Infection and Drug Resistance

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ORIGINAL RESEARCH

Predicting Antibiotic Tolerance in hvKP and cKP Respiratory Infections Through Biofilm Formation Analysis and Its Resistance Implications

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Introduction: Respiratory infections are a major global health concern, with Klebsiella pneumoniae standing out due to its evolving antibiotic resistance. This study compares the resistance profiles of hypervirulent Klebsiella pneumoniae (hvKP) and classical Klebsiella pneumoniae (cKP), aiming to shed light on their clinical implications.

Methods: We analyzed 86 cases, comprising 42 hvKP and 44 cKP strains, using comprehensive antimicrobial susceptibility testing and clinical data evaluation to assess antibiotic tolerance and resistance mechanisms.

Results: Our findings reveal distinct resistance patterns between hvKP and cKP, highlighting the role of chromosomal mutations and plasmid-mediated gene transfer in conferring antibiotic resistance. Notably, hvKP strains exhibited unique resistance trends, including the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases, differing from those of cKP.

Discussion: This research underscores the importance of continuous surveillance and the development of targeted therapies against antibiotic-resistant Klebsiella pneumoniae. It emphasizes the critical need for judicious antibiotic use and novel therapeutic approaches to combat respiratory infections caused by these increasingly resistant pathogens.

Keywords: Klebsiella pneumoniae, antibiotic resistance, hypervirulent *Klebsiella pneumoniae*, hvKP, classical *Klebsiella pneumoniae*, cKP, public health

Introduction

In the realm of infectious diseases, respiratory infections remain a formidable challenge, accounting for significant morbidity and mortality worldwide.[1] Among the diverse array of pathogens implicated in respiratory tract infections, Klebsiella pneumoniae stands out due to its increasing prevalence and the remarkable ability to develop resistance against multiple antibiotic agents.[2,3] *K. pneumoniae* can be classified into two major groups: hypervirulent *K. pneumoniae* (hvKP) and classical *K. pneumoniae* (cKP), each with distinct pathogenic profiles and clinical implications.[4] Recent years have witnessed a surge in cases involving these two strains, underscoring the urgency to delve deeper into their resistance mechanisms and develop strategies to mitigate their impact on public health.[5,6]

hvKP, characterized by its hypermucoviscosity and the ability to cause invasive infections, has been associated with severe clinical manifestations such as liver abscesses, endophthalmitis, and meningitis.[7] On the other hand, cKP is typically linked to hospital-acquired infections, exhibiting a predilection for individuals with compromised immune systems.[8] Despite the differences in their virulence and clinical presentations, both hvKP and cKP pose significant challenges due to their capacity to acquire resistance to commonly used antibiotics.[9]

The emergence of antibiotic-resistant strains of K. pneumoniae has been documented globally, with alarming increases in the incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates.[10,11] This trend is particularly concerning given the limited therapeutic options available for treating infections caused by these resistant

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strains.[10] In this context, understanding the antibiotic tolerance profiles of hvKP and cKP becomes paramount, as it holds the key to developing targeted interventions and optimizing antimicrobial therapy.

A plethora of studies have shed light on the resistance mechanisms employed by *K. pneumoniae*, revealing a complex interplay of chromosomal mutations and plasmid-mediated gene transfer.[12] In particular, the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases has been identified as a major driver of antibiotic resistance in these strains.[13] These enzymes confer resistance to a wide range of β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, severely limiting the treatment options and contributing to the high mortality rates associated with these infections.[14,15]

In this study, we endeavor to unravel the intricate landscape of antibiotic tolerance in hvKP and cKP respiratory infections, with a particular focus on their resistance profiles and the underlying molecular mechanisms. By leveraging clinical data and conducting comprehensive antimicrobial susceptibility testing, we aim to provide a nuanced understanding of the resistance patterns exhibited by these strains and draw implications for clinical practice. Our findings underscore the need for continuous surveillance, judicious use of antibiotics, and the development of novel therapeutic agents to curb the spread of antibiotic-resistant K. pneumoniae and improve patient outcomes.

