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Research Article

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* PRODUCING EXTENDED-SPECTRUM B-LACTAMASE AND CARBAPENEMASE

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ABSTRACT

Objective: Antimicrobial resistance poses a worldwide health challenge with significant social and economic consequences. The increasing prevalence of extended-spectrum β-lactamases (ESBL) and carbapenemase (CP)-producing *Klebsiella pneumoniae* calls for urgent research and new drug development. These genes undermine infection control efforts because of their association with multidrug resistance (MDR).

Methods: This cross-sectional study was conducted from April 2022 to April 2025 at the microbiology department of MMIMSR, Mullana, Ambala. Identification and antibiotic susceptibility testing for *K. pneumoniae* were performed using the Vitek-2 Compact (BioMérieux). The presence of ESBL and CP-producing isolates was phenotypically confirmed using the combined disc method. Genes *KPC, NDM, IMP, VIM, OXA-48, TEM, CTX-M,* and *SHV* were detected using singleplex conventional polymerase chain reaction.

Results: Out of 612 *K. pneumoniae* isolates, 375 non-repetitive MDR strains were obtained from patients at M.M. Hospital, Ambala. The majority of these isolates came from males (57.6%), urine samples (61.3%), and hospitalized patients (75.5%). All isolates demonstrated complete resistance (100%) to β-lactams (Cephalosporins and carbapenems), with high resistance rates shown by cefepime (96.8%), ciprofloxacin (95.2%), gentamicin (93.6%), and trimethoprim-sulfamethoxazole (76%). Tigecycline (62%) and nitrofurantoin (53%) showed the most activity. ESBL production was identified in 51.2% of isolates, and CP production in 48.3%; Modified carbapenem inactivation method positivity was 70.1%, with 42.1% metallo-β-lactamase and 10.6% serine CPs. Among resistance genes, *CTX-M* (45.8%) and *TEM* (23.7%) were the most common ESBL determinants, while *NDM* (48.2%) and *OXA-48 (*37.8%) were the most prevalent CPs, followed by *KPC* (13.6%). The co-occurrence of genes was frequent, notably *NDM+OXA-48 (*16.2%) and triple *KPC+NDM+OXA-48 (*0.8%). All isolates were negative for *SHV, IMP*, and *VIM* genes.

Conclusion: The findings highlight the need for urgent infection control. Policymakers should establish guidelines for regulating antibiotic sales and require susceptibility testing before prescribing antibiotics. Nationwide monitoring of carbapenem resistance and gene profiling is essential for better antimicrobial use.

Keywords: Multi-drug Resistant, Extended-spectrum beta lactamase, Carbapenem-resistant *Klebsiella pneumoniae*, extensively drug-resistant.

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INTRODUCTION

Emerging global health concerns involve the swift dissemination of multidrug-resistant (MDR) bacteria, which are associated with increased mortality, longer illness durations, ineffective drugs, heightened vulnerability in immunocompromised individuals, and elevated medical expenses [1]. Antimicrobial resistance constitutes an escalating global concern. If unaddressed, the annual mortality attributable to this issue could rise from 700,000 to 10 million by the year 2050, resulting in an economic impact estimated at $100 trillion. Furthermore, the increasing occurrence of MDR bacterial infections poses a significant risk to patient safety worldwide [2].

Klebsiella pneumoniae is part of the ESKAPE group, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. This group emphasizes its exceptional capacity to "escape" from antibacterial treatments, posing a significant challenge for medical care [2].

K. pneumoniae, in particular, exhibits MDR, allowing it to increasingly resist last-line antimicrobials such as colistin, tigecycline, and carbapenems. This bacterium can colonize various human systems, including the gastrointestinal tract, nasopharynx, and skin, and can cause both hospital- and community-acquired infections [3].

Extended-spectrum cephalosporins were developed in the 1980s; they offered new hope for treating infections caused by bacteria producing beta-lactamases. However, enzymes called extended-spectrum β-lactamases (ESBLs) can still break down these antibiotics, making them less effective. ESBLs are enzymes encoded on plasmids that help bacteria resist certain antibiotics by breaking down penicillins, cephalosporins, and aztreonam, although they do not affect carbapenems. The main groups of ESBLs include *CTX-M*, *SHV*, and *TEM*. Interestingly, *CTX-M* shares only about 40% similarity with *TEM* or *SHV* and is more closely related to β-lactamases found in *Kluyvera* species [4].

Carbapenems remain some of the few effective treatments for *Enterobacteriaceae* producing ESBLs. However, overuse of carbapenems for ESBL-positive infections has led to an increase in bacteria that produce carbapenemases (CPs), resulting in carbapenem resistance. Carbapenem antibiotics are used as a last option to treat MDR bacteria. It is used when a patient is critically ill or has an infection caused by Gram-negative bacteria that are resistant to most conventional antibiotics [5]. Some studies indicate that *K. pneumoniae* in hospitals throughout Southeast and South Asia exhibits high resistance to various treatments, including carbapenems [6,7].

Emerging carbapenem-resistant *K. pneumoniae* (CRKP) strains present a significant public health challenge worldwide [8]. The World Health

Organization has classified it as a priority pathogen, emphasizing the urgent need for novel antibiotics [9]. The increasing prevalence of CRKP poses a substantial public health risk since these strains are usually classified as MDR, extensively drug-resistant (XDR), or pan-drug-resistant, complicating treatment choices. CRKP infections can cause increased mortality, greater healthcare costs, and socioeconomic consequences [10].

K. pneumoniae develops carbapenem resistance mainly through CP production, but also via OMP loss, overexpression of AmpC and ESBL enzymes, and efflux pump activation. Recognizing these mechanisms helps develop effective treatments and control strategies to curb CRKP spread [11].

Carbapenem resistance is primarily caused by CPs, enzymes that degrade carbapenems and β-lactam medicines. The CPs are classified into three categories: A, B, and D. Genes such as *IMI*, *NMC*, *SME*, *GES*, and *KPC* belong to Group A, whereas Group B includes metallo-β-lactamases (MBLs) such as *VIM, IMP, GIM, NDM, SIM*, and *DIM*. Group D contains oxacillinases, which include *OXA-48* [12].

Overuse of antibiotics in healthcare facilities and in community settings, inadequate infection prevention and control measures, prolonged hospital stays, the use of invasive medical equipment like central lines, urinary catheters, and ventilator tubes, living in nursing facilities, and immunosuppressive conditions are all factors that contribute to the spread of ESBLs and CP-producing bacteria [8].

This study investigates the extent of antimicrobial resistance among clinical *K. pneumoniae* isolates, with particular emphasis on CP and ESBL production. We aimed to assess their prevalence and analyze their antibiotic susceptibility patterns at a tertiary care hospital in North India.

METHODS

This cross-sectional study was conducted from April 2022 to April 2025. Various clinical samples collected from the Outpatient Department (OPD) and Inpatient Department (IPD) across multiplespecialties in the Department of Microbiology, MMIMSR, Mullana. Ethical approval was taken from the institutional ethics committee vide letter no MMIMSR/IEC/2427.

Various clinical samples, including sputum, blood, urine, ear swabs, pus, pleural fluid, and soft tissue from all age groups and departments (e.g., burns, medicine, intensive care unit, pediatric intensive care unit, neonatal intensive care unit, surgery, ear, nose, and throat, and tuberculosis), were included. Only MDR *K. pneumoniae* isolates were studied, excluding others.

