

EVALUATION OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE IN URINARY ISOLATES OF *KLEBSIELLA PNEUMONIAE* AT MARDAN MEDICAL COMPLEX, MARDAN.

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ABSTRACT

Background: *Klebsiella pneumoniae* is an important uropathogens associated with increasing antimicrobial resistance and a range of virulence-associated characteristics. The coexistence of antimicrobial resistance and virulence determinants may complicate the management of urinary tract infections. This study evaluated antimicrobial susceptibility and selected phenotypic and genotypic virulence characteristics among urinary *K. pneumoniae* isolates recovered at Mardan Medical Complex, Mardan, Pakistan.

Methods: A laboratory-based cross-sectional study was conducted from January 2025 to January 2026 in the Microbiology Laboratory of Mardan Medical Complex. A total of 250 confirmed urinary *K. pneumoniae* isolates were included using non-probability consecutive sampling. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method against selected antimicrobial agents. Capsule production was assessed by India ink staining, hypermucoviscosity by the string test, and the virulence-associated *rmpA* gene was detected by polymerase chain reaction. Associations between virulence characteristics and antimicrobial resistance were assessed using chi-square or Fisher's exact tests.

Results: Of the 250 isolates, 146 (58.4%) were recovered from females and 104 (41.6%) from males. The highest antimicrobial resistance was observed against ampicillin (94.8%), followed by cefotaxime (74.0%), ceftazidime (71.2%), ciprofloxacin (68.0%), amoxicillin-clavulanic acid (62.0%), and gentamicin (51.2%). Resistance to imipenem, nitrofurantoin, and fosfomycin was 23.2%, 43.6%, and 26.8%, respectively. Capsule production was detected in 203 (81.2%) isolates, while 91 (36.4%) exhibited a hypermucoviscous phenotype and 78 (31.2%) carried *rmpA*. Among *rmpA*-positive isolates, capsule production was significantly more frequent than among *rmpA*-negative isolates (92.3% vs. 76.2%, $p=0.002$). Similarly, hypermucoviscosity was significantly associated with *rmpA* carriage (70.5% vs. 20.9%, $p<0.001$).

Conclusion: Urinary *K. pneumoniae* isolates demonstrated a substantial burden of antimicrobial resistance together with frequent virulence-associated characteristics, particularly capsule production, hypermucoviscosity, and *rmpA* carriage. The significant associations of *rmpA* with capsule production and hypermucoviscosity support a relationship between this gene and capsule-associated phenotypes. Continued local antimicrobial-resistance surveillance and integrated monitoring of virulence characteristics are warranted.

KEYWORDS: *Klebsiella pneumoniae*; urinary tract infection; antimicrobial resistance; virulence factors; *rmpA*; hypermucoviscosity; capsule production; urinary isolates.

INTRODUCTION

Urinary tract infections (UTIs) are among the most frequently encountered bacterial infections worldwide and represent an important cause of morbidity in both community and healthcare settings. Although *Escherichia coli* remains the predominant uropathogens, *Klebsiella pneumoniae* is an important cause of complicated and healthcare-associated UTIs, particularly among hospitalized patients, individuals with urinary catheters, recurrent infections, and those with underlying comorbidities. The increasing occurrence of antimicrobial-resistant Gram-negative uropathogens has substantially complicated the management of UTIs, with resistance being particularly concerning when it involves third-generation cephalosporins and carbapenems [1,2]. *K. pneumoniae* is a Gram-negative, non-motile, facultatively anaerobic member of the Enterobacterales and can colonize the gastrointestinal tract and other mucosal surfaces without causing disease. However, pathogenic strains possess a diverse collection of virulence and resistance determinants that facilitate colonization, persistence, tissue invasion, and treatment failure [3].

The pathogenicity of *K. pneumoniae* is mediated by several interacting virulence mechanisms. The polysaccharide capsule is one of its major pathogenic determinants because it can reduce complement-mediated killing and phagocytosis and enhance bacterial survival within the host. Fimbrial adhesins, particularly type 1 and type 3 fimbriae, contribute to attachment to host epithelial cells and biofilm development, thereby facilitating colonization of the urinary tract and persistence on medical devices. In addition, siderophores such as enterobactin, yersiniabactin, and aerobactin enable the organism to acquire iron under nutrient-limited conditions, while lipopolysaccharide, outer-membrane components, and other surface-associated factors contribute to host interaction and immune evasion [3,4]. Biofilm formation is particularly relevant to urinary infections because biofilms can promote bacterial persistence and reduce susceptibility to antimicrobial agents and host immune responses [4].

The emergence of hypervirulent *K. pneumoniae* (hvKp) has added another dimension to the clinical significance of this organism. Hypervirulent strains may possess enhanced siderophore systems, increased capsule production, hypermucoviscous phenotypes, and specific virulence-associated genes. Importantly, virulence should not be considered synonymous with hypermucoviscosity or the presence of a single virulence gene, because pathogenic potential can vary according to the combination, expression, and genetic background of individual isolates [3,5]. More recently, strains combining antimicrobial resistance with hypervirulence have increasingly been reported. Such convergence may occur through the acquisition of resistance plasmids by virulent strains, acquisition of virulence determinants by multidrug-resistant classical strains, or horizontal transfer of hybrid plasmids containing both resistance and virulence-associated determinants [5,6].

Antimicrobial resistance is therefore a major concern in *K. pneumoniae*. The organism has an exceptional capacity to acquire mobile genetic elements carrying resistance determinants, including extended-spectrum β -lactamases (ESBLs), AmpC β -lactamases, carbapenemases, and resistance mechanisms affecting fluoroquinolones, aminoglycosides, and other antimicrobial classes. Consequently, infections caused by multidrug-resistant *K. pneumoniae* may leave clinicians with limited therapeutic options [2,7]. The World Health Organization's 2024 Bacterial Priority Pathogens List specifically identifies carbapenem-resistant *K. pneumoniae* and third-generation-cephalosporin-resistant *Klebsiella* spp. among important antimicrobial-resistant bacterial threats requiring surveillance, research, and development of improved interventions [8]. The simultaneous presence of resistance and virulence determinants is particularly concerning because it may allow strains to survive antimicrobial pressure while maintaining the capacity to colonize and cause disease.