Methods

Clinical Sample Collection and Classification

The study involved collecting 86 strains *of Klebsiella pneumoniae* (KP) isolated from inpatients with respiratory tract infections at the First Affiliated Hospital of Guangxi Medical University between January 2019 and January 2021. All strains underwent identification and drug sensitivity testing using the VITEK-2 Compact system. The strains were initially classified into two groups based on the positive string test: hypervirulent *Klebsiella pneumoniae* (hvKP) as the observation group and classic *Klebsiella pneumoniae* (cKP) as the control group. Clinical data of patients infected with hvKP were analyzed to identify the risk factors and clinical characteristics associated with hvKP infections.

Identification of Klebsiella Pneumoniae Strains

In this study, the clinical isolates were subjected to meticulous microbiological procedures to ensure precise identification of Klebsiella pneumoniae strains. Following overnight incubation at 37°C, single colonies were isolated and identified using an automated rapid microbial mass spectrometry system. This state-of-The-art technology guarantees a high level of accuracy, essential for the reliability of subsequent analyses.[16]

Preservation and Revival of Bacterial Strains

To maintain the viability and integrity of the Klebsiella pneumoniae strains, a systematic preservation protocol was employed. Single colonies were cultured on MH agar/broth plates and in MH broth, followed by storage in a −80°C ultra-low temperature freezer with a specific preservation fluid. The revival process was meticulously carried out, involving streaking the bacteria onto blood agar plates and incubating them under optimal conditions, ensuring the strains remained true to their original form for the experimental analyses.

Antimicrobial Susceptibility Testing

The VITEK-2 Compact system from bioMérieux, France, was utilized for the antimicrobial susceptibility testing, encompassing a comprehensive range of antibiotics. The interpretation of results was strictly based on the 2019 Clinical and Laboratory Standards Institute (CLSI) guidelines, ensuring the data's relevance and accuracy.[17] This thorough approach is crucial for understanding the resistance patterns of the Klebsiella pneumoniae strains and guiding effective clinical treatment.

Mucoviscosity String Test and Clinical Data Collection

The mucoviscosity string test was conducted to differentiate hvKP from cKP strains, following established protocols and criteria.[18] Simultaneously, a comprehensive collection of clinical data from patients was undertaken, ensuring the inclusion of a wide range of relevant parameters. This data collection process was exhaustive, with stringent inclusion and exclusion criteria, ensuring the study's validity and reliability.

Crystal Violet Assay for the Evaluation of KP Biofilm Formation Ability

In-Vitro Biofilm Model Establishment: *Klebsiella pneumoniae* (KP) strains were revived from −80°C deep freeze storage and streaked onto MH agar plates using a sterile inoculating loop. After overnight incubation at 37°C, a single colony was transferred into 5mL of MH broth and incubated at 37°C with shaking (220 rpm) for 18 hours to form a bacterial suspension. The concentration of the bacterial suspension was adjusted to OD600=0.01 using MH broth. 200μL of the adjusted suspension was added to each well of a 96-well plate, with three replicates per strain. Sterile MH broth served as the negative control. The 96-well plate was then incubated at 37°C for 24 hours.

Crystal Violet Staining for Biofilm Detection

After incubation, the supernatant in the 96-well plate was discarded, and the wells were washed three times with sterile distilled water to remove planktonic bacteria. Following drying at room temperature, each well was stained with 0.1% crystal violet for 10 minutes. Excess stain was removed by washing gently with sterile distilled water. After air drying, 200μL of 95% ethanol was added to each well to dissolve the stain, and the plate was left for 10 minutes. The optical density (OD) at 550nm was measured three times using a microplate reader. According to the standards in the referenced literature, a well was considered positive for biofilm formation if the OD value was greater than or equal to the sum of the negative control's OD value plus three times the standard deviation of the negative control. All experiments were repeated independently three times.

Scanning Electron Microscopy (SEM) Observation of KP Biofilm Formation

The preparation of biofilm samples and SEM observation followed the protocols outlined in the referenced literature,[19] with slight modifications.