Pus sample and respiratory samples

All samples were Gram-stained and cultured on blood and MacConkey agar (HiMedia, India). For blood cultures, two blood samples from different veins were collected and inoculated into blood culture bottles, then incubated in an automated system (BD BACTEC FX40) for 5 days. Other sterile fluids, about 3–5 mL, were incubated for 48 h. When bottles tested positive, they were subcultured on Blood and MacConkey agar to identify species and perform antibiotic testing. Only those bottles that showed positive results in both sets were considered true positives; bottles with only one positive result or no growth after 5 days were considered contaminated [13].

Bacterial identification, antibiotic sensitivity testing, and categorization of multidrug-resistant *K. pneumoniae*

All bacterial isolates were identified using a Gram-negative ID card in the Vitek-2 Compact system. Antibiotic sensitivity of *K. pneumoniae* was tested using N405 and N235 AST cards (urine only) according to the manufacturer's instructions. Results were interpreted according to the 2022 Clinical and Laboratory Standards Institute guidelines [14]. In the current study, MDR was defined as bacteria resistant to at least one antibiotic in three or more antibiotic classes, as recommended by the Centers for Disease Control and Prevention and European Centre for Disease Prevention and Control [15].

Disc diffusion test for ESBL production

To fulfill this objective, four antibiotic discs were used: Cefotaxime (30 μg), ceftazidime (30 μg), cefotaxime–clavulanic acid (30/10 μg), and ceftazidime–clavulanic acid (30/10 μg) (HiMedia Laboratories Pvt Ltd., Mumbai, India). The discs were placed 25 mm apart on a Mueller–Hinton agar (MHA) plate inoculated with a 0.5 McFarland suspension. The plates were then incubated at 37°C for 18–24 h under optimal conditions. After incubation, zone diameters were measured. A ≥5 mm difference between the zones of cephalosporin discs and their respective cephalosporin/clavulanic acid discs was regarded as positive. Control strains included *K. pneumoniae* ATCC 700603 (ESBL-positive), BAA-2146 (MBL-positive), and MTCC 109 (ESBL-negative) [14].

Disc diffusion test for MBL production

The CP Nordmann–Poirel (Carba NP) test-

Two solutions were prepared: solution A, containing phenol red at pH 7.8 and $ZnSO_4$, and solution B, prepared by adding freshly prepared imipenem to solution A. Both had the same indicator concentration. Equal volumes (e.g., 100 μL) of each were dispensed into labeled microtubes, with positive and negative controls. A standardized inoculum – either lysate from 18–24 h growth in extraction buffer or colonies suspended directly – was added. Tubes were vortexed briefly, incubated at 37°C for up to 2 h, and observed every 10–30 min. Results were recorded promptly to prevent late shifts. A positive result showed solution B shifting from red to orange/yellow, indicating hydrolysis by CP; weak producers may turn yellow after 2 h. Negative: both stayed red, showing no activity. If both turned yellow, the test was invalid and needed to be repeated [14].

Modified carbapenem inactivation method (mCIM)

A loopful of CP-positive *K. pneumoniae* isolates was gently inoculated into Tryptic Soy Broth (TSB), and then a meropenem disc was added. After incubation, the disc was carefully placed on an MHA plate inoculated with meropenem-susceptible *Escherichia coli* ATCC 25922. The diameter of the inhibition zone was used to interpret the results: 6–15 mm indicated a positive result, 16–18 mm an intermediate result, and ≥19 mm a negative result. This test demonstrated high sensitivity and specificity, but it may sometimes miss CP genes. In addition, the mCIM does not differentiate between serine β-lactamases and metallo-β-lactamases; therefore, an EDTA-Modified Carbapenem Inactivation Method (eCIM) was performed for confirmation [14].

eCIM

To conduct the test, prepare 2 mL of TSB culture medium in a test tube, add either 0.1 or 5 mM EDTA, and inoculate with a 1 μL loopful of the tested bacteria. Place a 10 μg meropenem disc and incubate at 37°C for 4 h. After incubation, remove the disc and transfer it to an MHA plate inoculated with a 0.5 McFarland suspension of *E. coli* ATCC 25922. Incubate for 18–24 h at 37°C. An increase of ≥5 mm in the inhibition zone compared to mCIM indicates Metallo-β-lactamase activity, while 4 mm or less suggests the absence of serine β-lactamases [14].

Molecular characterization of ESBLs and CP genotype by polymerase chain reaction (PCR)

Bacterial DNA was extracted using Geno Sen's Genomic DNA extraction kit (Genome Diagnostics Pvt Ltd. (as per the extraction kit protocol). The singleplex conventional PCR method was used to detect ESBL genes (*CTX-M, SHV, TEM*) and CP genes (*KPC, NDM, IMP, VIM, OXA-48*). The primer sequences (Eurofins Scientific, Bengaluru) and the PCR program for each reaction, including denaturation, annealing temperature, extension, and the number of PCR cycles, are shown in Table 1. In our study, each PCR reaction mixture consisted of 25 μL, containing 5 μL of DNA template, 1.5 μL of each forward and reverse primer, 12.5 μL of DNA polymerase master mix (HiMedia, Mumbai), and 4.5 μL of DNase- and RNase-free water. *K. pneumoniae* 700603, and BAA-2146 were used as positive controls.

Table 1: List of primers used in the study

Name of the genes	Primersequence (5'-3')	Amplicon (bp)	PCR program						Ref
			Initial denaturation	Denaturation	Annealing	Initial extension	Final extension	PCR Cycle	
SHV	SHV-F: CGCCTGTGTATTATCTCCCT SHV-R: CGAGTAGTCCACCAGATCCT	294bp	94°C 5 min	94°C 30 sec	60°C 30 sec	72°C 50 sec	72°C 5 min	35	[16]
TEM	TEM-F: TTTCGTGTCGCCCTTATTCC TEMR: ATCGTTGTCAGAAGTAAGTTGG	404bp	94°C 5 min	94°C 30 sec	60°C 30 sec	72°C 50 sec	72°C 5 min	35	[16]
CTX-M	CTX-M-F: CGCTGTTGTTAGGAAGTGTG CTX-M-R: GGCTGGGTGAAGTAAGTGAC	754bp	94°C 5 min	94°C 30 sec	60°C 30 sec	72°C 50 sec	72°C 5 min	35	[16]
KPC	KPC F- CGTCTAGTTCTGCTGTCTTG KPCR-CTTGTCATCCTTGTTAGGCG	798bp	95°C 5 min	95°C 1 min	56°C 31 sec	72°C 1 min	72°C 5 min	35	[17]
IMP	IMPF-GGAATAGAGTGGCTTAAYTCTC IMPR-GGTTTAAYAAAACAACCACC	232bp	95°C 5 min	95°C 30 sec	52°C 40 sec	72°C 50 sec	72°C 5 min	35	[17]
VIM	VIMF-GATGGTGTTTGGTCGCATA VIMR-CGAATGCGCAGCACCAG	390bp	95°C 5 min	95°C 1 min	56°C 31 sec	72°C 1 min	72°C 5 min	35	[17]
NDM	NDMF-GGTTTGGCGATCTGGTTTTC NDMR-CGGAATGGCTCATCACGATC	621bp	95°C 5 min	95°C 30 sec	52°C 40 sec	72°C 50 sec	72°C 5 min	35	[17]
OXA-48	OXA48F-GCGTGGTTAAGGATGAACAC OXA48R-CATCAAGTTCAACCCAACCG	438bp	95°C 5 min	95°C 1 min	56°C 31 sec	72°C 1 min	72°C 5 min	35	[17]

PCR: Polymerase chain reaction

Gel electrophoresis and visualization under ultraviolet lights by transilluminator

After amplification, the products were gently loaded onto a 1.5% agarose gel containing ethidium bromide and a 50-base-pair ladder (HiMedia, Mumbai, India) to estimate molecular weights and subjected to electrophoresis at 70 volts for 1 h. The gel images were taken using an imaging system.

