Karthik et al | Article 9517
J Pure Appl Microbiol. 2025;19(1):245-254. doi: 10.22207/JPAM.19.1.15
Received: 30 April 2024 | Accepted: 18 November 2024
Published Online: 06 February 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Molecular Profile of Emerging Hypervirulent *Klebsiella pneumoniae* Clinical Isolates in Diabetic Patients

A. Karthik[1]*, V.M. Vinodhini[2] and K.V. Leela[1]

[1]Department of Microbiology, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, Kattankulathur, Kancheepuram District, Tamil Nadu, India.
[2]Department of Biochemistry, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, Kattankulathur, Kancheepuram District, Tamil Nadu, India.

Abstract

The emergence of multidrug-resistant hypervirulent *Klebsiella pneumoniae* (MDR-hvKp) strains has become a significant concern in healthcare settings worldwide. This study aims to elucidate the current landscape of MDR-hvKp infections in diabetic patients, shedding light on the challenges posed by these pathogens and highlighting the urgent need for concerted efforts in surveillance, prevention, and treatment to mitigate their impact on public health. This is the prospective study conducted over a period of 12 months. This study consisted all non-duplicate n = 500 different clinical samples from diabetic patients which were received for bacterial culture in the microbiology department during the study period. Determination of antimicrobial susceptibility and drug resistance was performed by conventional and molecular methods. Among *Klebsiella pneumoniae* Extended Spectrum Beta-Lactamase (ESBL) positive isolates of *K. pneumoniae*, 53 isolates showed presence of bla_{SHV} (n = 53, 77.9%), bla_{TEM} (n = 51, 75%) and bla_{CTX-M} (n = 42, 61.7%), bla_{TEM} with bla_{SHV} positive for 31 isolates, bla_{TEM} with bla_{CTX-M} positive for 27 isolates and 19 isolates were positive for bla_{TEM} with bla_{SHV} and bla_{CTX-M}. Among 32 Metallo-β-lactamase (MBL) positive *K. pneumoniae*, bla_{KPC} was positive for (n = 32, 47%), bla_{VIM} + bla_{IMP} (n = 31, 45.5%), bla_{VIM} (n = 28, 41.1%), bla_{IMP} (n = 24, 35.2%) and bla_{KPC} + bla_{VIM} (n = 23, 33.8%) were identified. The increasing prevalence of antibiotic resistance is limiting the potential treatment choices for diseases caused by bacteria that have developed resistance to drugs.

Keywords: *Klebsiella pneumoniae*, Multidrug-resistant, ESBL, MBL, Hyper-virulent

***Correspondence:** karthickmicro7@gmail.com

Citation: Karthik A, Vinodhini VM, Leela KV. Molecular Profile of Emerging Hypervirulent *Klebsiella pneumoniae* Clinical Isolates in Diabetic Patients. *J Pure Appl Microbiol.* 2025;19(1):245-254. doi: 10.22207/JPAM.19.1.15

INTRODUCTION

The emergence of multidrug-resistant hypervirulent *Klebsiella pneumoniae* (MDR-hvKp) strains has become a significant concern in healthcare settings worldwide.[1] *Klebsiella pneumonia* (*K. pneumoniae*), a common Gram-negative bacterium, has long been associated with various infections, ranging from urinary tract infections to severe pneumonia.[2]

K. pneumoniae represents a critical challenge in healthcare, particularly among diabetic patients. Diabetic patients, already predisposed to infections due to their compromised immune status and impaired wound healing, are particularly vulnerable to the deleterious effects of MDR-hvKp infections. The intertwining of multidrug-resistance and hypervirulence in these strains not only amplifies the difficulty in treating infections but also exacerbates the potential for rapid disease progression and mortality in diabetic individuals.[3]

Among the diverse patient populations affected by MDR-hvKp, individuals with diabetes mellitus are particularly vulnerable. Diabetes mellitus, characterized by hyperglycemia and impaired immune function, creates a conducive environment for bacterial infections, making diabetic patients prone to a higher risk of acquiring and experiencing severe complications from MDR-hvKp infections.[4]

Understanding the epidemiology, clinical manifestations, and mechanisms underlying the emergence and dissemination of MDR-hvKp in diabetic patients is imperative for guiding effective therapeutic strategies and infection control strategies.[5]

This study aims to elucidate the current landscape of MDR-hvKp infections in diabetic patients, shedding light on the challenges posed by these pathogens and highlighting the urgent need for concerted efforts in surveillance, prevention, and treatment to mitigate their impact on public health.