The situation is particularly relevant in Pakistan, where increasing antimicrobial resistance among uropathogens has been documented. A recent Pakistani study of *K. pneumoniae* isolates from community- and hospital-acquired UTIs demonstrated substantial resistance and identified clinically important resistance determinants, including blaCTX-M-15 and blaNDM, together with virulence-associated genes such as *fimH* and *mrkD* [9]. Similarly, a 2025 study involving clinical isolates from Khyber Pakhtunkhwa, including specimens from Mardan Medical Complex, reported considerable antimicrobial resistance and frequent virulence-associated phenotypes, including biofilm formation and hypermucoviscosity [10]. These findings demonstrate the importance of region-specific surveillance because the distribution of resistance and virulence characteristics can vary according to geographical location, healthcare setting, patient population, and local antimicrobial-use practices.

Despite these reports, there remains a need for focused characterization of *K. pneumoniae* recovered specifically from urinary specimens at individual healthcare institutions. Local determination of antimicrobial susceptibility and virulence characteristics can provide clinically relevant information for empirical treatment, infection-control strategies, antimicrobial stewardship, and epidemiological surveillance. Furthermore, evaluating resistance and virulence together is important because the clinical significance of an isolate cannot necessarily be understood from its antimicrobial susceptibility profile alone. Therefore, the present study was designed to evaluate virulence factors and antibiotic resistance among urinary isolates of *Klebsiella pneumoniae* at Mardan Medical Complex, Mardan. The study aims to characterize the virulence profile and antimicrobial susceptibility of urinary *K. pneumoniae* isolates and to provide institution-specific evidence regarding the coexistence of pathogenicity-associated characteristics and antimicrobial resistance. Such evidence may contribute to improved understanding of the local epidemiology of *K. pneumoniae* UTIs and support more appropriate laboratory-guided management and infection-control practices.

MATERIALS AND METHODS

A laboratory-based cross-sectional study was conducted to evaluate antimicrobial resistance and selected phenotypic and genotypic virulence characteristics of urinary *Klebsiella pneumoniae* isolates. The study was conducted in the Microbiology Laboratory of Mardan Medical Complex, Mardan, Khyber Pakhtunkhwa, Pakistan, from January 2025 to January 2026. The study population comprised patients with suspected urinary tract infection whose urine specimens were submitted to the microbiology laboratory for routine bacterial culture. A total of 250 confirmed urinary *K. pneumoniae* isolates were included using non-probability consecutive sampling. Only one isolate per patient was included to avoid duplication. Urine specimens yielding significant pure growth of *K. pneumoniae* and confirmed by conventional identification methods and API 10E were included, whereas duplicate isolates, contaminated or improperly labeled specimens, mixed cultures in which *K. pneumoniae* could not be reliably identified, and isolates that failed to grow or could not be adequately confirmed were excluded. Urine specimens were collected using standard microbiological procedures in sterile, leak-proof containers. Midstream clean-catch urine was obtained from patients able to provide a specimen, while catheterized urine was

collected aseptically from the catheter sampling port. Specimens were transported promptly to the microbiology laboratory for processing. Urine samples were inoculated using a calibrated loop onto Cystine-Lactose-Electrolyte-Deficient (CLED) and MacConkey agar and incubated under standard aerobic conditions. Following incubation, bacterial growth was assessed for colony morphology, purity, and significant growth. Colonies suggestive of *Klebsiella* species were selected for further identification. Presumptive *K. pneumoniae* isolates were identified by colony morphology, Gram staining, and conventional biochemical testing. Typical isolates produced large, smooth, glistening and often mucoid colonies, with lactose fermentation on MacConkey agar, while Gram staining demonstrated Gram-negative bacilli. Biochemical identification included indole, citrate, urease, methyl red, Voges–Proskauer, oxidase, catalase, and triple sugar iron tests. Final identification was confirmed using the API 10E identification system according to the manufacturer's instructions. Confirmed isolates were maintained under appropriate storage conditions until further testing.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. A bacterial suspension equivalent to 0.5 McFarland was prepared from a fresh culture and uniformly inoculated onto the agar surface. Antibiotic disks were applied aseptically, followed by incubation under standard conditions. Zones of inhibition were measured in millimeters and interpreted according to the applicable antimicrobial susceptibility testing standards. Appropriate quality-control procedures were followed using a suitable reference strain. The antibiotics tested were ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, nitrofurantoin, fosfomycin, and polymyxin B.

Capsule production was assessed by negative staining using India ink. A visible clear halo surrounding the bacterial cell against the dark background was considered indicative of capsule production. The hypermucoviscous phenotype was assessed using the string test, in which a bacterial colony was lifted vertically from the agar surface using a sterile inoculating loop. Formation of a viscous string measuring more than 5 mm was considered a positive result.

For molecular detection, genomic DNA was extracted from confirmed *K. pneumoniae* isolates using a validated bacterial DNA extraction procedure. PCR amplification was performed using specific primers targeting the *rmpA* gene. Each reaction contained PCR master mix, forward and reverse primers, template DNA, and nuclease-free water, with a known positive control and a no-template negative control included in each PCR run. The primers used were forward 5'-ACTGGGCTACCTCTGCTTCA-3' and reverse 5'-CTTGCATGAGCCATCTTTCA-3', producing an expected amplicon of approximately 535 bp. PCR cycling was performed using the validated protocol for the selected primer pair, consisting of initial denaturation followed by repeated cycles of denaturation, annealing, and extension and a final extension step. PCR products were separated by agarose gel electrophoresis alongside an appropriate DNA molecular-weight marker and visualized using a suitable nucleic-acid detection system. Samples showing a band at the expected molecular size in the presence of a valid positive control and absence of amplification in the negative control were considered positive for the *rmpA* gene.

