

RESEARCH ARTICLE

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Molecular Epidemiology of Carbapenem-resistant *Klebsiella pneumoniae* in West Bengal, India

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Abstract

Overcoming resistance to carbapenem in *Klebsiella pneumoniae* is a major concern for public health worldwide, including in India. According to 2024 WHO Bacterial Priority Pathogens List, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is identified as the top-ranked bacterium. Although studies on this have been conducted in West Bengal, India, data on prevalence and geographical molecular distribution of CRKP remain limited. The study aims to investigate demographic distribution of CRKP isolates for proper epidemiological knowledge, to determine the geographical molecular distribution of CRKP isolates, to detect prevalence of carbapenemase-producing genes among those isolates in West Bengal, India, and to develop a Heat Map for strengthening the epidemiological surveillance system. 128 CRKP clinical isolates were collected from different clinical settings of Paschim Medinipur, Jhargram, Purba Medinipur, Kolkata, Purulia and Birbhum districts of West Bengal, from July 2023 to June 2025. We performed AST & biochemical analysis, followed by end-point multiplex PCR. The demographic distribution analysis was performed by R and SPSS software. CRKP prevalent zones among the sample collection sites were identified using ArcGIS. A Kernel Density Heat Map was used to strengthen the epidemiological surveillance system. From this study, through demographic distribution, it is observed that females and the "≥50" age group are most affected, and the difference is statistically significant (P-value < 0.05). Among the 6 districts, Kolkata exhibited highest prevalence with 40 cases (31.25%), whereas Birbhum showed the lowest prevalence, with 10 cases (7.8%). *bla*_{NDM} was the most prevalent carbapenemase-producing gene identified in this region. The Heat Map shows the spatial distribution. This study highlights the comprehensive epidemiological burden of CRKP infection in West Bengal, which might help the public health policy makers to strengthen the surveillance system and the clinicians in target therapy by optimizing the use of antibiotics. This will also help in outbreak situation control by proper epidemiological knowledge.

Keywords: Carbapenem-resistant *Klebsiella pneumoniae*, End-Point Multiplex PCR, Carbapenemase-producing Genes, Kernel Density Heat Map, Public Health Surveillance

INTRODUCTION

Antimicrobial resistance (AMR) is a significant global public health concern currently. The World Health Organization (WHO) reports that 1.27 million deaths in 2019 were directly caused by AMR, and an additional 4.95 million deaths were linked to resistant infections.¹ Among the most concerning pathogens, the ESKAPEE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species and *Escherichia coli*) are extremely virulent, multidrug-resistant bacteria that significantly increase morbidity, mortality, and healthcare costs. Drug inactivation, target alteration, decreased intracellular drug accumulation, and biofilm development are some of the mechanisms used by the ESKAPEE pathogens to avoid antimicrobial activity.^{2,3}

Klebsiella pneumoniae is one of the most important ESKAPEE pathogens, which is

a Gram-negative bacterium that belongs to the *Enterobacteriaceae* family and is frequently found in soil, water, and a variety of hosts, including humans and animals. Globally, *K. pneumoniae* is first in the WHO (World Health Organization) BPPL (Bacterial Priority Pathogens List) report, 2024. Hyper virulent *K. pneumoniae* infection's first case was found in Taiwan, China (Continent: Asia) in 1986. Later, cases of these infections were also found in North America, South America, Europe, Africa and Australia continuously.⁴ According to the NARS-Net (National Antimicrobial Resistance Surveillance Network), India yearly report of 2021, *Klebsiella* spp. take second position (21.2% of all most commonly isolated pathogens in India) in the pathogen priority list. Whereas, with respect to carbapenem-resistant *Enterobacterales* (CRE) in blood, 50% resistance was observed in *Klebsiella* spp.⁵ Emergence of carbapenem-resistant *Enterobacteriaceae*, particularly of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), is among the most significant challenges for public health

