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Original Article

Molecular Profiling of Antibiotic Resistance and Virulence in Multidrug-Resistant Uropathogenic *Klebsiella pneumoniae*

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Abstract

Background: Urinary tract infections (UTIs) constitute a serious public health concern, with *Klebsiella pneumoniae* being a prevalent cause worldwide. This bacterium is recognized for elevated recurrence rates and significant antibiotic resistance. The complex virulence mechanisms of *K. pneumoniae* contribute to its persistence within the host. Nevertheless, it is still unknown how particular virulence genes and antibiotic resistance relate to uropathogenic *K. pneumoniae*. This study examined the relationship between pathways of virulence and antibiotic resistance in the isolates of uropathogenic *K. pneumoniae*.

Methods: Overall, 207 uropathogenic *K. pneumoniae* isolates were analyzed for antibiotic susceptibility. Then, polymerase chain reaction (PCR) was used to identify resistance (*blaSHV*, *blaTEM*, *blaNDM*, *blaCTX-M*, *blaKPC*, and *blaOXA*) and virulence (*Ompk35*, *Ompk36*, *Mdtk*, *AcrAB*, *TolC*, *mrkD*, *allS*, *ybtS*, *entB*, *kfu*, and *iutA*) genes. Eventually, enterobacterial repetitive intergenic consensus (ERIC)-PCR was utilized to assess the genetic diversity of the strains.

Results: All multidrug-resistant strains (100%) carried the *Mdtk* and *AcrAB* efflux pump genes. Virulence genes (e.g., *mrkD* and *entB*) were common across the isolates. Several genes were shown to be prevalent in β-lactam resistance, including *blaSHV* (95.8%), *blaCTX-M* (70.8%), *blaNDM* (62.5%), *blaTEM* (41.7%), and *blaOXA* (33%). Finally, ERIC-PCR produced distinct banding profiles between 50 and 1500 base pairs, with each isolate demonstrating between one and eight bands.

Conclusion: Our findings emphasize the connection between *K. pneumoniae* virulence genotypes and antibiotic resistance. Understanding these associations is crucial for managing and preventing *K. pneumoniae*-related UTIs effectively.

Keywords: Antimicrobial resistance, ERIC-PCR, *K. pneumoniae*, Uropathogenic, Virulence genes

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Introduction

Recently, many centres around the world have undergone outbreaks of multidrug-resistant (MDR) *Enterobacteriaceae* infections, along with *Klebsiella pneumoniae*, highlighting the urgent need for effective control and treatment strategies on a global scale. The most important pathogen among the genus is *K. pneumoniae*, which causes a variety of diseases in hospitals, assisted living homes, and communities worldwide. These infections can affect the lungs, urinary tract, abdominal cavity, surgical sites, and soft tissues and can even lead to bacteremia (1). Despite being the second most frequent cause of urinary tract infections (UTIs) after *Escherichia coli*, *K. pneumoniae* is more harmful than a related species (2). *K. pneumoniae* is a major opportunistic pathogen involved in a wide spectrum of infections, including those affecting the respiratory tract, urinary system, bloodstream, and soft tissues, particularly in healthcare environments (3). Approximately 12% of UTIs are attributed to *K. pneumoniae*, with this number rising rapidly worldwide, particularly in Asia. The spread of bacteria that produce extended-spectrum beta-lactamases (ESBLs) and are resistant to antibiotics is mostly due to this increase (4). Drug-resistant *K. pneumoniae* is becoming more common, which has increased its therapeutic effect and made treatment more difficult. Most uropathogenic *K. pneumoniae* isolates have developed resistance mechanisms against various antimicrobial

