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ORIGINAL ARTICLE

# Phylogenetic analysis of *bla*$_{NDM}$ genes of carbapenem resistant uropathogens isolated from federal tertiary care hospital, Pakistan: insights into the evolution and dissemination of drug resistance

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## ABSTRACT

**Background and Objectives:** Global health is seriously threatened by the rise of carbapenem-resistant Enterobacterales (CRE). The *bla*$_{NDM}$ gene, a key carbapenemase coding gene, causes global health concern due to its multidrug resistance and easy spread through mobile genetic elements. This study aimed to identify and genetically characterize the *bla*$_{NDM}$ genes from uropathogens, its antibiotic susceptibility, and its correlation with global sequences.

**Materials and Methods:** Urine samples were processed following microbiological guidelines. Isolates were identified using API-20E. Antibiotic susceptibility was tested using disc diffusion method, and bacterial DNAs were extracted for *bla*$_{NDM}$ gene sequencing for phylogenetic analysis.

**Results:** CREs were detected in 11.92% (n=51) of the 428 Enterobacterales. Among CRE isolates, 45% (n=23) were positive for *bla*$_{NDM}$ gene harbored by *Klebsiella pneumoniae* (57%), followed by *Escherichia coli* (26%). Uropathogenic CRE, harboring *bla*$_{NDM}$, revealed susceptibility of 34.78%, 60.87%, and 65.22% to amikacin, nitrofurantoin, and fosfomycin respectively. The *bla*$_{NDM-5}$ variant was most common (69.57%), followed by *bla*$_{NDM-1}$ (26.09%) and *bla*$_{NDM-7}$ (4.35%). Phylogenetic analysis revealed that *bla*$_{NDM}$ variants exhibit diverse relationships with Pakistani and worldwide sequences.

**Conclusion:** The significant presence of *bla*$_{NDM}$ in uropathogens, along with extensive antibiotic resistance, underscores the urgent need for continuous monitoring and antibiotic stewardship programs to manage the growing threat of CRE infections.

**Keywords:** Urine; NDM; Carbapenem resistance; Colistin; Enterobacteriaceae

## INTRODUCTION

Enterobacterales, a large order of Gram-negative bacteria, include notorious pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species. These bacteria are frequently involved in various types of infections such as urinary tract infections (UTIs), bloodstream infections, and

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pneumonia (1). These infections can lead to severe complications and are a major cause of hospital-acquired infections (HAI) globally. It is projected that the number of deaths caused by antibiotic-resistant bacterial infections may surpass 10 million by 2050 (2). Antibiotic resistance due to the overuse and misuse of antibiotics over time has led to high rates of morbidity, mortality and healthcare costs. It is estimated that more than half of *E. coli* and *K. pneumoniae* will develop resistance against cephalosporins very soon (3). It is also observed that members of Enterobacterales also exhibit multidrug resistance (MDR), extremely-drug resistance (XDR) and even pan-drug resistance (4).

Bacteria have devised several mechanisms to acquire antibiotic resistance. Plasmids carrying resistance genes are particularly important, as they can be transferred by horizontal gene transfer from one bacterium to another, resulting in the dissemination of genes, which encode enzymes that can degrade the antibiotics (5). Among these enzymes, β-lactamases capable of degrading β-lactam antibiotics (e.g. penicillin, cephalosporins and even carbapenems) are the most significant and they include disastrous carbapenemases. Carbapenems have been the cornerstone and the last resort drugs in treating MDR infections. However, the emergence of carbapenem-resistant Enterobacterales (CRE) has become a global health crisis in the past decade. This resistance has severely limited the treatment options for MDR Enterobacterales infections (6).

Among carbapenemases, New-Delhi metallo-β-lactamase (NDM) is one such enzyme encoded by the $bla_{NDM}$ gene that can typically degrade β-lactam antibiotics including carbapenems except monobactams (7). The $bla_{NDM}$ gene was first identified in Sweden, in a Swedish patient of Indian origin who had been hospitalized in New-Delhi, India for gluteal abscess (8). Now, it has been reported in a wide range of bacterial species worldwide. It is commonly found in members of Enterobacterales especially, *E. coli*, *K. pneumoniae*, and *Enterobacter* species (7). Significant challenges are faced while treating these infections. To date, several different variants have been identified including $bla_{NDM-1}$, $bla_{NDM-2}$, $bla_{NDM-3}$, and many more (9). This diversity is driven by mutations, which leads to the emergence and development of new variants demonstrating enhanced antibiotic resistance. Therefore, it is important to understand the genetic diversity of $bla_{NDM}$ gene to establish surveillance strategies and tailored antibiotic therapies to effectively combat the spread of antibiotic resistance.

