Fighting the Dual Threat: Hypervirulent *Klebsiella pneumoniae* and Carbapenem Resistance in Diabetics

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Abstract
The prevalence of diabetes is rising these days as a result of hypervirulent Klebsiella pneumonia (hvKp), multidrug-resistant (MDR) and carbapenem-resistant bacteria. The burden of drug-resistant Klebsiella pneumonia bacteria is due to diabetic infections. This study was carried out at the SRM Institute of Science and Technology from January 2022 to December 2023 with Human Institutional Ethics Committee approval. All clinical samples underwent bacterial identification and antibiotic susceptibility testing. The combined disc test for metallo-beta-lactamase producers was carried out using conventional microbiological methods. The string test was performed to determine hvKp.

The identification of hvKp and carbapenem-resistant genes was accomplished using PCR amplification. The most significant number of pathogens (70.2%) isolated from various clinical samples was around 350. Of the 104 Klebsiella pneumoniae strains discovered, 47% (n=49) were drug-resistant. Of these, around 32 (30.7%) produced MBL, 11 had the rmpA-hypervirulent gene positive and 15 had the bla VIM gene positive. Pathogens had a much greater prevalence of MDR and MBL producers. The results of this study highlight the necessity of preventive measures against drug-resistant pathogen colonization and infection.

Keywords: MDR-Multidrugresistant, MBL-Metallo Beta Lactamase, *Carbapenem, hvKp-Hypervirulent Klebsiella pneumoniae.*

Introduction
In recent years, there have been worries about hypervirulent *Klebsiella pneumoniae* (hvKp) strains' tendency to cause serious infections in otherwise healthy persons. When combined with antibiotic resistance, specifically carbapenemase synthesis, the harm posed by these strains is amplified, particularly in diabetic individuals who are already prone to infections.[13,14]

Patients with diabetes usually have weaker immune systems, making them more susceptible to infections, particularly those caused by antibiotic-resistant bacteria like *Klebsiella pneumoniae* (CP-Kp) which generated carbapenemase. The severity of infections can be particularly problematic when hvKp strains are present.[12,19]

Because carbapenem antibiotics are commonly considered as the last line of defence against germs that are resistant to numerous medications, bacteria produce enzymes called carbapenemases that confer resistance to these antibiotics.[16] When carbapenemase-producing bacteria emerge, healthcare providers face significant challenges, particularly in hypervirulent *Klebsiella pneumoniae* infections with limited treatment options. K. pneumoniae strains are hypervirulent and antibiotic resistant, highlighting the importance of stringent infection control methods, careful antibiotic administration and the development of alternative treatment techniques such as alternate therapies or new medicines. This is especially crucial for persons with diabetes.[3]

Healthcare facilities must focus on preventative measures to limit the risk of transmission while also implementing effective surveillance techniques to monitor the presence and spread of these strains. To further tackle this threat to public health, research into novel therapeutic methods and vaccines that target hypervirulent and antibiotic-resistant *K. pneumoniae* strains is required.[4]

Material and Methods
This prospective study was done at the SRM Institute of Science and Technology from January 2022 to December 2023 with Human Institutional Ethical Committee permission. Patients having a history of infections were the topic of clinical samples obtained from both the outpatient and inpatient units. All clinical samples were grown and pathogen identification was performed using standard microbiological techniques.

Antimicrobial Susceptibility Testing: The antibiotic pattern for commonly used antibacterial medications was determined using Kirby- Bauer's disc diffusion method as recommended by the Clinical and Laboratory Standard Institute (CLSI). Antibiotics including cefuroxime, ampicillin, amikacin, cefazolin, ceftazidime, cefotaxime, cefepime, pipercillin-tazobactam, gentamicin, tobramycin, co-trimoxazole, ciprofloxacin, imipenem and meropenem were tested and the results were compared to the ATCC reference strain.[8] The production of MBL (metallo-beta-lactamase) was determined using the double disc diffusion method and the combination disc test.This test involved incubating an imipenem (10ul) disc alone or with 10 µl of

100mM EDTA at 37˚C for 16-24 hours. MBL was considered positive if the imipenem-EDTA disc's zone of inhibition was at least 7 mm larger than that of the imipenem disc alone.[2,6,7,11,17]

Detection of Virulence Factors - Blood Hemolysis: The isolates were placed in blood agar plates containing 5% sheep blood for the plate haemolysis test. After 24 hours of incubation at 37°C, haemolysis was identified.[10,18]

Phenotypic Detection of Hypermucoviscosity: The mucoviscous string-stretching ability of one colony from each strain was tested. When a produced string is longer than 10 mm, it is classified as the HMV phenotype.[9,10] Figure 1 illustrates the string test used to identify the hypervirulent strain of *Klebsiella pneumoniae*.

Note: Positive "string test" on a hypervirulent strain of *Klebsiella pneumonia* stretching
(Colonies results information of a string of >5mm)
Figure 1: String test to detect hypervirulent strain of *Klebsiella pneumoniae*

PCR Amplification of Carbapenem resistant gene and Hypervirulent Associated Genes: Following phenotypic confirmation, the isolates underwent genotypic investigation. The multiplex PCR amplification approach was utilised to identify resistance genes.The DNA template was created using 17 bacterial isolates. The colonies were removed and suspended in 100 µl of mili-Q water. They were then cooled at -20°C for 10 minutes before boiling for 30 minutes. The bacterial debris was separated by centrifugation for seven minutes at 13,000 rpm. The supernatant was then extracted and used as a template DNA. UV spectrophotometry was used to determine DNA concentration. Table 1 lists the PCR primers that were utilized to find the target resistance genes. Table 2 illustrates the cycling conditions for the PCR reaction.

