Pathogenic Leap: Molecular Profiling of Emerging *Hypervirulent Klebsiella pneumoniae* in Diabetics

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Abstract

The rise of multidrug-resistant hypervirulent Klebsiella pneumoniae (MDR-hvKp) strains has become a significant concern in healthcare settings globally. This study seeks to understand the current picture of MDR-hvKp infections in diabetic patients, shedding light on the challenges posed by these pathogens and emphasizing the critical need for coordinated efforts in surveillance, prevention and treatment to limit their influence on public health. 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 ESBL positive isolates of K.pneumoniae, 53 isolates showed presence of blaSHV (n = 53, 77.9%), blaTEM (n = 51,75%) and blaCTX-M (n = 42, 61.7%), blaTEM with blaSHV positive for 31 isolates, blaTEM with blaCTX-M positive for 27 isolates and 19 isolates were positive for blaTEM with blaSHV and blaCTX-M. Among 32 MBL positive K.pneumoniae, blaKPC was positive for (n = 32, 47%). BlaVIM + blaIMP (n = 31, 45.5%), blaVIM (n = 28, 41.1%), blaIMP (n = 24, 35.2%) and blaKPC + blaVIM (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 resistant to drugs.

Keywords: *Klebsiella pneumonia*, Multidrug-resistant, ESBL, MBL and Hyper-virulent.

Introduction

The rise of multidrug-resistant hypervirulent *Klebsiella pneumoniae* (MDR-hvKp) strains has become a significant concern in healthcare settings globally.[1] *Klebsiella pneumonia* (*K.pneumoniae*), a common Gram-negative bacterium, has long been connected with numerous diseases, ranging from urinary tract infections to serious pneumonia.[7] *K. pneumoniae* poses a significant difficulty in healthcare, especially among diabetic patients. Diabetic patients are already prone to infections due to their reduced immunological status and delayed wound healing, making them more vulnerable to the detrimental effects of MDR-hvKp infections. The interweaving of multidrug resistance and hypervirulence in these strains not only increases the complexity of treating infections but also exacerbates the possibility for rapid disease progression and mortality in diabetic patients.[1]

Among the numerous patient populations impacted by MDR-hvKp, persons with diabetes mellitus are most vulnerable. Diabetes mellitus, characterised by hyperglycemia and reduced immune function, presents a permissive environment for bacterial infections, making diabetic individuals more susceptible in acquiring and experiencing severe consequences from MDR-hvKp infections.[29]

Understanding the epidemiology, clinical symptoms and mechanisms that underpin the formation and spread of MDR-hvKp in diabetic patients is critical for guiding effective treatment and infection control efforts.[27] This study seeks to understand the current picture of MDR-hvKp infections in diabetic patients, shading light on the challenges posed by these pathogens and emphasising the critical need for coordinated efforts in surveillance, prevention and treatment to limit their influence on public health.

Material 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 of 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 overnight incubation at 37°C, the isolated colony was cultivated in pure culture. The colony was then discovered through a normal process that included biochemical evaluations of the putative colonies. These isolates underwent antibiotic susceptibility testing with a Gram-negative antibiotic panel.[19]

Determination of antimicrobial susceptibility: A pattern of antimicrobial susceptibility was identified. They were

tested for zones of inhibition using the CLSI criteria. The antibiotics tested included amikacin, ampicillin, cefepime, cefazolin, ceftazidime, cefuroxime, cefotaxime, gentamicin, pipercillin-tazobactam, ciprofloxacin, co-trimoxazole, meropenem and imipenem and the results were compared to the ATCC strain.[9]

Detection of Drug resistant *K. pneumoniae*: The disc diffusion approach revealed MDR, XDR and PDR strains.

AmpC Beta-lactamases:
- Cefoxitin (30µg) disc was utilized for screening.
- Cefoxitin may be a source of AmpC-beta-lactamases if the zone diameter exceeds 14 mm after incubation.[25,33]

Double disk synergy test:
- We investigated third-generation cephalosporin-resistant bacterial isolates for ESBL formation.
- Cefotaxime (30µg) was added 15 mm from the edge of piperacillin/tazobactam and incubated at 37˚ for 24 hours. The zone of inhibition grew by more than 5 mm, confirming the production of ESBL.[4]

Metallo-β-lactamase – MBL:
- The combined disc test used imipenem (10µg) and 0.1 M anhydrous EDTA (10µl).
- A zone diameter of over 5 mm around the EDTA disc is regarded positive compared to the imipenem disc.[11]

Hypermucoviscosity (HMV):
- The colony's tendency to spread like a mucoviscous string.
- A longer-than-10 mm string extension is indicative with the HMV phenotype.