MATERIALS AND METHODS

This is the prospective study conducted over a period of 12 months (January 2023 to December 2023). Institutional Ethical Committee (IEC) approval was obtained. This study consisted all non-duplicate n = 500 different clinical samples from diabetic patients which were received for bacterial culture in the microbiology department during the study period. This study included various clinical samples (n = 500) from diabetic patients that were received for bacterial culture in the microbiology department during the course of the study.

Microbiological analysis

After being incubated at 37 °C overnight, the isolated colony was grown in pure culture. The colony was then identified using a routine process that included evaluating the suspected colonies biochemically. Antibiotic susceptibility testing was done on these isolates using a Gram-negative antibiotic panel.[6]

Determination of antimicrobial susceptibility

The pattern of antimicrobial susceptibility was identified. They were evaluated for zones of inhibition in accordance with the CLSI guidelines. Amikacin, ampicillin, cefepime, cefazolin, ceftazidime, cefuroxime, cefotaxime, gentamicin, pipercillin-tazobactam, ciprofloxacin, co-trimoxazole, meropenem and imipenem were among the antibiotics that were tested and findings were compared with the ATCC strain.[7]

Detection of Drug-resistant *K. pneumoniae*

Based on the findings of the disc diffusion method, Multi Drug Resistant (MDR), Extensively Drug Resistant (XDR), and Pan Drug Resistant (PDR) strains were identified.

AmpC beta-lactamases

- Cefoxitin (30 µg) disk was used for screening.
- Following incubation, if the zone's diameter is greater than 14 mm, cefoxitin may be a potential source of AmpC beta-lactamases.[8,9]

Double disk synergy test

- Third-generation cephalosporin-resistant bacterial isolates were tested for ESBL production.
- Cefotaxime (30 µg) at a distance of 15 mm from piperacillin/tazobactam edge to edge; incubated at 37 °C at 24 hours; zone of inhibition increased by more than 5 mm in

diameter, indicating the presence of ESBL formation.[10]

Metallo-β-lactamase - MBL

- Combined disc test: imipenem (10 µg) and 0.1 M anhydrous EDTA (10 µl) were employed in one.
- An increase in the zone diameter surrounding the EDTA disc of more than 5 mm is considered positive when compared to the imipenem disc.[11]

The process of identifying virulence factors

Hypermucoviscosity (HMV)

- The colony's propensity to expand like a mucoviscous string
- A longer-than-10 mm string expansion is indicated of the HMV phenotype.

Figure 1 shows a hypervirulent strain of *K. pneumoniae* that passed the string test (Figure 2).[12]

Blood Hemolysis

- The isolates were placed in blood agar plates that had 5% sheep blood in order to perform the hemolysis test.
- After incubation for 24 hours at 37 °C, hemolysis was observed.[13]

Biofilm forming assay

- The biofilm productions were evaluated using the microtiter plate method. *Klebsiella*

Figure 1. Hypermucoviscous *K. pneumoniae*

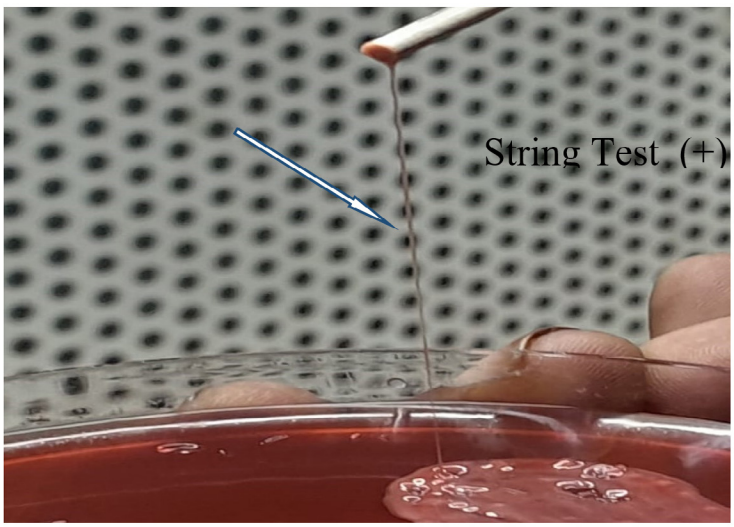

Figure 2. String test positive for *K. pneumoniae*

isolates attachment to an inert substrate was investigated.

