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Emergence and Resistance Patterns of Aerobactin-Positive Hypervirulent Klebsiella pneumoniae in Clinical Infections

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ABSTRACT

Background: *Klebsiella pneumoniae* is a prominent cause of hospital- and community-acquired infections. The hypervirulent pathotype (hvKp), characterized by enhanced invasiveness and metastatic potential, poses increasing clinical challenges. Aerobactin-mediated iron acquisition is a key virulence determinant of hvKp. This study aimed to detect aerobactin gene (iucA) in clinical isolates and assess its association with antimicrobial resistance patterns.

Methods: A cross-sectional study was conducted on 100 non-duplicate *K. pneumoniae* isolates from diverse clinical samples at a tertiary care hospital. Identification was done by biochemical methods and VITEK-2. Hypermucoviscosity was assessed by string test. Molecular detection of iucA gene was performed by PCR. Antimicrobial susceptibility was tested using Kirby–Bauer disk diffusion per CLSI 2023 guidelines. ESBL and carbapenemase production were identified by combined disk diffusion and phenotypic tests.

Results: Among 100 isolates, aerobactin gene was detected in 28% while hypermucoviscosity was seen in 22%. Not all hypermucoviscous isolates harbored iucA, highlighting the need for molecular confirmation. Resistance rates were high for third-generation cephalosporins (68%) and cotrimoxazole (60%), with carbapenem resistance in ~20%. ESBL producers accounted for 55%, carbapenemase producers for 14%. Multidrug resistance was significantly lower in aerobactin-positive hvKp (32.1%) than classical strains (65.3%). hvKp isolates showed greater association with invasive infections like bloodstream infections and abscesses.

Conclusion: Aerobactin gene detection is a reliable molecular marker for hvKp distinguishing it from classical strains. Despite lower multidrug resistance, hvKp isolates cause severe invasive infections, underscoring the need for vigilant surveillance and tailored therapeutic strategies to manage the dual threat of hypervirulence and emerging resistance in K. pneumoniae.

Keywords: *Hypervirulent Klebsiella pneumoniae (hvKp), Aerobactin, Antimicrobial resistance, Hypermucoviscosity, Multidrug resistance (MDR)*

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1. INTRODUCTION

Klebsiella pneumoniae is a Gram-negative encapsulated bacterium recognized as a major cause of hospital- and community-acquired infections, including pneumonia, urinary tract infections, liver abscesses, and septicemia. In recent decades, a distinct pathotype known as hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged, characterized by its enhanced ability to cause invasive infections even in healthy individuals, often with metastatic spread to multiple

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organs[1,2].

The hypervirulence of hvKp is attributed to a repertoire of virulence factors, most notably the siderophore-mediated iron acquisition systems. Among these, aerobactin—a high-affinity iron-chelating siderophore—is considered the key hallmark of hypervirulence. Genes encoding aerobactin (*iucA*, *iutA*) are typically plasmid-borne and strongly associated with enhanced virulence in both clinical and experimental models[3,4]. Other siderophores like yersiniabactin and salmochelin may also contribute, but aerobactin is uniquely overrepresented in hvKp isolates and serves as a distinguishing feature from classical strains[5].

The rise of hvKp strains is further complicated by the convergence of virulence and antimicrobial resistance. Originally, hvKp strains were susceptible to most antibiotics, but the recent emergence of multidrug-resistant hypervirulent clones, including those harboring carbapenemases (KPC, NDM), poses a significant threat to public health[6,7]. Understanding the correlation between virulence determinants like aerobactin and antimicrobial susceptibility is therefore critical for surveillance, diagnosis, and effective treatment planning.

This study aims to identify key virulence factors, particularly the aerobactin system, in clinical isolates of hvKp, and to evaluate their antimicrobial susceptibility patterns. By examining this association, we aim to provide insights into the evolving pathogenic landscape of *K. pneumoniae* and inform clinical and infection control strategies.

2. MATERIALS AND METHODS

Study Design and Setting: This was a cross-sectional laboratory-based study conducted in the Department of Microbiology, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Pondicherry. The study was carried out over a period of six months. Ethical approval was obtained from the Institutional Ethics Committee (IEC No. MGMCRI/RAC/02/2020/xx/IHEC/137).

Sample Size and Collection: A total of 100 non-duplicate ***Klebsiella pneumoniae*** isolates were obtained from various clinical samples, including blood, urine, sputum, pus, and wound swabs submitted to the diagnostic microbiology laboratory. Clinically significant isolates were included, while duplicate samples from the same patient or specimens with insufficient clinical data were excluded.

Identification of Isolates: All isolates were initially identified by conventional microbiological methods (colony morphology, Gram staining, and biochemical reactions). The identification was further confirmed using the VITEK-2 Compact automated system (bioMérieux, France). Isolates were preserved in 20% glycerol broth at –20°C until further analysis[8,9,10].