UTIs are among one of the most prevalent bacterial infections affecting millions of individuals every year. These infections can lead to severe life-threatening conditions if left untreated without use of potent antibiotics (10). This study was performed to genetically characterize the $bla_{NDM}$ gene from CRE, isolated from UTI patients. By utilizing molecular techniques (PCR and sequencing), we aimed to demonstrate the genetic diversity of the $bla_{NDM}$ gene variants and their distribution in uropathogens and further to explore possible therapeutic options. Based on these genetic characteristics, effective treatment strategies can be developed. In addition, this study provides valuable insights that can help in the implementation of antibiotic stewardship programs and infection control practices.

## MATERIALS AND METHODS

**Bacterial strains.** The study was carried out over a period of one year from February 2022 to January 2023. Bacterial isolation and susceptibility testing were performed in the microbiology laboratory of Pakistan Institute of Medical Sciences (PIMS), Islamabad. Further bacterial DNA isolation and genetic testing were performed at Comsats University Islamabad (CUI), Islamabad. Ethical approval was acquired by the ethical committees of Shaheed Zulfiqar Ali Bhutto Medical University affiliated with PIMS (No. F.1-1/2015/ERB/SZABMU/678 dated 25-11-2020) and CUI, Islamabad.

Urine samples of patients were collected from various wards, including surgical wards, medical wards, intensive care units, and OPDs. Clinical details of these patients were collected as presented in Table 1. These samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) media and incubated overnight at 36 + 1°C. After incubation, all positive cultures were identified by phenotypic characteristics of bacterial colonies, Gram staining, oxidase, catalase and biochemical tests using API-20E.

**Phenotypic detection of carbapenemases.** Carbapenemase and metallo-β-lactamase (MBL) screening was performed through the modified Carbapenem Inactivation Method (mCIM) and EDTA-modified Carbapenem Inactivation Method (eCIM) (11).

**Antimicrobial susceptibility testing.** All positive cultures with Enterobacterales were processed for antibiotic susceptibility testing (AST) on Mueller-Hinton (MH) agar plates. Imipenem (IMP), meropenem (MEM), and ertapenem (ETP) were used to screen for CRE. Other antibiotics for AST included amoxicillin (AML), amoxicillin/clavulanic acid (AMC), ceftriaxone (CRO), cefixime (CFM), cefepime (FEP), gentamicin (CN), tobramycin (TOB), amikacin (AK), aztreonam (ATM), norfloxacin (NOR), ciprofloxacin (CIP), levofloxacin (LEV), sulfamethoxazole/trimethoprim (SXT), nitrofurantoin (F), fosfomycin (FOT), tazobactam/piperacillin (TZP), cefoperazone/sulbactam (SCF), and tetracycline (TE). Results were interpreted according to CLSI guidelines (11). Minimum inhibitory concentration (MIC) of colistin (CT) was determined through micro-broth dilution method. MIC breakpoints for colistin susceptibility have not been established by CLSI to date. However, intermediate MIC breakpoint is ≤2 µg/ml for colistin in Enterobacterales (11). Cefoxitin (FOX) was used for AmpC screening on disk diffusion method. Isolates resistant to cefoxitin were interpreted as AmpC producers. Isolates resistant to carbapenems were included in the study.

***bla*$_{NDM}$ variant determination and phylogenetic analysis.** Bacterial DNA extraction was performed using a bacterial DNA extraction kit according to manufacturer instructions (FavorPrep™, Favorgen Biotech Corp. Taiwan). Extracted DNA was subjected to PCR for 16s rRNA gene using F (5-AGAGTTTGATCCTGGCTCAG-3) and R (5-TACGGTTACCTTGTTACGACTT-3) primers, to confirm the presence and integrity of bacterial DNA. PCR of *bla*$_{NDM}$ gene was carried out using F (5-ATGGAATTGCCCAATATTATG-3) and R (5-TCAGCGCAGCTTGTCGGCC-3) primers amplifying 813 bp amplicon (Fig. 1), which were sequenced from Macrogen Inc. (South Korea), a commercial DNA sequencing facility. DNA dragon software was used to assemble forward and reverse amplified sequences into a consensus sequence. These sequences were aligned using MAFFT software. These consensus sequences were subjected to NCBI database and aligned with reference *bla*$_{NDM}$ sequences using NCBI blast facility to determine *bla*$_{NDM}$ variants. Additionally, the *bla*$_{NDM}$ gene sequences already reported in different parts of the world were retrieved from GenBank, using Taxonomy Browser. The *bla*$_{NDM}$ gene sequences from urine samples were subjected to Molecular Evolutionary Genetics Analysis (MEGA-6) software for phylogenetic analysis to understand the worldwide phylogenetic relation of *bla*$_{NDM}$ gene prevalent in Pakistani population experiencing UTI.