RESULTS

During the study, 612 *K. pneumoniae* isolates were obtained from OPD and IPD patients at Maharishi Markandeshwar Multispeciality Hospital, Mullana, Ambala, and examined; of these, 375 non-repetitive MDR *K. pneumoniae* isolates were further processed. As depicted in Table 2, out of these 375 MDR isolates, 216 (57.6%) came from male patients, while 159 (42.4%) were from female patients. The most common source was urine specimens (230, 61.3%), followed by respiratory secretions (40, 10.7%), pus (37, 9.9%), wound swabs (35, 9.3%), and sterile body fluids (33, 8.8%) (Figure 1-5).

The average age of the research participants was 50 years, with ages ranging from 1 day to 100 years. Among them, 251 (66.93%) were over 45 years old, 98 (26.13%) were between 18 and 45 years old, 7 (1.86%) were between 5 and 18 years old, and 19 (5.06%) were under the age of 05. Majority of the isolates (321, 75.46%) were collected from hospitalized patients (IPD), whereas 54 (24.53%) were from outpatients (OPD).

Antimicrobial resistance profile of MDR *K. pneumoniae* isolates

In this study, the resistance pattern of 375 MDR *K. pneumoniae* isolates is presented in Table 3, 100% resistance rate was seen in amoxicillin/clavulanic acid (375/375, 100%), piperacillin+tazobactam (375/375, 100%), cefuroxime (375/375, 100%), ceftazidime (375/375, 100%), ceftriaxone (375/375, 100%), imipenem (375/375, 100%), meropenem (375/375, 100%), and ertapenem (375/375, 100%).

Intense resistance was observed to commonly used antimicrobials agents such as cefepime (363/375, 96.8%), ciprofloxacin (357/375, 95.2%), norfloxacin (217/230, 94.3% [U]), gentamicin (351/375, 93.6%), cefoxitin (187/230, 81.3% [U]), and trimethoprim–sulfamethoxazole (285/375, 76%). Besides this, a notable intermediate resistance was observed to nitrofurantoin (17/230, 7.3% [U]) and amikacin (79/375, 21%).

K. pneumoniae isolates showed the highest susceptibility to tigecycline (90/145, 62%), followed by nitrofurantoin (122/230, 53%) (U),

Table 2: Distribution of *Klebsiella pneumoniae* in demographic and clinical characteristics of study participants

Variables	Category	Frequency n=375 (%)
Sex	Female	159 (42.4)
	Male	216 (57.6)
Age	Birth to<5 years	19 (5.06)
	5 years to<18 years	7 (1.86)
	18 years to<45 years	98 (26.13)
	≥45 years	251 (66.93)
Specimen type	Pus	37 (9.8)
	Urine	230 (61.3)
	Wound swabs	35 (9.3)
	Blood and sterile fluids	33 (8.8)
	Respiratory secretions	40 (10.6)
Patient setting	Inpatient	321 (85.6)
	Outpatient	54 (14.4)

amikacin (173/375, 46.1%), and cefoperazone/sulbactam (44/145, 30.3%), with trimethoprim–sulfamethoxazole at 24% (90/375). The lowest sensitivity rates were observed with cefepime (3.2%), ciprofloxacin (4.8%), norfloxacin (5.6%), and gentamicin (6.4%).

Of the total 375 MDR *K. pneumoniae*, 192 (51.2%) were ESBL-positive, and 183 (48.8%) were ESBL-negative.

Whereas, in the CarbaNP Test, 181 (48.26%) were CP producers, 154 (41%) were negative, and 40 (10.6%) showed an invalid result. Whereas 263 (70.13%) were mCIM Positive and 112 (29.86%) were negative. From the total number of mCIM-positive *K. pneumoniae*, eCIM was performed, with 158 (42.13%) positive (MBL), 28 (10.6%) negative (serine), and 77 (29.27%) indeterminate.

Prevalence of MDR genes in *K. pneumoniae* isolates

45.66% (172 of 375) carried only the *CTX-M* gene, while 23.73% (89 of 375) had only the *TEM* gene. In addition, 10.6% (40 of 375) exhibited co-resistance in both *CTX-M* and *TEM*. The occurrence of isolates with only *CTX-M* was significantly higher than that of isolates with only *TEM*, as confirmed by a two-proportion z-test ($p<0.001$). *NDM* was detected alone in 48.26% (181/375) of isolates, followed by *OXA-48* alone in 37.87% (142/375), and *KPC* alone in 13.60% (51/375). *NDM*-only isolates were notably more frequent than those with only *OXA-48* ($p=0.004$) or only *KPC* ($p<0.001$), according to two-proportion z-tests. Co-occurrence of CP genes was also observed: 1.06% (4/375) carried *KPC* and *OXA-48*, 0.8% (3/375) carried *NDM* and KPC and 16.2%

Table 3: Antimicrobial resistance patterns of MDR *Klebsiella pneumoniae* isolates

Antimicrobial categories	Antimicrobial agents	S n (%)	I n (%)	R n (%)
Tetracyclines	Tigecycline (n=145)	90 (62)	-	55 (37.9)
Nitrofuran	Nitrofurantoin (n=230) (U)	122 (53)	17 (7.3)	91 (39.5)
Folic acid synthesis inhibitors	Trimethoprim sulfamethoxazole	90 (24)	-	285 (76)
Aminoglycosides	Gentamicin	24 (6.4)	-	351 (93.6)
	Amikacin	173 (46.1)	79 (21)	123 (32.8)
Fluoroquinolones	Ciprofloxacin	18 (4.8)	-	357 (95.2)
	Norfloxacin (n=230) (U)	13 (5.6)	-	217 (94.3)
Antipseudomonal penicillin's+β-lactamase inhibitors	Piperacillin/Tazobactam	-	-	375 (100)
Penicillin's β-lactamase inhibitors	Amoxicillin/Clavulanic acid	-	-	375 (100)
Folate pathway inhibitors	Cefoperozone/sulbactam (n=145)	44 (30.3)	-	101 (69.6)
β-lactam				
Cephalosporins	Cefuroxime	-	-	375 (100)
	Ceftriaxone	-	-	375 (100)
	Ceftazidime	-	-	375 (100)
	Cefepime	12 (3.2)	-	363 (96.8)
Carbapenems	Imipenem	-	-	375 (100)
	Meropenem	-	-	375 (100)
	Ertapenem	-	-	375 (100)

