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ORIGINAL RESEARCH

Clinical and Molecular Profile of Carbapenem Resistant *Klebsiella pneumoniae* Infections in a Tertiary Care Hospital –Mangalore

Athira Pattolath[1], Prabha Adhikari[1], Vidya Pai[2]

[1]Department of Geriatric Medicine, Yenepoya Medical College Hospital, Yenepoya Deemed to be University, Mangalore, Karnataka, India; [2]Department of Microbiology, Yenepoya Medical College Hospital, Yenepoya Deemed to be University, Mangalore, Karnataka, India

Correspondence: Prabha Adhikari, Department of Geriatric Medicine, Yenepoya Medical College Hospital, Yenepoya Deemed to be University, University Road, Deralakatte, Mangalore, Karnataka, 575018, India, Tel +91 9880991290, Fax +91 0824-2204664, Email prabha.raghuveer@gmail.com

Purpose: Carbapenemase producing *Klebsiella pneumoniae* infection has increased in recent years, leading to limitations in treatment options. The present study was undertaken to detect the Carbapenemase-producing genes in *K. pneumoniae*, the risk factors for acquiring them, and their impact on clinical outcomes.

Patients and Methods: This prospective study included 786 clinically significant *K. pneumoniae* isolates. Antimicrobial susceptibility testing was done by conventional method, carbapenem-resistant isolates were screened by carba NP test, and positive isolates were further evaluated by multiplex PCR method. The patient's clinical and demographic details, co morbidity, and mortality were collected. Multivariate analysis was performed to check risk factors for acquiring CRKP infection.

Results: The results of our study showed high prevalence of CRKP (68%). The variables subjected to the multivariate analysis found that diabetes, hypertension, cardiovascular disease, COPD, use of immunosuppressants, previous hospitalization history, previous surgery, and parenteral nutrition are found to be significantly associated with carbapenem resistant *K. pneumoniae* infection. Clinical outcomes revealed that patients in the CRKP group had higher risk of mortality and were discharged against medical advice, and they also had higher rate of septic shock. Most of the isolates carried blaNDM-1 and blaOXA-48 carbapenemase genes. Additionally, the co-existence of blaNDM-1 and blaOXA-48 was found in our isolates.

Conclusion: The prevalence of CRKP was alarmingly high in our hospital with the limited choice of antibiotics. This was associated with high mortality and morbidity with the increase in health care burden. While this information is important to treat critically ill patients with higher antibiotics, strict infection control practices need to be in place to prevent the spread of these infections in the hospital. Clinicians need to be aware of this infection to use appropriate antibiotics to save the lives of critically ill patients with the infection.

Keywords: *K. pneumoniae*, antimicrobial resistance, carbapenemase, risk factors

Introduction

Klebsiella pneumoniae is a common *Enterobacteriaceae* in hospitals and the general public that can cause several disorders, including cardiovascular diseases, cystitis, and respiratory diseases.[1] When the host's immune system is weakened or skin tissue is injured, the pathogen can easily enter the host and cause infections through the respiratory tract, blood, urinary system, etc., leading to an extended hospital stay and greater medical costs. Additionally, *K. pneumoniae* multi-drug resistant (MDR) strains are one of the most significant bacteria causing nosocomial infections (hospital acquired illnesses) and pose a substantial threat to patient life.[2]

According to the World Health Organization's assessment of the global status of antibiotic resistance, *K. pneumoniae* is one of the top three bacteria of global concern because many antibiotics used to treat bacterial diseases are losing their efficacy.[3] Furthermore, among the top ten MDR bacteria in intensive care units, *K. pneumoniae* strains are now the second most prevalent MDR bacteria.[4]

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Carbapenem antibiotics, which include meropenem, ertapenem, and imipenem, are β-lactam antibiotics with a β-lactam ring and a broad spectrum of activity and utility. These antibiotics are used as a last resort to treat infections brought on by extended-spectrum β-lactamases (ESBL) producing organisms and MDR-GNBs.[5] However, as carbapenem antibiotics become more widely used, the status of MDR *K. pneumoniae* worsens, resulting in more significant clinical treatment failure and death.[6]

Carbapenem resistance can be due to porin mutations, efflux pumps, or carbapenemase synthesis. Although there is an expansion in the number of emerged carbapenemases, five important members that belong to three Ambler classes are the most studied, namely, class A; *Klebsiella pneumoniae* carbapenemases (blaKPC), Class B; New Delhi metallo β-lactamases (blaNDM), Verona integron-encoded metallo β-lactamases (blaVIM), Active on imipenem metalloβ-lactamases (blaIMP), and class D; Oxacillinase-48-like carbapenemases (blaOXA-48-like).[7] According to the Antimicrobial Resistance Reference Gene database maintained by the National Centre for Biotechnology Information (NCBI), these carbapenemases have several variations. The "big five" carbapenemases also vary in their geographic distribution and epidemiological status, which might be either endemic or limited to reported cases.[8] These newly-emerging Carbapenem resistant *K. pneumoniae* (CRKP) classes in *K. pneumoniae* linked to life-threatening illnesses have been widely reported.[9] This pathogen poses a significant risk to human health,[10] and it is also a distinct risk factor for nosocomial infection and mortality.[11] The emergence of MDR strains has become a critical issue that must be addressed, notably in the case of CRKP. Prevention, inhibition of CRKP infection, and investigation of drug resistance mechanisms have emerged as a contemporary problem in research focus.

CRKP has spread globally, but there are regional variations in the strains.[12,13] An observational study in the United States found spatial variance in the CRKP incidence rate, which indicated general emergence and dispersion.[14] In China, the frequency of CRKP ranges from 0.9% to 23.6% in different provinces,[15] and hence drug resistance status may vary endemically.[16] Furthermore, among carbapenemase-producing strains, the incidence of *K. pneumoniae* carbapenemase (KPC) is lower in Spain than in Greece, and the prevalence of New Delhi metallo β-lactamase (NDM) is notably high in India.[17]

Despite the large number of research on carbapenem resistant *K. pneumoniae* infections, the clinical consequences for specific regional areas have not been thoroughly investigated. Therefore, we proposed this study, in which the prevalence of CRKP, risk factors for developing CRKP infection, and clinical profile, which include outcomes of severity and mortality, as well as phenotypical and genotypical characteristics of carbapenemase production were detected.

Materials and Methods

Study Design

Prospective study.

Study Setting

Yenepoya Medical College Hospital, Mangalore, South Karnataka, India

Study Duration

Two years and three months (April 2019-March 2021).