- The strains were kept for 24 hours at 37 °C in Brain Heart Infusion Broth (BHIB). 48-well polystyrene microtiter plate with flat bottom wells was filled with 50 µl of the culture dilution, and it was incubated for 48 hours.
- Following incubation wells were gently washed three times using sterile saline and methanol fixation was carried out for 20 minutes.
- Following a crystal violet stain, each well was washed. 1 ml of ethanol was used to decolorize the biofilm-associated crystal violet. The optical density was measured at 620 nm (Figure 3).[14]

Extraction and amplification of 16S rRNA

DNA was extracted using DNA Extraction kit. Absorbance at 280 nm wavelengths was used to measure the concentration and purity of DNA.[15]

For the amplification of the 16S rRNA gene, universal primers were used. The reverse primer was R 5'-ACGGTTACCTTGTTACGACTT-3', and the forward primer was F 5'-AGAGTTTGATCCTGGCTCAG-3'. 3 ml of template DNA, 2 ml of forward and reverse

Table 1. Primers for the identification of target genes

PCR Target gene		Sequences of Primer (5'-3')	Product Size (bp)
Resistant genes			
1.	CTX-M	CGCTTTGCGATGTGCAG-ACCGCGATATCGTTGGT	550
2.	NDM	GGGCAGTCGCTTCCAACGGT-GTAGTGCTCAGTGTCGGCAT	476
	IMP	TTGACACTCCATTTACDG-GATYGAGAATTAAGCCACYCT	139
	VIM	GATGGTGTTTGGTCGCATA-CGAATGCGCAGCACCAG	390
	KPC	CATTCAAGGGCTTTCTTGCTGC-ACGACGGCATAGTCATTTGC	538
Biofilm gene			
3.	wcaG	GGTTGGKTCAGCAATCGTA-ACTATTCCGCCAACTTTTGC	169
Hyper virulent gene			
4.	rmpA	ACT GGG CTA CCT CTG CTT CA-ACT GGG CTA CCT CTG CTT CA	550
5.	magA (K1)	GGTGCTCTTTACATCATTGC-GCA ATG GCC ATT TGC GTT AG	282
6.	Wzy (K2)	GACCCGATA TTC ATA CTT GAC AGA G-CCT GAA GTA AAA TCG TAA ATA GAT GGC	641

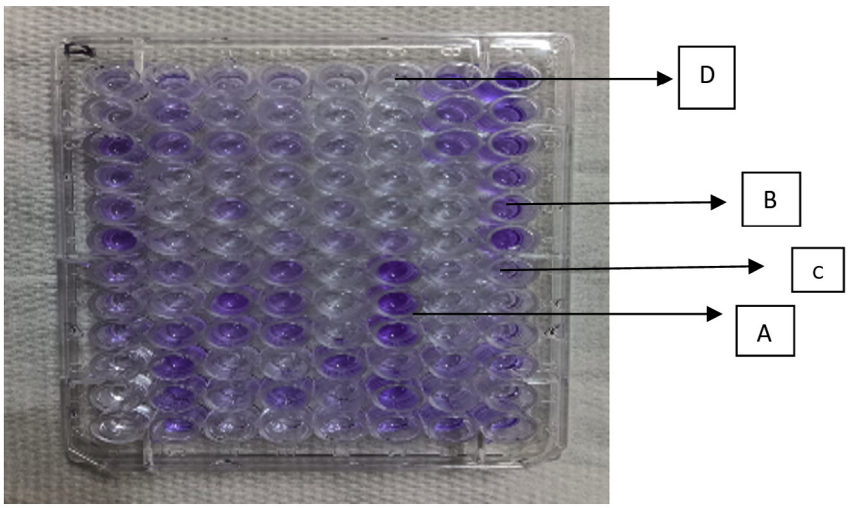

Figure 3. Biofilm formation in microtitre plate assay
Note: A - Strong, B - Moderate, C - Weak, D - None

Table 2. PCR-Amplification conditions

PCR	Initial Denaturation	Denaturation	Annealing	Elongation	Final Elongation	Cycle
1.	95 °C 7 min	95 °C 1 min	58 °C 1 min 30 sec	72 °C 2 min	72 °C 5 min	40 cycles
2.	95 °C 5min	95 °C 1 min	55 °C 2 min	72 °C 1 min	72 °C 7 min	35 cycles
3.	95 °C 10 min	95 °C 1 min	56 °C 1 min 45 sec	72 °C 1 min	72 °C 10 min	45 cycles
4.	97 °C 7 min	97 °C 1 min	59 °C 1 min 45 sec	72 °C 2 min	72 °C 7 min	40 cycles
5.	95 °C 10 min	95 °C 1 min	56 °C 1 min 30 sec	72 °C 1 min	72 °C 10 min	45 cycles
6.	95 °C 7 min	95 °C 1 min	52 °C 1 min 45 sec	72 °C 2 min	72 °C 7 min	35 cycles

primer, 5.5 ml of dH2O, and 12.5 ml of master mix make up the total volume of a 25 ml PCR reaction mixture.[16]

The PCR technique was standardised to include an initial denaturation at 94 °C for 15 minutes, followed by 35 cycles of denaturation for 1 minute at 94 °C, annealing for 1 minute at 52 °C, extension for 1 minute and 30 seconds at 72 °C, and final extension for 5 minutes at 72 °C. With the amplified PCR sample, 1% Agarose gel electrophoresis was carried out using 1X TAE (Tris-Acetate-EDTA) buffer containing ethidium bromide.