agents, particularly β-lactams, cephalosporins, and polymyxins. Antimicrobial-resistant uropathogenic *K. pneumoniae* surveillance is essential to enhance infection control measures, particularly in the healthcare sector, and to aid clinicians in the selection of empirical therapy. According to previous research, virulence gene profiling and genotyping may provide some insights into *K. pneumoniae* infections (5). Siderophores, fimbriae, outer membrane porins, and a protective capsule are important components of the pathogenicity of this pathogen. These features support the growth of drug resistance, the attachment of bacteria to uromucosal surfaces, the initiation of inflammatory reactions, and the spread of disease throughout the urogenital tract (6,7). The bacteria are protected from phagocytosis by the bacterial capsule, which is made up of strain-specific capsular polysaccharides called K antigens. Notably, K1 and K2 are associated with serious human infections out of the 77 capsular serotypes that have been discovered (8). Genes linked to virulence, such as those governing siderophore production (*entB* and *ybtS*), adhesins (*mrkD* and *fimH*), iron uptake (*kfu* and *iutA*), and allantoin metabolism (*allS*), play an essential role in enabling *K. pneumoniae* to adhere to and invade host tissues. The harmful effects of *K. pneumoniae* infections, especially those contracted in hospital settings, are significantly influenced by these genes (9,10). Six virulence genes (*mrkD*, *ycfM*, *fimH*, *ybtS*, *entB*, and *kfu*) and three β-lactamase-encoding genes (*blaKPC*, *blaCTX-M-Gp1*, and *blaOXA-1-like*) were observed in *K. pneumoniae* in a prior study (11). ESBLs result from the production of enzymes encoded by the *blaSHV*, *blaTEM*, and *blaCTX-M* genes, with nearly 300 variations described (12). Additionally, *AcrAB-TolC* efflux pumps (*mdtk*, *AcrAB*, and *TolC*) and outer membrane porins (*Ompk35* and *Ompk36*) are crucial for antimicrobial resistance in *K. pneumoniae*. The presence of these virulence factor genes influences the pathogenicity and genetic diversity of uropathogenic isolates. Prior research has highlighted the prevalence of various genotypes with distinct virulence profiles in MDR uropathogenic *K. pneumoniae* strains (13). Detailed knowledge of genotype, antibiotic resistance, and virulence pattern of uropathogenic *K. pneumoniae* is necessary for reducing the risk of outbreaks. Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), the amplification of repetitive sequences in the bacterial genome, is a cheaper and faster genotyping method for determining clonal diversity among uropathogenic *K. pneumoniae* isolates. Differences in the positions and the number of repetitive sequences will result in a different banding pattern by gel electrophoresis (14). Therefore, the current study aims to examine the connection between uropathogenic MDR *K. pneumoniae*'s genetic diversity and virulence genes.

Materials and Methods

Sample Collection and Study Design

This prospective analytical study was conducted at the Department of Microbiology, Dr. ALM Postgraduate Institute of Basic Medical Sciences, University of Madras, from September 2017 to January 2021. A total of 207 laboratory-archived uropathogenic *K. pneumoniae* isolates were collected from patients with severe UTIs and kept at -20 °C. Standard biochemical analysis was used to identify and resuscitate the isolates.

Antibiotic Susceptibility Test

The antibiotic susceptibility of *K. pneumoniae* isolates was evaluated using the Kirby-Bauer disc diffusion method with commercially available antibiotic discs (Himedia, Mumbai, India). Initially, ESBL production was screened using the discs of ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). Confirmation was performed using the combined disc method with ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg), considering a ≥ 5 mm zone difference as indicative of ESBL production. In addition, metallo-beta-lactamase (MBL) production was detected in imipenem-resistant strains using the combined disc test with imipenem (10 µg) discs, one supplemented with 10 µL of 0.5 M ethylenediaminetetraacetic acid (pH: 8.0), and a ≥ 7 mm zone difference indicating MBL production. This procedure was similarly applied to meropenem. Moreover, *AmpC* beta-lactamase production was detected using cefoxitin (30 µg) discs. Fluoroquinolone resistance was confirmed using nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), and levofloxacin (5 µg) discs, while aminoglycoside resistance was tested with gentamicin (10 µg) and amikacin (30 µg) discs. Finally, zone diameters were determined and reported in accordance with the Clinical and Laboratory Standards Institute's (CLSI's) recommendations.

Colistin Resistance Screening

For the initial screening of colistin resistance, *K. pneumoniae* isolates were inoculated in 0.9% sodium chloride and adjusted to 0.5 McFarland standards. Then, eosin methylene blue agar plates containing 3.5 µg/mL colistin were streaked with a 10 µL inoculum. Next, the plates were incubated in an aerobic environment for 24–48 hours at 37 °C. Colistin susceptibility was then determined by the absence of growth or limited growth in the primary streak (susceptible) versus growth in all three streaks (resistant), following CLSI–the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Suitable control strains were present. Subsequently, colistin-resistant isolates were subjected to further testing in accordance with CLSI standards (2018) using the broth microdilution method. Minimum inhibitory concentration values were interpreted based on CLSI-EUCAST criteria: ≤ 2 µg/mL as susceptible and > 2 µg/mL as resistant.

Deoxyribonucleic Acid Extraction

Using the boiling lysis method, DNA was extracted based

on our previously published research (15). A Nanodrop spectrophotometer (Nanovue Plus; GE Healthcare Life Sciences, United States) was used to measure the amount and quality of the extracted DNA and then stored at -20°C for further use.

Detection of Virulent and Multidrug Resistance Genes

The PCR was utilized to identify capsular serotypes (K1 and K2) and virulence genes (*ybtS*, *mrkD*, *rmpA*, *entB*, *iutA*, *kfu*, and *allS*) (10), cephalosporin resistance genes (*blaTEM*, *blaSHV*, and *blaCTX-M*), carbapenem resistance genes (*blaNDM*, *blaKPC*, and *blaOXA*) (16), transport system genes (*Ompk35*, *Ompk36*, *TolC*, *mdtk*, and *AcrAB*), and *mcr* genes (*mcr*1-8) (17). The specific primers employed in this study are presented in Supplementary file 1, and Table 1 provides the PCR programs for different genes. Amplicons were confirmed using 1.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized with a Carestream Gel Logic 212 Pro gel documentation system (USA).

Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction

The diversity of *K. pneumoniae* isolates was assessed using ERIC-PCR with universal primers: forward (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and reverse (5′-AAGTAAGTGACTGGGGTGAGCG-3′). The PCR mixture comprised 10×PCR buffer, 10 pmol of each primer, 200 µM of deoxyribonucleotide triphosphate, 0.5 units of Taq polymerase, template DNA, and deionized water. Additionally, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 47°C for 1 minute, and extension at 72 °C for 4 minutes were part of the PCR process, which began with an initial denaturation at 95 °C for 2.5 minutes. The final extension was conducted at 72 °C for 4 minutes. The resulting amplicons were separated on a 1.8% (wt/vol) agarose gel at 100 V for 35 minutes. Ultimately, the genetic relatedness of the strains was assessed by creating a dendrogram from the ERIC-PCR patterns using GelJ software, version 2.0.

Statistical Analysis

Descriptive statistics were determined using Microsoft Excel. Statistical significance was measured using chi-square analysis which was performed to assess associations between ERIC-PCR clusters, antibiotic resistance profiles, and virulence genes ($P<0.05$).

Results

Antibiotic Susceptibility Testing

Phenotypic screening demonstrated that 78.74% (163/207) of the isolates were suspected ESBL producers, with 75.36% (156/207) confirmed positive. Moreover, most isolates were resistant to multiple antibiotics. The combined disc test identified 54.58% (113/207) as MBL producers (Figure 1a), with 61.94% (70/113) resistant to imipenem and 95.57% (108/113) resistant to meropenem. Overall, 95.65% (198/207) of the isolates were resistant to penicillin and ampicillin, while 77.77% (161/207) showed resistance to fluoroquinolones. In addition, sensitivity to chloramphenicol and fosfomycin was observed in 53.62% (111/207) and 54.0% (111/207) of the isolates, respectively (Figure 1b). Among the 207 uropathogenic *Klebsiella* isolates, 6.8% (14/207) were extensively drug-resistant (XDR), 68.1% (141/207) were MDR, 20.8% (43/207) were resistant to one or two classes, and 4.3% (9/207) were fully susceptible. Most XDR and MDR isolates were identified as *K. pneumoniae* subsp. *pneumoniae* (Figure 1c).

Colistin Resistance

Initial screening using eosin methylene blue agar detected colistin resistance in 6.8% (14/207) of the isolates, all of which were *K. pneumoniae* subsp. *pneumoniae*. The minimum inhibitory concentration assay confirmed complete colistin resistance in all 14 isolates (CLSI-EUCAST). All 14 colistin-resistant isolates corresponded to the XDR category, consistent with their classification in the overall resistance analysis. None of the isolates belonged to the MDR-only group.

Capsular K1/K2 Serotype Detection

In a broader analysis of 207 isolates, molecular detection revealed that 4.3% (9/207) were positive for the K1 serotype, and 1.4% (3/207) were positive for the K2 serotype. Most K1 and K2 serotype isolates were *K. pneumoniae* subsp. *pneumoniae* (91.6%, 11/12). Among

Table 1. Multiplex PCR Protocols and Conditions Used for Detecting Capsular Serotypes, Virulence Factors, Antibiotic Resistance Genes, and Transport System Genes

Target Genes	PCR Mix (µL)	Conditions
Capsular *K1* and *K2*	10: 5 PCR master mix, 2 DNA templates, 1 milliQ water, 0.5 of each primer	95 °C for 15 minutes; 30 cycles of 94 °C for 30 seconds, 60 °C for 90 seconds, and 72 °C for 60 seconds; final extension at 72°C for 10 minutes
Virulence genes (*mrkD*, *rmpA*, *ybtS*, *entB*, *iutA*, *kfu*, and *allS*)	25: 10 PCR master mix, 2 DNA templates, 6 milliQ water, 0.5 of each primer	95 °C for 15 minutes; 30 cycles of 94 °C for 30 seconds, 60 °C for 90 seconds, and 72 °C for 60 seconds; final extension at 72 °C for 10 minutes
Cephalosporin resistance (*blaTEM*, *blaSHV*, and *blaCTX-M*)	10: 5 PCR master mix, 2 DNA templates, 2 milliQ water, 0.5 of each primer	94 °C for 5 minutes; 37 cycles of 94 °C for 30 seconds, annealing for 30 seconds, 72 °C for 50 seconds; final extension at 72 °C for 5 minutes
Carbapenem resistance (*blaNDM*, *blaKPC*, and *blaOXA*)	10: 5 PCR master mix, 2 DNA templates, 2 milliQ water, 0.5 of each primer	94 °C for 4 minutes; 36 cycles of 94 °C for 30 seconds, annealing for 40 seconds, and 72 °C for 50 seconds; final extension at 72 °C for 5 minutes
Transport system genes (*Ompk35*, *Ompk36*, *TolC*, *mdtk*, and *AcrAB*)	10: 5 PCR master mix, 2 DNA templates, 2 milliQ water, 0.5 of each primer	95 °C for 15 minutes; 30 cycles of 94 °C for 30 seconds, specific annealing for 90 seconds, 72°C for 60 seconds; final extension at 72 °C for 10 minutes
Colistin resistance genes (*mcr* 1-8)	15: 5 PCR master mix, 2 DNA templates, 2 milliQ water, 0.5 of each primer	94 °C for 15 minutes; 25 cycles of 94 °C for 30 seconds, annealing for 90 seconds, and 72 °C for 60 seconds; final extension at 72 °C for 10 minutes