Selection Criteria

Inclusion Criteria

- Patient sample which shows the presence of *K. pneumoniae* isolates which are clinically significant.
- Age >18 years.
- A single strain of *K. pneumoniae* per patient.
- Only those isolates of *K. pneumoniae* obtained as pure growth from clinical samples.
- Inpatients.

Exclusion Criteria

- Polymicrobial infection (Isolation of *K. pneumoniae* along with other bacteria or fungal organism).

Ethical Statement

The study was conducted according to the guidelines of the World Medical Association Helsinki Declaration for studies on human subjects. It was approved by the Institutional Ethics Committee Board (protocol number YEC-1 2019/038, Dated 11-04-2019) of Yenepoya Deemed to be University. Only participants who gave their informed consent were enrolled in the study.

Isolation & Identification of Bacterial Strain

A total of 786 *K. pneumoniae* isolates were isolated and identified using the standard manual conventional method from routine clinical samples, such as blood, urine, pus, sputum, endotracheal tube aspirate, and broncho-alveolar lavage (BAL) fluid culture. The samples were inoculated into blood agar, MacConkey agar, and Nutrient agar. Blood and respiratory samples were inoculated on chocolate agar as well. Gram staining was done for the growth on the plates following an overnight incubation at 37 °C. In order to identify *K. pneumoniae*, biochemical tests, including the catalase test, oxidase test, indole test, citrate utilization test, urea hydrolysis test, motility test, triple sugar iron test, decarboxylase test, methyl red, Voges–Proskauer test, Hugh and Leifson OF test, and nitrate reduction test were carried out.[18] The HiMedia Laboratories Pvt. Ltd., Mumbai, India, provided all the media and reagents. The BD Phoenix M50 system (BD Diagnostic Systems, Oxford, UK) was used for additional confirmation.

Antibiotic Susceptibility Testing

According to recommendations from the Clinical Laboratory Institute (CLSI), bacterial susceptibility to antimicrobial drugs was assessed using the traditional Kirby Bauer's disc diffusion method (in vitro) with Mueller Hinton agar (MHA) plates.[19] The *K. pneumoniae* suspension adjusted to 0.5 McFarland turbidity standards ($1x10^8$ CFU/mL) was added to the MHA plates. Amikacin, ampicillin, aztreonam, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin-tazobactam, colistin, and tetracycline were among the antimicrobial discs evaluated. At 37°C, the plates were incubated overnight. The inhibition zones were measured, and the outcomes were compared to the chart of a typical assessment. The ATCC25922 strain of *E. coli* was chosen as a quality control strain. *K. pneumoniae* isolates resistant to three or more classes of antibiotics were considered multidrug resistant (MDR).[20]

Collection of Clinical Information & Risk Factor Analysis

Medical records were reviewed and collected if *K. pneumoniae* was discovered 48 hours after admission. The following information was documented for the analysis: demographics; chronic illnesses and pathological conditions, such as diabetes mellitus, a solid tumour, cardiovascular disease, respiratory disease, renal disease, hepatic disease, and central nervous system disease; history of hospitalisation within the previous six months; antibiotic therapy administered in the 30 days prior to the positive culture; and recent (1 month) surgical procedure and recent invasive procedures (Parenteral nutrition, central venous, urinary or gastric insertion, and mechanical ventilation).

All patients were monitored for the following outcomes: Mortality, DAMA (Discharged Against Medical Advices), development of sepsis (blood stream infection), length of stay in ICU, evaluation of SOFA score in ICU patients, and duration of mechanical ventilation for the patients.

MIC Determination by Broth Micro-Dilution Method

According to CLSI recommendations, the broth micro-dilution method was carried out. 0.5 McFarland suspension of the isolates was made and diluted 100-fold with cation-adjusted Mueller–Hinton broth (CAMHB). About 50 µL of the bacterial solution was seeded into a 96-well plate with 50 µL of CAMHB and serial antibiotic concentrations. There were roughly 5×10^5 CFU/mL of bacteria in the final inoculum. The final concentrations of meropenem were 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL and 0.125 µg/mL. The suspension was cultured at 35°C for 18 to 20 hours.[19]

Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL

Phenotypic assays among probable ESBL generating isolates verified ESBL production. The third-generation cephalosporins ceftazidime (30μg) and ceftazidime clavulanic acid (30/10 μg) discs were positioned 25 mm apart on a lawn growth of the microorganism. The zone of inhibition for ceftazidime-clavulanic acid increased by around 5mm when contrasted to ceftazidime, indicating that it produces ESBLs. For ESBL test quality control, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used.[19]

Phonotypical Detection of Carbapenemase Production by Carba NP Test

The test was performed in accordance with Nordmann, Poirel, and Dortet's description of how it should be done: "A change in color of the pH indicator is indicative of carbapenem hydrolysis induced by carbapenemase producing bacteria, which create acid".[8] The steps were carried out according to the manufacturer's instructions. API suspension media (25 μL; bioMérieux, New Delhi, India) was added to the wells, and 5–6 colonies from the fresh culture plate were collected and placed in the prescribed well. The turbidity of the inoculum was compared to that of the supplied strip. Following that, 10 μL of inoculum was added to two wells, one of which contained imipenem. Imipenem was utilized as the carbapenemase zinc substrate for metallo β-lactamases (MBL)-producing gram-negative bacteria, and the results were considered positive if the color changed from red to yellow, orange, or thick orange in comparison to the control well.[19,21] Further tests were performed on organisms that tested positive for carbapenemase.

Molecular Detection of Carbapenemase Genes

Extraction of Bacterial DNA

DNA was extracted using HiPurA® Genomic DNA Purification kits (Hi-Media®, India), which rely primarily on the reversible nucleic acid-binding characteristics of the improved silica gel membrane and the speed and versatility of Miniprep spin columns to produce high-quality DNA. The process consists of three fundamental steps. First, test DNA was allowed to bond to the silica-gel membrane of the HiEluteMiniprep spin column after being extracted from the log-phase bacterial culture by centrifugation and lysed by lysozymes. The second phase involved removing leftover contaminants as a contaminant passed through the membrane, and the third step involved eluting and collecting pure genomic DNA in a tube. DNA from the bacteria was extracted, eluted from the columns in 200 μL elution buffer, and then kept at −20°C in a small Eppendorf tube.

Multiplex PCR Assay

To find particular regions of the gene that encode the carbapenemase enzyme, such as blaVIM, blaNDM-1, blaKPC, blaOXA-48, and blaIMP, the carbapenemase gene (multiplex) probe-based Hi-Media Hi-PCR kit has been used. This kit can precisely identify one or a combination of carbapenemase genes in a single-tube reaction with a wide range of organisms. The company's recommendations were followed using positive, internal, and negative controls.