Fig. 1 shows a hypervirulent strain of *K.pneumoniae* that passed the string test (Fig. 2).[6]

Blood Hemolysis:
- The isolates were placed on blood agar plates containing 5% sheep blood for the haemolysis test.
- After 24 hours of incubation at 37°C, hemolysis was observed.[8]

Biofilm Forming Assay:
- Microtiter plates were used to evaluate biofilm formation. Klebsiella isolates' adhesion to an inert substrate was studied.
- The strains were incubated in BHIB for 24 hours at 37°C. A 48-well polystyrene microtiter plate with flat bottoms was filled with 50µl of culture dilution and incubated for 48 hours.

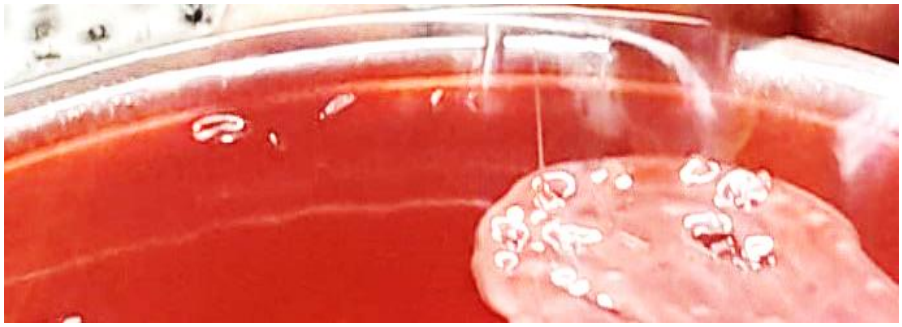


Fig. 1: Hypermucoviscous *K. pneumoniae*

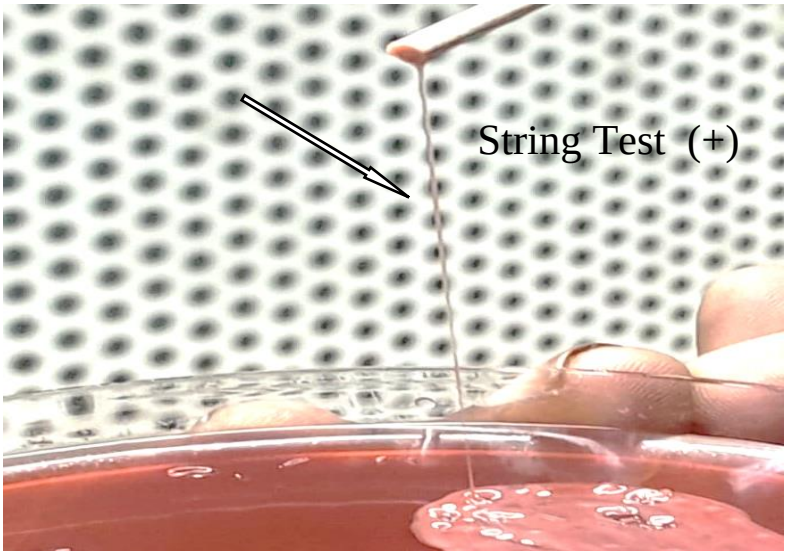


Fig. 2: String test positive for *K. pneumoniae*

- After incubation, wells were gently rinsed three times with sterile saline and fixed in methanol for 20 minutes.
- After applying a crystal violet dye, each well was rinsed. 1ml of ethanol was employed to decolorize the biofilm-associated crystal violet. The optical density was measured at 620 nm.[22]

Extraction and amplification of 16S rRNA: DNA was extracted using the DNA extraction kit. The content and purity of DNA were measured using absorbance at wavelengths of 280 nm.[13] We utilised universal primers to amplify the 16S RNA gene. The reverse primer was R 5'-ACGGTTACCTTGTTACGACTT-3', while the forward primer was F 5'-AGAGTTTGATCCTGGCTCAG-3'. A 25 ml PCR reaction mixture includes 3 ml of template DNA, 2 ml of forward and reverse primers, 5.5 ml of DH_2O and 12.5 ml of master mix.[17]