Note. PCR: Polymerase chain reaction.

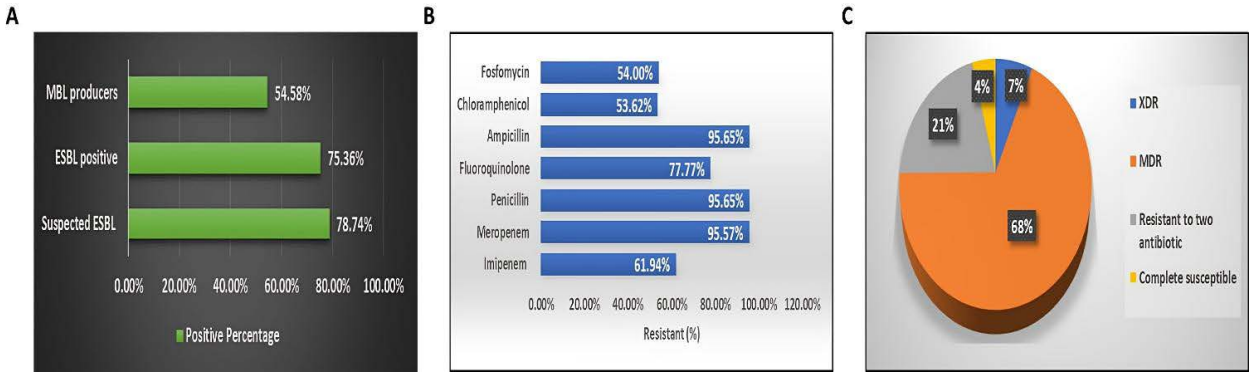

Figure 1. (A) Identification of ESBL-Producing and MBL-Producing *Klebsiella pneumoniae* Isolates, **(B)** Percentage Distribution of Drug-Resistance Profiles Among the *K. pneumoniae* Isolates, and **(C)** Comparative Analysis of Multidrug-Resistant Isolates, Extensively Drug-Resistant Isolates, Isolates Resistant to Two Antibiotic Classes, and Susceptibility Rates of *K. pneumoniae*. *Note*. ESBL: Extended-spectrum beta-lactamase; MBL: Metallo-beta-lactamase

them, 66.7% (8/12) were MDR, and 83.3% (10/12) were strong biofilm producers (Figure 2).

Virulence Gene Detection

The detection of virulence genes in 207 isolates demonstrated varied profiles. The *mrkD* gene was present in 97.5% of isolates, indicating its widespread role as a fimbrial adhesin. In addition, the *entB* gene, involved in enterobactin synthesis, was found in 93.7% of isolates, highlighting its importance in iron acquisition. Further, the *ybtS* gene was detected in 42.9% of isolates, showing moderate prevalence. Furthermore, the *kfu* and *iutA* genes, both related to iron uptake, were observed in 21.7% of isolates each. Moreover, the *allS* gene, associated with allantoin metabolism, was present in 8.2% of isolates (Figure 3). The comparison of virulence gene distribution between MDR and non-MDR isolates, as well as between XDR and non-XDR isolates, revealed no statistically significant associations for any of the tested genes (all $P > 0.05$), emphasizing that the presence of virulence determinants is independent of MDR or XDR status.

Molecular Detection of Antibiotic Resistance Genes, Outer Membrane Porins, and Efflux Pumps

Genotypic analysis was performed on 24 isolates, comprising all XDR and colistin-resistant *K. pneumoniae* recovered in this study. Based on the results, 41.7% (10/24), 95.8% (23/24), and 70.8% (17/24) were positive for the *blaTEM*, *blaSHV*, and *blaCTX-M* genes, respectively. Additionally, 62.5% (15/24) and 33.3% (8/24) were positive for the *blaNDM* and *blaOXA-48* genes, respectively, but none of them were positive for *blaKPC*. Additionally, none of the isolates carried any *mcr* gene variants. The molecular detection of outer membrane porins (omp) and efflux pump genes showed universal positivity for *Ompk36*. Eventually, the *AcrAB*, *TolC*, and *Ompk35* genes were present in 95.8% of the isolates, and the *mdtk* gene was found in 79.2% (Figure 4).

Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction Analysis

A total of 207 DNA samples were collected for analysis. Of them, 24 samples (12%) had no ERIC pattern, but 183 samples (88%) had ERIC patterns. The online program GelJ (version 2.0) was used to perform the ERIC-PCR analysis. One to eight bands, ranging in size from 50 bp to 1500 bp, were found in each sample by the ERIC-PCR gel analysis (Figure 5). Although 88% of the isolates showed ERIC patterns, clustering analysis revealed no significant association between ERIC profiles and resistance categories. To ascertain the genetic relatedness of the strains, a dendrogram and clustering analysis were created using the ERIC patterns (Figure 6).

Discussion

With an emphasis on XDR strains, the molecular mechanisms behind antibiotic resistance in MDR *K. pneumoniae* strains, as well as their potential for pathogenicity, were examined in this study. Due to the extensive use of antimicrobial drugs, the incidence of MDR *K. pneumoniae* infections has increased throughout the past ten years (18). Considering that there are few effective antibiotic treatment options available, this trend presents a serious health risk. According to the findings, *K. pneumoniae* (98.1%) was the most common species among the uropathogenic *Klebsiella* isolates, which is in line with the results of previous research, indicating that *K. pneumoniae* was the primary perpetrator of UTIs (19,20). In this investigation, 76.8% of the *Klebsiella* isolates produced ESBLs, which is extremely high in comparison to previous investigations. Our findings contradict those of a prior South Indian study, demonstrating that 35.7% of the *Klebsiella* isolates were resistant to carbapenem (21). Colistin resistance was observed in 6.8% of *K. pneumoniae* isolates in our study, which does not match the results of other studies, reporting colistin resistance in 2.1%, 11.1%, 18.5%, and 19% of the *K. pneumoniae* isolates. Nearly 25% of XDR and colistin-resistant *K. pneumoniae* isolates were found to be significant ESBL producers using the E-strip MIC test, which is consistent with previous findings. MBL, AmpC, or KPC were not produced by any of the XDR isolates in our study, which conforms to the findings of other studies (22,23).

In this study, the K1-4.3% serotype was shown to be more

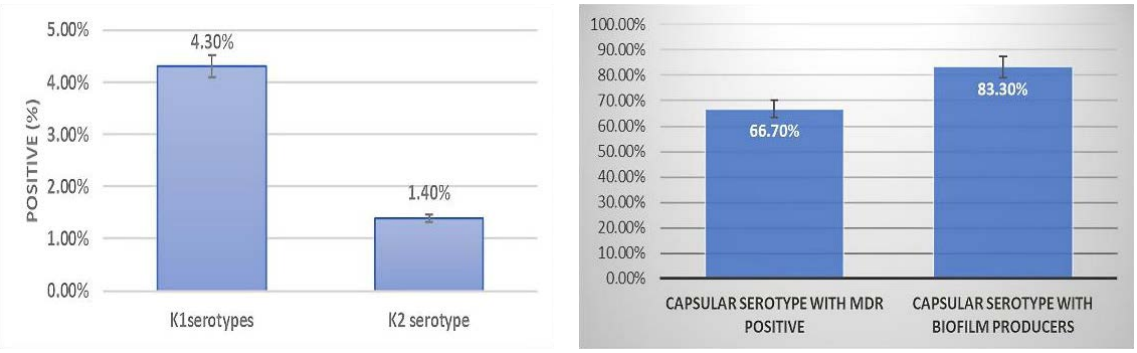

Figure 2. (A) Graphs Representing the Proportion of Identified Capsule Serotypes (K1 and K2) Among the *K. pneumoniae* Isolates and **(B)** Classification of Capsule-Producing *Klebsiella pneumoniae* Strains Based on Their Multidrug-Resistance Status and Biofilm Production Capability

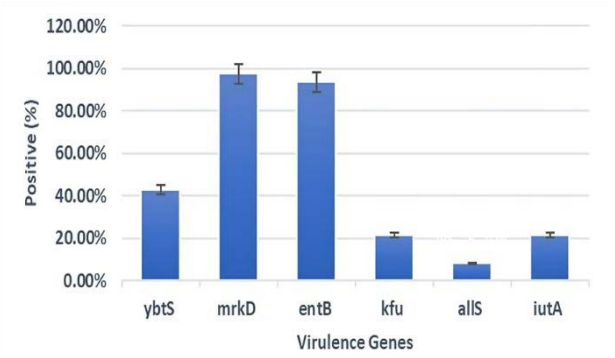

Figure 3. Mapping and Prevalence of Virulence-Associated Genes Across the Clinical Isolates of *K. pneumoniae*. *Note*. This figure illustrates the identification of specific virulence genes and their frequency among the tested isolates