The image was generated using a UV transilluminator while the amplified bands were visible using the gel documentation.[17]

Determination of multidrug-resistance genes

Specific primers and PCR reaction were used to identify the following genes: bla_{VIM}, bla_{CTX-M}, bla_{NDM}, rmpA, WCaG, bla_{KPC}, IMP, magA (K1), and wzy (K2). These genes are described in Table 1 and Table 2 provides the amplification conditions.

RESULTS

In the current investigation, 500 non-duplicate clinical samples in diabetic patients were obtained from a tertiary care hospital. Among the 500 samples, 386 bacterial isolates were identified (386/500), 20.7% (n = 80/386) were in blood, 27.4% (n = 106/386) in urine, 14.5% (n = 56/386) in pus, 8% (n = 31/386) in stool, 15.2% (n = 59/386) in wound swab and 13.9% (n = 54/386) in sputum sample. Distribution of bacterial pathogens is shown in Table 3.

The majority of these MDR *K. pneumoniae* isolates were found in wound swab/exudate samples (n = 21), urine (n = 11), pus (n = 14), and blood (n = 6). From a total of 152 non-duplicate isolates of *K. pneumoniae,* 68 isolates were identified as MDR and these were further included for characterization.

The majority of MDR isolates were found in males (n = 41), followed by females (n = 27). *Klebsiella s*pp. were isolated more frequently from urine samples (n = 28; 34.47%), pus samples (n = 27; 17.37%), sputum samples (n = 14; 12.63%), blood samples (n = 22; 9.74%) and wound swabs (n = 48; 8.68%). The distribution analysis of *Klebsiella* spp. around clinical wards indicated that approximately n = 83; 36.6% isolates were from the OPD. Approximately (n = 48; 13%), (n = 21; 13%) of *Klebsiella* spp. were isolated from the IPD and ICU, respectively (Table 4). The majority of MDR *Klebsiella* isolates (36.7%) were collected from wounds, with pus (22%) and blood (19.1%), urine (13.2%), and sputum (6%). According to this, the majority of MDR *K. pneumoniae* isolates were obtained from IPD (n = 21), OPD (n = 46), and ICU (n = 13). Hospital-acquired infections (n = 8), community-acquired infections (n = 15), and diabetic infections (n = 59) were linked to the majority of MDR *K. pneumoniae* cases.

Resistance profile of *Klebsiella pneumonia* isolates

Table 5 represents the percentage of isolates exhibiting resistance toward thirteen antibiotics. During the study period ciprofloxacin, cefazolin, and Nitrofurantoin appeared to be the most effective drugs, as 93%, 92.1% and 90% of ESBL-producing *K. pneumonia* were screened. The highest resistance observed among MBL-producing *K. pneumoniae* were cefazolin (95.5%), followed by Nitrofurantoin (90.7%) and Tigecycline (83.8%). We found similar antibiotic resistance trends in MDR *K. pneumoniae* isolated

Table 3. Distribution of pathogens obtained from clinical specimems

Source of the organism	*E. coli* (%)	*Klebsiella* sp. (%)	*A. baumanii* (%)	*Pseudomonas* sp. (%)	*Citrobacter* sp. (%)	*Shigella* sp. (%)	*Enterobacter* sp. (%)	*Salmonella* sp. (%)	*Proteus* sp. (%)
Blood	10	22	12	4	-	-	-	2	-
Urine	23	28	14	18	6	-	7	-	2
Pus	4	27	9	15	6	-	4	-	-
Stool	7	13	-	-	-	7	-	2	-
Wound swab	6	48	14	17	2	-	-	-	6
Sputum	2	14	14	21	-	-	-	-	-
Total	52	152	63	75	14	7	11	4	8

Table 4. Occurrence of *Klebsiella* spp. in clinical sample

	Klebsiella spp. n = 152	MDR *Klebsiella* spp. n = 68
Gender		
Male	93	41
Female	59	27
Community-acquired Infections (CAIs)	36	15
Hospital-acquired Infections (HAIs)	19	8
Diabetic Infections	97	59
Clinical sources		
Urine	28	9
Blood	22	13
Pus swab	27	15
Sputum	14	6
Wound swab	48	25
Hospital sites		
ICU	21	13
IPD	48	21
OPD	83	46

Note: IPD- in-patient department; OPD- out-patient department; ICU- intensive care unit

from different clinical sources. Although the percentage of *K. pneumoniae* strains that were resistant to Meropenem and Imipenem was lower. A comparison between the resistance pattern of isolates from Diabetic infections versus community-acquired infections indicated similar resistance patterns toward the antibiotics mentioned in Table 5.

