ICU	9 (10.5)	1 (4)	0.006
Medicine and allied *	30 (34.9)	16 (64)	0.023
Surgery and allied **	13 (15.1)	6 (24)	0.539
Diagnosis			
Sepsis	27 (31.4)	6 (24)	<0.001
CNS disorders	15 (17.4)	3 (12)	<0.001
Respiratory tract infections	14 (16.3)	6 (24)	0.043
Neoplasms	11 (12.8)	4 (16)	0.054
Trauma	6 (7)	3 (12)	0.306
PUO	4 (4.6)	-	0.043
Miscellaneous	9 (10.5)	3 (12)	0.068
Prior antibiotic intake			
Ceftriaxone	57 (66.3)	15 (60)	0.729
Piperacillin-tazobactam	30 (34.9)	13 (52)	0.189
Colistin	30 (34.9)	12 (48)	0.340
Imipenem	20 (23.3)	5 (20)	0.920
Meropenem	27 (31.4)	12 (48)	0.059
Length of hospital stay			
< 1 week	16 (18.6)	13 (52)	<0.001
> 1 week	70 (81.4)	12 (48)	
Outcome			
Recovery	63 (73.3)	22 (88)	0.205
Death	23 (26.7)	3 (12)	

* Medicine and allied: General medicine, Haematology, Medical gastroenterology, Radiotherapy, Nephrology, Medical oncology, Endocrinology, Critical care unit.
** Surgery and allied: Neurosurgery, Surgical gastroenterology, CVTS, Surgical oncology, Urology, Plastic surgery, Pediatric surgery, Kidney transplant unit, Surgical ICU.

Table 3. Correlation of MIC values of carbapenems in *bla*$_{NDM-1}$ producing and non-producing *K. pneumoniae* isolates.

bla$_{NDM-1}$ PCR	MIC of imipenem (µg/mL)					MIC of meropenem (µg/mL)				
	2	4	8	16	32	2	4	8	16	32
Positive	-	5	5	67	9	-	9	13	54	10
Negative	-	13	-	10	2	1	12	3	9	-

DISCUSSION

Emergence and global spread of carbapenem-resistant *Enterobacterales* (CRE), particularly carbapenem-resistant *K. pneumoniae* (CRKP), represents a calamitous event that can undermine progress made by scientific institutions in the fight against antimicrobial resistance (12). Most of the CRKP now encountered across the globe carry the bla_{NDM-1} gene. The present study is the first from Kashmir to find out the extent to which bla_{NDM-1} is prevalent in *K. pneumoniae* isolates recovered from blood samples of patients admitted to a 1250-bedded tertiary care hospital.

A total of 130 *K. pneumoniae* isolates were included. Of these, 111 (85.4%) were carbapenem-resistant. This represents a substantial increase from a previous report of 22% carbapenem resistance among *K. pneumoniae* isolates at our institution, indicating a significant shift in the local antimicrobial resistance profile (13). According to the Center for Disease Dynamics, Economics and Policy (CDDEP), 60% of the Indian *K. pneumoniae* isolates are resistant to carbapenems (14). Similarly, a review by Das et al. reported high national prevalence rates of carbapenem resistance, exceeding 70% in *Acinetobacter baumannii* and 50% in *K. pneumonia* (15).

A total of 105 (94.6%) isolates were MBL positive with CDDT, and 85 (76.6%) isolates were MBL positive by DDST. All the isolates found to be MBL producers by DDST were positive for MBL production by CDDT also. Thus, CDDT was more sensitive in detecting MBL-positive *K. pneumoniae* isolates. These results are in concordance with an earlier study conducted at our institution, where the CCDT performed better than DDST for the detection of MBL in *Acinetobacter baumannii* isolates (16). In another study by Manoharan et al., the authors found that CDDT had the highest sensitivity and specificity of 87.8% and 84.4% when compared to the E-test for the detection of MBL in *P. aeruginosa* (17). Gautam et al., in their study done in Nepal, reported a better concordance between CDDT and PCR for the detection of the NDM-1 gene as compared to DDST and PCR, as measured using the kappa agreement tool (18).