The multiplex probe PCR kit is made to find specific areas of the genes that produce different carbapenemase enzymes. This technique is used to amplify the targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides, that have a fluorescent reporter dye attached to the 5'end and a quencher dye to the 3'end. The details of primers and probes[22] for blaNDM, blaKPC, blaIMP, blaOXA-48, and blaVIM are shown in Table 1. We used two multiplex assays to identify two combinations of five carbapenemase genes and an internal control; Set 1 and Set 2 contained blaNDM, blaKPC, blaIMP, blaVIM, and blaOXA-48, respectively. In this kit, there are two master mixes. Master mix-1 detects blaNDM-1, blaKPC, blaIMP, and blaVIM in the FAM, HEX, Texas Red, and Cy5 channels, while master mix-2 detects blaOXA-48 in the Texas Red channel. In both final mixes, the Cy5.5 channel contained evidence of internal control.

A 25μL reaction mixture consisting of 5 μL sample DNA and 20 μL master mix was used for this assay. For each reaction, the master mix contained 12.5 μL Hi-Quanti 2X Realtime PCR master mix (MBT180); 4μL of each CRGI/CRG2Primer-Probe Mix (DS0949/DS0950); 1μL internal control Primer-Probe Mix (DS0498); 1μL Internal control B DNA (DS0385A); 5μLTemplate DNA and 1.5μL Molecular biology-grade water (ML065). The two master mixes (CRG1 and CRG2) were prepared as described in Table 2. Then, 10μL of CRG1 and CRG2 were transferred to the PCR cartridge, and run the PCR. The cycling procedure included 10 minutes of initial denaturation at 95°C, followed by 5 seconds of denaturation at 95°C, 45 cycles of annealing and extension at 60°C for one minute, and a final holding stage in SLAN-96P real-time PCR system. A cycle threshold value of ≤40

Table 1 Primers and Probes Used in the Multiplex, Real-Time PCR Test

Gene	Primer Names	Probe	Sequences (5'-3')	Wavelength (nm)
blaNDM	NDM Carbapenemase	FAM labeled hydrolysis probe	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	FAM (465–510)
blaKPC	KPC Carbapenemase	HEX labeled hydrolysis probe	F: ATGTCACTGTATCGCCGTCT R: TTTTCAGAGCCTTACTGCCC	610 (540–610)
blaIMP	IMP Carbapenemase	Texas Red labelled hydrolysis probe	F: GAATAGRRTGGCTTAAYTCTC R: CCAAACYACTASGTTATC	640 (610–645)
blaOXA	OXA-48 Carbapenemase	Texas Red labeled hydrolysis probe	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGATGGC	640 (610–645)
blaVIM	VIM Carbapenemase	Cyan500 labeled hydrolysis probe	F: GTTTGGTCGCATATCGCAAC R: AATGCGCAGCACCAGGATAG	FAM (465–510)

Abbreviations: NDM, New Delhi metallo β-lactamases; KPC, *Klebsiella pneumoniae* carbapenemases; IMP, Active on imipenem metallo β-lactamases; VIM, Verona integron-encoded metallo β-lactamases; OXA-48, Oxacillinase-48-like carbapenemases.

Table 2 Composition of Master Mix for Detecting blaNDM, blaKPC, blaVIM, blaIMP, and blaOXA48gene

Components	Product Code	Volume to be Added for 1R (for a 25 µL Reaction)	
		CRG1 Tube	CRG2 Tube
Hi-Quanti 2X Realtime PCR Master Mix	MBT180	12.5 µL	12.5 µL
CRG1 Primer-Probe Mix	DS0949	4 µL	–
CRG2 Primer-Probe Mix	DS0950	–	4 µL
Internal Control Primer-Probe Mix	DS0498	1 µL	1 µL
Internal Control B DNA	DS0385A	1 µL	1 µL
Molecular Biology Grade Water for PCR	ML065	1.5 µL	1.5 µL
Positive Control / Negative Control / Template DNA	–	5 µL	5 µL
Total volume	–	Upto 25 µL	Upto 25 µL

was considered positive. In a single-tube reaction, the kit enables the accurate and precise detection of both single and co-present carbapenemase-encoding genes.[22,23]

Statistical Analyses

Continuous variables were reported as means and standard deviations (SDs) if they were normally distributed or as medians and interquartile ranges (IQRs) when they were not. Categorical variables have been given as frequencies and percentages. Depending on how they were distributed, categorical variables were compared using chi-square or Fisher's exact tests. Continuous variables were compared using Student's t-tests or Mann–Whitney *U*-tests. The significant risk factor for acquiring CRKP was determined by using univariate analysis. For testing the null hypothesis, a P-value of 0.5 was selected as the level of significance. Stepwise multiple logistic regression analyses of the risk factor for CRKP infection were also computed for variables deemed significant on standard statistical analysis to discover independent risk factors. IBM SPSS STATISTICS (version 23.0, New York, USA) was used to analyze all the data.

Results

In the current investigation, 786 consecutive nonduplicate *K. pneumoniae* isolates were obtained from a tertiary care teaching hospital in Mangalore between April 2019 and March 2021. Among all the *K. pneumoniae* identified (n = 786), 42% (n = 330/786) were in pus, 26% (n = 204/786) in respiratory specimens, 20.3% (n = 160/786) in urine, and 11.7% (n = 92/786) in blood. Figure 1 depicts the distribution of *K. pneumoniae* isolates from clinical samples in the various treatment units. The most significant

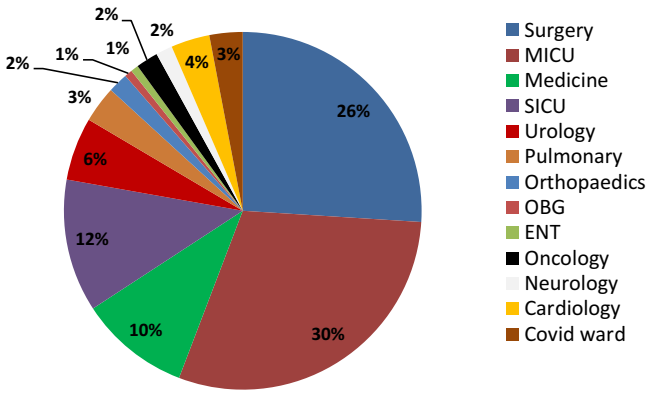

Figure 1 Distribution of CRKP isolates in various departments of hospital.

contributor to CRKP (29.8%, 160/536) was the medical ICU, whereas most CSKP isolates (31.6%, 79/250) were found in the surgical ward (Figure 2).