A total of 68 MDR *K. pneumoniae* isolates were found to exhibit reduced susceptibility to meropenem and imipenem. These 68 isolates were further studied for ESBL and MBL production. Among ESBL positive isolates of *K. pneumoniae*, 53 isolates showed presence of bla_{SHV} (n = 53, 77.9%), bla_{TEM} (n = 51, 75%) and $bla_{CTX\text{-}M}$ (n = 42, 61.7%), bla_{TEM} with bla_{SHV} positive for 31 isolates, bla_{TEM} with $bla_{CTX\text{-}M}$ positive for 27 isolates and 19 isolates were positive for bla_{TEM} with bla_{SHV} and $bla_{CTX\text{-}M}$. Among 32 MBL positive *K. pneumoniae*, bla_{KPC} was positive for (n = 32, 47%), bla_{VIM} + bla_{IMP} (n = 31, 45.5%), bla_{VIM} (n = 28, 41.1%), bla_{IMP} (n = 24, 35.2%) and bla_{KPC} + bla_{VIM} (n = 23, 33.8%) were identified. Similarly, MBL resistant genes were also represented in Table 6.

Table 5. Antimicrobial resistance patterns of multidrug-resistance *Klebsiella* sp.

Antibiotics	ESBL-producing *Klebsiella pneumoniae* (%)	MBL-producing *Klebsiella pneumoniae* (%)
Cefazolin	92.6	95.5
Cefepime	55.8	77.9
Imipenem	7.3	11.7
Meropenem	5.5	8.2
Ciprofloxacin	92.1	80.7
Moxifloxacin	72.0	67.6
Gentamycin	64.7	61.7
Amikacin	35.2	30.8
Nitrofurantoin	90	90
Norfloxacin	72	67
Tetracycline	81	75
Chloramphenicol	85.2	72
Citrimoxazoles	75	80.8
Tigecycline	90	83.8

Table 6. Distribution of ESBL and MBL resistant genes in *Klebsiella pneumomiae*

MDR *K. pneumoniae* (n = 68)	
Name of Gene	n (%)
ESBL	
bla_{TEM} alone	51
bla_{SHV} alone	53
bla_{CTX-M} alone	42
bla_{TEM} + bla_{SHV}	31
bla_{TEM} + bla_{CTX-M}	27
bla_{TEM} + bla_{SHV} + bla_{CTX-M}	19
MBL	
bla_{KPC} alone	32
bla_{VIM} alone	28
bla_{IMP} alone	24
bla_{OXA-48} alone	26
bla_{NDM} alone	18
bla_{KPC} + bla_{IMP}	23
bla_{VIM} + bla_{IMP}	31
bla_{IMP} + bla_{NDM}	17
bla_{KPC} + bla_{IMP}	21
bla_{OXA-48} + bla_{NDM}	11
bla_{KPC} + bla_{VIM} + bla_{IMP}	17
bla_{KPC} + bla_{OXA-48} + bla_{NDM}	19
bla_{VIM} + bla_{IMP} + bla_{OXA-48}	13

DISCUSSION

The increasing prevalence of antibiotic resistance is limiting the potential treatment choices for diseases caused by bacteria that have developed resistance to drugs. Greece has been shown to have the highest rate of carbapenem resistance globally, at 68%, followed by India and the eastern Mediterranean regions at 54%.[18] Table 1 lists the most frequently reported ESBLs in India, which include SHV, TEM, and CTX-M. Manoharan *et al*. and Goyal *et al*. have documented co-existence of SHV, TEM, and CTX-M in *Enterobacteriaceae*, which is similar to this study's observation.[19,20] Study by Tsay *et al*. revealed that 49% of diabetes mellitus patients had bacteremia acquired in the community as a result of a *K. pneumoniae* infection.[21]

Gram-negative bacteria are more deadly than gram-positive bacteria and have been linked to infection in healthcare settings.[22] North East India might be concerned about the increasing number of hospital-acquired and community-acquired multidrug-resistant bacterial infections. The current investigation also shows that the stains that were isolated from different clinical cases had a high level of resistance to all widely used antibiotics. As with previous studies from this region, the least amount of resistance was observed against imipenem, gentamicin, and amikacin.[23] As a result of treatment with carbapenem, there is a correlation between increased production of ESBL 77.9% and carbapenem resistance.[24] According to the current investigation, 11% of the bacteria had both ESBL and MBL genes, and 47% of the isolates were MBL producers. The increased incidence was similarly found by Devi *et al*.[25] Multidrug-resistant pathogen colonization is becoming more prevalent in ICU patients due to their main illnesses, which frequently require recurrent hospital stays.[26]