## RESULTS

**Demographics of CRE isolates.** In all, 1002 urine samples were isolated. Enterobacterales were found in 42.71% (n=428) of the samples. Of these, 11.92% (n=51) of the cultures were found to be CRE (Table 1). The M/F ratio of the CRE isolates was 1.2:1, with 54% (n=28) males and 45% (n=23) females. The age distribution of CRE isolates showed that 24% of these isolates were in the 21-30 age range, followed by the 31-40 and 61-70 age groups, with 18% each. Patients from Emergency (37%) accounted for the majority of the CRE isolates (Fig. 2a). Urinary CRE isolates were predominantly associated with HAIs (80.39%) and UTI (39.22%) as primary illnesses. These patients were 54.90% residents of Islamabad territory (Table 1). According to isolate-wise distribution, 45% were CRE positive, isolated from *K. pneumoniae* and *E. coli*, followed by *Enterobacter* species (10%) (Fig. 2b).

**Carbapenemase and MBL screening.** The mCIM test showed that 90.20% (46/51) CRE produced carbapenemases. The remaining isolates might have non-enzymatic mechanism of carbapenem resistance including reduced outer membrane permeability, increased efflux pump activity, which were not explored in this study. However, the eCIM test on CRE isolates showed that 63.04% (29/46) of them produced MBL. In eCIM negative isolates, the synthesis of serine-β-lactamase may have caused carbapenem resistance (Table 2).

***bla*$_{NDM}$ screening and their demographics.** Among eCIM positive isolates, 79.31% (n=23/29) were *bla*$_{NDM}$ gene harboring isolates. Among *bla*$_{NDM}$ positive isolates, 57% (n=13) were isolated from males and 43% (n=10) were from females with a M/F ratio of 1.3:1. The age-wise distribution shows that 30.43% (n=7) of the isolates with *bla*$_{NDM}$ were from the age range of 21-30 years, followed by 21.74% (n=5) from the age group of 11-20 years. Patients from the emergency department accounted for 35% (n=8) of the iso-

**Table 1.** Clinical data of CRE, *bla*$_{NDM}$ positive and *bla*$_{NDM}$ negative isolates.

| | CRE (%) % (N=51) | *bla* $_{NDM}$ positive (%) % (N=23) | *bla*$_{NDM}$ negative (%) including N/A % (N=28) |
|---|---|---|---|
| **a. Primary Illness** | | | |
| Carcinoma/Tumor | 9.80 (5) | 4.35 (1) | 14.29 (4) |
| Cardiac Related | 3.92 (2) | 4.35 (1) | 3.57 (1) |
| Kidney Related | 9.80 (5) | 21.74 (5) | 0 |
| Liver cirrhosis | 1.96 (1) | 0 | 3.57 (1) |
| Meningitis | 3.92 (2) | 0 | 7.14 (2) |
| Ortho Related/RTA | 7.84 (4) | 8.70 (2) | 7.14 (2) |
| Pneumonia/VAP | 7.84 (4) | 8.70 (2) | 7.14 (2) |
| Septicemia/Fever | 15.69 (8) | 17.39 (4) | 14.29 (4) |
| UTI | 39.22 (20) | 34.78 (8) | 42.86 (12) |
| **b. Categorization of community or hospital acquired infections** | | | |
| HAI | 80.39 (41) | 82.61 (19) | 78.57 (22) |
| CAI | 19.61 (10) | 17.39 (4) | 21.43 (6) |
| **c. City** | | | |
| Abbottabad | 3.92 (2) | 4.35 (1) | 3.57 (1) |
| Azad Jummu Kashmir | 9.80 (5) | 17.39 (4) | 3.57 (1) |
| Gilgit Baltistan | 3.92 (2) | 4.35 (1) | 3.57 (1) |
| Islamabad Territory | 54.90 (28) | 39.13 (9) | 67.86 (19) |
| Punjab Province | 9.80 (5) | 8.70 (2) | 10.71 (3) |
| Rawalpindi nearby cities | 17.65 (9) | 26.09 (6) | 10.71 (3) |