Results

This study was conducted to screen and to identify the drug resistance pattern of the infection-causing *Klebsiella pneumonia* in the tertiary care hospital. All categories of clinical samples were included in the study: The sources of the 350 clinical samples that were divided up, were as follows: bodily fluids (n=3) 0.83%, swabs (n=1) 0.27%, pus (n=11) 3%, urine (n=44) 12%, tissue (n=3) 83% and blood (n= 288) 82%. Pseudomonas species (n=84)24%), *Klebsiella pneumonia* (118; 34%) and *Acinetobacter baumannii complex* 40 (11%) identified the largest number of pathogens.

Other isolates included 21 (6%), 5 % and 1 % of *Haemophilus influenza*, *Streptococcus pyogenes*, *Citrobacter species*, 8 (2%) and *Enterobacter species*, *Proteus species* and *Escherichia coli*. Out of the isolated *Pseudomonas species*, 72 were *Pseudomonas aeruginosa*, 7 *Pseudomonas alginate*, 3 *Pseudomonas stutzeri* and 2 *Pseudomonas putida*.

There were 42 (36%) and 76 (64%) pathogens identified from the outpatient and inpatient departments respectively. The inpatient department, which includes general ward (19), medicine ward (11), semiprivate ward (11) and private ward (7), was the next most common source of isolates, with ICU (28) being the largest source. Of the total isolates, 28 (24%) produced MBL, while 40 (40%) of the *Klebsiella pneumonia* isolates were multidrug resistant. Table 3 displays the antibiogram pattern for *Klebsiella pneumonia*. Nine Carbapenemes-resistant strains tested positive for VIM, eight tested positive for both CTX-M and KPC, four tested positive for IMP and two tested positive for the NDM gene. A string test was performed on 104 isolates of *Klebsiella pneumoniae* to validate the phenotypic presence of hvKp and 54 (46%) of the strains tested positive.

Discussion

The rise of multidrug-resistant *K. pneumoniae* is a major concern in the treatment of acute infections around the world. Several events and mechanisms contribute to the formation and spread of antibiotic resistance in bacterial strains. The role of virulence factors in MDR *K. pneumoniae* infections is widely recognised. The bacterial pathogens were primarily isolated from the inpatient department (64.40%), with the majority coming from sputum samples (n=254). A similar pattern was observed in sputum samples (n = 299). Notably, *Klebsiella pneumoniae* was the most frequently isolated pathogen, which is consistent with our findings. Amudan et al[1] also observed comparable pathogen yields across various departments and wards, reinforcing our findings. Among these isolates, 33% of *K. pneumoniae* displayed multidrug resistance.

Pandya et al[17] reported a resistance rate of 6% to imipenem, ertapenem, meropenem and third-generation cephalosporins, with 96% of isolates testing positive for metallo-beta-lactamase (MBL) by Carba NP test, mirroring our study's results.According to Zheng et al[23], 20% of *Klebsiella pneumoniae* isolates exhibited carbapenem resistance, a finding consistent with our study. Gharrah et al[10] found only 3% of *Klebsiella pneumoniae* isolates positive for blood hemolysis, whereas in our study, 22% were positive for this virulence factor. This significant difference aligns with our findings. Barwa et al[5] also detected the blaVIM gene in a manner consistent with our study.

Table 1
Oligonucleotide primers used for detecting capsular and Carbapenem resistant and Hypervirulent gene.

Target gene	Sequences of Primer(5'-3')	Amplified product(bp)
PCR-I - Carbapenem Resistant Gene		
CTX-M	CGCTTTGCGATGTGCAG - ACCGCGATATCGTTGGT	550
PCR-II		
NDM	GGGCAGTCGCTTCCAACGGT - GTAGTGCTCAGTGTCGGCAT	476
IMP	TTGACACTCCATTTACDG - GATYGAGAATTAAGCCACYCT	139
VIMF	GATGGTGTTTGGTCGCATA - CGAATGCGCAGCACCAG	390
KPCF	CATTCAAGGGCTTTCTTGCTGC - ACGACGGCATAGTCATTTGC	500
PCR-III - Hypervirulent gene		
rmpA	ACT GGGCTACCTCTGCTTCA - ACT GGGCTACCTCTGCTTCA	550

Table 2
Multiplex PCR –Amplification

PCR Panel	Initial Denaturation	Denaturation	Annealing	Elongation	Final Elongation	Cycle
PCR-I	95°C 10 min	95°C 1 min	51°C 1 min	72°C 1 min	72°C 5 min	40 cycles
PCR-II	95°C 10 min	95°C50 sec	55°C 1 min	72°C 1 min	72°C 7 min	35 cycles
PCR-III HvKp	95°C 10 min	95°C30 sec	59°C30 sec	72°C 1 min	72°C 7 min	40 cycles

Our study found 54 (46%) isolates positive for hypermucoviscosity, as determined by the string test, a proportion similar to that reported by Shankar et al[21,22] and Shah et al[20] who reported percentages of 31.3% and 40% respectively. Additionally, our study revealed that 14 (50%) strains lacked both K1 and K2 capsular types, a proportion comparable to findings reported by Shankar et al.[21,22]

Conclusion

Klebsiella pneumonia, a prevalent cause of bacterial illnesses, is more likely to acquire resistance and virulence genes. Multiple drug resistance is strongly associated with clinical isolates. Using molecular markers to identify hypervirulent Kp in the early stages is critical for averting outbreaks and minimising mortality. *Klebsiella pneumonia* has become increasingly common in hospital settings and it is the leading cause of nosocomial infections. Improved creative and early therapeutic methods rely on identifying resistance genes and conducting additional study on virulence factors.

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