worldwide,^{6,7} linked to a higher death rate and healthcare costs due to increased use of antibiotics, medication without doctors' suggestion, which was made by patients themselves and failure to implement antibiotic policies in clinical settings.^{8,9} Carbapenems serve as the last option antibiotics to handle infections caused by CRKP.¹⁰ Currently, limited antibiotic options are available for the treatment of CRKP infections. Humans typically have *K. pneumoniae* in their urinary, respiratory, cutaneous, and gastrointestinal systems. These bacteria can cause a variety of diseases, including potentially fatal infections like cystitis, parenteral infections, pneumonia, sepsis, and endocarditis, when virulence factors, such as pili, capsules, and iron carriers, or siderophores, are released by them. Additionally, the majority of hospital-acquired infections are caused by them.¹¹⁻¹³ The synthesis of carbapenemases among them is the main mechanism by which *K. pneumoniae* develops carbapenem resistance.^{14,15} IMP, VIM, KPC, OXA-48, and NDM-1 are the most prevalent carbapenemases, which are encoded by *bla*_{IMP}, *bla*_{VIM}, *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48} genes, respectively, known as carbapenem resistance-determining genes.^{16,17} But resistance can also be caused by other mechanisms, such as overexpression of AmpC or ESBLs β -lactamases, loss or alteration of outer membrane porins, efflux pumps' overactivity, biofilm development, and persistence phenotypes.^{18,19} A study in a tertiary care hospital in Indore, India, reported carbapenem resistance in 25% of the total isolated *K. pneumoniae* isolates from different clinical specimens having *bla*_{KPC}, *bla*_{IMP}, and *bla*_{NDM} genes.²⁰ Here, we studied the molecular epidemiology of CRKP infection in the six districts of West Bengal, India. In another study, including the ICU of ten distinct tertiary care facilities in Kolkata, West Bengal, India, reported the development of *bla*_{KPC-2} in *K. pneumoniae*, along with other carbapenemases like *bla*_{NDM} and *bla*_{OXA-48} like variants, poses a major health risk to vulnerable populations, particularly ICU patients.²¹ Despite this, the geographical molecular epidemiology of CRKP in West Bengal, integrating antimicrobial resistance patterns with spatial distribution, remains limited. Therefore, this study aims to ascertain the geographical molecular distribution

of CRKP isolates, identify the prevalence of carbapenemase-producing genes among those isolates in West Bengal, India, investigate the demographic distribution of CRKP isolates for appropriate epidemiological knowledge, and develop a heat map to strengthen the epidemiological surveillance system.

MATERIALS AND METHODS

Collection of CRKP isolates from the study area

Six different districts of West Bengal, namely Paschim Medinipur, Jhargram, Purba Medinipur, Kolkata, Purulia, and Birbhum, comprised the study area. We collected CRKP clinical isolates from different healthcare facilities in those districts from July 2023 to June 2025 in the nutrient agar stab, maintaining the aseptic condition. The source samples of those isolates were urine, blood, pus, sputum, and throat swabs. The information about the source samples and demographic data was collected from the lab registers of those healthcare facilities. Those isolates were further investigated for this study.

Identification of the CRKP isolates

The clinical isolates were cultured on MacConkey agar plates and incubated overnight at 37 °C. After incubation based on the colony morphology, like colour, size, texture, and biochemical analysis, the bacterial isolates were identified. Triple sugar iron agar, indole, Simmons' citrate agar, urease, motility, and PPA biochemical tests were done to verify the bacterial type.

Antibiotic Sensitivity Testing (AST) of the CRKP isolates

The susceptibility of the bacterial isolates to certain antibiotics was assessed using the Kirby-Bauer disk diffusion (KBDD) method following standard guidelines.²² Here in this study, Amoxycillin + Clavulanic acid (β -lactam combination agent) (10 μ g), Amikacin (Aminoglycosides) (30 μ g), Ampicillin (Penicillin, β -lactam agent) (10 μ g), Gentamicin (Aminoglycosides) (10 μ g), Cefixime (β -lactam third generation cephalosporin) (5 μ g), Ciprofloxacin (Fluoroquinolones) (5 μ g), Ofloxacin (Fluoroquinolones) (5 μ g), Meropenem (Carbapenem) (10 μ g), and Imipenem

(Carbapenem) (10 µg) discs were used to check the susceptibility of the collected bacterial isolates and also to determine the CRKP isolates.

End-point multiplex PCR

End-point multiplex PCR was performed to detect the carbapenemase-producing genes among those CRKP isolates by isolating their genomic DNA through the boil template method, following standard PCR program. Table 1 comprises the list of the primers utilized here. Appropriate positive and negative controls were used during the experiment.²³⁻²⁶

Statistical analysis

Data regarding the age-sex-wise demographic distribution and distribution of the carbapenemase-producing genes among the CRKP isolates were analysed by using R software (v.4.x) and IBM SPSS Statistics (v.26). The chi-square test was used to compare the age-sex-wise demographic distribution. Statistical significance

was defined as a P-value of less than 0.05. The effect size for gene distribution was assessed using Cramer's V.

A chi-square test and Cramer's V tests were also performed to find the association with age, sex, and the related genes in the CRKP isolates. All tests were two-sided, and statistical significance was defined as $P < 0.05$.

GIS analysis

District-level choropleth maps were generated using ArcGIS version 10.8 to visualize the distribution of CRKP isolates. Districts were categorized into low, moderate, and high burden groups based on the number of CRKP clinical isolates.

Heat map analysis

Advanced spatial statistics, such as kernel density estimation, was developed to show the spatial distribution.