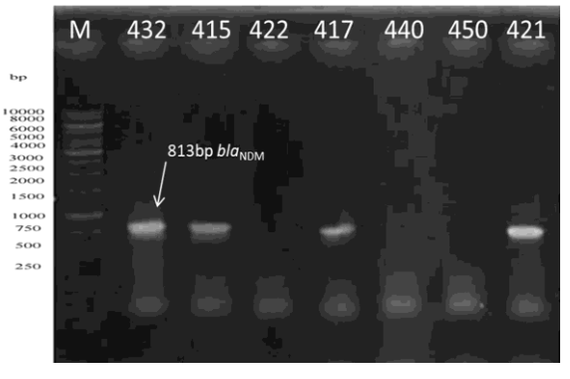


**Fig. 1.** Detection of *bla*$_{NDM}$ gene by PCR and its visualization on gel electrophoresis. M is 1K bp ladder. Lane 432, 415, 417, 421 gave positive results with 813 bp band and Lane 422, 440 and 450 gave no results.

lates, whereas SICU accounted for 17% (n=4) of them (Fig. 2a). It was revealed that 45% (n=23/51) of CRE were positive for *bla*$_{NDM}$ belonging to *K. pneumoniae* (57%), *E. coli* (26%), and *Enterobacter* species (17%).

Genetic characterization of *bla*$_{NDM}$ gene from our study revealed three variants including *bla*$_{NDM-5}$, the most common variant (69.5%), followed by *bla*$_{NDM-1}$ (26%) and *bla*$_{NDM-7}$ (4%) (Table 2). *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ were 50% (n=3) and 62.50% (n=10), 33.33% (n=2) and 25% (n=4), 16.67% (n=1) and 12.50% (n=2) associated with *K. pneumonia*, *E. coli* and *Enterobacter* species respectively. *bla*$_{NDM-7}$ was exclusively (n=1) harbored by *Enterobacter* species in our study.

**Antibiotic susceptibility testing.** CRE showed complete resistance to, ceftriaxone, cefixime, cefepime, aztreonem and amoxicillin/clavulanic acid. Among aminoglycosides, CRE were comparatively less resistant to amikacin (50.98%) than other group members. Levofloxacin (90.20%), ciprofloxacin (98.04%), and norfloxacin (98.04%) all exhibited significant resistance in CRE isolates. Co-trimoxazole and tetracycline were 82.35% and 80.39% resistant respectively. Whereas, fosfomycin and nitrofurantoin were 54.90% and 50.98% sensitive respectively (Fig. 3a). Piperacillin/tazobactam and cefoperazone/sulbactam were 94.12% and 92.16% resistance respectively. No CRE isolate was resistance to colistin (Table 2). Cefoxitin, which was used to test for AmpC screening, showed

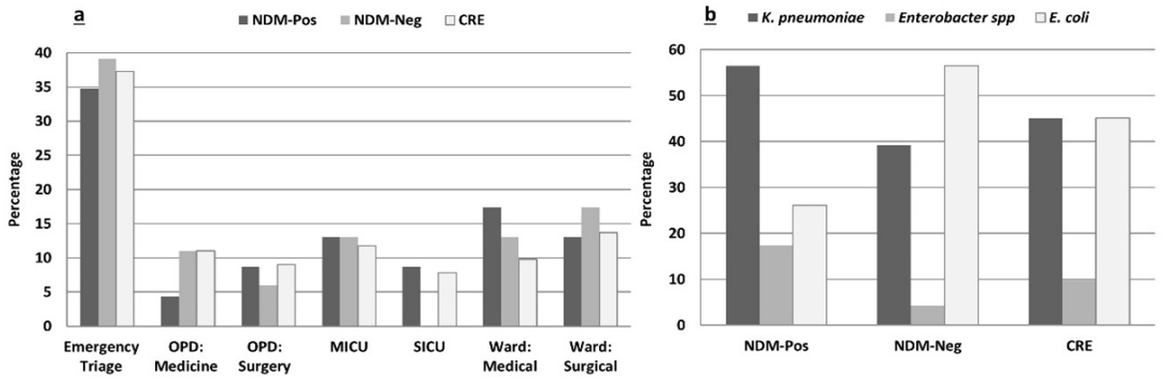


**Fig. 2.** (a) Location wise distribution of CRE, *bla*$_{NDM}$ positive and *bla*$_{NDM}$ negative isolates. (b) Bacterial species distribution among CRE, *bla*$_{NDM}$ positive and *bla*$_{NDM}$ negative isolates.