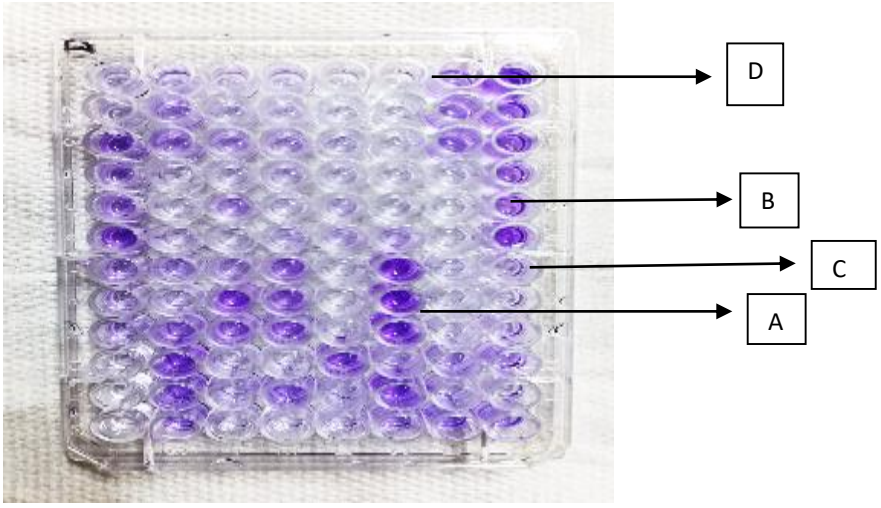


Fig. 3: Biofilm formation in microtitre plate assay.
Note: A-Strong, B-Moderate C-Weak, D-None.

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

Table 2
PCR – Amplification conditions

PCR	Initial Denaturation	Denaturation	Annealing	Elongation	Final Elongation	Cycle
1	95°C 7 minutes	95°C 1 min	58°C 1 min 30 sec	72°C 2 min	72°C 5 min	40cycles
2	95°C 5min	95°C 1 min	55°C 2 min	72°C 1 min	72°C 7 min	35cycles
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

The PCR technique was standardised to include a 15-minute initial denaturation at 94°C, 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 a final extension for 5 minutes at 72°C. 1% agarose gel electrophoresis was performed on the amplified PCR sample using 1X TAE (Tris-Acetate-EDTA) buffer containing ethidium bromide. The images were obtained with a UV transilluminator, when the amplified bands were visible, utilising the gel documentation.[28]

Determination of Multidrug Resistance Genes: Specific primers and PCR reactions were utilised to identify the following genes: blaVIM, blaCTX-M, blaNDM, rmpA, WCaG, blaKPC, IMP, magA(K1) and wzy(K2). Table 1 describes these genes, while table 2 lists 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.pneumonia*, 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).