Table 1. Primers used to detect the target genes

Target gene	Primer sequence (5'-3')	Amplicon size (bp)	Approx molecular weight (Daltons)
NDM-F	GGGCAGTCGCTTCCAACGGT	188	188 × 660 Dalton
NDM-R	GTAGTGCTCAGTGTCGGCAT		
OXA-48-F	TTGGTGGCATCGATTATCGG	390	390 × 660 Dalton
OXA-48-R	GAGCACTTCTTTGTGATGGC		
KPC-F	TGTCAGTGTATCGCCGTC	1000	1000 × 660 Dalton
KPC-R	CTCAGTGCTCTACAGAAAACC		

Total Number of Bacterial Isolates (n=8424)

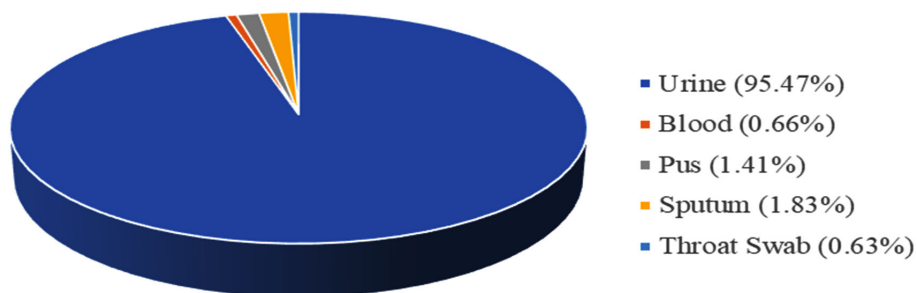


Figure 1. Distribution of the source samples of the total collected clinical isolates

RESULTS

The present study included a total of 8,424 clinical isolates from July 2023 to June 2025 from various healthcare facilities across six districts of West Bengal, India, namely Paschim Medinipur, Jhargram, Purba Medinipur, Kolkata, Purulia, and Birbhum. The clinical isolates were from 95.47% urine samples, 0.66% blood samples, 1.41% pus

samples, 1.83% sputum samples, and 0.63% throat swab samples (Figure 1). Notably, urine was the primary source for most of the clinical isolates.

Distribution of pathogenic bacterial clinical isolates

From 8424 isolates, after subculturing on the MacConkey agar plate, 1374 showed bacterial growth. The formation of distinct bacterial colonies

Table 2. Type of bacteria present in the collected isolates

	Name of the bacterial isolates				Total no. of clinical isolates (n)
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Proteus</i> spp.	Others	
Number of isolates	724	504	19	127	1374

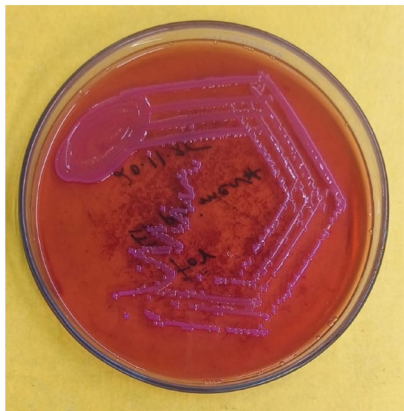


Figure 2. Growth of *Klebsiella pneumoniae* in the MacConkey agar plate

and biochemical analysis were used to identify the pathogenic bacteria. The colonies varied in size, texture, and colour. It revealed that among the 1374 bacterial isolates, 52.69% *E. coli*, 36.68% *K. pneumoniae* (Figure 2), 1.38% *Proteus* spp., and 9.24% were other bacteria (Table 2, Figure 3). Among the 127 others, *Acinetobacter* spp., *Alcaligenes* spp., *Enterobacter*, and *Pseudomonas* spp. were present.

Antimicrobial sensitivity testing of *K. pneumoniae* clinical isolates

Among the 504 *K. pneumoniae* isolates, 128 (25.40%) were resistant to the carbapenem group of antibiotics as per CLSI guidelines, at

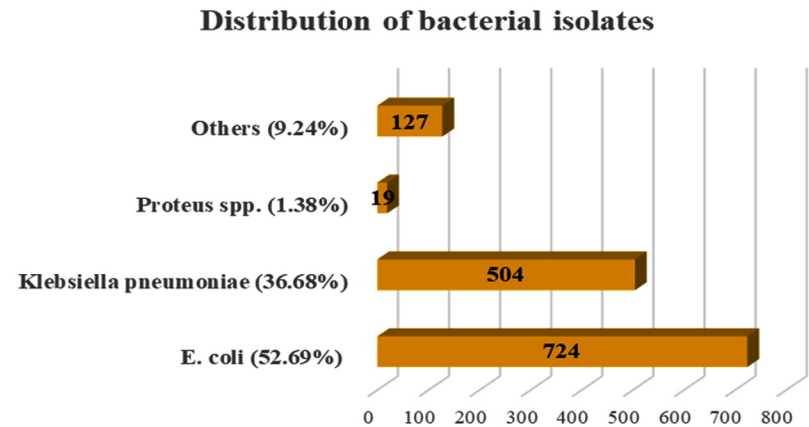


Figure 3. Type of bacteria present in the collected isolates

the time of AST through the Kirby-Bauer Disc Diffusion method. Out of the 128 *K. pneumoniae* isolates, meropenem resistance was detected in 122 (95.31%) of the isolates, while imipenem resistance was present in all 128 (100%). The complete antibiogram of the 128 CRKP isolates is revealed in Table 3.