Comparison of Clinical Characteristics, Risk Factor Analysis, and Outcome

Patients with CRKP isolates had a mean age of 62.78± 6.7 years. Thirty-four percent of participants who had an infection with *K. pneumoniae* were female, while 66% were male. Nearly half of the patients infected with CRKP had an underlying condition such as diabetes, poor glycemic control, or hypertension. The majority of CRKP patients had undergone invasive procedure. Patients in the CRKP group had a greater rate of exposure to antibiotics and prior hospitalization when compared to the CSKP group.

According to univariate analysis, key risk factors included: smoking habit (P = 0.04), having diabetes (P = 0.001), having poor glycemic control (P = 0.001), hypertension (P = 0.001), having a tuberculosis infection (P = 0.001), having chronic kidney disease (CKD) (P = 0.001), having cardiovascular disease (CVD) (P= 0.001), having COPD (P= 0.003), having used immunosuppressive therapy (P = 0.001), and having had surgery in the past (P = 0.001). The univariate analysis also determined that indwelling devices such as urine catheters, nasogastric insertion, parenteral nutrition, and central venous catheter were significantly associated with CRKP infection. Table 3 displays the findings of the univariate analysis of the clinical traits in *K. pneumoniae* infected participants.

Outcome measurements in *K. pneumoniae* patients found that infection-related mortality was considerably greater in CRKP patients compared to CSKP patients. We found that the SOFA score (P = 0.001) was higher in the CRKP group with regard to the ICU admission status. There were 107 fatalities, of which 97 (90.6%) involved patients with CRKP infections and 10 (9.4%) patients with CSKP infections. Among the 169 participants who were discharged against medical advice (DAMA), 134 (79.2%) of them were from the CRKP group, and 35 (21%) belonged to the CSKP group. A total of 510 participants recovered, with 305

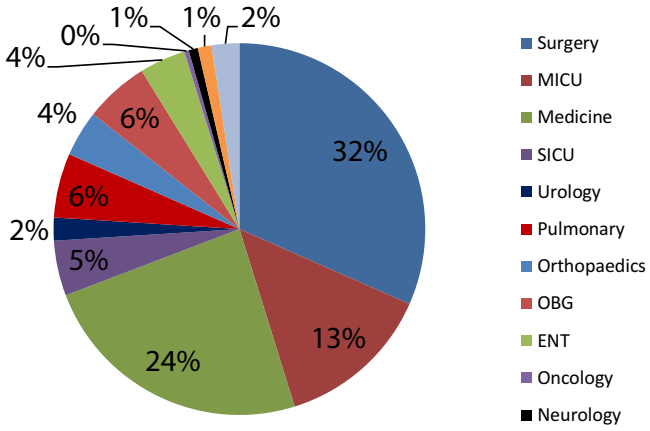

Figure 2 Distribution of CSKP isolates in various departments of hospital.

Table 3 Comparison of Clinical Characteristics of CSKP and CRKP Isolates by Univariate Analysis

Variables	CRKP (536) n%	CSKP (250) n%	TOTAL	OR (95% C.I)	P
Smoking	22 (4.1)	19 (7.6)	41	0.52 (0.276–0.980)	0.04
Alcohol	24 (4.5)	18 (7.2)	42	0.60 (0.322–1.135)	0.114
Diabetes	311 (58.0)	66 (26.4)	377	3.853 (2.772–5.357)	0.001
Poor glycemic control	270 (50.4)	57 (22.8)	327	3.437 (2.445–4.831)	0.001
Hypertension	240 (44.8)	43 (17.2)	283	3.903 (2.696–5.651)	0.001
COPD	93 (17.4)	23 (9.2)	116	2.072 (1.277–3.361)	0.003
Asthma	52 (9.7)	17 (6.8)	69	1.473 (0.833–2.602)	0.181
TB	16 (3.0)	26 (10.4)	42	0.265 (0.139–0.504)	0.001
Immunosuppression	156 (29.1)	20 (8.0)	176	4.721 (2.882–7.733)	0.001
CKD	209 (39.0)	43 (17.2)	252	3.077 (2.122–4.461)	0.001
CLD	29 (5.4)	11 (4.4)	40	1.243 (0.61–2.53)	0.548
Cardiovascular disease	195 (36.4)	29 (11.6)	224	4.358 (2.848–6.667)	0.001
Pulmonary disease	212 (39.6)	106 (42.4)	318	0.889 (0.655–1.206)	0.449
Neurological disease	53 (9.9)	24 (9.6)	77	1.033 (0.622–1.716)	0.899
Autoimmune disease	16 (3.0)	9 (3.6)	25	0.824 (0.359–1.891)	0.647
Prior history of surgery	321 (59.9)	72 (28.8)	393	3.691 (2.67–5.102)	0.001
Tumour	32 (6.0)	9 (3.6)	41	1.7 (0.799–3.618)	0.164
Use of broad spectrum antibiotics	310 (57.8)	45 (18.0)	355	6.249 (4.336–9.005)	0.001
Previous hospitalizations	323 (60.3)	49 (19.6)	372	6.22 (4.353–8.889)	0.001
Urinary Catheter	329 (61.38)	106 (42.4)	435	2.159 (1.591–2.93)	0.001
Central venous catheter	92 (17.2)	20 (8.0)	112	2.383 (432–3.965)	0.001
Nasogastric insertion	281 (52.4)	82 (32.8)	363	2.258 (1.65–3.09)	0.001
Parenteral nutrition	216 (40.3)	64 (25.6)	280	1.962 (1.407–2.735)	0.001
Outcome factors					
ICU admission	160 (29.9)	34 (13.6)	194	2.703 (1.801–4.058)	0.001
SOFA's >12	121 (22.6)	20 (8.0)	141	3.353 (2.034–5.527)	0.001
Mechanical ventilation	143 (26.7)	20 (8.0)	163	4.184 (2.55–6.867)	0.001
Septic shock	86 (16.0)	6 (2.4)	92	7.772 (3.348–18.041)	0.001
Recovered	305 (56.9)	205 (82.0)	510	0.29 (0.201–0.418)	0.001
DAMA	134 (25.0)	35 (14.0)	169	2.048 (1.363–3.077)	0.001
Mortality	97 (18.1)	10 (4.0)	107	5.303 (2.714–10.36)	0.001

Notes: *P*<0.05 is significant (if *P*<0.05 [0.02–0.05]=95%; *P*<0.01 [0.002–0.01]=99%; *P*<0.001 [0.000–0.001]=99.9%).