Globally, the majority of cases are found in intensive care units.[27] The majority of the time, ESBL development and antimicrobial resistance in *Klebsiella* were connected. The WHO classified ESBL-producing *Klebsiella* as highly pathogenic superbugs in 2017.[28] Our isolates of *K. pneumoniae* showed multidrug-resistance, with an increase in carbapenem resistance. Imipenem and meropenem are the two medicines that are effective in treating CRKP. It may be advantageous to use a combination of antibiotics in along with removing invasive devices.[29]

In our research work, Klebsiella exhibited resistance to second- and third-generation cephalosporins. This shows the importance of screening gram-negative bacteria for cephalosporin resistance and the development of ESBL and MBL.[30] Based on the genes, carbapenemases are considered to be the main mechanism that causes the development of CRKP isolates. In this study, bla_{KPC} gene was present in majority, according to MBL resistance gene analysis. We identified a higher incidence of bla_{OXA-48} and no bla_{VIM} in any of the isolates we examined, compared to another study by Ghaith *et al*.[31] with a different genotypic profile. Regarding ESBL genes, 42 isolates received our investigation with bla_{CTX-M}. In an investigation by Amer *et al*., similar isolates were found, which is in accordance with this observation.[32]

The isolates in this study were found to be detected with other carbapenemases, including bla_{KPC}, bla_{VIM}, and bla_{OXA-48}. In bacteremic patients with MDR *K. pneumoniae*, showing indicates that a combination treatment may be associated with better prognosis than monotherapy alone.[33] The emergence of multidrug resistance has usually been attributed to the rapid dissemination and fast spread of resistant *Klebsiella* spp. The hypervirulent strains of *K. pneumoniae* have emerged; these strains were initially predominant among isolates that were susceptible to antibiotics. They have recently been observed in MDR isolates, as the study also showed. This presents further treatment challenges because virulence associated with antibiotic resistance can be highly concerning, possibly resulting in extremely high rates of mortality.[34] Hospital-acquired infections were more common than community-acquired infections, according to our overall analysis, but the higher isolation percentages from the OPD indicated an increased probability that the infections were diabetic infections.

CONCLUSION

In summary, there has been a rise in the frequency and antibiotic resistance of *K. pneumoniae* isolates. Antibiotic stewardship is necessary to prevent diabetic patients from overusing antibiotics, as *K. pneumoniae* represents a severe threat to the healthcare system. Strict infection control measures must be implemented in hospital settings. It can be challenging to diagnose and treat because of how different the indications and symptoms can be. The development of appropriate healthcare standards to combat AMR resistance and subsequently decrease the death rate requires a thorough understanding of the bacteria that cause infections, their antibiotic susceptibility profile, the specific cause of resistance, and the geographic pattern of resistance. The present study demonstrated that the isolates showed higher levels of drug resistance as well as greater prevalence of ESBL and MBL producers. The development of appropriate healthcare standards to combat AMR resistance requires a thorough understanding of the bacteria that cause infections, their antibiotic susceptibility profile, the precise cause of resistance, and the distribution pattern of resistance. The current investigation demonstrated an increase in drug resistance in the isolates as well as a higher frequency of ESBL and MBL producers. Effective therapy is currently being delayed because there are currently few available therapeutic options due to the increasing emergence of multidrug-resistant organisms. Developing protection and management methods should be used in hospitals. Information obtained from this study may be significant in developing new community treatment strategies and in providing baseline information that may be used to implement antibiotic guidelines that will decrease the development of drug resistance. To detect new MDR-Kp infections as soon as possible, infection management measures including antibiotic stewardship programs with continuous monitoring are essential.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Jagan V for invaluable suggestions and support during the course of our work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethical Committee, SRM Medical College Hospital and Research Centre, Chengalpattu, India, vide reference number 2937/IEC/2021.