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

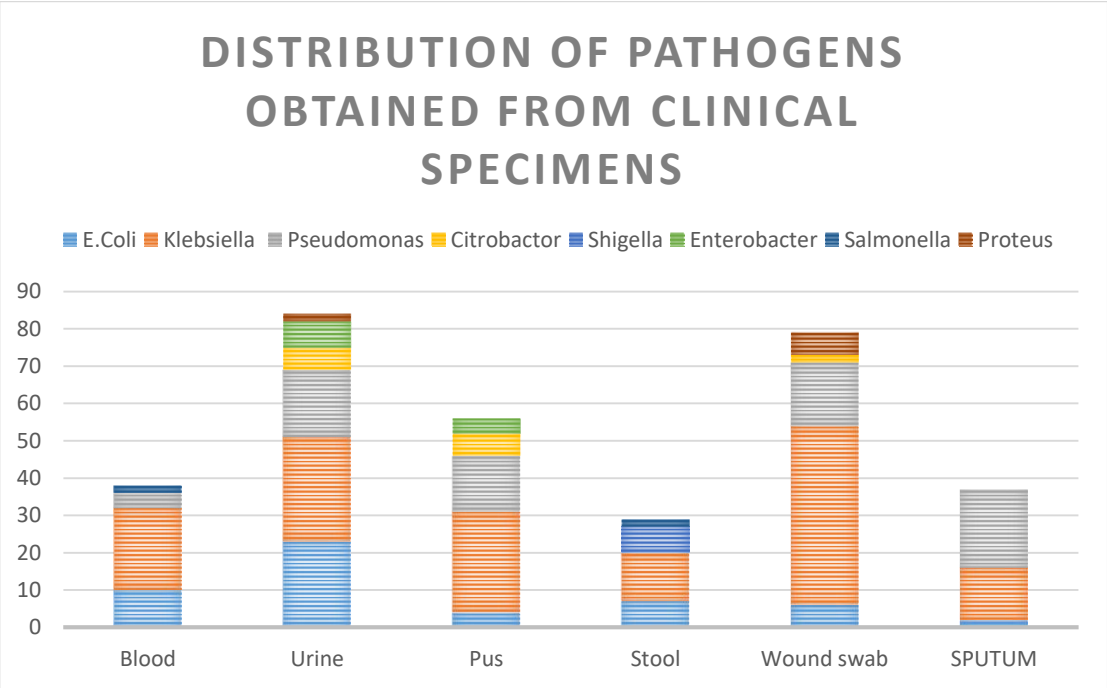
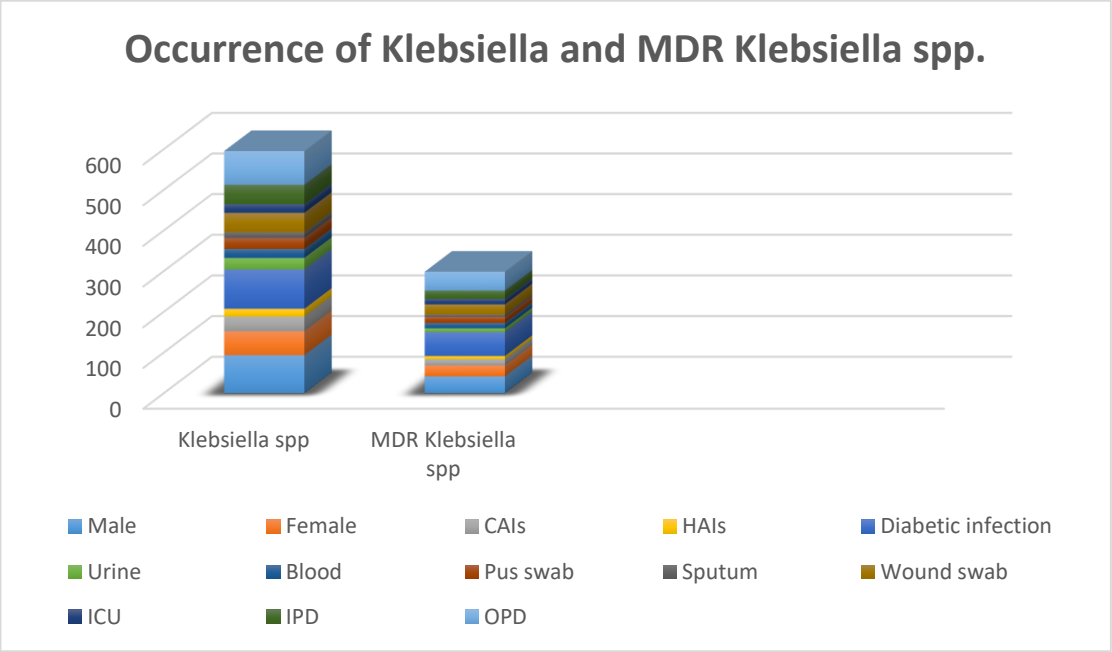


Table 4
Occurrence of Klebsiella spp. in clinical samples

	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.



Klebsiella spp. 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. 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%), following table 4. 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: The percentage of isolates exhibiting resistance toward thirteen antibiotics is represented in table 5. 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* was cefazolin (95.5 %), followed by nitrofurantoin (90.7 %) and tigecycline (83.8 %). We found similar antibiotic resistance trends in MDR *K.pneumoniae* isolated from different clinical sources. Although the percentage of *K. pneumoniae* strains 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 above.

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 blaSHV (n = 53, 77.9%), blaTEM (n = 51,75%) and blaCTX-M (n = 42, 61.7%), blaTEM with blaSHV positive for 31 isolates, blaTEM with blaCTX-M positive for 27 isolates and 19 isolates as positive for blaTEM with blaSHV and blaCTX-M as in table 6.