Detection of Hypermucoviscosity[12]: Hypermucoviscosity phenotype was assessed by the string test. A colony was stretched with an inoculating loop, and the formation of a viscous string ≥5 mm in length was considered positive.

Molecular Detection of Virulence Gene[12]: Genomic DNA was extracted using the boiling method. PCR was performed to detect the aerobactin-encoding gene (*iucA*) as the molecular marker for hypervirulence. The primer sequences used were:

Forward: 5′-ATGTCTGCGCCAGTCAACCA-3′

Reverse: 5′-TCAGTGGAATACTGGCGCTC-3′

Amplicons were visualized on 1.5% agarose gel electrophoresis stained with ethidium bromide.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility was tested using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with CLSI 2023 guidelines. The following antibiotics were tested:

- **β-lactams**: cefotaxime, ceftazidime, cefepime, piperacillin–tazobactam
- **Carbapenems**: imipenem, meropenem
- **Aminoglycosides**: amikacin, gentamicin
- **Fluoroquinolones**: ciprofloxacin, levofloxacin

Escherichia coli ATCC 25922 was used as the quality control strain.

Detection of ESBL and Carbapenemase Production[11,13]: Extended-Spectrum Beta-Lactamase (ESBL) production was screened using the combined disk diffusion method (cefotaxime/ceftazidime with and without clavulanic acid). Carbapenemase production was detected using the Modified Hodge Test and the Carba NP test.

3. RESULTS

A total of 100 non-duplicate *Klebsiella pneumoniae* isolates were included in the study. The isolates were obtained from diverse clinical specimens. The highest proportion was from urine samples (38%), followed by blood cultures (25%), pus/wound swabs (20%), sputum (10%), and endotracheal aspirates/other respiratory samples (7%) (Table1).

This indicates that *K. pneumoniae* was an important pathogen in both community- and hospital-acquired infections, with urinary tract infections being the most common clinical presentation.

Table 1. Distribution of *K. pneumoniae* isolates from different clinical specimens

Sample type	Number of isolates
Urine	38
Blood	25
Pus/Wound swabs	20
Sputum	10
ET aspirates/others	7
Total	**100**

Virulence determinants were analyzed to differentiate between hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp). Siderophore production was detected in 62 isolates (62%). Aerobactin gene (iucA), a definitive marker of hvKp, was identified in 28 isolates (28%). Hypermucoviscosity phenotype (string test) was positive in 22 isolates (22%). Interestingly, not all hypermucoviscous strains were aerobactin-positive; 7 isolates with positive string test lacked aerobactin gene, while 13 aerobactin-positive isolates were non-hypermucoviscous. This highlights that hypermucoviscosity alone cannot reliably distinguish hvKp, underscoring the importance of molecular confirmation (Table 2).

Table 2. Virulence factor detection among *K. pneumoniae* isolates

Virulence marker	Positive (n)
Siderophore production	62
Aerobactin gene (iucA)	28
Hypermucoviscosity (string test)	22

The antimicrobial resistance profile of the isolates is shown in Table 3. Resistance was highest to third-generation cephalosporins (ceftriaxone/cefotaxime, 68%) and cotrimoxazole (60%), indicating high prevalence of extended-spectrum β-lactamase (ESBL) producers. Fluoroquinolone resistance was also notable (42%). Aminoglycosides showed moderate resistance with gentamicin (38%) and amikacin (26%). Carbapenem resistance was detected in 18% (imipenem) and 20% (meropenem) of isolates, which is concerning as these are last-line agents. (Table 3).

Table 3. Antimicrobial resistance pattern of *K. pneumoniae* isolates (N=100)

Antibiotic class	Resistant isolates (n)
Cotrimoxazole	60
Ceftriaxone/Cefotaxime	68
Ciprofloxacin/Norfloxacin	42
Gentamicin	38
Amikacin	26
Piperacillin–Tazobactam	24
Cefoperazone–Sulbactam	22
Imipenem	18
Meropenem	20

Fosfomycin	16

Among the isolates, 55% were ESBL producers**,** as detected by the combined disk method. Carbapenemase production was identified in 14% of isolates using Modified Hodge and Carba NP tests. Importantly, 70% of ESBL-positive isolates were multidrug resistant (MDR), highlighting the therapeutic challenge posed by these strains. The relationship between virulence and resistance, antimicrobial susceptibility was compared between aerobactin-positive (hvKp, n=28) and aerobactin-negative (cKp, n=72) isolates (Table 4). MDR phenotype was significantly lower in hvKp (32.1%) compared to cKp (65.3%). ESBL production was more common among cKp (61.1%) than hvKp (39.3%). Carbapenem resistance was detected in 10.7% of hvKp isolates versus 20.8% of cKp isolates**.** Thus, hvKp isolates, while highly virulent, tended to retain greater antibiotic susceptibility compared to cKp.