2.4.1 Biofilm Preparation: KP strains were revived and cultured as described in section 2.3.1. After adjusting the bacterial suspension to OD600=0.1 using MH broth, 2mL of the suspension was added to each well of a 24-well plate, along with a piece of polyvinyl chloride (PVC) carrier. Three replicates were prepared for each strain. The 24-well plate was incubated at 37°C for 24 and 72 hours. The PVC carriers with biofilm were washed three times with sterilized PBS (PH=7.4) to remove planktonic bacteria. The biofilm was then fixed with 2.5% glutaraldehyde for 2 hours at 4°C. After washing three times with sterilized PBS (PH=7.4), the samples were dehydrated in a series of ethanol solutions of increasing concentration (50%, 70%, 80%, 90%, and 100%, with three changes at 100% and one change at each of the other concentrations) for 10 minutes each. The samples were then coated with gold in a vacuum and observed under a scanning electron microscope.

Statistical Analysis

The statistical analyses in this study were conducted using the SPSS 22.0 software package. Quantitative data conforming to a normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$)and comparisons between two groups were performed using the *t*-test. Non-normally distributed data were represented by median and interquartile range [M (P25-P75)], and the Mann–Whitney *U*-test was applied for intergroup analysis. Categorical data were described using frequencies and percentages, and comparisons were made using the chi-square test or Fisher's exact test, as appropriate. All tests were two-tailed, and a P-value of less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics

The study analyzed 86 cases of *Klebsiella pneumoniae* (KP) infections, comprising 42 cases of hypervirulent *Klebsiella pneumoniae* (hvKP) and 44 cases of classic *Klebsiella pneumoniae* (cKP). The gender distribution across the groups showed no significant difference, with 68 males and 18 females in the total KP group, 35 males and 7 females in the hvKP group, and 33 males and 11 females in the cKP group (P=0.342). The age distribution was also similar across the groups, with a median age of 54.65 years in the KP group, 54.38 years in the hvKP group, and 54.91 years in the cKP group (P=0.707). In terms of clinical presentation, a significant difference was observed in the incidence of community-acquired

pneumonia, with 35 cases in the KP group, of which 12 were hvKP and 23 were cKP (P=0.025). However, hospital-acquired pneumonia was more prevalent in the hvKP group, with 30 cases compared to 21 in the cKP group, indicating a higher hospital-associated infection rate in patients with hvKP (Table 1).

Underlying Diseases and Risk Factors in Klebsiella Pneumoniae Infections

In a comparative analysis of 86 patients with *Klebsiella pneumoniae* infections, encompassing 42 cases of hypervirulent *Klebsiella pneumoniae* (hvKP) and 44 cases of classic *Klebsiella pneumoniae* (cKP), the study revealed distinct differences in the prevalence of certain comorbidities (Table 2). Notably, diabetes mellitus, malignant tumors, post-surgical complications within one week, and hypoproteinemia were more prevalent in the hvKP group. Specifically, diabetes mellitus was found in 18 cases of hvKP compared to 9 in cKP, malignant tumors in 12 hvKP patients versus 5 in cKP, post-surgery complications within one week in 17 hvKP cases against 9 in cKP, and hypoproteinemia in 11 hvKP patients compared to 4 in cKP, all showing statistically significant differences (P<0.05). These findings highlight the increased association of certain underlying diseases and postoperative complications with hypervirulent Klebsiella pneumoniae infections, suggesting a trend towards more severe health outcomes in the hvKP patient group.

Inflammatory Indices in Respiratory System Infection Patients with hvKP and cKP

In the analysis of 86 patients clinically diagnosed with *Klebsiella pneumoniae* (KP) infections, comprising 42 hvKP and 44 cKP cases, the study evaluated various inflammatory indices. The assessment revealed that there were no

Table 1 Baseline Characteristics of 86 KP-Infected Patients with Respiratory System Infection

Parameter	KP (n=86)	hvKP (n=42)	cKP (n=44)	P
Gender				0.342
Male	68	35	33	
Female	18	7	11	
Age (years)	54.65 (46.75–65.25)	54.38 (45.75–63.50)	54.91 (48.50–66.00)	0.707
Community-Acquired Pneumonia	35	12	23	0.025
Hospital-Acquired Pneumonia	51	30	21	

Abbreviation: KP, Klebsiella pneumoniae.