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

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

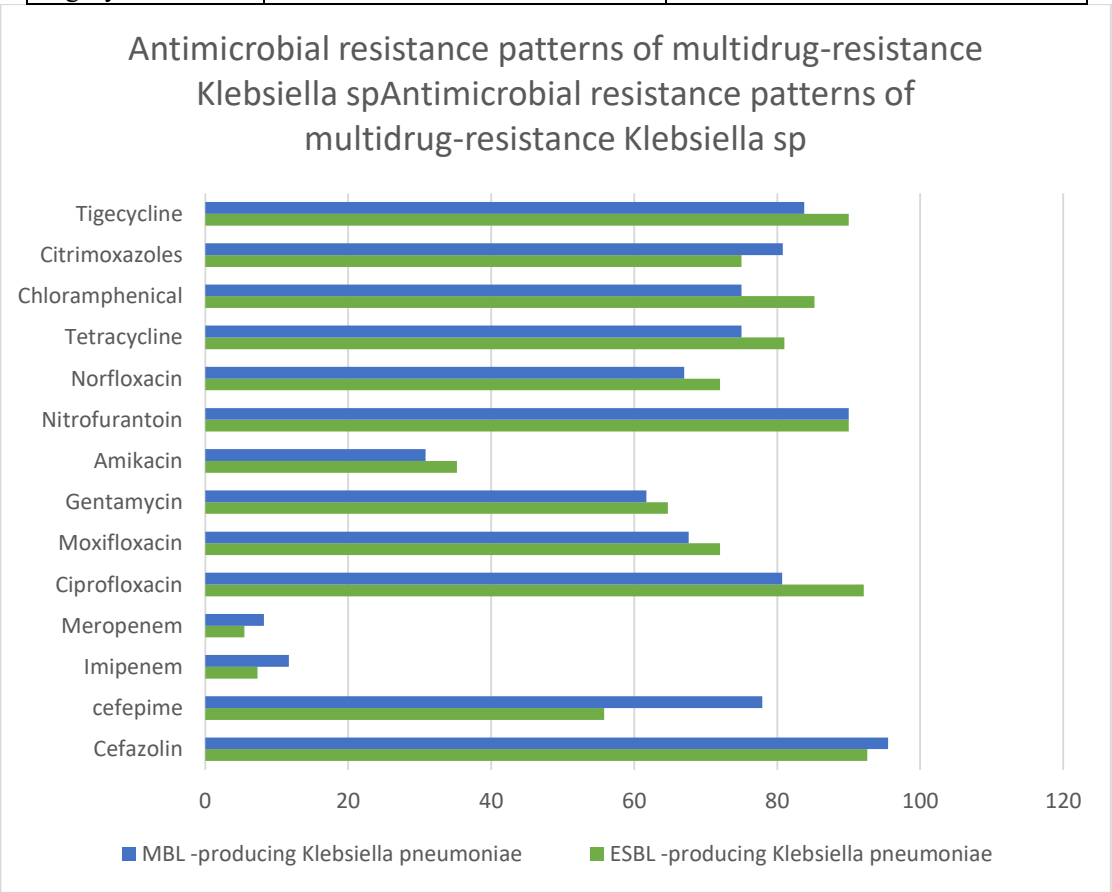


Table 6
Distribution of ESBL and MBL Resistant genes in *Klebsiella pnemomiae*

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 $_{VIM}$	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

Among 32 MBL positive *K.pneumoniae*, blaKPC was positive for (n = 32, 47%), blaVIM + blaIMP (n = 31, 45.5%), blaVIM (n = 28, 41.1%), blaIMP (n = 24, 35.2%) and blaKPC + blaVIM (n = 23, 33.8%) were identified. Similarly, MBL resistant genes are also represented in table 6.

Discussion

The growing prevalence of antibiotic resistance is narrowing the range of effective treatment options for diseases caused by drug-resistant bacteria. Greece currently has the highest rate of carbapenem resistance worldwide at 68% with India and the eastern Mediterranean regions following at 54%.[32] Table 1 identifies the most commonly reported extended-spectrum beta-lactamases (ESBLs) in India, which include SHV, TEM and CTX-M. Manoharan et al[2] and Goyal et al[16] have observed the co-occurrence of SHV, TEM and CTX-M in Enterobacteriaceae, a finding that aligns with the observations in this study. Tsay et al[30] found that 49% of patients with diabetes mellitus developed community-acquired bacteremia due to *K. pneumoniae* infection.