Data were analyzed using IBM SPSS Statistics [version 26]. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were summarized using appropriate descriptive statistics. Associations between antimicrobial resistance, phenotypic virulence characteristics, and *rmpA* detection were assessed using the chi-square test or Fisher's exact test, as appropriate. A two-sided *p* value <0.05 was considered statistically significant.

RESULTS

A total of 250 confirmed urinary *Klebsiella pneumoniae* isolates were included in the study. The isolates were evaluated for antimicrobial susceptibility, capsule production, hypermucoviscosity, and the presence of the *rmpA* virulence-associated gene.

Table 1. Demographic characteristics of patients from whom *K. pneumoniae* was isolated

Characteristic	n	%
Sex		
Male	104	41.6
Female	146	58.4
Age group (years)		
≤20	32	12.8
21–40	71	28.4
41–60	82	32.8
>60	65	26.0
Total	250	100.0

Among the 250 urinary *K. pneumoniae* isolates were recovered from 146 female patients (58.4%) and 104 (41.6%) from male patients. The largest proportion of isolates was observed among patients aged 41–60 years (32.8%), followed by those aged 21–40 years (28.4%).

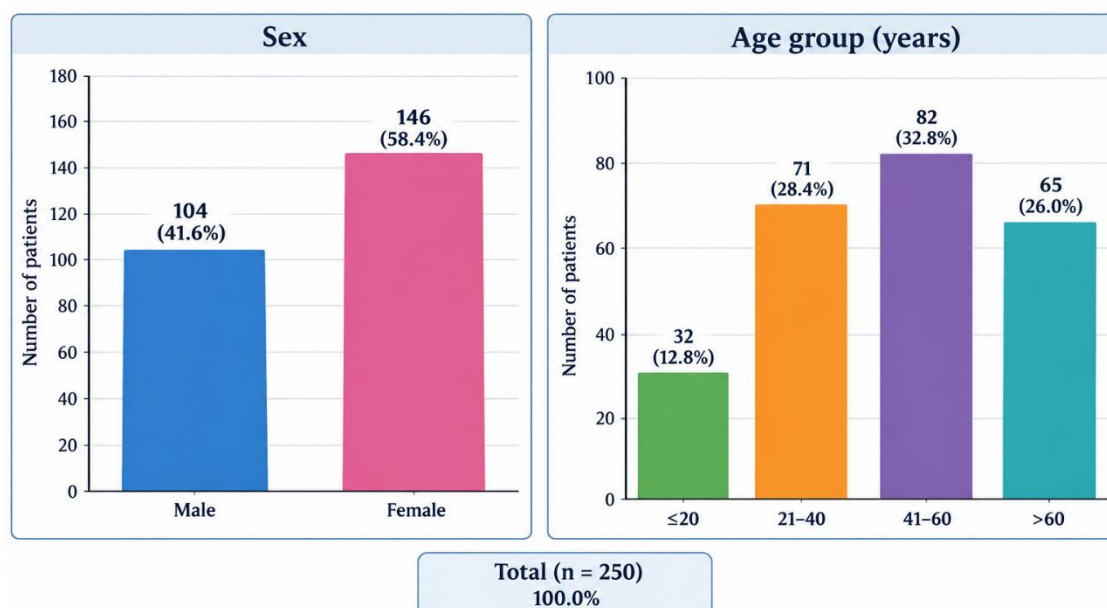
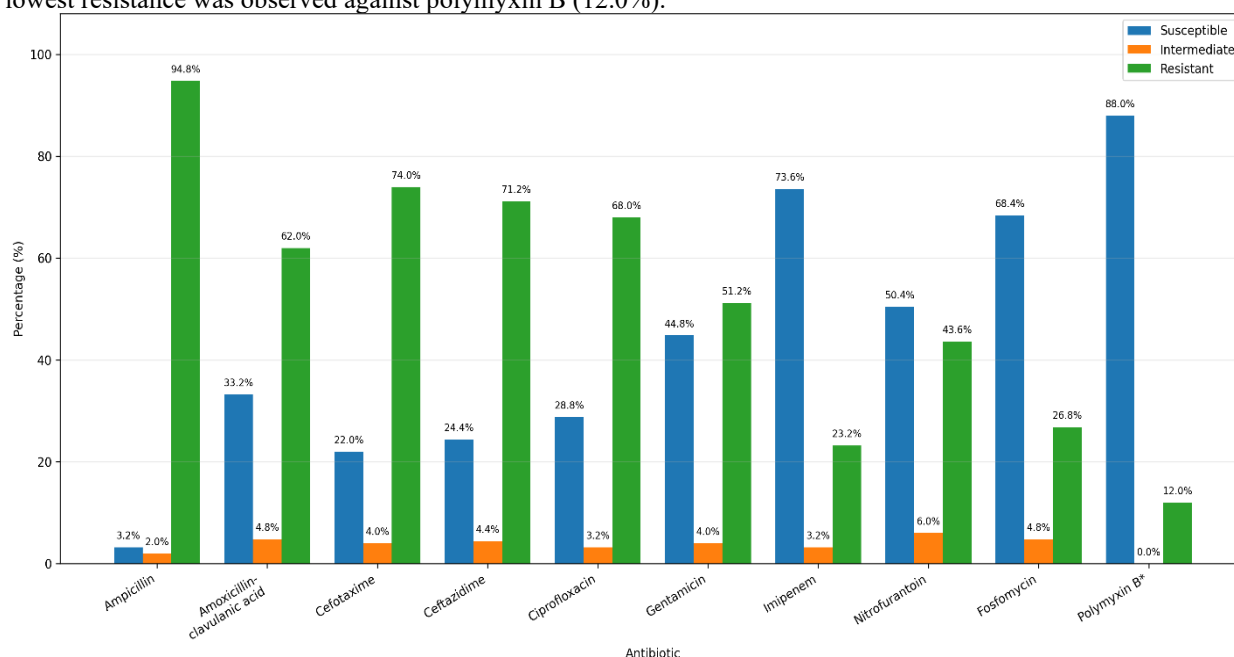