Abbreviations: CRKP, Carbapenem resistant *K. pneumoniae* isolates; CSKP, Carbapenem susceptible *K. pneumoniae* isolates; COPD, Chronic obstructive pulmonary disease; CKD, Chronic Kidney Disease; CLD, Chronic Liver Disease; SOFA's, Sequential organ failure assessment; ICU, Intensive Care Unit; DAMA, Discharged against medical advice.

(60%) coming from the CRKP group and 205 (40%) from the CSKP group. Univariate analysis revealed that all of the outcome factors, including recovered (P = 0.001), DAMA (P = 0.003), and mortality (P = 0.001), were significantly associated with severe outcomes in CRKP infection.

When the factors were exposed to multivariate analysis; diabetes (P = 0.001), hypertension (P = 0.001), immunosuppressive therapy (P = 0.001), cardiovascular disease (P = 0.004), prior hospitalization (P= 0.001), prior history of surgery (P= 0.001), COPD (P = 0.001) and parenteral nutrition (P = 0.001) were all significant risk factors affecting the development of CRKP infections. The findings of the multivariate logistic regression study of how risk factors affect participants with *K. pneumoniae* infection are shown in Table 4.

Antimicrobial Susceptibility of *K. pnemumoniae* Isolates

There were *786 K. pneumoniae isolates*, 536 (68%) of which were carbapenem-resistant strains and 250 (32%) of which were carbapenem-sensitive strains. 77.4% (415/536) of the 536 CRKP isolates were multidrug resistant, meaning they were resistant to more than three classes of antibiotics, compared to 7.2% (18/250) of the CSKP isolates. Figures 3 and 4 depict the phenotypic antimicrobial resistance pattern of isolated carbapenem resistant and carbapenem sensitive

Table 4 Multivariate Analysis of Factors Influencing the Acquisition of Carbapenem Resistance in *K. pneumoniae* Isolates

Variables	OR	95% CI	P
Diabetes	3.160	2.008–4.971	0.001
Hypertension	3.412	2.132–5.461	0.001
COPD	3.193	1.620–6.294	0.001
Immunosuppression	4.369	2.305–8.282	0.001
Cardiovascular disease	2.386	1.326–4.293	0.004
Prior history of surgery details	2.263	1.436–3.566	0.001
Use of broad spectrum antibiotics	1.804	0.943–3.453	0.075
Previous hospitalizations	4.314	2.240–8.308	0.001
Central venous catheter	0.521	0.242–1.121	0.095
Parenteral nutrition	0.284	0.154–0.521	0.001
Septic shock	3.627	1.229–10.706	0.020
Recovered	0.487	0.268–0.885	0.018

Notes: *P*<0.05 is significant (if *P*<0.05 [0.02–0.05]=95%; *P*<0.01 [0.002–0.01]=99%; *P*<0.001 [0.000–0.001]=99.9%).
Abbreviations: OR, Odds ratio; CI, Confidence interval.

K. pneumoniae isolates. Ampicillin (100%) had the most significant level of resistance among the 536 CRKP isolates examined, followed by cefepime (93%), cefotaxime (93%), ceftazidime (93%), amoxicillin-clavulanic acid (90.70%), cotrimoxazole (90%), Aztreonam (88%), and chloramphenicol (88%). Both the CRKP and CSKP isolates showed good sensitivity to colistin and tigecycline. In the CSKP group, all antibiotics studied showed greater susceptibilities except for ampicillin (Figure 4). 87.3% of the CRKP isolates were positive for ESBL.

Determination of Meropenem MIC

The MIC of meropenem showed a varied range. Most of the *K. pneumoniae* isolates showed a MIC of 8µg/mL (48.5%, 260/536). 27.5% (145/536) of isolates had MIC at 4 µg/mL. Eighteen isolates (3.3%) showed maximum MICs of 128µg/mL. The remaining isolates (20.7%, 113/536) showed 16 to 64 µg/mL MIC levels (Table 5).

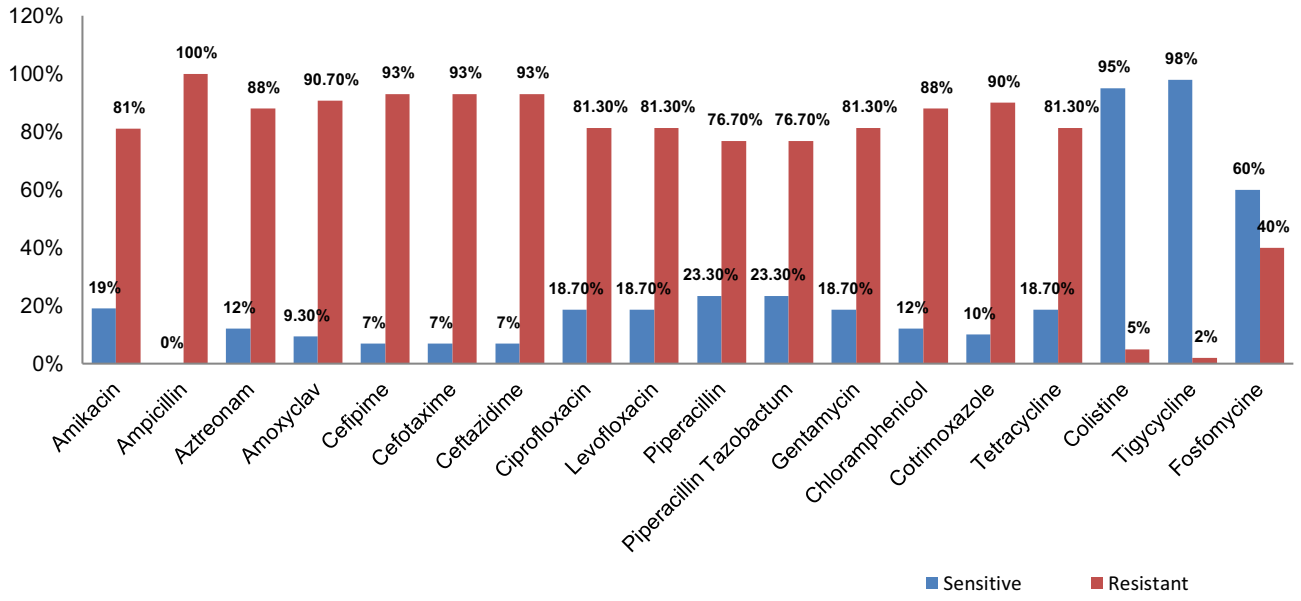

Figure 3 Antibiotic susceptibility pattern of CRKP (Carbapenem resistant *K.pneumoniae*) isolates.The interpretation of the results was according to the Clinical and Laboratory Standards Institute guidelines, CLSI(2020).