Gram-negative bacteria are generally more lethal than Gram-positive bacteria and are frequently associated with infections in healthcare settings.[2] Northeast India may be facing concerns due to the rising number of hospital-acquired and community-acquired multidrug-resistant bacterial infections. The current study also found that the strains isolated from various clinical cases exhibited high levels of resistance to all commonly used antibiotics. Consistent with previous studies from this region, the lowest resistance was observed against imipenem, gentamicin and amikacin.[12] As a result of treatment with carbapenem, there is a correlation between increased production of ESBL 77.9% and carbapenem resistance.[15]

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.[10] Multidrug-resistant pathogen colonization is becoming more prevalent in ICU patients due to their main illnesses, which frequently require recurrent hospital stays.[20] Globally, the majority of cases are found in intensive care units.[23] The majority of the time, ESBL development and antimicrobial resistance in *Klebsiella* were connected. The WHO[32] classified ESBL-producing *Klebsiella* as highly pathogenic superbugs. 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 along with removing invasive devices.[18]

Klebsiella in our investigation showed resistance to cephalosporins of the second and third generations. This shows the importance of screening Gram-negative bacteria for cephalosporin resistance and the development of ESBL and MBL.[5] Based on the genes, carbapenemases are considered to be the main mechanism that causes the development of CRKP isolates. According to MBL resistance gene analysis, the blaKPC gene was present in majority. We identified a higher incidence of bla OXA–48

and no blaVIM in any of the isolates we examined, compared to another study by Ghaith et al[14] with a different genotypic profile. Regarding ESBL genes, 42 isolates received our investigation with blaCTX–M. In an investigation by Amer et al[3] similar isolates were found which is in accordance with this observation.

In the isolates according to study, other carbapenemases, including blaKPC, blaVIM and blaOXA-48, were identified. In bacteremic patients with MDR *K. pneumoniae*, a combination treatment may be associated with better prognosis than monotherapy alone.[24] 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.[26] 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

There has been an increase in both the frequency and antibiotic resistance of *K. pneumoniae* isolates. Antibiotic stewardship is crucial to prevent overuse, especially among diabetic patients, as *K. pneumoniae* poses a significant threat to the healthcare system. Implementing strict infection control measures in hospitals is essential. The varied signs and symptoms of *K. pneumoniae* infections can make diagnosis and treatment challenging. To effectively combat antimicrobial resistance (AMR) and reduce mortality rates, it is important to have a comprehensive understanding of the bacteria causing infections, their antibiotic susceptibility profiles, specific resistance mechanisms and the geographic distribution of resistance.

The present study found that the isolates exhibited high levels of drug resistance and a greater prevalence of ESBL and MBL producers. This increase in multidrug-resistant organisms is leading to delays in effective therapy, as there are limited treatment options available. Hospitals need to adopt protective and management strategies. The findings from this study could be valuable for developing new community treatment strategies and providing baseline information for implementing antibiotic guidelines to curb the development of drug resistance. Continuous monitoring and antibiotic stewardship programs are essential for early detection of new multidrug-resistant *K. pneumoniae* infections.

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References

1. Ali M.R., Yang Y., Dai Y., Lu H., He Z., Li Y. and Sun B., Prevalence of multidrug-resistant hypervirulent Klebsiella pneumoniae without defined hypervirulent biomarkers in Anhui, China: A new dimension of hypervirulence, *Frontiers in Microbiology*, **14**, 1247091 **(2023)**

2. Agyepong N., Govinden U., Owusu-Ofori A. and Essack S.Y., Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana, *Antimicrobial Resistance & Infection Control*, **7**, 1-8 **(2018)**

3. Amer R., El-Baghdady K., Kamel I. and El-Shishtawy H., Prevalence of extended spectrum Beta-Lactamase Genes among Escherichia coli and Klebsiella pneumoniae clinical isolates, *Egyptian Journal of Microbiology*, **54(1)**, 91-101 **(2019)**

4. Aminul P., Anwar S., Molla M.M. and Miah M.R., Evaluation of antibiotic resistance patterns in clinical isolates of Klebsiella pneumoniae in Bangladesh, *Biosafety and Health*, **3(06)**, 301-6 **(2021)**