Figure 1: Demographic characteristics of patients from whom *K. pneumoniae* was isolated

Table 2. Antimicrobial susceptibility pattern of urinary *K. pneumoniae* isolates

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	8 (3.2)	5 (2.0)	237 (94.8)
Amoxicillin-clavulanic acid	83 (33.2)	12 (4.8)	155 (62.0)
Cefotaxime	55 (22.0)	10 (4.0)	185 (74.0)
Ceftazidime	61 (24.4)	11 (4.4)	178 (71.2)
Ciprofloxacin	72 (28.8)	8 (3.2)	170 (68.0)
Gentamicin	112 (44.8)	10 (4.0)	128 (51.2)
Imipenem	184 (73.6)	8 (3.2)	58 (23.2)
Nitrofurantoin	126 (50.4)	15 (6.0)	109 (43.6)
Fosfomycin	171 (68.4)	12 (4.8)	67 (26.8)
Polymyxin B	220 (88.0)	—	30 (12.0)

The isolates demonstrated substantial resistance to several commonly used antimicrobial agents. The highest resistance was observed against ampicillin (94.8%), followed by cefotaxime (74.0%), ceftazidime (71.2%), ciprofloxacin (68.0%), and amoxicillin-clavulanic acid (62.0%). Gentamicin showed resistance in 51.2% of isolates. Resistance to imipenem, nitrofurantoin, and fosfomycin was 23.2%, 43.6%, and 26.8%, respectively. The lowest resistance was observed against polymyxin B (12.0%).



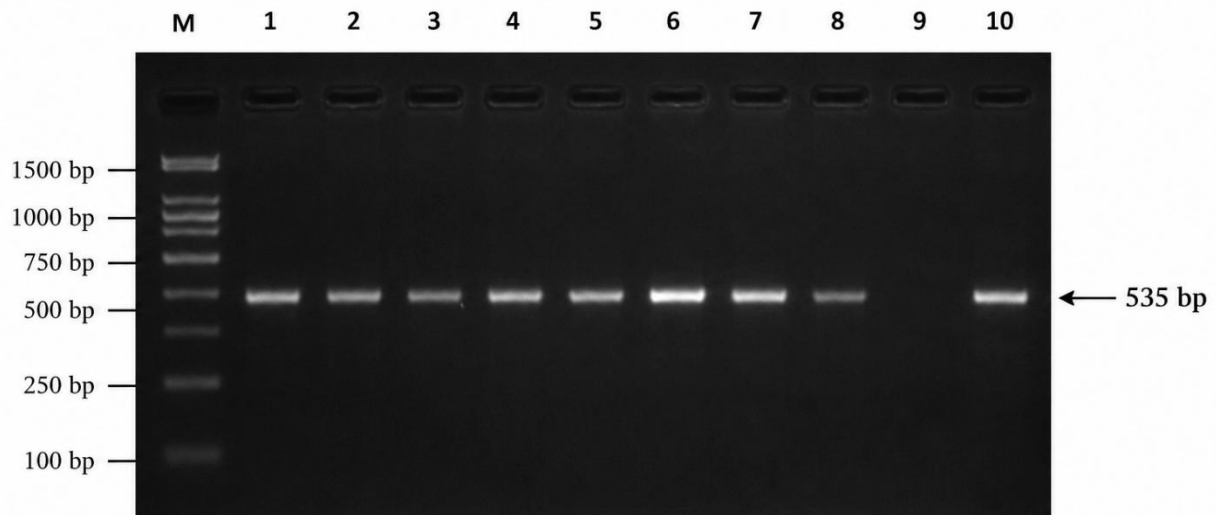
Labels show percentage values; n values from the supplied table are retained in the source data.

Figure 2: Antimicrobial susceptibility pattern of urinary *K. pneumoniae* isolates

Table 3. Distribution of phenotypic and genotypic virulence characteristics

Virulence characteristic	Positive n (%)	Negative n (%)
Capsule production	203 (81.2)	47 (18.8)
Hypermucoviscosity (string test)	91 (36.4)	159 (63.6)
<i>rmpA</i> gene	78 (31.2)	172 (68.8)

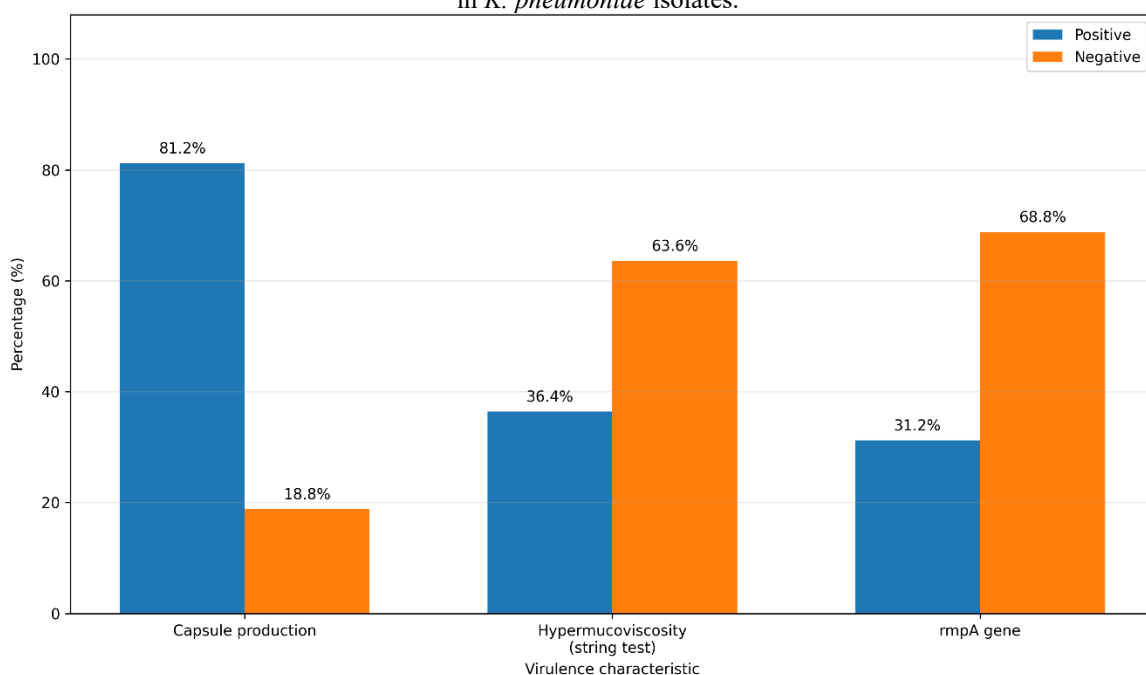
Capsule production was detected in 203 (81.2%) isolates, whereas 47 (18.8%) were non-capsulated by the applied phenotypic test. A positive string test indicating a hypermucoviscous phenotype was observed in 91 (36.4%) isolates. The *rmpA* gene was detected by PCR in 78 (31.2%) isolates.