**Table 2.** Phenotypic screening tests

| | Phenotypic Screening Tests | | MBL gene | *bla* NDM variants | | |
|---|---|---|---|---|---|---|
| | mCIM | eCIM | *bla* NDM | *bla* NDM-1 | *bla* NDM-5 | *bla* NDM-7 |
| Positive | 46/51 (90.20%) | 29/46 (63.04%) | 23/29 (79.31%) | 6/23 (26.09%) | 16/23 (69.57%) | 1/23 (4.35%) |
| Negative | 5/51 (9.80%) | 17/46 (36.96%) | 6/29 (20.69%) | - | - | - |
| Invalid (NA) | 0 | 5/51 (9.80%) | - | - | - | - |

84.31% resistance, indicating that AmpC β-lactamases may have been co-harbored in addition to carbapenem resistance.

Isolates harboring *bla*$_{NDM}$ were generally more resistant than *bla*$_{NDM}$ negative isolates except for fosfomycin and nitrofurantoin which were 65.22% and 60.87% sensitive for *bla*$_{NDM}$ positive isolates respectively, as compared to 46.43% and 42.86% sensitive respectively against *bla*$_{NDM}$ negative isolates (Fig. 3a). *bla*$_{NDM-5}$ harboring isolates were relatively more susceptible as compared to *bla*$_{NDM-1}$ harboring isolates. Whereas, there was only one *bla*$_{NDM-7}$ harboring isolate which was more resistant to *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ harboring isolates (Fig. 3b).

**Phylogenetic analysis of *bla*$_{NDM}$ genes.** Phylogenetic analysis of *bla*$_{NDM}$ (Fig. 4) with worldwide sequences using the maximum likelihood method revealed distribution in different groups. *bla*$_{NDM-1}$ genes from our study showed an association with genes from Pakistan (CP078034.1, CP346080, OP346087) and from Vietnam (CP098372.1) in four groups (Fig. 4a). *bla*$_{NDM-5}$ of our study were divided in three groups. These had a close association with Portugal (OP046713.1), Pakistan (OP346106), USA (CP054412.1), and South Korea (MK986791.1) genes (Fig. 4b). *bla*$_{NDM-7}$ sequence from our study showed an association with USA (CP021759.1) (Fig. 4c). It is observed that study sequences have a diverse relationship with worldwide sequences.

## DISCUSSION

UTIs present a significant health hazard to the global health sector due to their high prevalence and potential for the development of severe complications if left untreated. The emergence and spread of *bla*$_{NDM}$ gene have added to the challenges of managing UTIs since NDM enzymes degrade carbapenem antibiotics, often considered as last resort, leading to increased rates of morbidity, mortality, and healthcare costs. Therefore, continuous monitoring of carbapenem resistance and genetic determinants causing resistance in uropathogens is crucial. In Pakistan, a limited number of studies have been conducted on this subject (12, 13). In this study, we targeted CRE isolates from urine. We isolated and genetically characterized *bla*$_{NDM}$ genes from urine isolates, depicted the phylogenetic relation of *bla*$_{NDM}$ variants with worldwide sequences and narrated the therapeutic options. This is the first study of its kind which