REFERENCES

1. Ali MR, Yang Y, Dai Y, et al. Prevalence of multidrug-resistant hypervirulent *Klebsiella pneumoniae* without defined hypervirulent biomarkers in Anhui, China: A new dimension of hypervirulence. *Front Microbiol.* 2023;14:1247091. doi: 10.3389/fmicb.2023.1247091
2. Chen L, Kreiswirth BN. Convergence of carbapenem-resistance and hypervirulence in *Klebsiella pneumoniae*. *Lancet Infect Dis*. 2018;18(1):2-3. doi: 10.1016/S1473-3099(17)30517-0
3. 3. Pattolath A, Adhikari P, Pai V. Carbapenemase-Producing *Klebsiella pneumoniae* Infections in Diabetic and Nondiabetic Hospitalized Patients. *Cureus*. 2024;16(1):e52468. doi: 10.7759/cureus.52468
4. Solgi H, Shahcheraghi F, Bolourchi N, Ahmadi A. Molecular characterization of carbapenem-resistant serotype K1 hypervirulent *Klebsiella pneumoniae* ST11 harbouring bla_{NDM-1} and bla_{OXA-48} carbapenemases in Iran. *Microb Pathog.* 2020;149:104507. doi: 10.1016/j.micpath.2020.104507
5. Shen D, Ma G, Li C, *et al*. Emergence of a multidrug-resistant hypervirulent *Klebsiella pneumoniae* sequence type 23 strain with a rare $bla_{CTX-M-24}$ -harboring virulence plasmid. *Antimicrob Agents Chemother.* 2019;63(3):e02273-18. doi: 10.1128/AAC.02273-18
6. Kar B, Sharma M, Peter A, et al. Prevalence and molecular characterization of β-lactamase producers and fluoroquinolone resistant clinical isolates from North East India. *J Infect Public Health.* 2021;14(5):628-637. doi: 10.1016/j.jiph.2021.02.007
7. Das A, Sahoo RK, Gaur M, et al. Molecular prevalence of resistance determinants, virulence factors and capsular serotypes among colistin resistance carbapenemase producing *Klebsiella pneumoniae*: a multi-centric retrospective study. *3 Biotech*. 2022;12(1):30. doi: 10.1007/s13205-021-03056-4
8. Ravi V, Vijay DD, Sujhithra A, Jayanthi S, Subramanian TK, Harish N. Neonatal Sepsis: The impact of Hypervirulent *Klebsiella pneumonia* in a Tertiary Care Hospital. *J Pure Appl Microbiol.* 2022;16(3):2035-2044. doi: 10.22207/JPAM.16.3.56
9. Younas S, Ejaz H, Zafar A, Ejaz A, Saleem R, Javed H. AmpC beta-lactamases in *Klebsiella pneumoniae*: An emerging threat to the paediatric patients. *J Pak Med Assoc.* 2018;68(6):893-897.
10. Aminul P, Anwar S, Molla MM, Miah MR. Evaluation of antibiotic resistance patterns in clinical isolates of *Klebsiella pneumoniae* in Bangladesh. *Biosaf Health*. 2021;3(06):301-306. doi: 10.1016/j.bsheal.2021.11.001
11. Dhungana K, Awal BK, Dhungel B, Sharma S, Banjara MR, Rijal KR. Detection of *Klebsiella pneumoniae* carbapenemase (KPC) and metallo betalactamae (MBL) producing Gram negative bacteria isolated from different clinical samples in a Transplant Center, Kathmandu, Nepal. *Acta Scientific Microbiology.* 2019;2(12):60-69. doi: 10.31080/ASMI.2019.02.0432
12. Shankar CS, Nabarro LE, Anandan SA, et al. Extremely high mortality rates in patients with carbapenem resistant, hypermucoviscous *Klebsiella pneumoniae* blood stream infections. *J Assoc Physicians India*. 2018;66(12):13-16.
13. Das A, Behera BK, Acharya S, et al. Genetic diversity and multiple antibiotic resistance index study of bacterial pathogen, *Klebsiella pneumoniae* strains isolated from diseased Indian major carps. *Folia Microbiol.* 2019;64(6):875-887. doi: 10.1007/s12223-019-00701-7
14. Nirwati H, Sinanjung K, Fahrunissa F, et al. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. *BMC Proc.* 2019;13(Suppl 11):20. doi: 10.1186/s12919-019-0176-7
15. Ghaffarian F, Hedayati M, Ebrahim-Saraie HS, Roushan ZA, Mojtahedi A. Molecular epidemiology of ESBL-producing *Klebsiella pneumoniae* isolates in intensive care units of a tertiary care hospital, North of Iran. *Cell Mol Biol.* 2018;64(7):75-79. doi: 10.14715/cmb/2018.64.7.13
16. He Y, Guo X, Xiang S, et al. Comparative analyses of phenotypic methods and 16S rRNA, khe, rpoB genes sequencing for identification of clinical isolates of *Klebsiella pneumoniae*. *Antonie Van Leeuwenhoek*. 2016;109(7):1029-1040. doi: 10.1007/s10482-016-0702-9
17. Singh SK, Mishra M, Sahoo M, et al. Antibiotic resistance determinants and clonal relationships among multidrug-resistant isolates of *Klebsiella pneumoniae. Microb Pathog.* 2017;110:31-36. doi: 10.1016/j.micpath.2017.06.013
18. World Health Organization. Antimicrobial resistance: global report on surveillance. World Health Organization. 2014.
19. Manoharan A, Premalatha K, Chatterjee S, Mathai D, SARI Study Group. Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among *Enterobacteriaceae* with their *in vitro* antimicrobial susceptibility. *Indian J Med Microbiol.* 2011;29(2):161-164. doi: 10.4103/0255-0857.81799
20. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A. Extended spectrum β-lactamases in *Escherichia coli* & *Klebsiella pneumoniae* & associated