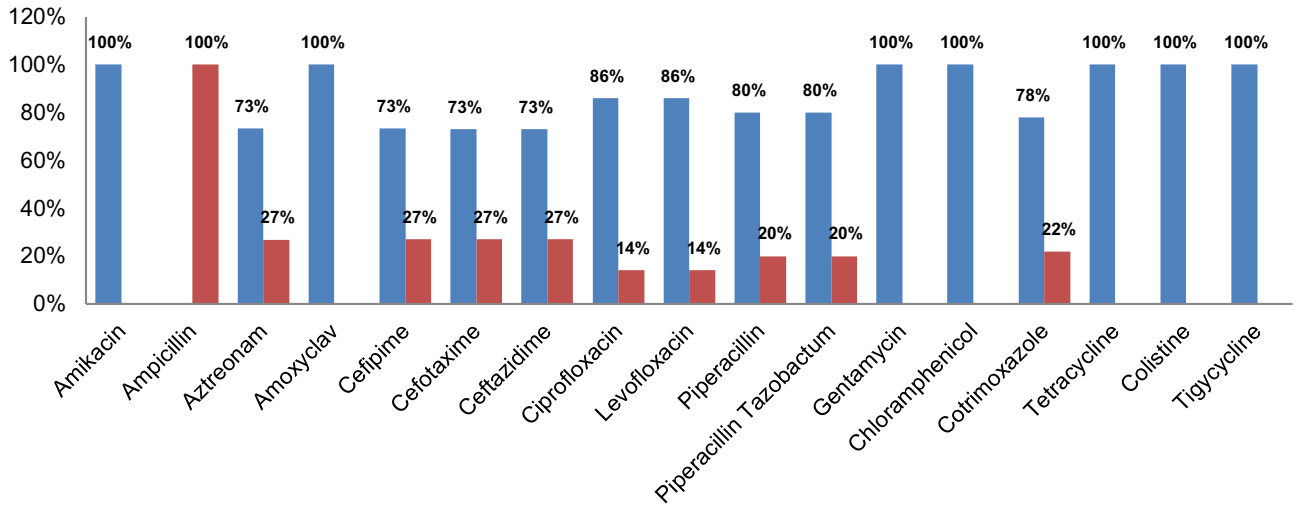

Figure 4 Antibiotic susceptibility pattern of CSKP (Carbapenem susceptible *K. pneumoniae*) isolates.The interpretation of the results was according to the Clinical and Laboratory Standards Institute guidelines, CLSI(2020).

In order to determine whether the distribution of MICs varied among carbapenemase types, the MIC profiles for the CRKP isolates were further examined. The data showed that 74.5% (104/140) of blaNDM-1-producing isolates and 84.5% (148/175) of blaOXA-48-producing isolates had a meropenem MIC of 8 µg/mL. In isolates producing harboring genes (blaOXA48-and blaNDM-1), 58% of the isolates (67/115) showed a meropenem MIC of 16 µg/mL. None of the carbapenemase-positive isolates collected had meropenem MICs lower than 4 µg/mL.

Resistance Mechanism of Carbapenem

Of the CRKP examined, the carbaNP test was positive in 81.7% (n = 438/536) of the cases. Four hundred and thirty (80.22%) of the 536 isolates of the CRKPs had carbapenemase genes, according to real-time PCR. The mean Ct value of the blaNDM-1 results was 17, and the range was from 13 to 25. The mean C_t value of the blaoxa-48 results was 15.2, ranging from 11 to 21. Table 6 lists the findings of Multiplex PCR for the carbapenemase genes. The distribution of the genes was as follows: blaNDM-1 alone accounted for 32% (175/536), while blaOXA-48 alone accounted for 26.11% (140/536). Among the carbapenemases, the coexistence of blaNDM-1 and blaOXA-48 like was found in 21% (115/536) of the carbapenemases. blaKPC, blaVIM and blaIMP were noticeably absent. The findings of the real-time PCR amplification for the carbapenemase genes are shown in Figure 5.

In our investigation, the most common genes in sputum isolates were blaOXA-48 (47.4%), followed by blaNDM-1 (36.4%), and blaOXA-48 with blaNDM-1 (16%). Fifty percent of the genes in the pus sample were blaNDM-1. The coexistence of blaOXA-48 and blaNDM-1 was predominant (48%) in urine samples. The most often found gene in the blood sample was blaNDM-1 (71%). Table 7 lists the distribution of carbapenemase genes amongst isolates of *K. pneumoniae* from various clinical origins.

Table 5 Determination of Meropenem MIC by Broth Micro-Dilution Method

Antibiotic	MIC Concentrations (µg/mL)											
	0.12	0.25	0.5	01	02	04	08	16	32	64	128	256
Meropenem	–	–	–	–	–	145(27.5%)	260(48.5%)	67(12.5%)	28(5.2%)	18(3%)	8(1.5%)	10(1.8%)

Notes: The MIC of meropenem determined by Broth Micro-Dilution Method in CRKP(536) isolates. The numeric in the bracket represents the percentage of *K. pneumoniae* strains.

Table 6 Multiplex Quantitative Real-Time PCR Assay for the CRKP Isolates Carrying Carbapenemase Genes

PCR Set	Carbapenemase Genes	No of Carbapenemase Genes	C_t value(Mean)
1	blaNDM-1	175	17
	blaKPC	0	Not detected
	blaIMP	0	Not detected
	blaVIM	0	Not detected
2	blaOXA-48	140	15.2

Notes: Shown are Cycle threshold value (mean). PCR set 1 targets blaNDM, blaKPC, blaVIM, and blaIMP, while PCR set 2 targets blaOXA48. C_t value less than 40 considered as positive for both blaNDM-1 and blaOXA-48 like.

Discussion

Antimicrobial resistance (AMR) is on the rise, notably among gram-negative bacteria such as *Klebsiella* spp. Numerous studies have revealed an increase in the incidence of antibiotic resistance among *Klebsiella* species. It is problematic since it causes several different acute infections. This widespread resistance could be attributed to the injudicious use of higher antibiotics without sufficient sensitivity guidelines. Furthermore, India's increased population density may have contributed to isolating more multidrug resistant *K pneumoniae* species.[24] Carbapenems are currently the drug of choice for treating severe hospital acquired infections. Recent research has revealed extremely high levels of carbapenem resistance in India and the Indian subcontinent, necessitating the adoption of alternative therapies.[25] It would be interesting to identify the microorganisms that produce carbapenemase accurately, and this would require phenotypic and genotypic research to identify every gene responsible for making carbapenemase.