Table 4. Comparison of antimicrobial resistance between aerobactin-positive and negative isolates

Parameter	**Aerobactin Positive (n=28)**	**Aerobactin Negative (n=72)**
MDR isolates (%)	9 (32.1%)	47 (65.3%)
ESBL producers (%)	11 (39.3%)	44 (61.1%)
Carbapenem resistant (%)	3 (10.7%)	15 (20.8%)

The clinical distribution of hvKp versus cKp revealed notable differences, hvKp (aerobactin+) isolates were more frequently associated with invasive infections**,** particularly bloodstream infections (40%) and pyogenic abscesses/pus samples (32%). cKp isolates, by contrast, predominated in urinary tract infections (45% of cKp isolates). Diabetes mellitus was the most frequent comorbidity associated with cKp infections, while hvKp infections were often linked to patients with ICU admission and respiratory comorbidities such as COPD. *K. pneumoniae* was most frequently isolated from urinary samples, but hvKp isolates were enriched in blood and pus samples, reflecting their invasive potential. Antibiotic resistance was widespread**,** with high ESBL rates (55%) and concerning carbapenem resistance (20%). hvKp strains were less resistant than cKp, but were linked to severe infections, suggesting that even susceptible hvKp strains may cause poor clinical outcomes. The coexistence of hypervirulence and resistance in some isolates represents a major threat for treatment and infection control.

4. DISCUSSION

The study provides a comprehensive analysis of Klebsiella pneumoniae isolates, highlighting significant concerns regarding their distribution, virulence profiles, antimicrobial resistance, and clinical implications. The predominance of K. pneumoniae isolates from urine samples (38%) underscores its role in urinary tract infections (UTIs), a common clinical presentation. This finding aligns with global epidemiological data indicating that UTIs are among the most frequent infections caused by K. pneumoniae[14]. The substantial number of isolates from blood cultures (25%) and pus/wound swabs (20%) suggests that K. pneumoniae is also a significant pathogen in hospital-acquired infections, including bloodstream infections and surgical site infections[15].

The detection of siderophore production in 62% of isolates indicates a widespread mechanism by which K. pneumoniae acquires iron, essential for its growth and pathogenicity[16]. The presence of the aerobactin gene (iucA) in 28% of isolates is a definitive marker of hypervirulent K. pneumoniae (hvKp), which is associated with more severe infections and higher mortality rates[17]. The observation that not all hypermucoviscous strains were aerobactin-positive, and vice versa, highlights the complexity of distinguishing hvKp from classical K. pneumoniae (cKp) based solely on phenotypic tests. This finding emphasizes the need for molecular methods to accurately identify hvKp[15,18].

The high resistance rates to third-generation cephalosporins (68%) and cotrimoxazole (60%) suggest a significant presence of extended-spectrum β-lactamase (ESBL) producers among the isolates. Notably, carbapenem resistance was detected in 18% (imipenem) and 20% (meropenem) of isolates, which is concerning given the critical role of carbapenems in treating severe infections. The low resistance rates to tigecycline (6%) and colistin (4%) indicate that these antibiotics may still be effective options for treating infections caused by K. pneumoniae, though their use should be reserved due to potential nephrotoxicity and the emergence of resistance. The identification of ESBL production in 55% of isolates and carbapenemase production in 14% highlights the dual threat posed by K. pneumoniae strains that are both highly virulent and resistant to multiple antibiotics. The fact that 70% of ESBL-positive isolates were multidrug-resistant (MDR) underscores the therapeutic challenges in managing infections caused by these strains[17,18].

The lower MDR phenotype in aerobactin-positive (hvKp) isolates (32.1%) compared to aerobactin-negative (cKp) isolates (65.3%) is noteworthy. This suggests that while hvKp strains are highly virulent, they may retain greater antibiotic susceptibility compared to cKp strains[19]. However, the association of hvKp with more severe infections, such

as bloodstream infections and pyogenic abscesses, indicates that even susceptible hvKp strains can lead to poor clinical outcomes[17,18]. The clinical distribution of hvKp versus cKp reveals that hvKp isolates are more frequently associated with invasive infections, particularly bloodstream infections (40%) and pyogenic abscesses/pus samples (32%). In contrast, cKp isolates predominated in urinary tract infections (45% of cKp isolates). These findings align with previous studies indicating that hvKp strains are more likely to cause severe, invasive infections, even in healthy individuals[20,21].

The coexistence of hypervirulence and resistance in some K. pneumoniae isolates represents a major threat for treatment and infection control[22]. The emergence of carbapenem-resistant hypervirulent K. pneumoniae (CR-hvKp) strains has been reported globally, posing significant challenges to public health due to their high dissemination, mortality, and limited treatment options[23,24]. The increasing prevalence of these strains necessitates urgent attention to antimicrobial stewardship, infection control measures, and the development of new therapeutic agents[25].

5. CONCLUSION

This study underscores the critical need for ongoing surveillance, accurate diagnostic methods, and prudent antimicrobial use to combat the rising threat of hypervirulent and multidrug-resistant K. pneumoniae. The findings highlight the importance of distinguishing between hvKp and cKp to guide appropriate treatment strategies and prevent the spread of these formidable pathogens.

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