MDR: Multidrug-resistant, S: Sensitive, I: Intermediate, R: Resistance

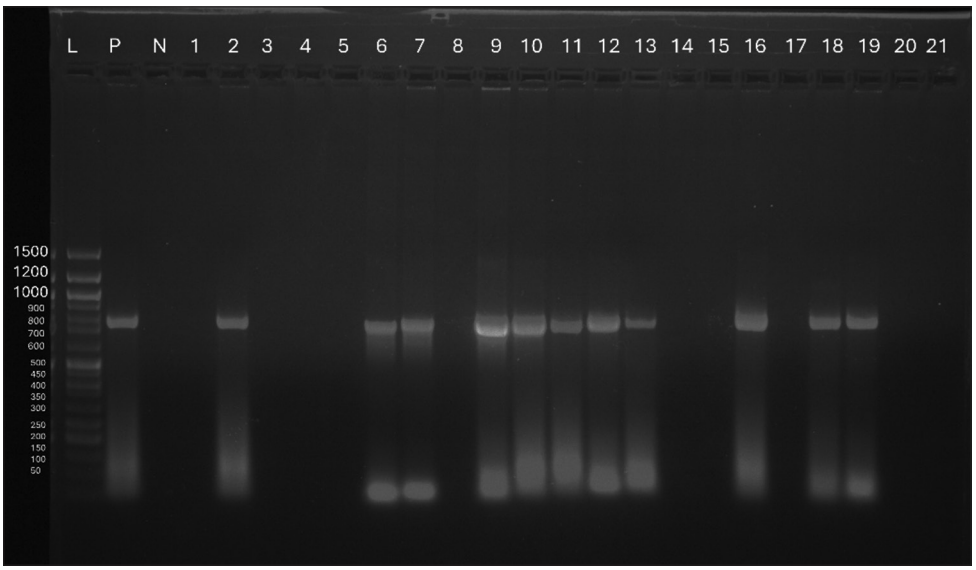

Fig. 1: Electrophoresis agarose gel showing the polymerase chain reaction amplified product of the *CTX-M* gene. Lane 1=50 bp ladder (HiMedia, India). Lane 2- positive control, Lane 3- Negative control (no template DNA added): lanes5,9,10,12,13,14,15,16,19,21 and 22=*CTX-M* positive amplicons

(61/375) carried both *NDM* and *OXA-48*. A small percentage (0.8%, 3/375) harbored *KPC*, *OXA-48*, and *NDM* simultaneously. Notably, no isolates tested positive for the *IMP, VIM,* or *SHV* genes (Table 5).

Co-occurrence of multiple resistance genes

The frequent co-occurrence of multiple resistance determinants. Plausible gene constellations included KPC+*NDM* (0.8%), *NDM*+*OXA-48 (*16.2%), *KPC*+*OXA-48 (*1.06%), and the tripartite *KPC*+*NDM*+*OXA-48 (*0.8%), with additive ESBL genotypes *CTX-M+TEM* (10.6%). This combinatorial genetic loading underscores a significant genotypic plasticity, facilitating both horizontal and vertical dissemination of resistance determinants.

Antibiotic resistance profile of ESBL and CP producing genes in *K. pneumoniae*

Table 4 presents the antimicrobial susceptibility of MDR *Klebsiella pneumoniae* isolates, including ESBL producers (n=192), CP producers (n=186), and isolates carrying genes such as *CTX-M* (n=172), *TEM* (n=89), *NDM* (n=181), *KPC* (n=51), and *OXA-48* (n=142).

All isolates showed 100% resistance to carbapenems (imipenem, meropenem, ertapenem) and cephalosporins (cefuroxime, ceftriaxone, ceftazidime), indicating widespread β-lactam resistance and reduced carbapenem effectiveness. β-lactam/β-lactamase inhibitor combinations were absolute for piperacillin–tazobactam and amoxicillin–clavulanic acid, likely attributable to β-lactamase activity. Resistance rates to cefoperazone–sulbactam varied: 12.2% among *CTX-M* producers, 30.9% among *NDM* producers, and 30.9% among *OXA-48* producers. Resistance to fluoroquinolones was considerably high, with ciprofloxacin resistance ranging from 93.6% (*CTX-M*) to 98.8% (*TEM*). For norfloxacin (evaluated exclusively in urinary isolates), resistance ranged from 54.6% (*NDM*) to 64.7% (*KPC*).

Aminoglycoside susceptibility patterns exhibited notable heterogeneity. Gentamicin resistance was nearly absolute, ranging from 92.8% (*NDM*) to 98.9% (ESBL producers). Conversely, amikacin retained relatively greater activity, with resistance rates ranging from 24.7% (*TEM*) to 37.7% (*CTX-M*).

Table 4: Antimicrobial susceptibility patterns of *Klebsiella pneumoniae* in isolates ESBL and CP producers' genes.

Antimicrobial categories	Antimicrobial agents	ESBL producers (n=192)	CP producers (n=186)	*CTX-M* (n=172)	*TEM* (n=89)	*NDM* (n=181)	*KPC* (n=51)	*OXA-48* (n=142)
Tetracyclines	Tigecycline	33 (17.1)	28 (15)	29 (16.8)	13 (14.6)	31 (17.1)	6 (11.7)	25 (17.6)
Nitrofuran	Nitrofurantoin (U)	49 (25.5)	43 (23.1)	44 (25.5)	24 (26.9)	42 (23.2)	14 (27.4)	35 (24.6)
Folic acid synthesis inhibitors	Trimethoprim sulfamethoxazole	147 (76.5)	142 (76.3)	130 (75.5)	66 (74.1)	141 (77.9)	38 (74.5)	97 (68.3)
Aminoglycosides	Gentamicin	190 (98.9)	175 (94)	169 (98.2)	86 (96.6)	168 (92.8)	49 (96)	133 (93.6)
	Amikacin	65 (33.8)	62 (33.3)	59 (37.7)	22 (24.7)	62 (34.2)	18 (35.2)	45 (31.6)
Fluoroquinolones	Ciprofloxacin	181 (94.2)	179 (96.2)	161 (93.6)	88 (98.8)	173 (95.5)	50 (98)	138 (97.1)
	Norfloxacin (U)	110 (57.2)	108 (58)	99 (57.5)	54 (60.6)	99 (54.6)	33 (64.7)	79 (55.6)
Antipseudomonal Penicillin's+β-lactamase inhibitors	Piperacillin/ Tazobactam	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
Penicillin's β-lactamase inhibitors	Amoxicillin/ Clavulanic acid	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
Folate pathway inhibitors	Cefoperozone/ sulbactam	52 (27)	53 (28.4)	21 (12.2)	19 (12.3)	56 (30.9)	9 (17.6)	44 (30.9)
Cephalosporins	Cefuroxime	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
	Ceftriaxone	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
	Ceftazidime	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
	Cefepime	-	-	-	86 (96.6)	-	47 (92.1)	137 (96.4)
Carbapenems	Imipenem	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
	Meropenem	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)
	Ertapenem	192 (100)	186 (100)	172 (100)	89 (100)	181 (100)	51 (100)	142 (100)

ESBL: Extended-spectrum β-lactamases, CP: Carbapenemase

Table 5: Distribution of genes responsible for carbapenem and cephalosporin resistance

Gene	Total no. of genes n (%)
KPC	51 (13.6)
NDM	181 (48.26)
OXA-48	142 (37.8)
CTX-M	172 (45.86)
TEM	89 (23.73)
Co-occurrence of genes	**Total no. of genes n (%)**
KPC+NDM	3 (0.8)
NDM+OXA-48	61 (16.2)
KPC+OXA-48	4 (1.06)
KPC+NDM+OXA-48	3 (0.8)
CTX-M+TEM	40 (10.6)

Alternative agents such as tigecycline and nitrofurantoin showed relatively maintained activity. Tigecycline resistance (n=78) varied from 11.7% (*KPC*) to 17.6% (*OXA-48*), while nitrofurantoin resistance (urinary isolates) ranged from 23.2% (*NDM*) to 27.4% (*KPC*). Resistance to trimethoprim–sulfamethoxazole was consistently high across all groups, between 68.3% (*OXA-48*) and 77.9% (*NDM*) .