common than the K2-1.4% serotype, which corroborates the results of a study conducted by Yeh et al, in which the K1 serotype (46.6%) was more common than the K2 serotype (20.5%) (24). Our findings confirmed high prevalence rates of virulence genes *mrkD* (98%) and *entB* (94%) in *K. pneumoniae* isolates, aligning with the findings of Rastegar et al (25), demonstrating 94.5% and 95%, respectively, for K1 and K2 serotypes. These findings are also in conformity with those of Compain et al (10). None of our isolates tested positive for the *rmpA* gene, and most did not exhibit the hypermucoviscous phenotype. This supports the findings, which associated the *rmpA* gene with hypermucoviscosity in *K. pneumoniae*. In a study conducted by Mirzaie and Ranjbar, the virulence-associated gene *entB* showed 75% resistance to ciprofloxacin in 80% of the isolates, and the efflux pump genes (*AcrAB*, *TolC* , and *mdtk*) were observed in 41%, 33%, and 26% of the strains (26), which matches our findings . Despite the high prevalence of *mrkD* and *entB* and the moderate/low frequencies of other virulence genes, our statistical analysis revealed no significant association between virulence gene distribution and MDR or XDR status (all $P > 0.05$). This indicates that virulence determinants are broadly distributed across resistant and susceptible isolates. The majority of ESBL-producing isolates were positive for the *blaSHV* gene (95.8%), which is in line with the results of other studies that identified the *blaSHV* gene in 87% and 62% of the isolates, respectively. Similarly, our results demonstrated that 71% of the isolates had the *blaCTX-M* gene, which is consistent with the findings of other studies, indicating that 44.6%, 69%, and 89.4% of the isolates carried the *blaCTX-M* gene (27, 28). ESBL genes *blaTEM*, *blaSHV*, and *blaCTX-M* (29.79%, 27.66%, and 17.02%) in ESBL-producing isolates were found positive, along with K1 and K2 serotypes and the *kfu* virulence gene among the isolates in another study by Zhong et al (28). Based on our findings, 41.7% of the isolates had the *blaTEM* gene, while a previous study reported that 36% of the isolates had the *blaTEM* gene (28). The presence of the *blaCTX-M*, *blaTEM*, *blaOXA*, and *blaSHV* (100%, 97%, 86%, and 83%) ESBL genes in similar genetic contexts has been confirmed in a South African study. The *blaNDM* gene was found in 62.5% of the carbapenemase-producing *K. pneumoniae* isolates compared to 7.1% and 37% in previous studies. In a study in Bangladesh, the analysis showed that the samples carried 64% and 38% *blaNDM* and *blaSHV* genes, and 19% carried both genes (28). The *blaOXA-48* gene was identified in 33% of the carbapenemase-producing *K. pneumoniae* isolates. Nonetheless, reports from other studies showed that 6%, 9%, 67%, and 71% of the isolates carried *blaOXA-48*, respectively. In our study, *Ompk36* was detected in all the isolates (100%), followed by *Ompk35* (96%), which conforms to the results of a study performed in Iran (28). The frequency of the *AcrAB* gene (96%) was higher than that of the *mdtk* gene (73%) in our XDR and colistin-resistant *K. pneumoniae* isolates, which is consistent with the results of a study conducted in Egypt (28). In the present study, 96% of XDR and colistin-resistant *K. pneumoniae* isolates were positive for the *TolC* gene, which corroborates the findings of the Brazilian study, where, in addition to the *TolC* gene, efflux pump-related genes *AcrAB* (100%), *mdtk* (88%), *Ompk35* (60%), and *Ompk36* (28%) were also found positive. Among ESBL producers, *blaKPC* (100%), *blaTEM* (100%), *blaSHV* (96%), *blaOXA* (84%), and *blaCTX-M* (72%) *amr* genes were the carriers. The genotyping results of colistin-resistant *K. pneumoniae* isolates revealed that the absence of a plasmid encodes resistance genes (*mcr*). In the absence of *mcr* genes, the