Table 2 Analysis of Underlying Diseases and Risk Factors in Patients with hvKP and cKP in Respiratory

Parameter	KP (n=86)	hvKP (n=42)	cKP (n=44)	P
Diabetes Mellitus	27	18	9	0.025
Malignant Tumor	17	12	5	0.045
Post-Surgery within 1 Week	26	17	9	0.043
Hypoproteinemia	15	11	4	0.037
Hypertension	31	13	18	0.336
Cerebrovascular Disease	25	12	13	0.921
Cardiovascular Disease	23	15	8	0.066
Connective Tissue Disease	3	1	2	——
MODS	6	4	2	0.629
Bronchiectasis	4	2	2	——
COPD	3	3	0	0.112
Anemia	12	8	4	0.183
Long-term Use of Immunosuppressants or Steroids	10	3	7	0.352
Liver Dysfunction	10	7	3	0.277
Renal Dysfunction	11	5	6	0.81

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; cKP, Classical Klebsiella pneumoniae.

significant statistical differences in body temperature, respiration rate, heart rate, white blood cell count (WBC), neutrophil percentage (N%), and procalcitonin levels between patients with hvKP and cKP respiratory system infections. The average body temperature was similar between the hvKP (37.37±0.97°C) and cKP (37.20±0.86°C) groups, as were the respiration rates (21.55±5.70 for hvKP vs 20.68±2.12 for cKP), heart rates (91.76±20.13 for hvKP vs 90.18±17.70 for cKP), WBC counts (10.58±6.19 for hvKP vs 10.67±6.96 for cKP), neutrophil percentages (74.60 ±14.11% for hvKP vs 75.54±13.42% for cKP), and procalcitonin levels (3.26±6.14 for hvKP vs 2.98±6.14 for cKP). These findings suggest that the inflammatory response in hvKP and cKP infections presents similarly in respiratory system infections (Table 3).

Prognosis Analysis in Respiratory System Infections

In the study of 86 patients with *Klebsiella pneumoniae* (KP) infections, it was observed that patients with hypervirulent *Klebsiella pneumoniae* (hvKP) experienced significantly longer hospital stays, averaging 30.80±21.89 days, compared to classic *Klebsiella pneumoniae* (cKP) patients, who averaged 19.60±13.03 days ($P<0.05$). The overall mortality rate among these patients was 5.81% (5/86), with a slightly higher rate in the hvKP group (7.14%, 3/42) than in the cKP group (4.55%, 2/44). Furthermore, the incidence of septic shock, severe pneumonia, ICU admission, mechanical ventilation, tracheal intubation or tracheostomy, and fungal coinfections was significantly higher in the hvKP group compared to the cKP group, indicating a more severe clinical course in hvKP infections (Table 4).

Table 3 The Levels of Inflammatory Indexes in Patients with hvKP and cKP in Respiratory System Infection

Parameter	KP (n=86)	hvKP (n=42)	cKP (n=44)	P
Temperature (°C)	37.29±0.91	37.37±0.97	37.20±0.86	0.394
Respiration Rate	21.10±4.26	21.55±5.70	20.68±2.12	0.349
Heart Rate	90.95±18.83	91.76±20.13	90.18±17.70	0.701
WBC (10^9)	10.62±6.55	10.58±6.19	10.67±6.96	0.952
Neutrophil Percentage (N%)	75.08±13.69	74.60±14.11	75.54±13.42	0.754
Procalcitonin	3.12±5.98	3.26±6.14	2.98±6.14	0.824

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; cKP, Classical Klebsiella pneumoniae.

Table 4 Analysis of the Prognosis of Patients with Respiratory System Infection hvKP and cKP

Parameter	KP (n=86)	hvKP (n=42)	cKP (n=44)	P
Hospital Stay (days)	25.33±18.86	30.80±21.89	19.60±13.03	0.005
Improved	81	39	42	0.957
Death	5	3	2	
Septic Shock	14	11	3	0.032
Severe Pneumonia	30	19	11	0.049
Admission to ICU	37	23	14	0.032
Mechanical Ventilation	30	19	11	0.049
Tracheal Intubation or Tracheostomy	30	19	11	0.049
Central Venous Catheter	45	21	24	0.673
Urinary Catheter	48	21	27	0.289
Gastric Tube	46	18	28	0.053
Drainage Tube	33	15	18	0.62
Fungal Coinfection	33	21	12	0.03

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; cKP, Classical Klebsiella pneumoniae.