This research was conducted in a tertiary care facility in Karnataka, India. During the study period, 786 consecutive, non-repeat, distinct *K. pneumoniae* isolates were recovered from various clinical samples collected in our laboratory, with pus showing the highest recovery, followed by respiratory specimens, urine, and blood. Previous research from India has revealed that urine samples isolate the most *K. pneumoniae*, followed by sputum or exudative specimens.[26,27] Furthermore, the majority of CRKP samples isolated in this investigation were from ICU patients (29.8%, 160/536), followed by the Surgery ward (26%, 139/536) and SICU (12%, 64/536) patients. Similarly, several investigations[28,29] found that CRKP isolates were substantially linked with ICU admitted patients. In the current study, the prevalence of CRKP was 68%, whereas it was 77% in a study carried out in a tertiary care hospital in Haryana, India.[30] Pawar et al discovered a prevalence of CRKP of roughly 31.77%,[31] while Kumari et al discovered it to be 16.8% in a study in Bihar.[25]

Several risk factors for CRKP have been reported in previously released reviews, which include severe serious illnesses, the use of broad-spectrum antibiotics, the use of carbapenem antibiotics, a lengthy hospital stay, malignancies, hematopoietic stem cell transplantation, tracheotomies, mechanical ventilation, and indwelling catheters.[32,33] In our study, the results of the univariate analysis demonstrated that smoking, diabetes, hypertension, COPD, tuberculosis infections, chronic kidney disease, cardiovascular disease, indwelling catheters, prior exposure to broad-spectrum antibiotics, prior hospitalizations, prior history of surgery and use of immunosuppressant's were all possible causes for CRKP infections. These findings are consistent with those of earlier research conducted in other regions of the world.[34,35]

Regarding ICU admission status and treatment procedures performed, we found that the SOFA score (>12 points) was higher in the CRKP group. The findings indicate that the severity of the disease is the primary cause of mortality, which is explained by the extremely high baseline median SOFA Score. In-hospital mortality was greater in CRKP patients in univariate analysis (OR, 5.303; 95% CI, 2.714–10.36; P = 0.001); however, in multivariate analysis, it was not statistically significant.

According to multivariate regression analysis, diabetes, hypertension, COPD, cardiovascular disease, immunosuppressive treatment, past hospitalization, prior surgical history, and parenteral nutrition were found to be significantly associated with CRKP infection. According to this, having a severe coexisting co-morbid condition such as diabetes, hypertension, CKD, and cardiovascular disorders was a significant risk factor for CRKP infections. Therefore, patients

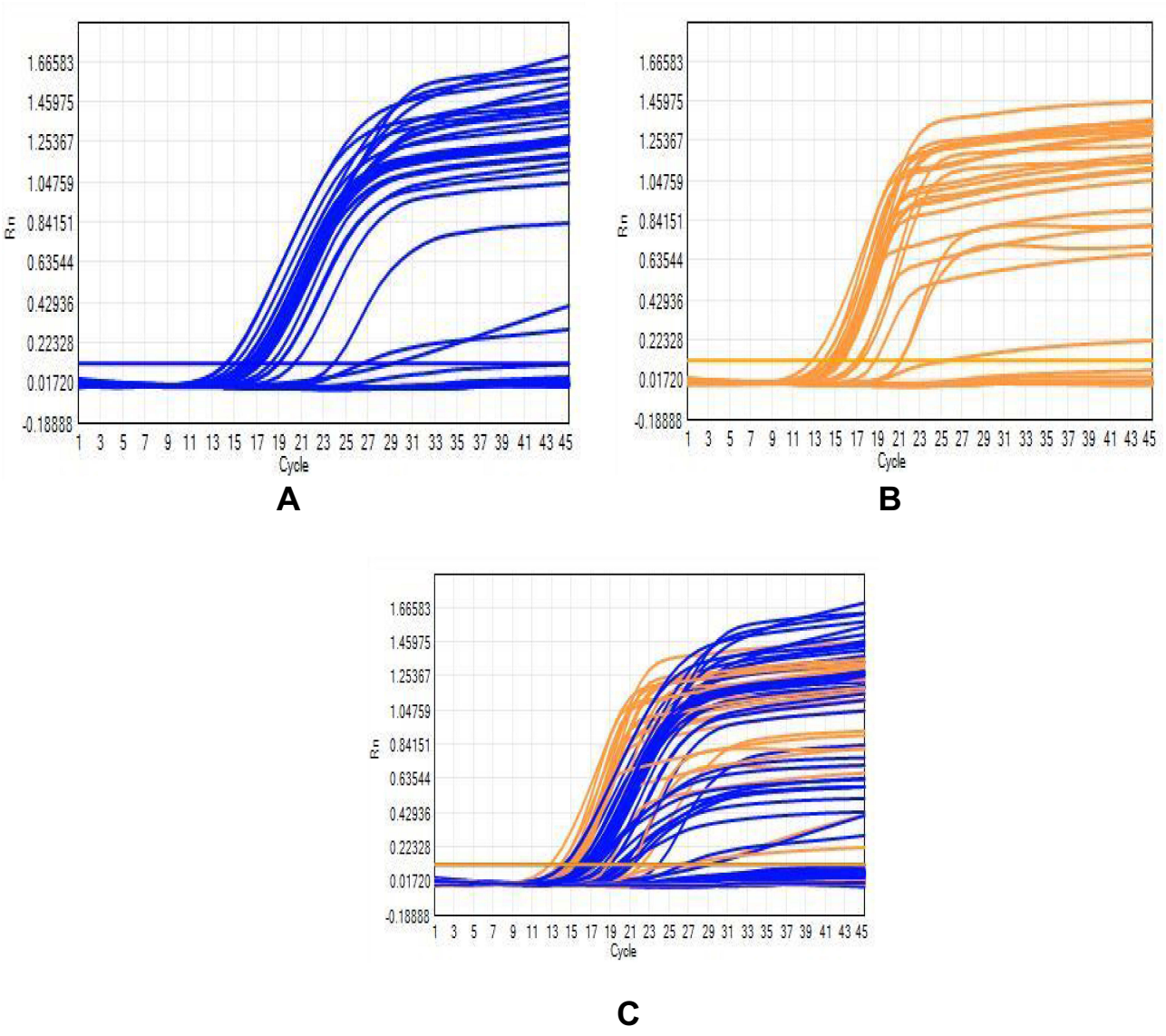

Figure 5 Real-time PCR amplification results for blaNDM-1 (**A**), blaOXA-48 (**B**), and coexistence of blaOXA48 and blaNDM-1 (**C**) obtained after 45 cycles on the SLAN-96P Real time PCR system. C_t value ≤40 is considered positive for blaNDM-1 and blaOXA-48 genes.

with more comorbidities, greater severity, and worse immunity were more likely to develop multi-drug resistance from sensitive *K. pneumoniae*. To accurately assess the true impact of antibiotic resistance, it is crucial to account for potential confounding factors such as underlying co-morbidities.