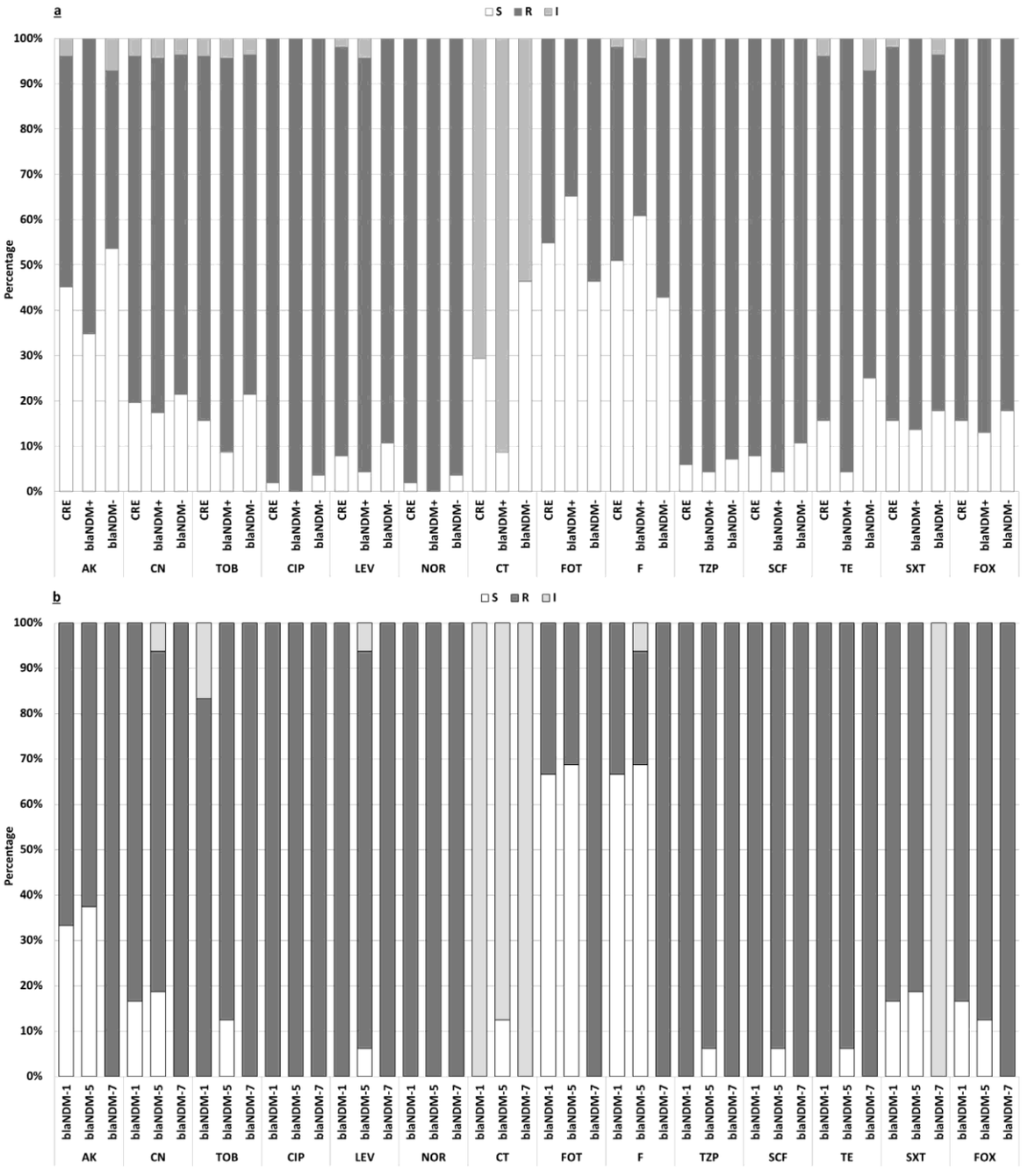


**Fig. 3.** (a) AST profile of CRE, *bla*$_{NDM}$ positive and *bla*$_{NDM}$ negative isolates. (b) AST profile of *bla*$_{NDM}$ variants -1, -5 and -7. For simplicity, exclusively resistant antibiotics (ATM, AML, AMC, CRO, CFM, and FEP) are not mentioned in Figure.

highlights the prevalence and clinical significance of these antibiotic-resistant determinants in UTI patients in Pakistan. Our findings not only emphasize the need for ongoing surveillance and control measures but also contribute to the growing body of evidence regarding the spread of *bla*$_{NDM}$genes in clinical settings.

Our study found that 11.92% of these Enterobacterales were CRE, which is similar to the findings of previous study conducted in Pakistan that found 19% CRE (14). Of these CRE, *K. pneumoniae* and *E. coli* were each found in 45% of our study's isolates. In another study from Pakistan, *E. coli* was found to be 68.3% and *K. pneumoniae* to be 31.7% (1). However, our results contradict with studies carried out in Libya, where *E. coli* was higher (57%), and Ethio-