Fungal and Other Bacterial Infections

Analysis of fungal infections revealed that 21 out of 42 hvKP patients and 12 out of 44 cKP patients had fungal coinfections, with a significantly higher prevalence in the hvKP group. Additionally, among patients with hvKP, 21 had solo KP infections, while 13 had only fungal coinfections. This contrasts with the cKP group, where 32 had solo KP infections and 5 had only fungal coinfections. These findings suggest a higher complexity in the infection patterns in the hvKP group compared to the cKP group (Table 5).

Antibiotic Resistance Analysis in hvKP and cKP

We conducted a comprehensive analysis to compare the drug resistance profiles between hvKP and cKP strains isolated from respiratory system infections. The analysis, presented in Table 6, highlights significant differences in resistance rates to various antibiotics. Notably, hvKP strains showed lower resistance percentages compared to cKP across most antibiotics tested. For instance, resistance to Ceftriaxone was observed in 14.29% of hvKP isolates compared to 38.64% in cKP, indicating a statistically significant difference (P=0.011). Similarly, notable differences were found in resistance to Cefuroxime, Ceftazidime, and Gentamicin, with P-values of 0.003, <0.001, and 0.005, respectively.

Table 5 Fungal Infections of hvKP and cKP

Parameter	hvKP (n=42)	cKP (n=44)	X2	P
Solo KP Infection	21	32	4.694	0.03
Fungal Coinfection	21	12		

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; cKP, Classical Klebsiella pneumoniae.

Table 6 Analysis of the Difference of Drug Resistance Between hvKP and cKP in Respiratory System Infection

Antibiotic	hvKP (n=42) R (%)	cKP (n=44) R (%)	P
Amoxicillin/Clavulanic Acid	5 (11.90)	13 (29.55)	0.044
Cefotaxime	5 (11.90)	13 (29.55)	0.044
Ceftriaxone	6 (14.29)	17 (38.64)	0.011
Cefuroxime	6 (14.29)	19 (43.18)	0.003
Ceftazidime	6 (14.29)	23 (52.27)	<0.001
Gentamicin	3 (7.14)	15 (34.09)	0.005
Levofloxacin	5 (11.90)	13 (29.55)	0.044
Piperacillin	14 (33.33)	25 (56.82)	0.029
Co-trimoxazole	6 (14.29)	18 (40.91)	0.006
Amikacin	2 (4.76)	2 (4.55)	——
Amikacin	5 (11.90)	12 (27.27)	0.074
Ciprofloxacin	7 (16.67)	15 (34.09)	0.064
Cefoperazone/Sulbactam	4 (9.52)	10 (22.73)	0.172
Ertapenem	2 (4.76)	3 (6.82)	——
Cefepime	4 (9.52)	8 (18.18)	0.397
Cefoxitin	4 (9.52)	9 (20.45)	0.266
Imipenem	1 (2.38)	3 (6.82)	0.642
Meropenem	1 (2.38)	3 (6.82)	0.642
Tigecycline	2 (4.76)	3 (6.82)	——
Tobramycin	3 (7.14)	6 (13.64)	0.528
Piperacillin/Tazobactam	2 (4.76)	5 (11.36)	0.469
ESBL-producing Strains	5 (11.90)	17 (38.64)	0.005

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; cKP, Classical Klebsiella pneumoniae.

Biofilm Formation and Virulence Genes Distribution in hvKP and cKP

Our comprehensive analysis highlighted the distinct biofilm formation capabilities and virulence gene distribution between hvKP and cKP strains. Notably, biofilm formation was observed in 69.77% of the 86 clinically isolated KP strains, with hvKP demonstrating significantly higher biofilm formation rates (85.71%) compared to cKP (54.54%). SEM results underscored that hvKP strains not only formed denser and more cohesive biofilms but also exhibited a more complex extracellular matrix, suggesting enhanced survival and antibiotic resistance mechanisms. These observations were corroborated by the dominance of capsule serotype K2 and the prevalence of virulence genes such as rmpA, iutA, and PEG-344 in hvKP strains, pointing to a robust virulence factor expression in these strains. Specifically, the rmpA gene was notably more expressed in hvKP strains under biofilm-forming conditions, highlighting a potential link between biofilm formation capabilities, virulence gene expression, and the observed increase in antibiotic tolerance (Figure 1).