5. Będzichowska A., Przekora J., Stapińska-Syniec A., Guzek A., Murawski P., Jobs K., Wróblewska B. and Kalicki B., Frequency of infections caused by ESBL-producing bacteria in pediatric ward–single center five-year observation, *Archives of Medical Science*, **15(3)**, 688-93 **(2019)**

6. Changwal R., Ameta R. and Ameta S.C., Oxidation of benzyl alcohols by molecular oxygen catalyzed by cobalt ferrite, *Res. J. Chem. Environ.*, **28(1)**, 91-97 **(2024)**

7. Chen L. and Kreiswirth B.N., Convergence of carbapenem-resistance and hypervirulence in Klebsiella pneumoniae, *The Lancet Infectious Diseases*, **18(1)**, 2-3 **(2018)**

8. Das A., Behera B.K., Acharya S., Paria P., Chakraborty H.J., Parida P.K. and Das B.K., Genetic diversity and multiple antibiotic resistance index study of bacterial pathogen, Klebsiella pneumoniae strains isolated from diseased Indian major carps, *Folia Microbiologica*, **64**, 875-87 **(2019)**

9. Das A., Sahoo R.K., Gaur M., Dey S., Sahoo S., Sahu A., Behera D.U., Dixit S., Jain P.S., Jain B. and Sahu K.K., Molecular prevalence of resistance determinants, virulence factors and capsular serotypes among colistin resistance carbapenemase producing Klebsiella pneumoniae: a multi-centric retrospective study, *3 Biotech.* **12(1)**, 30 **(2022)**

10. Devi U., Bora R., Das J.K. and Mahanta J., Extended-spectrum β-lactamase & carbapenemase-producing gram-negative bacilli in neonates from a tertiary care Centre in Dibrugarh, Assam, India, *Indian Journal of Medical Research*, **147(1)**, 110-4 **(2018)**

11. Dhungana K., Awal B.K., Dhungel B., Sharma S., Banjara M.R. and Rijal K.R., 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, *ASMI*, **2(12)**, 60-9 **(2019)**

12. Gajamer V.R., Bhattacharjee A., Paul D., Kapil J., Sarkar A., Singh A.K., Pradhan N. and Tiwari H.K., The first report of phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing uropathogens in Sikkim and Darjeeling hills of India, *Microbial Drug Resistance*, **24(9)**, 1284-8 **(2018)**

13. Ghaffarian F., Hedayati M., Ebrahim-Saraie H.S., Roushan Z.A. and Mojtahedi A., Molecular epidemiology of ESBL-producing Klebsiella pneumoniae isolates in intensive care units of a tertiary care hospital, North of Iran, *Cellular and Molecular Biology*, **64(7)**, 75-9 **(2018)**

14. Ghaith D., Morsy S.A., Sebak M. and Rabea R.A., Phenotypic and genotypic characterization of carbapenem-resistant Gram-negative organisms, Beni-Suef, Egypt, *Beni-Suef University Journal of Basic and Applied Sciences*, **12(1)**, 61 **(2023)**

15. Giriyapur R.S., Nandihal N.W. and Patil A.B., Comparison of disc diffusion methods for the detection of extended-spectrum beta lactamase-producing Enterobacteriaceae, *Journal of Laboratory Physicians*, **3(01)**, 033-6 **(2011)**

16. Goyal A., Prasad K.N., Prasad A., Gupta S., Ghoshal U. and Ayyagari A., Extended spectrum β-lactamases in Escherichia coli & Klebsiella pneumoniae & associated risk factors, *Indian Journal of Medical Research*, **129(6)**, 695-700 **(2009)**

17. He Y., Guo X., Xiang S., Li J., Li X., Xiang H., He J., Chen D. and Chen J., Comparative analyses of phenotypic methods and 16S rRNA, khe, rpoB genes sequencing for identification of clinical isolates of Klebsiella pneumoniae, *Antonie Van Leeuwenhoek*, **109**, 1029-40 **(2016)**

18. Ibrahim M.E., Abbas M., Al-Shahrai A.M. and Elamin B.K., 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, *Canadian Journal of Infectious Diseases and Medical Microbiology*, doi: 10.1155/2019/6054694 **(2019)**