Representative agarose gel (1.5%) showing PCR amplification of the *rmpA* gene (535 bp) in *K. pneumoniae* isolates.

Lane M: 100 bp DNA ladder; Lane 1–8: Test isolates (*rmpA* positive); Lane 9: Negative control (no template); Lane 10: Positive control (*rmpA* positive strain).

Figure 3(A). Representative agarose gel electrophoresis showing PCR amplification of the *rmpA* gene (535 bp) in *K. pneumoniae* isolates.



Positive/negative values are percentages; n values are 250 isolates for each characteristic.

Figure 3 (B): Distribution of phenotypic and genotypic virulence characteristics

Table 4. Association between *rmpA* gene and phenotypic virulence characteristics

Phenotypic characteristic	<i>rmpA</i> positive n (%)	<i>rmpA</i> negative n (%)	p-value
Capsule production			
Positive	72 (92.3)	131 (76.2)	0.002
Negative	6 (7.7)	41 (23.8)	
String test			
Positive	55 (70.5)	36 (20.9)	<0.001
Negative	23 (29.5)	136 (79.1)	

A significant association was observed between *rmpA* carriage and both phenotypic virulence characteristics. Capsule production was detected in 92.3% of *rmpA*-positive isolates compared with 76.2% of *rmpA*-negative isolates ($p=0.002$). Similarly, the hypermucoviscous phenotype was substantially more frequent among *rmpA*-positive isolates than among *rmpA*-negative isolates (70.5% vs. 20.9%, $p<0.001$).

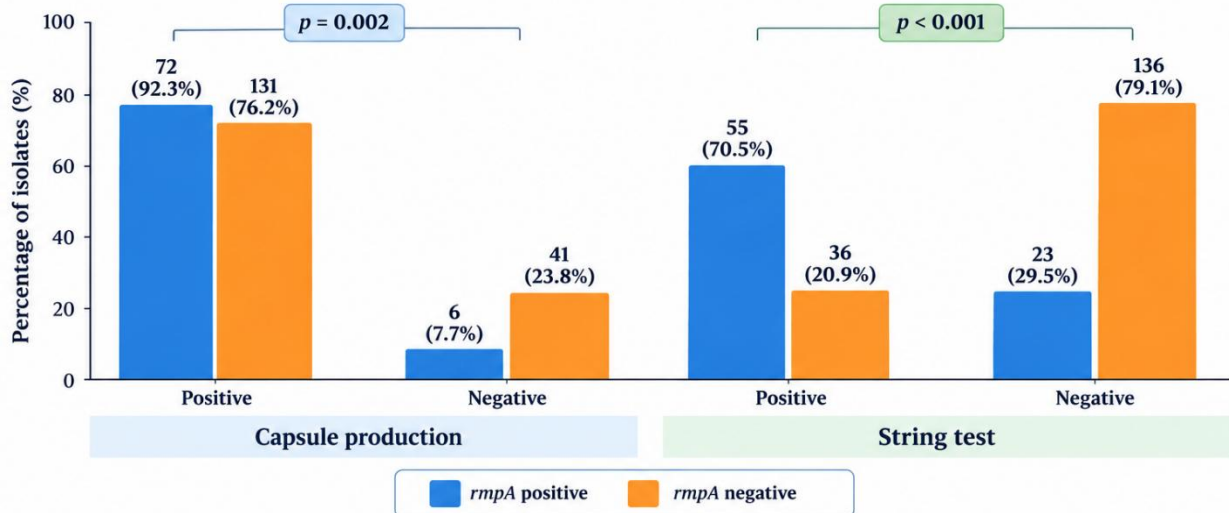


Figure 4: Association between *rmpA* gene and phenotypic virulence characteristics

Table 5. Association between *rmpA* carriage and antimicrobial resistance

Antibiotic	Resistant among <i>rmpA</i> positive n (%)	Resistant among <i>rmpA</i> negative n (%)	p-value
Ampicillin	76 (97.4)	161 (93.6)	0.273
Amoxicillin-clavulanic acid	54 (69.2)	101 (58.7)	0.114
Cefotaxime	62 (79.5)	123 (71.5)	0.177
Ceftazidime	58 (74.4)	120 (69.8)	0.454
Ciprofloxacin	57 (73.1)	113 (65.7)	0.237
Gentamicin	44 (56.4)	84 (48.8)	0.277
Imipenem	22 (28.2)	36 (20.9)	0.196
Nitrofurantoin	37 (47.4)	72 (41.9)	0.428
Fosfomycin	23 (29.5)	44 (25.6)	0.527
Polymyxin B	11 (14.1)	19 (11.0)	0.489

The proportion of antimicrobial resistance was generally higher among *rmpA*-positive isolates than among *rmpA*-negative isolates for most tested antibiotics.