Table 7 Distribution of Carbapenemases Genes Among Isolates of *K. pneumoniae* from Various Sources

Sl No	Source	blaOXA-48 (N=140)	blaNDM-1 (N=175)	blaOXA48+ blaNDM1 (115)
1	Sputum	65 (47.4%)	50 (36.4%)	22 (16%)
2	Pus /Swab	32 (22.8%)	70 (50%)	38 (27.2%)
3	Urine	33 (29%)	26 (23%)	55 (48%)
4	Blood	10 (29%)	25 (71%)	–
5	ET aspirate	–	4 (100%)	–

Note: Data presented as n (%).
Abbreviations: blaOXA-48, Oxacillinase; blaNDM-1, New Delhi metallo-beta -lactamase; ET aspirates, Endotracheal.

According to our study, chronic obstructive pulmonary disease (COPD) is a risk factor for CRKP infection, which is in agreement with earlier findings.[36,37] Patients with MDR-*K. pneumoniae* exhibited a greater prevalence of chronic pulmonary illness, which could explain why *K. pneumoniae* was the primary pathogen in patients with gram-negative bacillus pneumonia acquired in the hospital or the community.

Additionally, more patients in the CRKP group were found with septic shock and parenteral nutrition.

According to our research, the CRKP group had more patients who were receiving immunosuppressive medication than the CSKP group. Thus, individuals who are prone to infectious infections may have the option of using immunity enhancing treatments such intravenous immunoglobulin or thymosin application.

The prevalence of MDR *K. pneumoniae* clinical isolates has been continuously increasing over the world.[38] The current study found that *K. pneumoniae* is resistant to most commonly used antibiotics. In our study, *K. pneumoniae* showed notable antibiotic resistance, the same as in the Wang et al findings.[16] The CRKP strains in this investigation were extremely resistant to most antibiotics, which may be related to the duration and type of drug exposure, as well as the prevalence of antibiotic-resistance genes.[36] Surprisingly, the rate of antibiotic resistance in *K. pneumoniae* to the tested antibiotics observed in this investigation was higher than that found in the surveillance study conducted in India.[37] Similar to other studies; it was discovered that *K. pneumoniae* isolates in our study were entirely resistant to ampicillin.[16,38] Furthermore, tigecycline and colistin have good in vitro activity against our CRKP isolates, nevertheless, resistance concerns occur during therapy, posing a substantial risk to public health,[38] and colistin resistance is primarily connected with genetic mutations in lipid A modification.[39]

Four hundred and thirty-eight of the 536 isolates (81.7%) displayed phenotypic positive by Carba NP test, which was similar to what Vamsi et al observed.[22] According to the results of the genotypic detection for the carbapenemases, 32% of the isolates were found to carry the blaNDM-1 gene, and 26.11% of the isolates carried the bla OXA-48 gene. In our collection, we found the genes for blaNDM-1 and blaOXA-48, although other carbapenemase producing genes, such as blaKPC, blaIMP and bla VIM were not discovered in any of the *K. pneumoniae* isolates examined. This is consistent with the findings of Jaggi et al, who discovered that the NDM gene, alone or in combination with OXA-48, was present in 35.9% of CRKP isolates, while VIM and KPC were absent in all of their isolates.[40] 18.3% (98/536) of the carbapenem-resistant isolates examined in our study tested negative for all the examined genes. This can be explained by the fact that these strains exert their resistance through different mechanisms, such as those involving the presence or lack of porins or efflux pumps or a gene not examined in this work.

According to our investigation, blaNDM-1 and blaOXA-48 coexisted at a rate of 21% within the same isolate, which was equivalent to the 20.0% coexistence rate reported by Garg et al.[39] Because carbapenem-resistant genes can co-exist with beta-lactamases and other resistant genes on plasmids, treating infections caused by carbapenem-resistant strains becomes more difficult.[41] Additionally, coexistence with carbapenem results in the retention of genes that make organisms resistant to other antimicrobials, which poses a threat to the economy, patient recovery, and antibiotic chemotherapy globally.[42]

Study Limitation

Because the study was conducted in a single tertiary care center, our findings may not be generalizable to other settings, and only resistance to carbapenems via carbapenemase production was investigated. It has yet to be determined how other co-existing mechanisms, such as porin loss or efflux pumps, contribute to carbapenem resistance. The clonality of the isolates and the gene sequence were not examined in the study, which would have improved the evaluation of the spread of a carbapenemase or some clones to comprehend CRKP isolates in the clinical environment. Despite these drawbacks, the study has delivered information on the occurrence of common carbapenemase genes and the relevance of the issue.

Conclusion

In conclusion, this study demonstrated that the detection rate of CRKP in our hospital was greater than that of CSKP. The drug resistance condition must be highly valued because the CRKP in our investigation exhibits a multi-drug resistant phenotype and is resistant to several common antibiotics. The development of many resistance genes is linked to CRKP resistance. The primary carbapenemase genes recovered from clinical CRKP in our hospital were blaNDM-1 and blaOXA-48. The risk factors for contracting the CRKP infection were examined in our study. As a result, understanding the risks of acquisition and attempting to avoid them, if feasible, would aid in mitigating the spread of these organisms and reducing the burden of related infections on the health system and human lives. We identified eight independent factors associated with CRKP, including diabetes, hypertension,

COPD, cardiovascular disease, a history of hospitalization, a prior history of surgery, parenteral nutrition, and recent exposure to immunosuppressant's within 3 months of acquisition.

Strict and effective nosocomial infection management and control strategies should be developed, epidemiological investigations should be conducted on time, and antibiotic resistance monitoring should be strengthened. It is critical to prevent the spread and incidence of CRKP in hospitals.

Abbreviations

MDR, Multi-drug resistant; CRKP, Carbapenem resistant *Klebsiella pneumoniae*; CSKP, Carbapenem sensitive Klebsiella pneumoniae; ATCC, American Type Culture Collection; CLSI, Clinical Laboratory Standard Institute; MIC, minimum inhibitory concentration; NDM, New Delhi metallo β-lactamases; KPC, *Klebsiella pneumoniae* carbapenemases; IMP, Active on imipenem metalloβ-lactamases; VIM, Verona integron-encoded metallo β-lactamases; OXA-48, Oxacillinase-48-like carbapenemases.

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Disclosure

The authors report no conflicts of interest in this work.

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