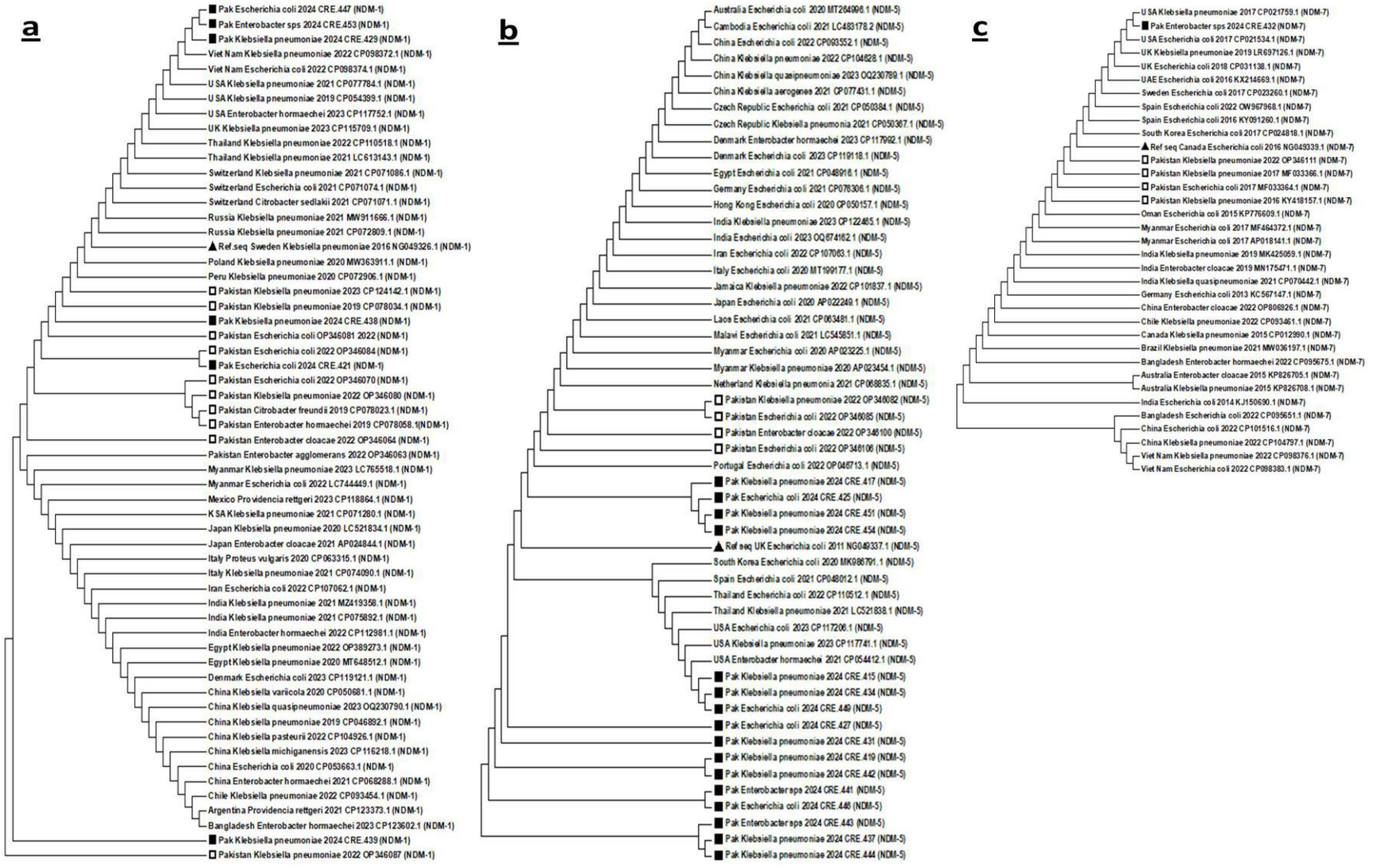


**Fig. 4.** Phylogenetic analysis of *bla*$_{NDM}$ variants with worldwide sequences. (a) *bla*$_{NDM-1}$ sequences; (b) *bla*$_{NDM-5}$ sequences; (c) *bla*$_{NDM-7}$ sequence. Present study sequences are tagged as black squares, previously reported Pakistani sequences are tagged as white squares and reference sequences are tagged as black triangles. Sequence of information in each sequence is as; "Country name-Bacterial name-(Bacterial ID #)-Year-Accession #-*bla*$_{NDM}$ variant".

pia, where *K. pneumoniae* was lower (29%) (15, 16). These variations in the prevalence of isolates in urine samples reflect regional differences in the distribution of CRE bacteria.

In our study, 45% of CRE, isolated from urine were positive for $bla_{NDM}$ gene, however another study from Pakistan reported very high rate of $bla_{NDM}$ gene among urinary CRE isolates (17). Variation may be due to difference in location and study design. Gender-wise distribution revealed that these $bla_{NDM}$ positive isolates are more common in men (57%). The findings correspond with past regional studies where prevalence in men was 66% (18). Age-wise distribution of these isolates demonstrates that these were most prevalent in the young age group 21-30. These results agree with the findings of a study conducted in Nepal where the most prevalence (30%) was reported in the age group 16-30 (19). Among these $bla_{NDM}$ positive isolates from urine, *K. pneumoniae* (57%) was the predominant bacteria. Our results agree with the results of previous studies where *K. pneumoniae* was reported to be 54% (20, 21).