19. Kar B., Sharma M., Peter A., Chetia P., Neog B., Borah A., Pati S. and Bhattacharya D., Prevalence and molecular characterization of β-lactamase producers and fluoroquinolone resistant clinical isolates from North East India, *Journal of Infection and Public Health*, **14(5)**, 628-37 **(2021)**

20. Kuo H.Y., Yang C.M., Lin M.F., Cheng W.L., Tien N. and Liou M.L., Distribution of blaOXAcarrying imipenem-resistant Acinetobacter spp. in 3 hospitals in Taiwan, *Diagn Microbiol Infect Dis*., **66(2)**, 195-9 **(2010)**

21. Manoharan A., Premalatha K., Chatterjee S. and 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 Journal of Medical Microbiology*, **29(2)**, 161-4 **(2011)**

22. Nirwati H., Sinanjung K., Fahrunissa F., Wijaya F., Napitupulu S., Hati V.P., Hakim M.S., Meliala A., Aman A.T. and Nuryastuti T., Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia, In BMC Proceedings, BioMed Central, **13**, 1-8 **(2019)**

23. Pillay D., Naidoo L., Swe Swe-Han K. and Mahabeer Y., Neonatal sepsis in a tertiary unit in South Africa, *BMC Infectious Diseases*, **21**, 1-0 **(2021)**

24. Qureshi Z.A., Paterson D.L., Potoski B.A., Kilayko M.C., Sandovsky G., Sordillo E., Polsky B., Adams-Haduch J.M. and Doi Y., Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens, *Antimicrobial Agents and Chemotherapy*, **56(4)**, 2108-13 **(2012)**

25. Ravi V., Vijay D.D., Sujhithra A., Jayanthi S., Subramanian T.K. and Harish N., Neonatal Sepsis: The impact of Hypervirulent Klebsiella pneumonia in a Tertiary Care Hospital, *J Pure Appl Microbiol*., **16(3)**, 2035-44 **(2022)**

26. Shankar C., Nabarro L.E., Devanga Ragupathi N.K., Muthuirulandi Sethuvel D.P., Daniel J.L. and Doss C., GP, Veeraraghavan B. Draft genome sequences of three hypervirulent carbapenem-resistant Klebsiella pneumoniae isolates from bacteremia, *Genome Announcements*, **4(6)**, 10-128 **(2016)**

27. Shen D., Ma G., Li C., Jia X., Qin C., Yang T., Wang L., Jiang X., Ding N., Zhang X. and Yue L., Emergence of a multidrug-resistant hypervirulent Klebsiella pneumoniae sequence type 23 strain with a rare bla CTX-M-24-harboring virulence plasmid, *Antimicrobial Agents and Chemotherapy*, **63(3)**, 10-128 **(2019)**

28. Singh S.K., Mishra M., Sahoo M., Patole S., Sahu S., Misra S.R. and Mohapatra H., Antibiotic resistance determinants and clonal relationships among multidrug-resistant isolates of Klebsiella pneumoniae, *Microbial Pathogenesis*, **110**, 31-6 **(2017)**

29. Solgi H., Shahcheraghi F., Bolourchi N. and Ahmadi A., Molecular characterization of carbapenem-resistant serotype K1 hypervirulent Klebsiella pneumoniae ST11 harbouring blaNDM-1 and blaOXA-48 carbapenemases in Iran, *Microbial Pathogenesis*, **149**, 104507 **(2020)**

30. Tsay R.W., Siu L.K., Fung C.P. and Chang F.Y., 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, *Archives of Internal Medicine*, **162(9)**, 1021-7 **(2002)**

31. Wang N., Zhan M., Liu J., Wang Y., Hou Y., Li C., Li J., Han X., Liu J., Chen Y. and Fan J., Prevalence of carbapenem-resistant Klebsiella pneumoniae infection in a Northern Province in China: clinical characteristics, drug resistance and geographic distribution, *Infection and Drug Resistance*, **15**, 569-79 **(2022)**

32. World Health Organization, Antimicrobial resistance: global report on surveillance, World Health Organization **(2014)**

33. Younas S., Ejaz H., Zafar A., Ejaz A., Saleem R. and Javed H., AmpC beta-lactamases in Klebsiella pneumoniae: An emerging threat to the paediatric patients, *JPMA*, **68(6)**, 893-7 **(2018)**.

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