Source specimen of the CRKP clinical isolates

The 128 CRKP clinical isolates were from 75% urine samples, 4.69% blood samples, 7.03% pus samples, 10.94% sputum samples, and 2.34% throat swab samples (Figure 4). For the CRKP

isolates, urine was also the source sample for most of the clinical isolates.

Demographic distribution of CRKP infection cases

Figure 5 represents the demographic breakdown of cases of CRKP infection during the study period. Results showed that 57 male patients (44.53%) and 71 female patients (55.47%) were diagnosed with CRKP infection. Also, it was found that 14 (10.94%) patients belonged to the “<15” age group, 44 (34.38%) patients belonged to the “15 - <50” age group, and 70 (54.69%) patients belonged to the “≥50” age group. From this study,

Table 3. Susceptibility pattern of CRKP isolates

Antibiotics	CRKP isolates (n = 128)					
	Susceptible (n)	%	Intermediate (n)	%	Resistant (n)	%
Amoxycillin + Clavulanic acid (β-lactam combination agent) (10 µg)	6	4.69	0	0	122	95.31
Amikacin (Aminoglycosides) (30 µg)	43	33.59	6	4.69	79	61.72
Ampicillin (Penicillin, β-lactam agent) (10 µg)	5	3.91	0	0	123	96.09
Gentamicin (Aminoglycosides) (10 µg)	48	37.5	0	0	80	62.5
Cefixime (β-lactam third generation cephalosporin) (5 µg)	12	9.38	0	0	116	90.63
Ciprofloxacin (Fluoroquinolones) (5 µg)	18	14.06	0	0	110	85.94
Ofloxacin (Fluoroquinolones) (5 µg)	21	16.41	0	0	107	83.59
Meropenem (Carbapenem) (10 µg)	6	4.69	0	0	122	95.31
Imipenem (Carbapenem) (10 µg)	0	0	0	0	128	100

Total number of CRKP isolates (n=128)

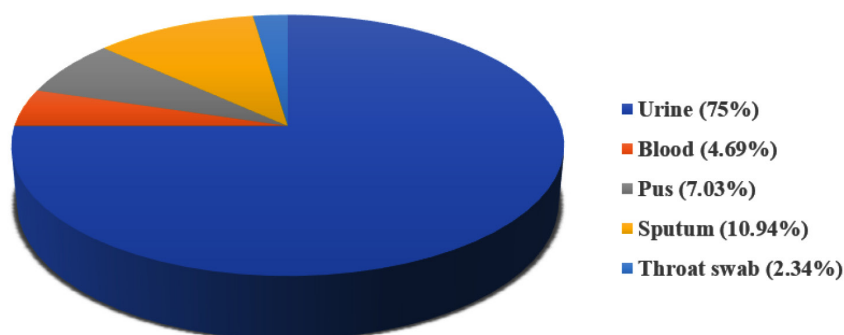


Figure 4. Distribution of the source samples of the CRKP isolates

through demographic distribution, it is observed that females and the “ ≥ 50 ” age group are most affected, with a difference of statistical significance (P-value <0.05).

Categorization of the districts using GIS mapping based on the CRKP isolate numbers

From geographical distribution analysis, it was found that Kolkata is the most prevalent zone among West Bengal with 40 (31.25%) CRKP

Table 4. District-wise distribution of the collected CRKP clinical isolates

Name of the district	No. of CRKP clinical isolates
Paschim Medinipur	29
Jhargram	13
Purba Medinipur	19
Kolkata	40
Purulia	17
Birbhum	10

Table 5. Distribution of the carbapenemase-producing genes in the collected CRKP clinical isolates

Carbapenemase producing genes	No. of Samples
NDM	64
OXA-48	29
KPC	12
NDM, OXA-48	23
Total	128

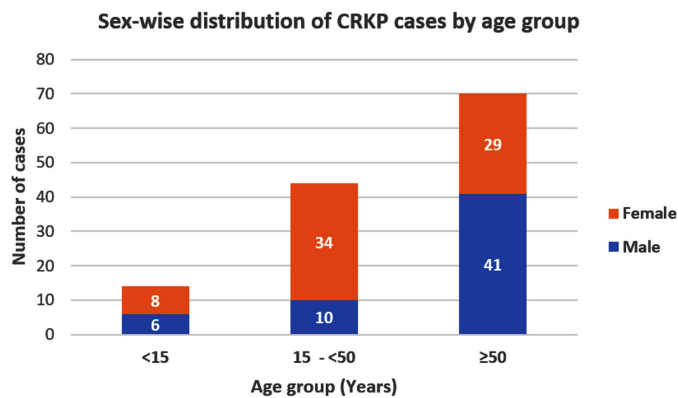


Figure 5. Demographic breakdown of CRKP-infected cases from July 2023 to June 2025