Discussion

This study presents a nuanced exploration of the clinical and microbiological disparities between hvKP and cKP strains in respiratory infections, offering valuable insights into their pathogenesis, resistance patterns, and implications for patient outcomes. Our findings underscore the heightened virulence of hvKP, as evidenced by longer hospitalization durations, increased severity of infections, and higher mortality rates compared to cKP. This aligns with emerging literature, highlighting hvKP's role in exacerbating respiratory infections, a trend that is increasingly reported in clinical settings globally.[20]

A pivotal aspect of our study was the examination of biofilm formation capabilities of these strains. Biofilms are known to play a critical role in the persistence and resistance of bacterial infections.[21] Our observations using Scanning Electron Microscopy (SEM) revealed distinct biofilm architectures between hvKP and cKP strains at different growth phases. The denser and more cohesive biofilm structure of hvKP, especially noted in the 72-hour cultures, can be correlated with the increased antibiotic tolerance and virulence seen in these strains. These findings contribute to the growing body of evidence

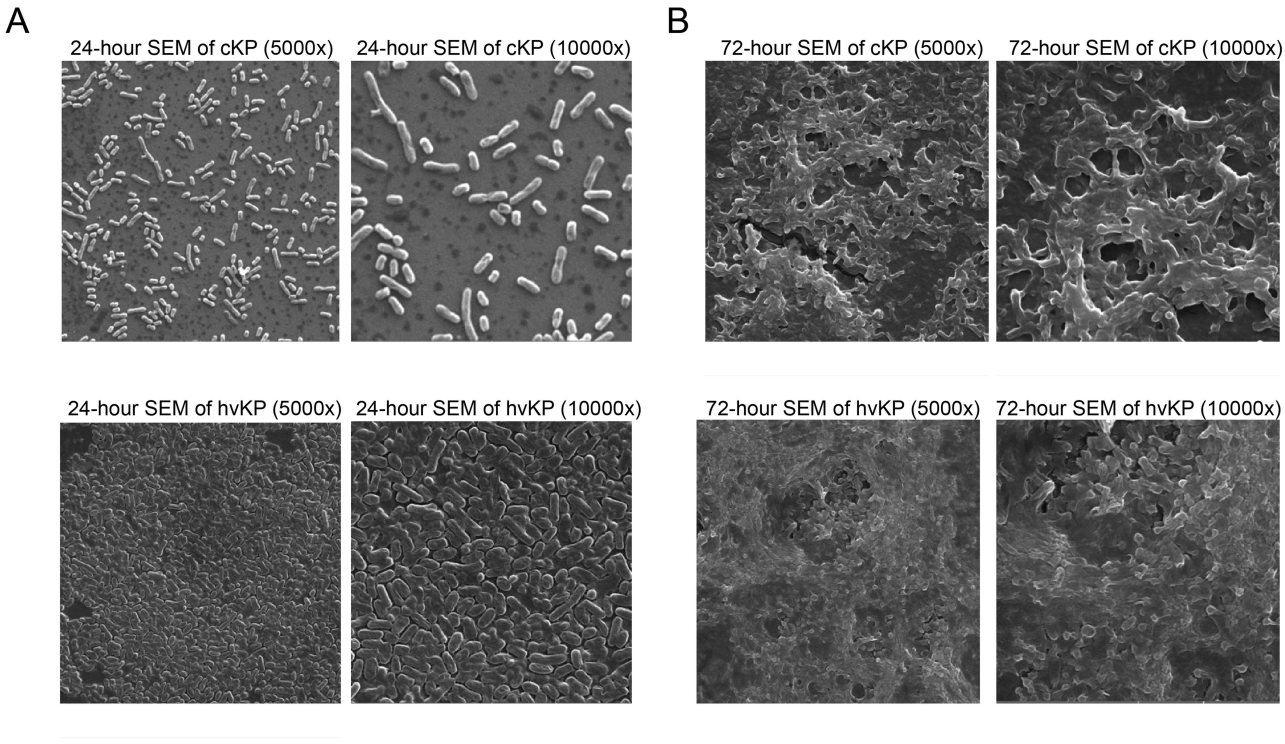

Figure 1 Scanning Electron Microscopy (SEM) Analysis of Biofilm Formation in hvKP and cKP Groups. (**A**) 24-Hour Biofilm Formation: This panel shows the SEM images illustrating the biofilm formation in both hypervirulent Klebsiella pneumoniae (hvKP) and classic Klebsiella pneumoniae (cKP) groups after 24 hours. The images provide a detailed view of the initial stages of biofilm development, highlighting the structural differences in the biofilm matrix between the hvKP and cKP strains. (**B**) 72-Hour Biofilm Formation: This panel depicts the SEM images at 72 hours, offering a comparative insight into the progression of biofilm formation in the hvKP and cKP groups. The images reveal more mature biofilm structures, showcasing the density, complexity, and architectural differences in the biofilms formed by the two groups over an extended period.

suggesting that biofilm formation is a key virulence factor in hvKP strains, a feature that complicates treatment strategies and underscores the need for targeted therapeutic approaches.[22] In the realm of antibiotic resistance, our study adds to the intricate narrative of the evolving resistance patterns in K. pneumoniae. While hvKP and cKP showed similar resistance patterns to a range of antibiotics, such as Amikacin and Ciprofloxacin, significant differences were noted in their susceptibility to other antibiotics including Amoxicillin/Clavulanic Acid, Ceftazidime, and Piperacillin. Interestingly, hvKP strains showed lower resistance rates to these antibiotics compared to cKP strains. This observation suggests that the mechanisms underlying antibiotic resistance may differ between hvKP and cKP. Rather than indicating a straightforward measure of