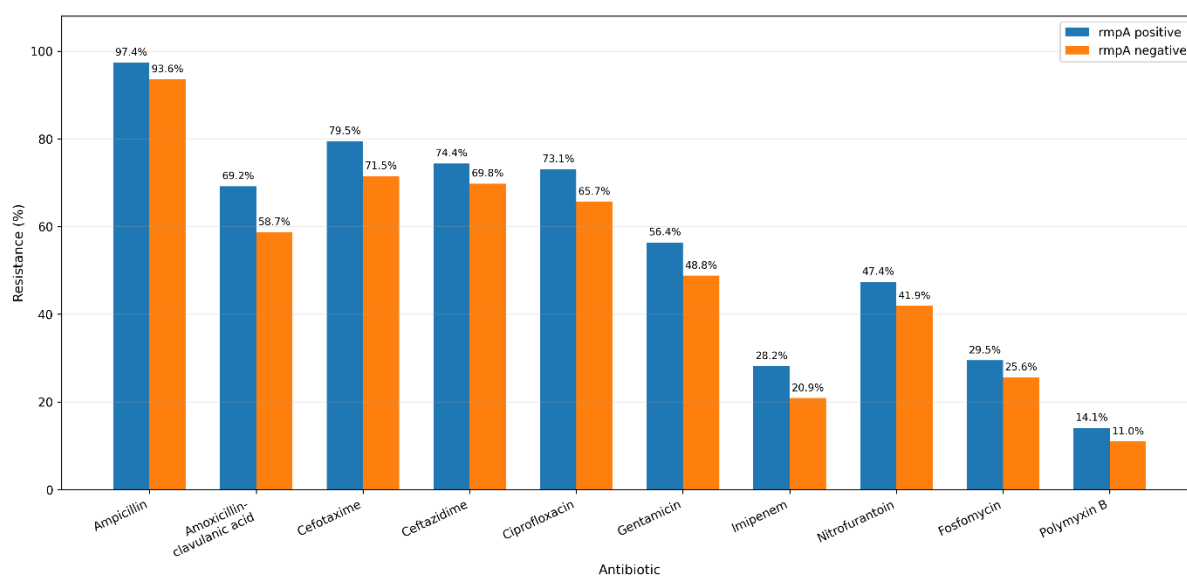


Figure 5: Association between *rmpA* carriage and antimicrobial resistance

Table 6. Association between hypermucoviscosity and antimicrobial resistance

Antibiotic	Resistant among string-positive n (%)	Resistant among string-negative n (%)	p-value
Ampicillin	88 (96.7)	149 (93.7)	0.338
Amoxicillin-clavulanic acid	60 (65.9)	95 (59.7)	0.344
Cefotaxime	70 (76.9)	115 (72.3)	0.440
Ceftazidime	67 (73.6)	111 (69.8)	0.531
Ciprofloxacin	65 (71.4)	105 (66.0)	0.374
Gentamicin	50 (54.9)	78 (49.1)	0.390
Imipenem	25 (27.5)	33 (20.8)	0.220
Nitrofurantoin	42 (46.2)	67 (42.1)	0.532
Fosfomycin	26 (28.6)	41 (25.8)	0.632
Polymyxin B	12 (13.2)	18 (11.3)	0.667

Comparison according to the string-test phenotype showed a generally higher proportion of resistance among hypermucoviscous isolates.

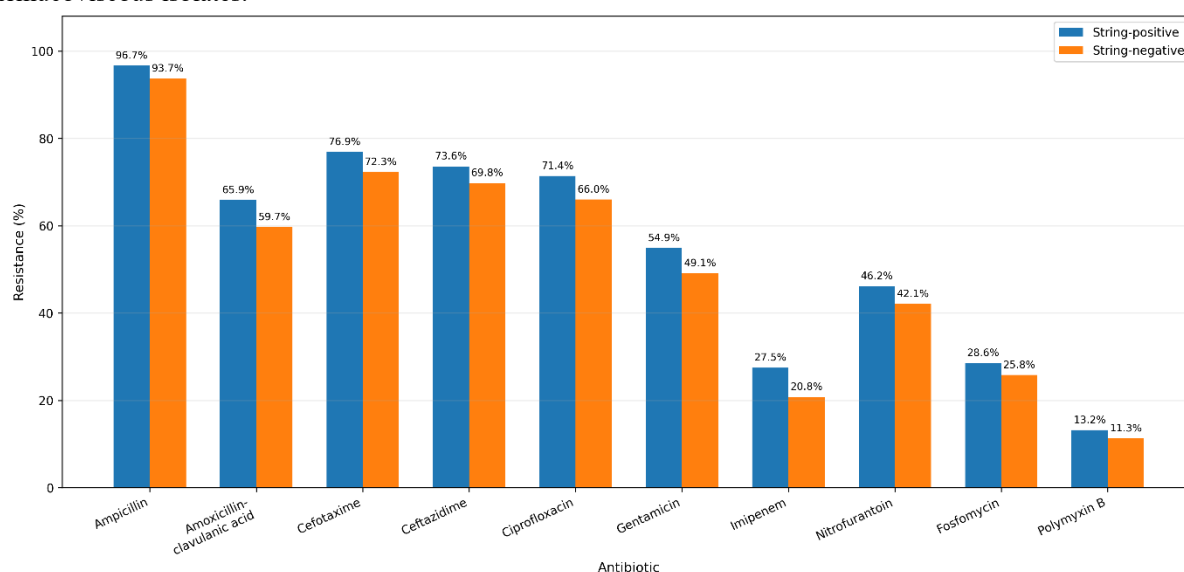


Figure 6: Association between hypermucoviscosity and antimicrobial resistance

Table 7. Overall distribution of major virulence-associated characteristics

Characteristic	Positive n (%)
Capsule production	203 (81.2)
Hypermucoviscosity	91 (36.4)
<i>rmpA</i> gene	78 (31.2)
<i>rmpA</i> positive + hypermucoviscous phenotype	55 (22.0)
<i>rmpA</i> positive + capsule positive	72 (28.8)

Overall, the findings indicate that capsule production was the most frequently observed virulence-associated characteristic (81.2%), followed by hypermucoviscosity (36.4%) and *rmpA* carriage (31.2%). The combination of *rmpA* carriage and a hypermucoviscous phenotype was observed in 22.0% of all isolates.