All the imipenem-resistant isolates were subjected to PCR for the detection of bla_{NDM-1} gene, out of which 86 (77.5%) isolates were bla_{NDM-1} positive. Results similar to ours have been reported previously from China by Zhu et al., wherein the authors reported NDM-1 gene positivity in 77.3% CRKP isolates (19). In a study from Indonesia done by Saharman et al., the authors reported bla_{NDM-1} gene positivity in 96% CRKP isolates (2). Studies carried out in India have reported extensive presence of bla_{NDM-1} gene in isolates of *K. pneumoniae* ranging from 11.8% to 100% (20-22). MIC value of both imipenem and meropenem, as given by VITEK-2 Compact, was ≥16 μg/mL for 77.9% and 62.8% of the bla_{NDM-1} –producing *K. pneumoniae* isolates (Table 3). Similar results were seen by Solanki et al. respectively (2014), where the authors found that most of the NDM-1-producing *Enterobacterales* had an MIC value of ≥16 μg/mL (11).

Recent literature highlights the global dissemination of bla_{NDM-1} –producing *K. pneumoniae*, driven largely by plasmid-mediated horizontal gene transfer rather than clonal expansion alone. Broad-host-range plasmids carrying bla_{NDM-1} have facilitated rapid inter- and intra-species spread across Asia, Europe, Africa, and the Middle East, with South Asia remaining a major endemic reservoir (6, 15). Of particular concern is the increasing association of bla_{NDM-1} with high-risk and hypervirulent clones of *Klebsiella pneumoniae* such as ST147, which has been reported globally in recent genomic surveillance studies and linked to both multidrug resistance and enhanced virulence traits (12, 23). The high prevalence of bla_{NDM-1} observed in the present study is therefore consistent with contemporary national and international data and underscores the convergence of antimicrobial resistance, plasmid mobility, and clonal virulence as a major threat to clinical management and infection control.

Cohen's Kappa was applied to assess agreement between CDDT and DDST with PCR for the detection of the bla_{NDM-1} gene. For CDDT, κ = 0.33 indicated fair agreement. Although CDDT achieved perfect sensitivity (100%) and negative predictive value (100%), its specificity was very low (24%), resulting in numerous false positives and limited reliability. For DDST, κ = 0.52 reflected moderate agreement. DDST showed a more balanced diagnostic profile, with sensitivity of 88.4%, specificity of 64%, positive predictive value of 89.4%, and negative predictive value of 61.5%. Overall, DDST correlated more consistently with PCR than CDDT, making it the more dependable phenotypic method. However, neither test reached strong agreement, underscoring the need for molecular confirmation to ensure accurate detection of bla_{NDM-1}-mediated resistance.

The discordance between phenotypic MBL detection and bla_{NDM-1} PCR results is expected, as phenotypic assays detect all MBL activity, while the molecular assay in this study was restricted to bla_{NDM-1}. Recent studies emphasized that CDDT and DDST remain useful as initial screening tools for MBLs in resource-limited settings. However, their diagnostic performance varies widely when compared with molecular methods (6, 8, 15, 24-27). Studies consistently report high sensitivity but limited specificity for EDTA-based phenotypic assays, attributed to the detection of non-NDM metallo-β-lactamases or non-specific EDTA effects. The high sensitivity and low specificity of CDDT observed in the present study, along with the comparatively lower sensitivity but better specificity of DDST, closely mirror these recent findings.

CDDT and DDST remain valuable tools for routine detection of MBL-producing *K. pneumoniae* in low-resource settings, due to their low cost, high sensitivity, ease of performance, and minimal infrastructure requirements (6, 15). However, their lack of genotypic specificity can result in false-positive results due to the detection of non-NDM MBLs. In contrast, PCR-based assays offer high specificity and accuracy for detecting bla_{NDM-1} and are essential for confirmation, surveillance, and outbreak investigations, but their widespread implementation is constrained by higher costs, the need for specialized equipment, and technical expertise (23, 24). Consequently, a pragmatic diagnostic approach in resource-limited laboratories involves the use of phenotypic methods for initial screening, supplemented by targeted molecular testing where feasible.