resistance, the lower resistance rates in hvKP strains could reflect unique genetic or phenotypic traits that influence how these strains acquire or express resistance to antibiotics. This distinction underlines the importance of designing antibiotic therapies that are specifically tailored to the strain of *K. pneumoniae* involved in the infection, acknowledging the complex landscape of antibiotic resistance that varies not only across bacterial species but also within strains of the same species. The clinical implications of our findings are significant. The increased virulence and complex resistance patterns of hvKP necessitate a more aggressive and tailored approach to treatment.[6] Early identification and differentiation of hvKP from cKP in clinical specimens should be prioritized to facilitate appropriate therapeutic interventions. Moreover, our results highlight the urgent need for novel therapeutic agents and strategies, particularly those targeting biofilm formation and antibiotic resistance mechanisms in hvKP.

In conclusion, our study elucidates distinct characteristics of hvKP and cKP strains in respiratory infections, emphasizing the critical role of biofilm formation in hvKP's heightened virulence and antibiotic tolerance. We have uncovered significant clinical and microbiological disparities, marking hvKP's association with more severe infections and outcomes. The differential antibiotic resistance patterns observed necessitate a nuanced approach to treatment, highlighting the importance of early strain identification for effective therapy. Our findings advocate for intensified research into targeted treatments, especially against biofilm-associated virulence and resistance mechanisms. Ultimately, addressing these challenges is vital for improving patient outcomes and combating the public health threat posed by these adaptable pathogens.

Data Sharing Statement

The original data supporting the conclusions of this article will be made available by the Dr.Zhongwei Wen, without undue reservation.

Ethics Statement

This research was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (as revised in 2013) concerning human rights. Ethics Committee Approval: The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Approval Number: 2022-E404-05). Informed Consent: Informed consent was obtained from all individual participants involved in the study. Participants were informed about the purpose of the research, the procedures to be undertaken, potential risks and benefits, and the confidentiality of their data. They were also informed of their right to withdraw from the study at any time without penalty. Data Handling and Confidentiality: All data collected during this study are stored securely and are accessible only to the research team. Personal identifiers have been removed to ensure the confidentiality and privacy of participants.

Disclosure

The authors declare no conflict of interest.

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