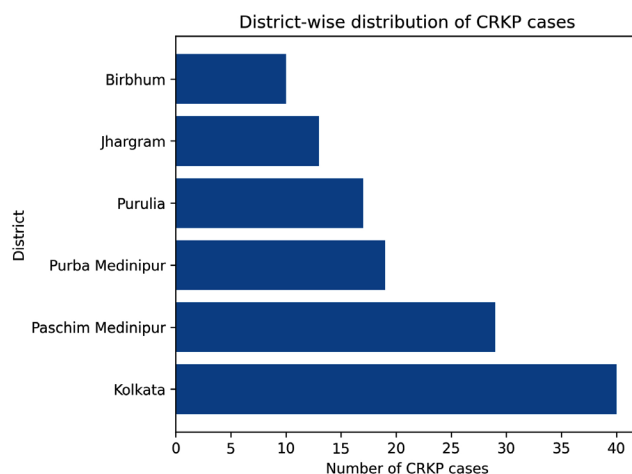


Figure 6. District-wise distribution of the collected CRKP clinical isolates

infective cases, and Birbhum has the lowest number of cases, i.e., 10 (7.81%). The district-wise geographical distribution is represented in Table 4 and Figure 6. Based on the case numbers, the districts were categorised into three categories: High, Moderate, and Low. The district has "<15" cases, belongs to the Low category; the district has "15-30" cases, belongs to the Moderate category; and the district has ">30" cases, belongs to the

High category. The categorisation of the sample collection sites was mapped using ArcGIS Software, version 10.8 (Figure 7).

Molecular characterization of CRKP isolates by PCR

The result of end-point multiplex PCR of the CRKP isolates revealed the presence of three types of carbapenemase producing genes

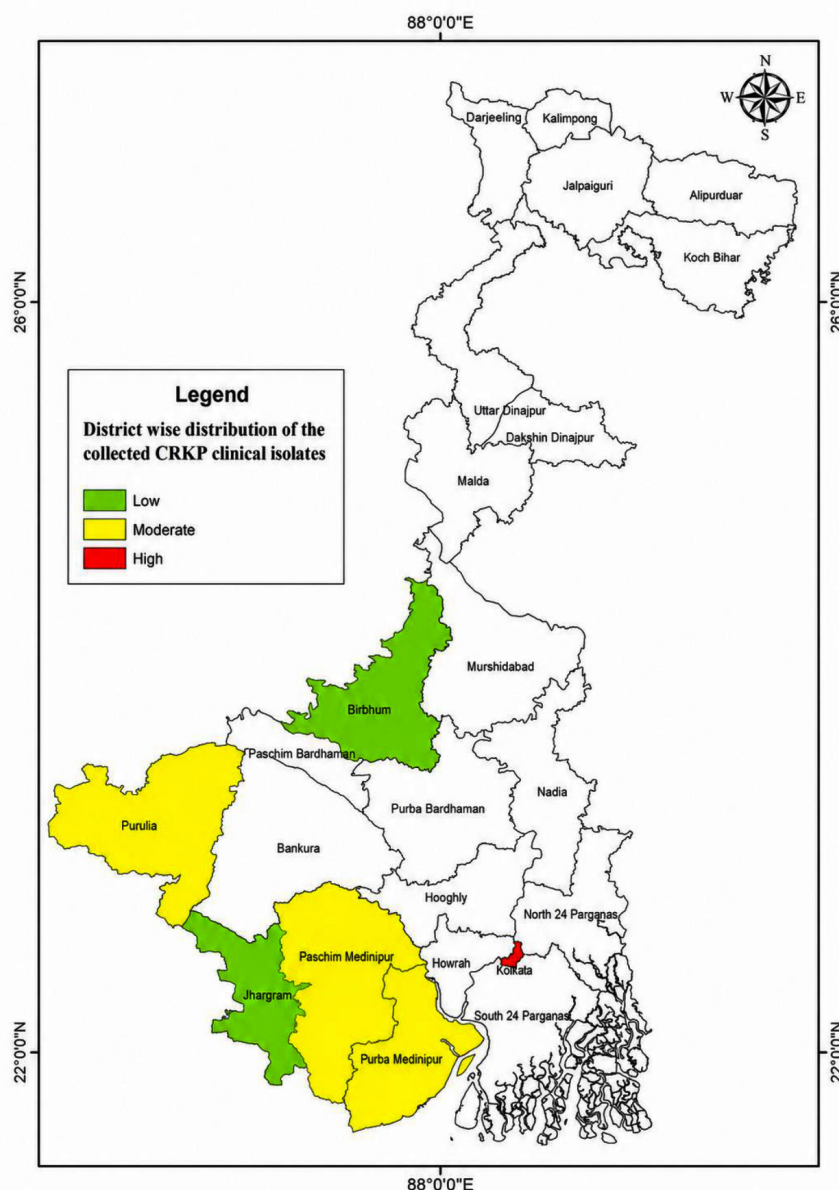


Figure 7. Categorisation of the CRKP clinical isolates collection sites in West Bengal (source: ArcMap V.10.8)