DISCUSSION

K. pneumoniae poses a significant challenge to the global healthcare system. It can easily spread among hospitalized patients, leading to hospital-acquired outbreaks. *K. pneumoniae* is an important pathogen in both community-acquired and hospital infections, with high mortality rates, particularly in immunocompromised individuals [18].

Globally, the drug resistance rate of *K. pneumoniae* has increased to alarming levels 70%, linked with infection-related fatality rates ranging from 40% to 70%. Carbapenem-resistant *K. pneumoniae* and MDR *K. pneumoniae* have become a significant and pressing global public health concern [19]. The first report about CRKP was published in 1996; since then, the occurrence of MDR strains has increased significantly. Resistance mainly arises from the production of acquired CPs such as *KPC, OXA-48, NDM, IMP*, and *VIM*, as well as from combined mechanisms involving ESBL activity [20].

MDR *K. pneumoniae* strains have acquired resistance to multiple antibiotics, including carbapenems, aminoglycosides, fluoroquinolones, and third-generation cephalosporins [21]. The current study investigates the extent of antimicrobial resistance among clinical *K. pneumoniae* isolates, with particular emphasis on CP and ESBL production. We aimed to assess their prevalence and analyze their antibiotic susceptibility patterns at a tertiary care hospital in North India.

In the current study, inpatients had a considerably higher infection rate (75.46%) than outpatients, exceeding the rate reported by Mustafai *et al.* (59.1%) [5]. A male predominance of 57.6% was observed, coherent with global studies by Rizvi *et al*. [22] and Ilyas *et al*. [23], which demonstrate similar male-to-female infection ratios.

This study found that 61.2% of *K. pneumoniae* strains are resistant to multiple drugs. Lagha *et al.* [24], Mirzaie and Ranjbar *et al.* [25], have reported higher MDR prevalence rates in *K. pneumoniae*, ranging from 83% to 92%, which exceed those in our study. On the contrary, our result was lower than that reported by Awoke *et al*. [2] reported 98.5% MDR *K. pneumoniae*, Teklu *et al.*[26] reported 83.5%, Moges *et al.* [27] reported 87.6% and Ilyas *et al.* [23] reported a high prevalence rate, 90.2% MDR *K. pneumoniae*.

In the current study, majority of the isolates were collected from urine specimens (61.3%), followed by respiratory secretions (10.7%), pus (9.9%), wound swabs (9.3%), and body and sterile fluids (8.8%). This distribution is similar to that described by Ilyas *et al.* [23], in which isolates were detected in urine (49.5%), blood (32.7%), pus (7.07%), pleural fluid (4.42%), tracheal samples (4.42%), and cerebral fluid (1.76%). Taha *et al.* [28] found that 50% of samples were urine, 25% were pus, 20% were sputum, and 6.25% were blood and bodily fluids. Urine remained the most prevalent specimen for isolation, as reported by Sahoo *et al.* [19], with a prevalence rate of 48.8%.

In this study, the mCIM testidentified CP activity in 70.13% of the tested CRKP isolates. The eCIM test determined that 42.13% of CP-positive CRKP isolates produced Metallo-CPs, while 10.6% produced Serine CPs. Kuchibiro *et al.* [29] observed that the carbapenem inactivation method

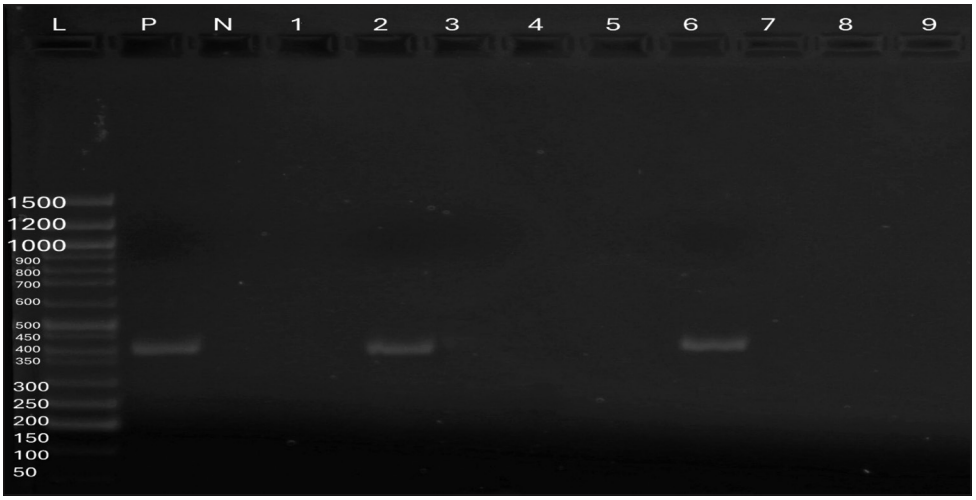

Fig. 2: Electrophoresis agarose gel showing the polymerase chain reaction amplified product of the *TEM* gene. Lane 1=50 bp ladder (HiMedia, India). Lane 2- positive control, Lane 3- Negative control (no template DNA added): lanes 5 and 9=*TEM* positive amplicons

Fig. 3: Electrophoresis agarose gel showing polymerase chain reaction amplified product of *KPC* gene. Lane 1=50 bp ladder (Himedia, India). Lane 2- positive control, Lane 3- Negative control (no template DNA added): lanes 8, 13, 16, 17 and 20=*KPC* positive amplicons

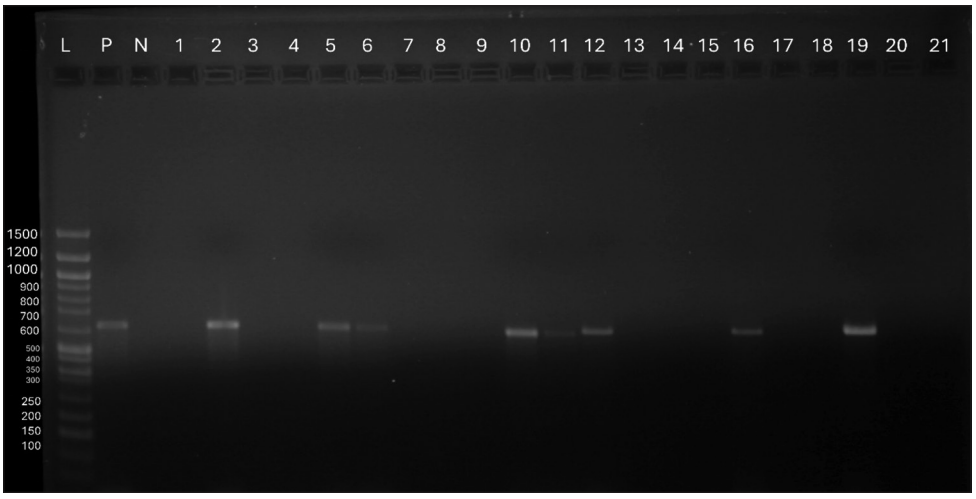

Fig. 4: Electrophoresis agarose gel showing the polymerase chain reaction amplified product of the *NDM* gene. Lane 1=50 bp ladder (Himedia, India). Lane 2- positive control, Lane 3- Negative control (no template DNA added): lanes 5, 7, 8, 12, 13, 14, 18 and 21=*NDM* positive amplicons

outperforms other phenotypic tests, such as the modified Hodge test and Carba NP test, achieving 100% sensitivity and specificity in detecting CP-positive isolates. In contrast, Song *et al.* [30] reported that the mCIM was positive in nearly all CP-producing CRE isolates, except for one *OXA-48*-like *Enterobacteriaceae* and 03 *K. pneumoniae* isolates producing *GES-5* (class A CPs).