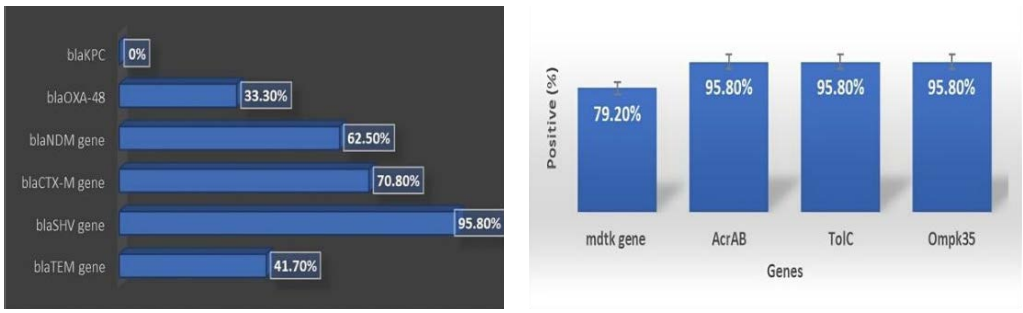

Figure 4. (A) Molecular Profiling of Antibiotic Resistance Genes in *Klebsiella pneumoniae* Isolates and **(B)** Proportional Representation of Genes Associated With Outer Membrane Porins and Efflux Pumps in the Isolates

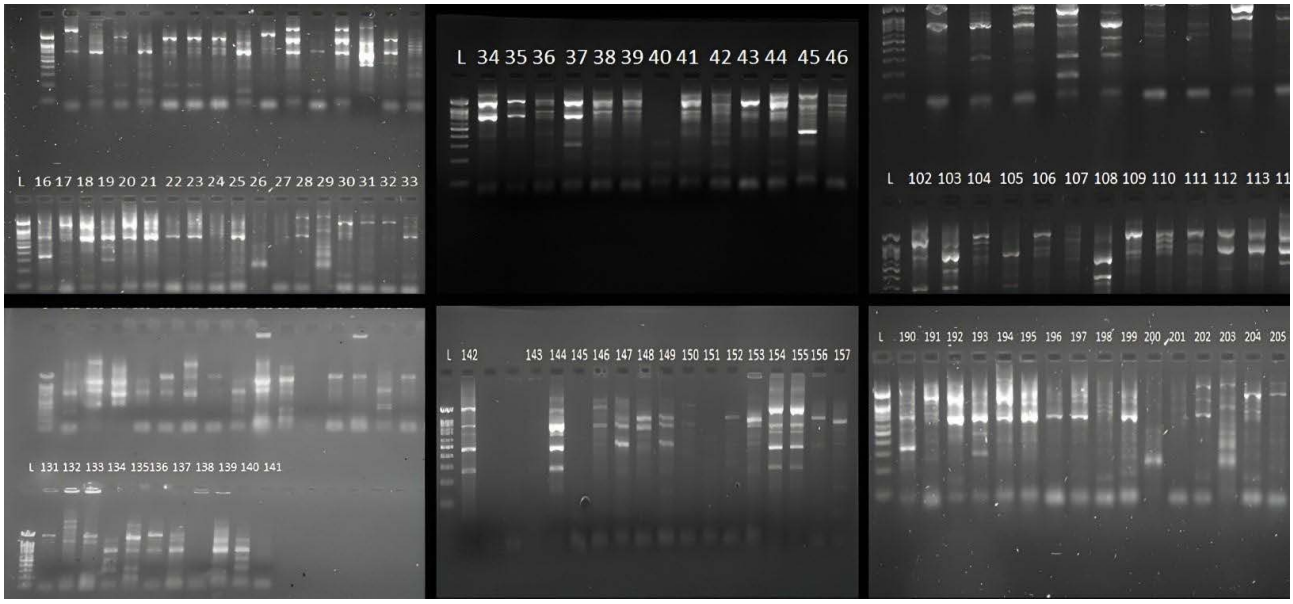

Figure 5. Gel Electrophoresis Results of Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction Fingerprinting, Showcasing Genetic Diversity Among *Klebsiella pneumoniae* Isolates