in them. These are NDM, OXA-48, and KPC. NDM was the most prevalent gene among them, found in 64 CRKP isolates (50%). KPC was rarely present, occurring in 12 cases (9.38%). In 29 samples (22.66%) OXA-48 was present and in 23 samples (17.97%) both NDM, and OXA-48 were present. The distribution of the carbapenemase-producing genes is represented in Table 5 and Figure 8. The gene distribution deviated significantly from uniformity (χ^2 test, $P < 0.05$; Cramer's V indicating moderate association).

To quantify the magnitude of deviation from uniformity, Cramer's V was calculated.

$$V = \chi^2 n(k-1) = 47.31128 \times 3 = 0.35V = \sqrt{\frac{\chi^2}{n(k-1)}} = \sqrt{\frac{47.31}{128 \times 3}} = 0.35V \\ = n(k-1)\chi^2 = 128 \times 347.31 = 0.35$$

Heat map analysis

A Kernel Density Heat Map based on this categorisation shows the spatial distribution (Figure 9).

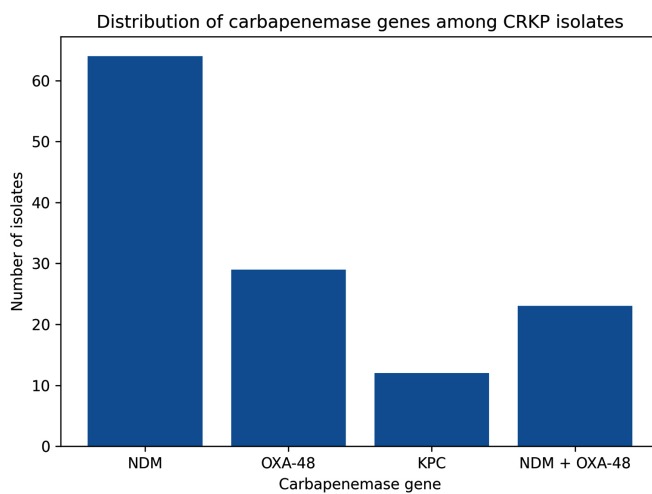


Figure 8. Distribution of the carbapenemase-producing genes among the collected CRKP clinical isolates

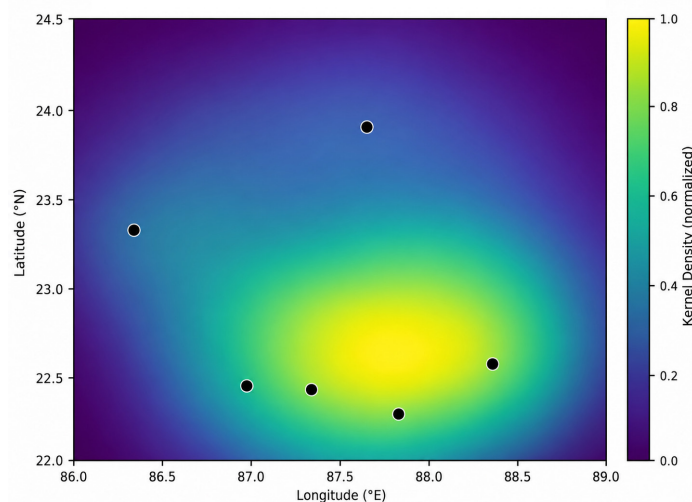


Figure 9. Kernel Density Heat Map of CRKP Cases

Statistical analysis of the association between age and sex of patients with carbapenemase-producing genes

NDM was slightly more frequent among females than males (36 vs 28) and was concentrated in the ≥ 50 year group (33/64, 51.6%). OXA-48 showed a similar pattern (16 females vs 13 males; 14/29, 48.3% in ≥ 50 years). KPC was more frequently observed among females (9/12, 75.0%) and was concentrated in the ≥ 50 year group (6/12, 50%). Whereas, the combined NDM + OXA-48 profile was somewhat more frequent among males (13/23, 56.5%) and was

strongly concentrated descriptively in the ≥ 50 year group (17/23, 73.9%) (Table 6).