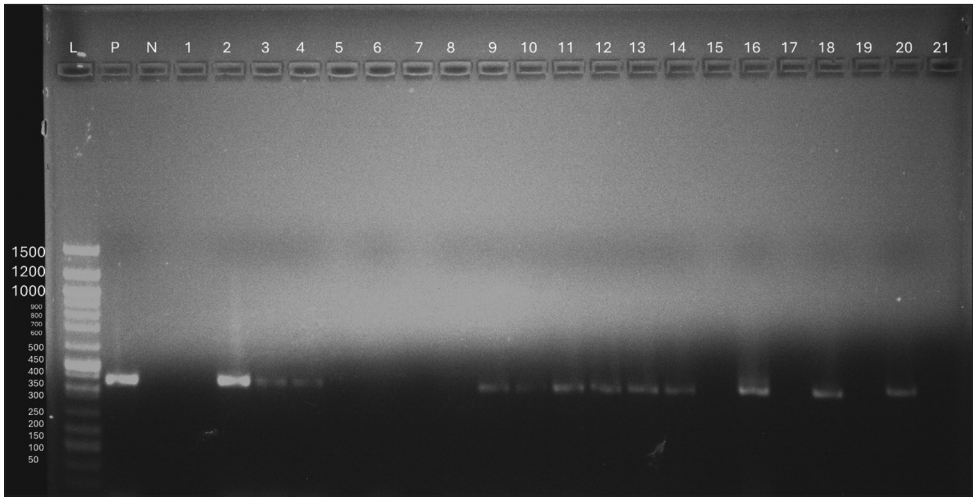

Fig. 5: Electrophoresis agarose gel showing polymerase chain reaction amplified product of *OXA-48* gene. Lane 1=50 bp ladder (Himedia, India). Lane 2- positive control, Lane 3- Negative control (no template DNA added): lanes 5, 6, 7, 12, 13, 14, 15, 16, 17, 19, 21 and 23=*OXA-48* positive amplicons

The current study found that all isolates were completely resistant to carbapenems (imipenem, meropenem, ertapenem) and cephalosporins (cefuroxime, ceftriaxone, ceftazidime), showing widespread β-lactam resistance and reduced carbapenem efficiency. Ethiopian studies showed high resistance: Awoke *et al.* [2] reported 97% to ceftriaxone and cefotaxime, 93.9% to sulfamethoxazole-trimethoprim, 84.1% to cefepime, and over 70% to carbapenems. Teklu *et al.* [26] found resistance rates of 62.2% to cefotaxime, 60.3% to cefepime, and 58.8% to norfloxacin, with lower levels of resistance to meropenem (5.2%) and amikacin (13.8%). Moges *et al.* [27] in Ethiopia reported 100% resistance to ampicillin, 96.6% to ceftazidime, 91.2% to cefepime, and 88.2% to ceftriaxone.

The prevalence of MDR *K. pneumoniae* producing ESBL was 51.2% in this study, aligning with Iranian (40.8%) [31] and Indian research (48.2%) [32]. Conversely, Ethiopia reported a much lower ESBL prevalence at 28.2% [33], but nearby regions showed higher rates, such as 54.5% in Ethiopia [34] and 58% in Burkina Faso. [35] Other studies reported higher ESBL rates, with Ilyas *et al.* [23] at 85%.

In our study, 45.66% of the cephalosporin genes exhibited the *CTX-M* gene alone, while 23.73% showed the *TEM* gene. Desai *et al.* [36] found that CTX-M-15 (88%), *SHV* (97%), and *TEM* (97%) – these rates exceed those observed in our research. Maleki *et al*. [37] observed the prevalence rates of CTX-M-3 at 56.5%, *SHV* at 85.5%, and *TEM* at 16.1%, aligning with our findings. Bora *et al.* [38] noted that *TEM* and SHV were each the least common ESBL genotypes, with 50.57%. Hassan *et al.* [39] observed a marked rise in ESBL genotypes, with CTX-M at 97.4%, surpassing *SHV* at 23.1%. While *CTX-M*-15 was most prevalent in ESBL, no *TEM* was identified (Table 5).

In our study, the co-occurrence of *CTX-M* and *TEM* was 10.6%, which was lower than that reported by Hassan *et al*. [39]. Whereas Bhaskar *et al.* [4] detected co-existence of *SHV* and *CTX-M* in 20.5% and 50%, respectively. Bora *et al.* [38] also reported that the combined presence of *SHV*, *CTX-M*, and *TEM* was found in the study, with a prevalence of 42.6%.

Our research revealed that among CRKP isolates, 48.26% carried the *NDM* gene, 37.8% harbored *OXA-48*, and 13.6% contained *KPC*. Greece has the highest global carbapenem resistance rate at 68%, driven by *KPC*, *OXA-48*-like, and *NDM* genes. The following are India and the eastern Mediterranean, with 54% resistance, primarily due to *NDM* and *OXA-48*-like genes. The USA, China (with *KPC*, *NDM*, and *OXA-48*-like), and Africa (*OXA-48*-like and *NDM*) display lower resistance rates of 11%, 11%, and 4%, respectively [40]. In Europe, *KPC* is the leading enzyme linked to resistance, followed by *OXA-48*-like and *NDM*. Conversely, in the USA, *KPC* remains the most common, followed by *NDM*, while *OXA-48*-like enzymes are rarely found. According to Spanish research, the majority of CP-producing *K. pneumoniae* harbor *OXA-48*-like or class B CPs, whereas *KPC* producers are uncommon, accounting for only 2–3%. [4]. Veeraraghavan *et al.*[41] found that among carbapenem-resistant isolates, 28% co-expressed *NDM* and *OXA-48*, 19% expressed *NDM* alone, 13% had *OXA-48*, and no isolates carried *KPC*.

Our genotypic analysis of CRKP isolates found that *NDM* was present in 48.26% of cases, *OXA-48* in 37.26%, and *KPC* in 13.6%. Taha *et al.* [28] identified *OXA-48* in 15.5%, *VIM* in 15%, *IMP* in 7.5%, *KPC* in 4%, and *NDM* in 3.8%. These figures are somewhat lower than ours, primarily because we omitted *IMP* and *VIM* from our analysis. Raheel *et al.* [42] discovered that the *OXA-48* gene (96.2%) was the most often identified, exceeding our findings, while the *KPC* gene (7.5%) was the least common.

The most prevalent CP on the Arabian Peninsula is *NDM-1*, which accounts for around 46.5% of cases. *OXA-48*-like enzymes are the second most prevalent, accounting for approximately 32.5% of all isolates [4]. Deshpande *et al.* [43] identified *NDM-1* in 75.2% of isolates from India. Another investigation found *NDM-1* in *K. pneumoniae* collected from a surgical site. Our findings revealed that 48.26% of isolates produced *NDM*. A hospital in Northeast India discovered that 8.67% of *K. pneumoniae* isolates have *NDM*.

In the current study, the combined prevalence of the genes *NDM* and *OXA-48* was 16.2%, which was the highest among all gene combinations. This rate exceeds the 4.52% reported by Sisay *et al.* [44]. The prevalence was higher in other studies, with Tula *et al.* [45] reporting 26.3% and Abbasi *et al.* [46] 29.8%. Conversely, Liu *et al.* [47] found a prevalence of only 6.6%, and Tanriverdi *et al.* [48] reported only 1.3%, both significantly lower than our findings.