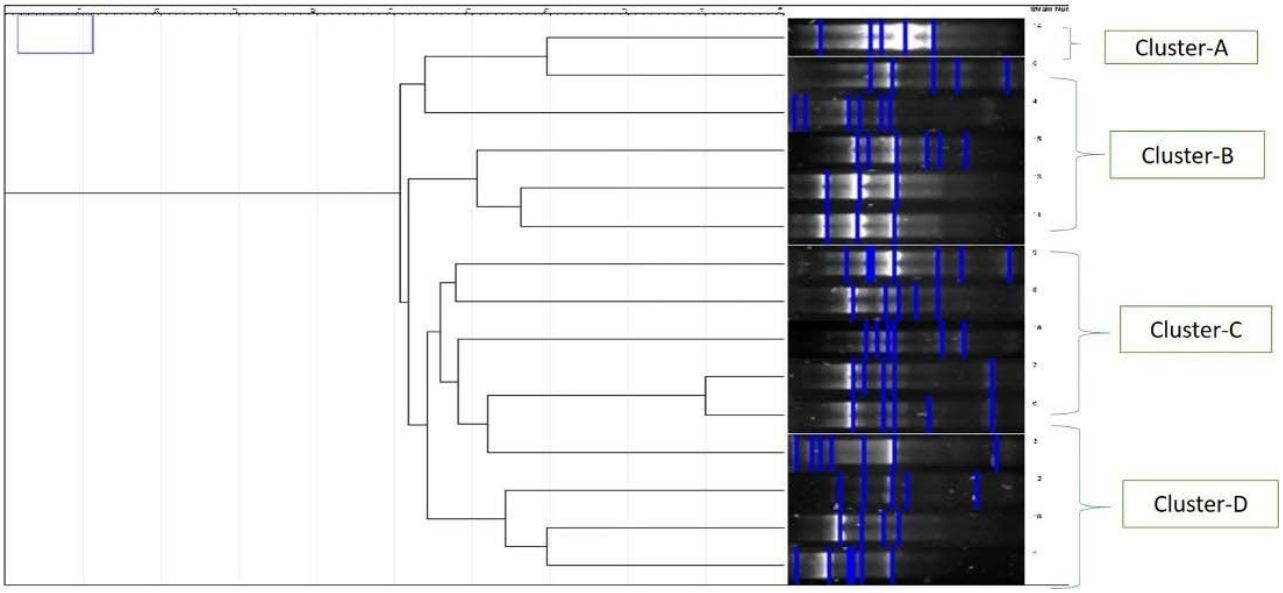

Figure 6. Dendrogram Illustrating the Clustering of *Klebsiella pneumoniae* Isolates Based on Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction Fingerprinting, Highlighting Genetic Relationships and Diversity Among the Isolates

presence of the chromosomally mediated gene *mgrB* may be linked to colistin resistance in *K. pneumoniae*. According to earlier research, mutations in the *mgrB* gene were the most frequent sources of colistin resistance in

K. pneumoniae. In this study, 207 DNA samples were analyzed using ERIC-PCR to assess genetic diversity and relatedness among bacterial strains. Our results showed that 183 samples (88%) exhibited ERIC patterns, while 24 samples (12%) did not display these patterns, which aligns with the results of Meacham et al (29), demonstrating variations in the genetic diversity of bacterial isolates by ERIC-PCR. They successfully used ERIC-PCR to construct a phylogenetic tree for *Klebsiella*, highlighting the method's effectiveness. Similarly, Barus et al identified 16 clusters from 61 *Klebsiella* spp. isolates based on ERIC-PCR profiles, revealing significant genetic diversity within *K. pneumoniae* (30), which is consistent with our findings, underscoring the utility of ERIC-PCR in discerning genetic diversity. The 1 to 8 bands per sample observed in our analysis are comparable to the diversity found in studies performed by Meacham et al (29) and Barus et al (30), further validating the robustness of ERIC-PCR for genetic analysis. Moreover, the dendrogram and clustering analysis in our study revealed distinct clusters, indicating significant genetic diversity among the bacterial strains, which corroborates the clustering patterns observed in the study by Barus et al (30), where different ERIC profiles described the genetic diversity within *Klebsiella* spp. In conclusion, our study, along with those of Meacham et al (29) and Barus et al (30), demonstrated the effectiveness of ERIC-PCR in revealing genetic diversity and relatedness among bacterial isolates. In accordance with our statistical analysis, no significant associations were found between ERIC-PCR clustering, antibiotic resistance, and virulence gene distribution ($P > 0.05$). This suggests that resistance and virulence determinants were not confined to specific clonal groups but rather dispersed across genetically diverse isolates. The lack of correlation may reflect the multifactorial nature of resistance and virulence acquisition in *K. pneumoniae*, where mobile genetic elements and horizontal gene transfer play a key role. One limitation of this study was that multivariate approaches (e.g., logistic regression) could not be applied due to the uneven distribution of virulence genes, some being nearly universal (*mrkD* and *entB*) and others rare (*rmpA* and *allS*). This imbalance restricted the robustness of regression modeling and may have limited deeper exploration of the predictors of MDR/XDR phenotypes. Future large-scale studies integrating whole genome sequencing and advanced statistical approaches may better delineate these complex relationships. Better resolution of such correlations may be possible with larger sample sizes and whole-genome-based methodologies. These consistent findings reinforce the method's reliability and robustness, making it a valuable tool for genetic analysis in microbial populations.

Conclusion

Our findings revealed the complex genetic landscape of *K. pneumoniae*, with multiple resistance and virulence factors coexisting, complicating infection control and treatment. The diverse virulence profiles highlighted the pathogen's ability to employ various pathogenic strategies. It is essential to comprehend these elements in order to create prevention and treatment plans that are effective. Moreover, continuous surveillance and molecular characterization are essential for informed clinical management and targeted therapies. Addressing the burden of MDR and XDR *K. pneumoniae* requires stringent infection control and the development of novel therapeutic options. Accordingly, future research should focus on the genetic diversity and evolution of resistance and virulence determinants.

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Competing Interests

The authors declare that they have no conflict of interests.

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Supplementary Files

The supplementary file contains Table S1.

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