The distribution of gene profiles did not differ significantly by sex ($\chi^2(3) = 3.209$, $P = 0.361$, Cramer's $V = 0.158$). Likewise, gene-profile distribution was not significantly associated with age category ($\chi^2(6) = 4.785$, asymptotic $p = 0.572$; Monte Carlo $P = 0.584$; Cramer's $V = 0.137$). Thus, although some gene profiles showed descriptive concentration in particular demographic groups, the available data do not support a statistically significant association between carbapenemase gene profile and either sex or age (Table 7).

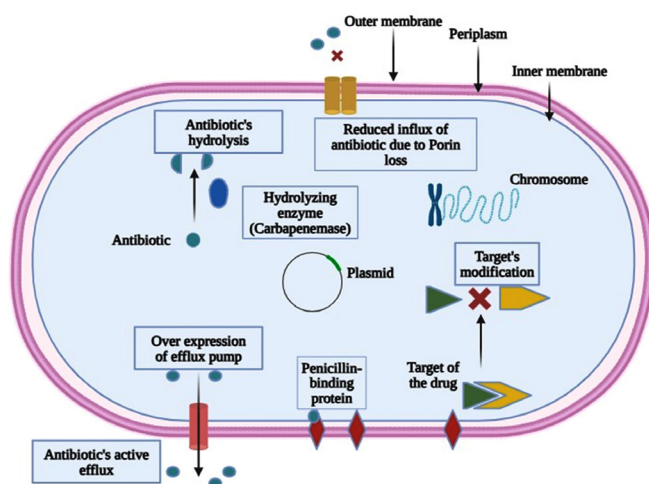


Figure 10. Structure of carbapenem-resistant *Klebsiella pneumoniae*

Table 6. Descriptive distribution of the association between age and sex of the patients with carbapenemase-producing genes in the collected CRKP clinical isolates

Gene profile	Male <15	Male 15 - <50	Male ≥ 50	Female <15	Female 15 - <50	Female ≥ 50	Total
NDM	3	6	19	4	18	14	64
OXA-48	2	3	8	2	8	6	29
KPC	1	0	2	1	4	4	12
NDM + OXA-48	0	1	12	1	4	5	23
Total	6	10	41	8	34	29	128

Table 7. Association tests

Comparison	Test statistic	d_f	P-value	Effect size	Interpretation
Gene profile x sex	$\chi^2 = 3.209$	3	0.361	Cramer's $V = 0.158$	No significant association
Gene profile x age	$\chi^2 = 4.785$	6	0.572; Monte Carlo $p = 0.584$	Cramer's $V = 0.137$	No significant association

DISCUSSION

CRKP is identified as the top-ranked bacterium according to the 2024 WHO Bacterial Priority Pathogens List, which has developed resistance to the carbapenem group of antibiotics, known as the last line of defence (Figure 10). For this reason, our molecular epidemiological study is the first study that aims to focus on strengthening the epidemiological surveillance system in public health by identifying the prevalent carbapenemase-producing genes through the geographical molecular distribution pattern in West Bengal, India, and also by developing a prediction model that will play a key role in the CRKP outbreak control.

We collected 8424 clinical isolates from six districts of West Bengal, India. After subculturing those isolates on MacConkey agar plates, as we aimed to investigate CRKP infection, bacterial growth was observed on 1374 plates. Among them, 724 *E. coli* (52.69%), 504 *K. pneumoniae* (36.68%), 19 *Proteus* spp. (1.38%), and 127 (*Acinetobacter* spp., *Alcaligenes* spp., *Enterobacter*, and *Pseudomonas* spp.) many other bacteria (9.24%) were found (Table 2 and Figure 3). Here, *E. coli* was the most prevalent isolate found in this study, as the burden of *E. coli* is predominant in India.²⁷⁻²⁹ When the 504 *K. pneumoniae* isolates were further investigated for AST, 128 CRKP were found. Meropenem resistance was detected in 95.31% of isolates, while imipenem resistance was observed in 100% of isolates (Table 3). For treating severe infections caused by *K. pneumoniae*, carbapenems such as imipenem and ertapenem are the primary options. *K. pneumoniae* produces enzymes called ESBLs (extended-spectrum β -lactamases), which can cause resistance to several antibiotics. Because of their efficacy, carbapenems are used to treat infections brought on by *K. pneumoniae* that produce ESBL. There are structural similarities between carbapenems, penicillins, and cephalosporins.³⁰ They have an impact by breaking down the bacterial cell wall, blocking penicillin-binding proteins (PBPs), and ultimately employing osmotic pressure to eradicate the bacteria. That's why resistance to carbapenems is a public health threat and a serious cause of concern and there are high mortality rates due to the resistance to this last