CONCLUSION

The current study highlights a worrying rise in antimicrobial resistance among *K. pneumoniae*, particularly ESBL and carbapenem-resistant strains, potentially leading to an increase in XDR bacteria. Notably, there was 100% resistance to 3rd and 4th-generation cephalosporins and carbapenems. Urgent measures are required to strengthen infection prevention and control efforts. Policymakers need to address rising antibiotic resistance by creating guidelines for managing infections caused by MDR, XDR, and CRKP bacteria. Regulations should control the sale, purchase, and prescription of antibiotics, with prescriptions issued solely based on bacterial culture and susceptibility reports. Moreover, national surveillance programs are crucial for tracking

carbapenem resistance, CP, and ESBL production, along with related genes in *Enterobacteriaceae* clinical isolates, to improve antimicrobial stewardship.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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REFERENCES

1. Salawudeen A, Raji YE, Jibo GG, Desa MN, Neoh HM, Masri SN, *et al*. Epidemiology of multidrug-resistant *Klebsiella pneumoniae* infection in clinical setting in South-Eastern Asia: A systematic review and meta-analysis. Antimicrob Resist Infect Control. 2023;12(1):142. doi: 10.1186/s13756-023-01346-5, PMID 38062531
2. Awoke T, Teka B, Seman A, Sebre S, Yeshitela B, Aseffa A, *et al*. High prevalence of multidrug-resistant *Klebsiella pneumoniae* in a tertiary care hospital in Ethiopia. Antibiotics (Basel). 2021;10(8):1007. doi: 10.3390/antibiotics10081007, PMID 34439057
3. Nguyen TN, Nguyen PL, Le NT, Nguyen LP, Duong TB, Ho ND, *et al*. Emerging carbapenem-resistant *Klebsiella pneumoniae* sequence type 16 causing multiple outbreaks in a tertiary hospital in southern Vietnam. Microb Genomics. 2021;7(3):mgen000519. doi: 10.1099/mgen.0.000519, PMID 33565955
4. Bhaskar BH, Mulki SS, Joshi S, Adhikary R, Venkatesh BM. Molecular Characterization of extended spectrum β -lactamase and carbapenemase Producing *Klebsiella pneumoniae* from a Tertiary Care Hospital. Indian J Crit Care Med. 2019;23(2):61-6. doi: 10.5005/jp-journals-10071-23118, PMID 31086448
5. Mustafai MM, Hafeez M, Munawar S, Basha S, Rabaan AA, Halwani MA, *et al*. Prevalence of carbapenemase and extended-spectrum β-lactamase producing enterobacteriaceae: A cross-sectional study. Antibiotics (Basel). 2023;12(1):148. doi: 10.3390/antibiotics12010148, PMID 36671350
6. Machado E, Costa P, Carvalho A, On Behalf of The Sarel Investigators. Occurrence of healthcare-associated infections (HAIs) by *Escherichia coli* and *Klebsiella* spp. producing Extended-Spectrum β-lactamases (ESBL) and/or carbapenemases in portuguese long-term care facilities. Pathogens. 2022;11(9):1019. doi: 10.3390/pathogens11091019, PMID 36145451
7. Idrees MM, Saeed A. Genetic and molecular mechanisms of multidrug-resistance in uropathogens and novel therapeutic combat. In: Ahmed S, Chandra Ojha S, Najam-ul-Haq M, editors. Biochemistry of Drug Resistance. Cham: Springer International Publishing; 2021. p. 505-38. doi: 10.1007/978-3-030-76320-6_20
8. Odewale G, Jibola-Shittu MY, Ojurongbe O, Olowe RA, Olowe OA. Genotypic determination of extended spectrum β-lactamases and carbapenemase production in clinical isolates of *Klebsiella pneumoniae* in Southwest Nigeria. Infect Dis Rep. 2023;15(3):339-53. doi: 10.3390/idr15030034, PMID 37367193
9. Di Pilato V, Pollini S, Miriagou V, Rossolini GM, D'Andrea MM. Carbapenem-resistant *Klebsiella pneumoniae*: The role of plasmids in emergence, dissemination, and evolution of a major clinical challenge. Expert Rev Anti Infect Ther. 2024;22(1-3):25-43. doi: 10.1080/14787210.2024.2305854, PMID 38236906
10. Karampatakis T, Tsergouli K, Behzadi P. Carbapenem-resistant *Klebsiella pneumoniae*: Virulence factors, molecular epidemiology and latest updates in treatment options. Antibiotics (Basel). 2023;12(2):234. doi: 10.3390/antibiotics12020234, PMID 36830145
11. Neelambike Sumana M, Maheshwarappa YD, Kalyani G, Mahale RP, Sowmya GS, Raghavendra Rao M, *et al*. A retrospective study of the antimicrobial susceptibility patterns of *Klebsiella pneumoniae* isolated from urine samples over a decade in South India. Front Microbiol. 2025;16:1553943. doi: 10.3389/fmicb.2025.1553943, PMID 40600137
12. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *enterobacteriaceae*. Emerg Infect Dis. 2011;17(10):1791-8. doi: 10.3201/eid1710.110655, PMID 22000347
13. Kumar H, Kaur N, Kumar N, Chauhan J, Bala R, Chauhan S. Achieving pre-eminence of antimicrobial resistance among non-fermenting Gram-negative bacilli causing septicemia in intensive care units: A single center study of a tertiary care hospital. Germs. 2023;13(2):108-20. doi: 10.18683/germs.2023.1374, PMID 38144242
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI Supplement M100. Wayne: Clinical and Laboratory Standards Institute; 2022.
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x, PMID 21793988
16. Hassan MI, Alkharsah KR, Alzahrani AJ, Obeid OE, Khamis AH, Diab A. Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. J Infect Dev Ctries. 2013;7(8):618-29. doi: 10.3855/jidc.2919, PMID 23949298
17. Booq RY, Abutarboush MH, Alolayan MA, Huraysi AA, Alotaibi AN, Alturki MI, *et al*. Identification and characterization of plasmids and genes from carbapenemase-producing *Klebsiella pneumoniae* in Makkah Province, Saudi Arabia. Antibiotics (Basel). 2022;11(11):1627. doi: 10.3390/antibiotics11111627, PMID 36421271
18. Mohammadpour D, Memar MY, Leylabadlo HE, Ghotaslou A, Ghotaslou R. Carbapenem-Resistant *Klebsiella pneumoniae*: A comprehensive review of phenotypic and genotypic methods for detection. Microbe. 2025;6:100246. doi: 10.1016/j.microb.2025.100246
19. Sahoo S, Mohanty J, Routray S, Sarangi A, Nayak D, Shah S, *et al*. Prevalence of multidrug-resistant *Klebsiella pneumoniae* in urinary tract infections: A retrospective observational study in eastern India. Microbes Infect Dis. 2024;6:299-307. doi: 10.21608/mid.2024.276619.1844
20. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015;59(10):5873-84. doi: 10.1128/AAC.01019-15, PMID 26169401
21. Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252-75. doi: 10.1093/femsre/fux013, PMID 28521338
22. Rizvi M, Khan N, Fatima A, Bushra R, Zehra A, Saeed F, *et al*. The microbiological characteristics and genomic surveillance of carbapenem-resistant *Klebsiella pneumoniae* isolated from clinical samples. Microorganisms. 2025;13(7):1577. doi: 10.3390/microorganisms13071577, PMID 40732086
23. Ilyas R, Asghar S, Zehra M, Usmani Y, Khan RM, Mirani ZA, *et al*. Molecular assessment of carbapenem-resistant and ESBL *Klebsiella pneumoniae* clinical isolates to decipher the correlation of antimicrobial resistance with virulence traits. Infect Genet Evol. 2025;133:105785. doi: 10.1016/j.meegid.2025.105785, PMID 40527416
24. Lagha R, Ben Abdallah F, ALKhammash AA, Amor N, Hassan MM, Mabrouk I, *et al*. Molecular characterization of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from King Abdulaziz specialist hospital at Taif City, Saudi Arabia. J Infect Public Health. 2021;14(1):143-51. doi: 10.1016/j.jiph.2020.12.001, PMID 33412373
25. Mirzaie A, Ranjbar R. Antibiotic resistance, virulence-associated genes analysis and molecular typing of *Klebsiella pneumoniae* strains recovered from clinical samples. AMB Express. 2021;11(1):122. doi: 10.1186/s13568-021-01282-w, PMID 34460016
26. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control. 2019;8:39. doi: 10.1186/s13756-019-0488-4, PMID 30815254
27. Moges F, Eshetie S, Abebe W, Mekonnen F, Dagnew M, Endale A, *et al*. High prevalence of extended-spectrum beta-lactamase-producing Gram-negative pathogens from patients attending Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara region. PLoS One. 2019;14(4):e0215177. doi: 10.1371/journal.pone.0215177, PMID 30986262
28. Taha MS, Hagras MM, Shalaby MM, Zamzam YA, Elkolaly RM, Abdelwahab MA, *et al*. Genotypic characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from an Egyptian University Hospital. Pathogens. 2023;12(1):121. doi: 10.3390/pathogens12010121, PMID 36678469
29. Kuchibiro T, Komatsu M, Yamasaki K, Nakamura T, Nishio H, Nishi I, *et al*. Evaluation of the modified carbapenem inactivation method for the detection of carbapenemase-producing Enterobacteriaceae. J Infect Chemother. 2018;24(4):262-6. doi: 10.1016/j.jiac.2017.11.010, PMID 29248418
30. Song W, Kim HS, Kim JS, Kim HS, Shin DH, Shin S, *et al*. Carbapenem inactivation method: accurate detection and easy interpretation of carbapenemase production in *Enterobacteriaceae* and *Pseudomonas*