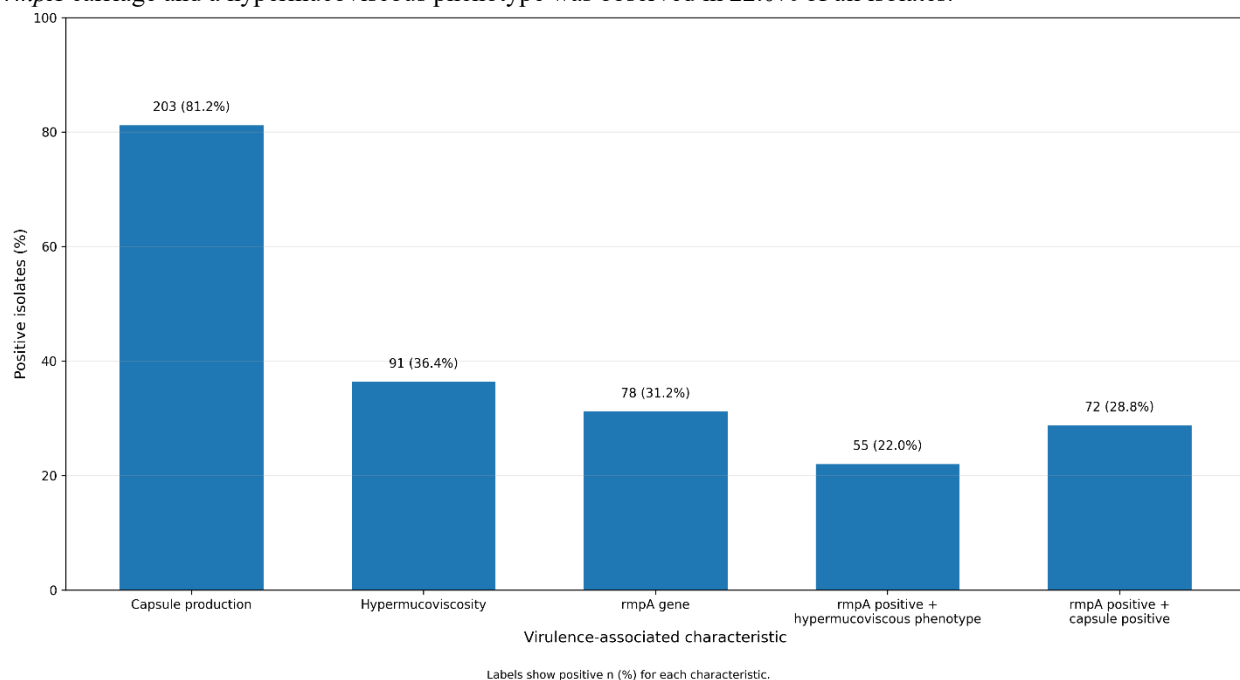


Figure 7: Overall distribution of major virulence-associated characteristics

DISCUSSION

The present study evaluated antimicrobial resistance and selected phenotypic and genotypic virulence characteristics among 250 urinary *Klebsiella pneumoniae* isolates recovered at Mardan Medical Complex. Overall, the findings demonstrate the coexistence of substantial antimicrobial resistance with important virulence-associated characteristics, particularly capsule production, hypermucoviscosity, and carriage of the *rmpA* gene. This combination is clinically relevant because *K. pneumoniae* has progressively evolved from a predominantly opportunistic healthcare-associated pathogen into a heterogeneous population containing classical multidrug-resistant strains and hypervirulent lineages with overlapping resistance and virulence determinants [11-13]. Recent genomic studies further indicate that antimicrobial resistance and virulence can converge through acquisition of mobile genetic elements, although the relationship is not necessarily consistent across individual isolates [14-16]. In the present study, urinary *K. pneumoniae* isolates were more frequently recovered from females than males, with females accounting for 58.4% and males for 41.6% of isolates. The predominance of female patients is compatible with the well-established epidemiology of urinary tract infection, in which anatomical and physiological factors contribute to a higher burden of UTI among females. The age distribution showed the largest proportion of isolates among patients aged 41–60 years (32.8%), followed by those older than 60 years (26.0%). The concentration of isolates in middle-aged and older patients may reflect the increasing contribution of comorbidities, recurrent urinary infections, healthcare exposure, catheterization, and other predisposing factors in these age groups. Recent studies of urinary *K. pneumoniae* similarly emphasize the importance of patient population, healthcare exposure, and local epidemiology when interpreting resistance patterns [17,18]. The demographic distribution should nevertheless be interpreted as describing the population from which isolates were recovered rather than as establishing independent demographic risk factors for *K. pneumoniae* infection. Because the present study was laboratory-based and did not include a non-infected comparison group, associations between age, sex, and the occurrence of *K. pneumoniae* UTI cannot be inferred directly.