resort of antibiotics.⁷ The source samples of these 128 clinical isolates were 96 from urine (75%), 6 from blood (4.69%), 9 from pus (7.03%), 14 from sputum (10.94%), and 3 from throat swab (2.34%) (Figure 4). This revealed that most of the CRKP cases came from patients having urinary tract infection (UTI). The UTI rate is generally higher in India. This may be linked to the poor healthcare system, lifestyle variations, lack of knowledge, and low water availability.²⁹ CRKP infection is found in all age groups in our study, as well as worldwide. But here, the infection cases were higher in individuals 50 years of age and older ($n = 70$, or 54.69% of the total cases). This higher level of cases in the older age group may be linked to their immunocompromised status, severe pneumonia, etc. This age group is predominantly affected by diabetes mellitus, which is another contributing factor to CRKP infection in them.^{31,32} 10.94% cases were found in the "<15" age group, and 34.38% cases were reported in the "15 - <50" age group. We also observed that CRKP infection was more predominant in females (55.47%) than in males, which could be related to their behaviour (Figure 5). Females, especially those in the reproductive/middle age group, are generally more prone to being affected by UTI due to various reasons, such as sanitation, lack of hydration, and deficiency in hygiene maintenance in them.^{33,34} Among the six districts from where the clinical isolates were collected, it was found that Kolkata had the highest number of CRKP infection cases, i.e., 40 (31.25%), and Birbhum had the lowest number of cases, i.e., 10 (7.81%) (Table 4, Figure 6). That's why Kolkata belongs to the High category, Paschim Medinipur, Purba Medinipur, and Purulia belong to the Moderate category, & Jhargram and Birbhum belong to the Low category based on the CRKP infection case numbers of the respective districts. This categorisation of the six districts was mapped using GIS mapping with a colour scale ranging from green (Low category) through yellow (Moderate category) to red (High category) (Figure 7) in the ArcGIS software. The GIS mapping facilitates early detection of CRKP infection outbreaks and controls them by identifying the hotspots.^{35,36} At the time of molecular characterisation, NDM was revealed as the most predominant carbapenemase-producing gene (50%) among the others. Whereas KPC was found to be the

lowest carbapenemase-producing gene (9.38%). 22.66% OXA-48 carbapenemase-producing gene was also found during the investigation. In 17.97% isolates, both NDM & OXA-48 were found (Table 5, Figure 8). Here, we did not identify a significant association between gene profile and either age or sex. Gram-negative bacterial strains that produce carbapenemase are rapidly emerging and demonstrate broad-spectrum β -lactam resistance, particularly New Delhi metallo- β -lactamase (NDM-1). Despite ongoing efforts to control it, NDM-1 and its variants continue to spread globally. Its ongoing evolution of novel variants makes clinical treatment difficult.³⁷

This integrated approach to geographical molecular epidemiology, involving hotspot identification through GIS mapping and determination of carbapenemase-producing genes by PCR, will help to strengthen the public health surveillance system, potentially aiding in the control of CRKP infection outbreaks. A Kernel Density Heat Map based on this categorisation shows the spatial distribution (Figure 9), which will help in strengthening the epidemiological surveillance system,³⁸ that will play an important role in CRKP infection outbreak control.

There are a number of limitations to this study that should be considered. Initially, the study was limited to six districts, which restricted West Bengal's actual CRKP infection scenario. Secondly, this study was conducted with a relatively small sample size. Hence, to confirm and expand on our findings, a study with a large-scale sample size is required. Additionally, this study did not investigate how climatic factors contribute to CRKP infection. Further investigation with sequence typing of the CRKP isolates is also required in future to strengthen the surveillance system more & also to guide clinicians in the appropriate direction for the treatment of CRKP infection.

CONCLUSION

This study is the first detailed molecular epidemiological study in which the six districts of West Bengal were categorized into Low, Moderate, and High categories based on the CRKP isolate numbers using GIS mapping, which will help in strengthening the surveillance system. The

comprehensive study regarding the demographic distribution of these six districts will also play an important role to make the surveillance system much stronger in future. On the other hand, molecular characterization revealed that *bla*_{NDM} is the predominant carbapenemase-producing gene, followed by *bla*_{OXA-48} and *bla*_{KPC}, highlighting the prevalence of NDM-mediated resistance in this region. This integrated approach of GIS mapping and molecular characterization will help to control the outbreak situation of CRKP infection in future and also help clinicians with targeted therapy by optimizing the usage of antibiotics. The spatial distribution, which is shown by the Kernel Density Heat Map, will help the public health policy makers to strengthen the epidemiological surveillance system. These findings provide critical evidence to support region-specific public health interventions, combating antimicrobial resistance (AMR), and targeted infection control strategies. Moreover, this study contributes valuable baseline data for West Bengal and establishes a model for region-specific surveillance to combat the growing threat of CRKP.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SMP, SuS, SP, and SaS conceptualized the study. SMP, SuS, and SP applied methodology. SMP and SKM performed formal analysis and investigation. SMP, SuS, SR, SP, SKM, and SaS performed data analysis. SuS and SaS supervised and visualized the study. SMP and SP wrote the original draft. SMP, ABM, SuS, SR, SP, DS, and SaS wrote, reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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