spp. Ann. Ann Clin Microbiol. 2016;19(4):83. doi: 10.5145/ACM.2016.19.4.83
31. Sharif MR, Soltani B, Moravveji A, Erami M, Soltani N. Prevalence and risk factors associated with extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates in Hospitalized Patients in Kashan (Iran). Electron Physician. 2016;8(3):2081-7. doi: 10.19082/2081, PMID 27123215
32. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Risk factors for acquisition of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in North-Indian hospitals. Saudi J Biol Sci. 2015;22(1):37-41. doi: 10.1016/j.sjbs.2014.05.006, PMID 25561881
33. Legese MH, Weldearegay GM, Asrat D. Extended-spectrum beta-lactamase- and carbapenemase-producing *Enterobacteriaceae* among Ethiopian children. Infect Drug Resist. 2017;10:27-34. doi: 10.2147/IDR.S127177, PMID 28182124
34. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing *Enterobacteriaceae* among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. Antimicrob Resist Infect Control. 2015;4:12. doi: 10.1186/s13756-015-0054-7, PMID 25908966
35. Ouedraogo AS, Sanou M, Kissou A, Sanou S, Solaré H, Kaboré F, *et al*. High prevalence of extended-spectrum β-lactamase producing Enterobacteriaceae among clinical isolates in Burkina Faso. BMC Infect Dis. 2016;16:326. doi: 10.1186/s12879-016-1655-3, PMID 27400864
36. Desai D, Misra RN, Das NK, Gandham NR, Vyawhare CR, Mirza S. Prevalence of extended spectrum β-lactamases *Klebsiella pneumoniae* in various clinical samples and characterization of the *bla* Genes in a tertiary care hospital of Western Maharashtra. Med J Dr Patil Vidyapeeth. 2023;16(3):398-402. doi: 10.4103/mjdrdypu.mjdrdypu_286_22
37. Maleki N, Tahanasab Z, Mobasherizadeh S, Rezaei A, Faghri J. Prevalence of CTX-M and TEM β-lactamases in *Klebsiella pneumoniae* isolates from patients with urinary tract infection, Al-Zahra Hospital, Isfahan, Iran. Adv Biomed Res. 2018;7:10. doi: 10.4103/abr.abr_17_17, PMID 29456981
38. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of *TEM*, bla_{SHV} and *CTX-M* genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian J Pathol Microbiol. 2014;57(2):249-54. doi: 10.4103/0377-4929.134698, PMID 24943758
39. Hassan H, Abdalhamid B. Molecular characterization of extended-spectrum beta-lactamase producing Enterobacteriaceae in a Saudi Arabian tertiary hospital. J Infect Dev Ctries. 2014;8(3):282-8. doi: 10.3855/jidc.3809, PMID 24619257
40. Oteo J, Saez D, Bautista V, Fernández-Romero S, Hernández-Molina JM, Pérez-Vázquez M, *et al*. Carbapenemase-producing Enterobacteriaceae in Spain in 2012. Antimicrob Agents Chemother. 2013;57(12):6344-7. doi: 10.1128/AAC.01513-13, PMID 24041898
41. 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;111:240-6. https://doi.org/10.1080/20477724.2017.1340128.
42. Raheel A, Azab H, Hessam W, Abbadi S, Ezzat A. Detection of carbapenemase enzymes and genes among carbapenem-resistant Enterobacteriaceae isolates in Suez Canal University Hospitals in Ismailia, Egypt. Microbes Infect Dis. 2020;1(1):24-33. doi: 10.21608/mid.2020.25702.1007
43. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: Treatment options with carbapenems compromised. J Assoc Physicians India. 2010;58:147-9. PMID 20848811
44. Sisay A, Kumie G, Gashaw Y, Nigatie M, Gebray HM, Reta MA. Prevalence of genes encoding carbapenem-resistance in *Klebsiella pneumoniae* recovered from clinical samples in Africa: Systematic review and meta-analysis. BMC Infect Dis. 2025;25(1):556. doi: 10.1186/s12879-025-10959-7, PMID 40251495
45. Tula MY, Enabulele OI, Ophori EA, Aziegbemhin AS, Iyoha O, Filgona J. A systematic review of the current status of carbapenem resistance in Nigeria: Its public health implication for national intervention. Niger Postgrad Med J. 2023;30(1):1-11. doi: 10.4103/npmj.npmj_240_22, PMID 36814157
46. Abbasi E, Ghaznavi-Rad E. High frequency of *NDM-1* and *OXA-48* carbapenemase genes among *Klebsiella pneumoniae* isolates in central Iran. BMC Microbiol. 2023;23(1):98. doi: 10.1186/s12866-023-02840-x, PMID 37038144
47. Liu Z, Gu Y, Li X, Liu Y, Ye Y, Guan S, *et al*. Identification and characterization of NDM-1-producing hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* in china. Ann Lab Med. 2019;39(2):167-75. doi: 10.3343/alm.2019.39.2.167, PMID 30430779
48. Tanriverdi Cayci Y, Biyik I, Korkmaz F, Birinci A. Investigation of *NDM*, *VIM*, *KPC* and *OXA-48* genes, blue-carba and CIM in carbapenem resistant Enterobacterales isolates. J Infect Dev Ctries. 2021;15(5):696-703. doi: 10.3855/jidc.13345, PMID 34106894