Most of the bla_{NDM-1} -positive *K. pneumoniae* in our study were recovered from male patients (n=49, 57%). This is similar to the earlier studies by Rahman et al. and Li et al., in which the authors found greater isolation of bla_{NDM-1} -positive isolates from male patients (28, 29). Furthermore, a significantly higher isolation of bla_{NDM-1} -producing *K. pneumoniae* was seen in patients in the age group of <10 years (n=34, 39.5%) and from patients admitted in the neonatal ICU (n=25, 29.1%). Huang et al. reported an outbreak of bla_{NDM-1} -producing *K. pneumoniae* in a neonatal unit of their hospital in China (30). Likewise, Zhu et al. reported the outbreak of bla_{NDM-1} -producing *K. pneumoniae* ST76 and ST37 isolates among neonates in China (19). In a study from India, Pathak et al. reported the outbreak of colistin-resistant bla_{NDM-1} -producing *K. pneumoniae* causing BSI among neonates (31).

Patients admitted to the neonatal intensive care unit (NICU) are at high risk for healthcare-associated infections. This vulnerability results from an interplay of factors, including immature immune systems, low birth weight, prematurity, and underlying conditions, which often lead to prolonged hospitalization, intensive antimicrobial exposure, and the use of indwelling devices. The risk is further amplified by the potential for cross-contamination via healthcare workers' hands or contaminated equipment.

All the bla_{NDM-1} -producing *K. pneumoniae* isolates (n=86) in our study were resistant to amoxicillin-clavulanic acid, cephalosporins, piperacillin-tazobactam, imipenem and meropenem. However, none of the isolates were resistant to colistin. Results similar to ours have been previously reported by many authors from India, in which NDM-producing Gram-negative isolates were highly resistant to commonly used antimicrobial agents (21, 28). It is a well-known fact that bla_{NDM-1} -producing bacteria carry plasmids with resistance determinants to major antibiotic classes like aminoglycosides, tetracyclines, macrolides, sulfamethoxazole and carbapenems (6). In our study, most of the bla_{NDM-1} -carrying *K. pneumoniae* isolates were recovered from blood samples of patients with sepsis (n=27, 31.4%). Kumarasamy et al. in their collaborative study conducted in India, Sweden, Pakistan and the UK, have reported bloodstream infection/sepsis to be the most common infection caused by bla_{NDM-1}-producing *K. pneumoniae* (4).

In the present study, the length of hospital stay of more than one week was seen in more patients from whom bla_{NDM-1} -positive *K. pneumoniae* were recovered (n=70, 81.4%). The most important risk factors for CRKP infection published earlier include prior exposure to antibiotics, ICU length of stay, use of invasive procedures and rectal colonization (32). The majority of the patients in our study recovered fully (n=63, 73.3%) and were discharged from the hospital, whereas 23 (26.7%) patients expired. In a 3-year prospective study conducted by Falcone et al. in Italy, the authors reported a 29.7% overall 30-day mortality among patients suffering from infection due to NDM-producing *Enterobacterales* (23).

From an epidemiological perspective, the high prevalence of the bla_{NDM-1} gene among CRKP is indicative of sustained endemic circulation rather than sporadic introduction. The predominance of

bla_{NDM-1} -positive isolates in intensive care and neonatal units, together with prolonged hospital stay and extensive prior exposure to broad-spectrum antibiotics, suggests healthcare-associated transmission under strong antimicrobial selection pressure (6, 15). Similar patterns have been reported internationally, where tertiary-care hospitals act as amplification hubs for NDM-producing *K. pneumoniae*, facilitated by plasmid-mediated horizontal gene transfer and silent colonization (23).

Limitations. As a single-center study, the results cannot be extrapolated to a larger population, as patient demographics, antimicrobial practices, and infection control measures tend to differ across various settings. Also, the current study focused on the prevalence of bla_{NDM-1} in carbapenem-resistant *K. pneumoniae* isolates recovered from blood samples only.

Multicentric studies need to be conducted to evaluate the extent of spread of these enzymes in various Gram-negative bacteria recovered from different clinical samples. Multivariate modelling to identify independent predictors of bla_{NDM-1} positivity, not done in the present study, is also acknowledged as a limitation.

CONCLUSION

This study highlights the high prevalence of bla_{NDM-1} -producing *K. pneumoniae* isolates in bloodstream infections within a tertiary care hospital, particularly affecting vulnerable populations such as neonates. A high resistance to carbapenems along with other categories of antimicrobial agents, is worrisome as the treatment options are severely limited. The results of our study call for continuous surveillance of such resistance determinants, the need to incorporate rapid diagnostic modalities with molecular confirmation as a part of routine diagnostics and a strict implementation of infection control guidelines along with antimicrobial stewardship to curb and limit the spread of such deadly pathogens.

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