Genetic characterization of $bla_{NDM}$ gene was performed to evaluate the genetic diversity of the variants. It was revealed that $bla_{NDM-5}$ was the most prevalent gene in 69.57% of $bla_{NDM}$ positive samples followed by $bla_{NDM-1}$ in 26.09%, and $bla_{NDM-7}$ in 4.35% samples. These findings are comparable with the results of a study carried out in Southwest China where $bla_{NDM-5}$ was 82% while these results contrast with a study conducted in 2014 in India where $bla_{NDM-1}$ was the most prevalent gene (77%) (18, 22). $bla_{NDM-1}$ and $bla_{NDM-5}$ were reported to be 50% and 62.50%, respectively harbored by *K. pneumonia*, which contradicted the findings from a previous study in which $bla_{NDM-5}$ and $bla_{NDM-7}$ were significantly associated with *E. coli* (18).

CRE isolates revealed highest susceptibility towards amikacin (45.10%) among aminoglycosides, which is lower than the study performed in China where it was 54.5% sensitive (23). Oral antibiotics, fosfomycin and nitrofurantoin, which are considered as first line options for uncomplicated UTIs also have relatively better susceptibility against CRE. According to a recent Indian study, fosfomycin has a susceptibility rate of 66.7%, compared to 30.7% for nitrofurantoin, suggesting that fosfomycin is more effective in-vitro (24). Due to the limited therapeutic scope of nitrofurantoin, which is primarily suggested for use against *E. coli* among GNRs (11), there are not many studies specifically examining nitrofurantoin susceptibility in (CRE). Colistin was exclusively susceptible to CRE as previously reported in Pakistan (25). The emergence of MDR and XDR pathogens, along with the lack of new drugs, has forced clinicians to use colistin for complicated UTI; nevertheless, it is nephrotoxic. Its effectiveness in treating UTIs is somewhat controversial because of the belief that colistin's active metabolites are not excreted into the bladder in sufficient concentrations. Despite this concern, colistin is still used alone or in combination in certain cases of UTIs, particularly when the clinicians are left with no other choice (26).

Urine samples containing $bla_{NDM}$ positive isolates showed 34.78% amikacin susceptibility, which was higher than the 22.22% rate reported in a recent Chinese study (27). $bla_{NDM}$ positive isolates were more resistant than $bla_{NDM}$ negative isolates, except for fosfomycin and nitrofurantoin which were relatively more effective against $bla_{NDM}$ positive isolates. Limited data are available on fosfomycin and nitrofurantoin susceptibilities in $bla_{NDM}$ positive isolates. The difference in susceptibility rates suggests that the spread of the $bla_{NDM}$ gene is dynamic and influenced by epidemiological factors. All $bla_{NDM}$ producing bacteria were also susceptible to colistin.

In this study, we amplified 813 bp of $bla_{NDM}$ sequence to provide an accurate picture of genetic relatedness of $bla_{NDM}$ gene with worldwide sequences as compared to previous studies in which only 475 bp amplicon was used (17). Phylogenetic analysis revealed diverse relationship with local and worldwide sequences depicting multiple origins of isolates from our health facility.

Several factors could account for the variation in the prevalence of resistant genes. Geographical and temporal factors are most likely contributors since these studies were conducted in different geographical areas and times reflecting different use of antibiotics and infection control practices. The selective pressure by these factors significantly influences the shift of prevalence of these antibiotic resistance genes. To interpret the variation and diversity of $bla_{NDM}$ gene prevalence, it is crucial to understand all the above stated factors. This study also highlights the need for continuous surveillance programs and effective strategies in every healthcare facility to combat the spread of antibiotic resistance. The present study also underscores the need for immediate action to regu-

late the use of antibiotics. Specifically, the statement calls for the infection control and healthcare regulatory authorities of Pakistan to establish a monitoring and evaluation system to curb the misuse of antibiotics. Additionally, it suggests exploring alternative therapies to treat and prevent infections that are resistant to carbapenems.

## CONCLUSION

UTIs present a serious health concern which is further complicated by the emergence of antibiotic resistance. The isolation of $bla_{NDM}$ genes from urine isolates in our study emphasizes the growing threat of antibiotic resistance in clinical settings. The notable presence of NDM-producing *K. pneumoniae* and *E. coli* in urine samples highlights the urgent need for improved surveillance systems, robust infection control measures, and antibiotic stewardship programs. Our findings contribute to the broader understanding of the dissemination of $bla_{NDM}$ genes and signify the importance of more studies and collaborative efforts to combat the spread of MDR organisms. In the face of widespread resistance, the higher susceptibility to fosfomycin seen in CRE clinical isolates is encouraging and justifies further susceptibility investigations to explore its potential for wider therapeutic usage. Given the rising prevalence of these resistant pathogens, it is imperative that healthcare systems adapt and implement effective strategies to safeguard public health and to use new antimicrobials wisely for treatment of UTIs.

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