risk factors. *Indian J Med Res*. 2009;129(6):695-700.
21. Tsay R, Siu LK, Fung C, Chang F. Characteristics of bacteremia between community-acquired and nosocomial *Klebsiella pneumoniae* infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. *Arch Intern Med.* 2002;162(9):1021-1027. doi: 10.1001/archinte.162.9.1021
22. Agyepong N, Govinden U, Owusu-Ofori A, Essack SY. Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana. *Antimicrob Resist Infect Control.* 2018;7:37. doi: 10.1186/s13756-018-0324-2
23. Gajamer VR, Bhattacharjee A, Paul D, et al. The first report of phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing uropathogens in Sikkim and Darjeeling hills of India. *Microb Drug Resist*. 2018;24(9):1284-1288. doi: 10.1089/mdr.2017.0159
24. Giriyapur RS, Nandihal NW, Krishna BVS, Patil AB, Chandrasekhar MR. Comparison of disc diffusion methods for the detection of extended-spectrum beta lactamase-producing *Enterobacteriaceae*. *J Lab Physicians.* 2011;3(1):33-36. doi: 10.4103/0974-2727.78561
25. Devi U, Bora R, Das JK, Mahanta J. Extended-spectrum β-lactamase & carbapenemase-producing gram-negative bacilli in neonates from a tertiary care Centre in Dibrugarh, Assam, India. *Indian J Med Res*. 2018;147(1):110-114. doi: 10.4103/ijmr.IJMR_1288_16
26. Kuo HY, Yang CM, Lin MF, Cheng WL, Tien N, Liou ML. Distribution of bla_{OXA} carrying imipenem-resistant *Acinetobacter* spp. in 3 hospitals in Taiwan. *Diagn Microbiol Infect Dis*. 2010;66(2):195-199. doi: 10.1016/j.diagmicrobio.2009.09.013
27. Pillay D, Naidoo L, Swe-Han KS, Mahabeer Y. Neonatal sepsis in a tertiary unit in South Africa. *BMC Infect Dis*. 2021;21:1-10. doi: 10.1186/s12879-021-05869-3
28. Wang N, Zhan M, Liu J, et al. Prevalence of carbapenem-resistant *Klebsiella pneumoniae* infection in a Northern Province in China: clinical characteristics, drug resistance, and geographic distribution. *Infect Drug Resist.* 2022;15:569-579. doi: 10.2147/IDR.S347343
29. Ibrahim ME, Abbas M, Al-Shahrai AM, Elamin BK. Phenotypic characterization and antibiotic resistance patterns of extended-spectrum β-Lactamase-and AmpC β-lactamase-producing Gram-negative bacteria in a referral hospital, Saudi Arabia. *Can J Infect Dis Med Microbiol.* 2019;2019(1):6054694. doi: 10.1155/2019/6054694
30. Bedzichowska A, Przekora J, Stapinska-Syniec A, et al. Frequency of infections caused by ESBL-producing bacteria in pediatric ward-single center five-year observation. *Arch Med Sci.* 2019;15(3):688-693. doi: 10.5114/aoms.2017.72407
31. Ghaith D, Morsy SA, Sebak M, Rabea RA. Phenotypic and genotypic characterization of carbapenem-resistant Gram-negative organisms, Beni-Suef, Egypt. *Beni-Suef Univ J Basic Appl Sci.* 2023;12(1):61. doi: 10.1186/s43088-023-00400-w
32. Amer R, El-Baghdady K, Kamel I, El-Shishtawy H. Prevalence of extended spectrum Beta-Lactamase Genes among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates. *Egypt J Microbiol*. 2019;54(1):91-101. doi. 10.21608/ejm.2019.16460.1113
33. Qureshi ZA, Paterson DL, Potoski BA, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. *Antimicrob Agents Chemother.* 2012;56(4):2108-2113. doi: 10.1128/AAC.06268-11
34. Shankar C, Nabarro LEB, Ragupathi NKD, et al. Draft genome sequences of three hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolates from bacteremia. *Genome Announcements.* 2016;4(6):10-128. doi: 10.1128/genomeA.01081-16