A prominent finding was the very high resistance to ampicillin, observed in 94.8% of isolates. This result is consistent with the well-recognized intrinsic and acquired β -lactam resistance mechanisms of *K. pneumoniae*. A recent systematic review and meta-analysis of hypervirulent *K. pneumoniae* reported an overall ampicillin resistance rate of approximately 95.5%, demonstrating that resistance to this older β -lactam remains extremely common even among strains characterized by hypervirulence-associated features [11]. Therefore, the 94.8% resistance observed in the present isolates is broadly comparable with contemporary international observations. Resistance to the third-generation cephalosporins was also high, with 74.0% of isolates resistant to cefotaxime and 71.2% resistant to ceftazidime. These findings indicate a substantial burden of resistance to important β -lactam agents used for treatment of Gram-negative infections. The magnitude of resistance is comparable with recent reports describing high rates of third-generation-cephalosporin resistance among *K. pneumoniae*. The 2024 systematic review by Beig et al. reported resistance rates of 65.8% for cefotaxime and 57.1% for ceftazidime among hypervirulent *K. pneumoniae*, although considerable geographical variation was identified [11]. The somewhat higher resistance observed in the present study may reflect differences in healthcare setting, antimicrobial exposure, circulating clones, and patient populations.

The findings are particularly relevant to the regional context. A 2025 study of urinary *K. pneumoniae* in Peshawar documented substantial resistance among multidrug-resistant isolates and emphasized the increasing resistance burden among UTI-associated *K. pneumoniae* in the local healthcare setting [19]. Similarly, a Pakistani study comparing community- and hospital-acquired UTI isolates found higher resistance among hospital-associated isolates, particularly to amoxicillin-clavulanate and third-generation cephalosporins, together with detection of clinically important ESBL and carbapenemase determinants [20]. Thus, the high cephalosporin resistance observed at Mardan Medical Complex is consistent with the broader resistance problem documented in Pakistan. Ciprofloxacin resistance was 68.0% in the present study. This finding is important because fluoroquinolones have historically been used extensively for urinary infections, creating strong selection pressure for resistance. Comparable findings have been reported in urinary *K. pneumoniae* studies. Xie et al. reported complete resistance to ciprofloxacin and levofloxacin among their selected multidrug-resistant urinary isolates, with quinolone resistance associated with determinants including *aac(6')-Ib-cr*, *oqxA*, and *oqxB* [21]. The high ciprofloxacin resistance in the current isolates may therefore reflect the cumulative effect of antimicrobial selection and acquisition of plasmid- or chromosome-associated resistance mechanisms.

Gentamicin resistance was 51.2%, indicating that resistance extended beyond β -lactams and fluoroquinolones to aminoglycosides. This multidrug resistance phenotype is concerning because simultaneous resistance across several antimicrobial classes can substantially reduce treatment options. Recent genomic investigations have demonstrated that *K. pneumoniae* can accumulate resistance determinants affecting β -lactams, aminoglycosides, quinolones, and other antimicrobial classes, frequently through mobile genetic elements [18,22].

In contrast, imipenem showed comparatively greater activity, with 73.6% of isolates susceptible and 23.2% resistant. Fosfomycin and nitrofurantoin also showed comparatively higher susceptibility, at 68.4% and 50.4%, respectively. These differences emphasize that antimicrobial susceptibility is strongly agent- and region-dependent. A large multicentre Indian study of urinary *K. pneumoniae* demonstrated substantial geographical variation in antimicrobial susceptibility, with national susceptibility estimates of 81% for meropenem and 89% for fosfomycin, while susceptibility to nitrofurantoin was considerably lower [23]. The comparatively lower susceptibility to nitrofurantoin in the present study may therefore reflect local epidemiological differences.

The comparatively greater activity of carbapenems and fosfomycin should not be interpreted as evidence that resistance to these agents is insignificant. Approximately one-quarter of the isolates were resistant to imipenem and fosfomycin. Contemporary surveillance indicates that carbapenem-resistant *K. pneumoniae* is an increasing concern, particularly because carbapenem resistance can coexist with virulence-associated determinants [15,24]. The clinical significance of this finding is therefore considerable, particularly in a hospital environment where resistant organisms may be exposed to repeated antimicrobial selection.

The present Study reports 12.0% resistance to polymyxin B. Recent Pakistani urinary surveillance has reported full susceptibility to colistin among its studied isolates, while global studies demonstrate substantial geographic variation in polymyxin resistance [20,25]. Any conclusion concerning polymyxin resistance should therefore be based on validated susceptibility testing.

Capsule production was the most frequently detected virulence-associated characteristic in the present study, occurring in 81.2% of isolates. This high frequency is biologically plausible because the polysaccharide capsule is a major virulence determinant of *K. pneumoniae*. It can interfere with complement-mediated killing and phagocytosis and contributes to bacterial persistence within the host [12,26]. Recent reviews continue to identify capsular polysaccharide as a central determinant of *K. pneumoniae* pathogenicity, while emphasizing that capsule structure and quantity differ considerably among lineages [27].

The high prevalence of capsule production in the present study is also consistent with studies demonstrating frequent capsule-associated characteristics among clinical *K. pneumoniae*. However, phenotypic capsule detection alone should not be interpreted as evidence that all capsule-positive isolates are hypervirulent. Hypervirulence is now understood as a multifactorial phenotype involving combinations of capsule-associated determinants, siderophore systems, regulatory genes, adhesins, and particular genetic backgrounds [12,13]. Stanton and Wyres specifically emphasized that no single phenotypic characteristic or gene should automatically be regarded as a definitive marker of hypervirulence [12].

Hypermucoviscosity was detected in 36.4% of isolates by the string test. This proportion is within the range reported in recent clinical investigations. For example, Azra et al. reported hypermucoviscosity in 32.8% of clinical *K. pneumoniae* isolates from Khyber Pakhtunkhwa [28], while other studies have reported substantial but variable frequencies depending on specimen type and patient population [14,29]. The relatively similar prevalence between the current study and the Khyber Pakhtunkhwa study strengthens the possibility that hypermucoviscous phenotypes are circulating in the regional *K. pneumoniae* population.

Nevertheless, the string test should be regarded as a phenotypic indicator rather than a definitive molecular definition of hypervirulence. Recent reviews have highlighted the limited specificity of hypermucoviscosity for identifying hypervirulent *K. pneumoniae* and recommend consideration of broader genetic and epidemiological characteristics [13,30]. Furthermore, recent experimental observations indicate that hypermucoviscous and non-hypermucoviscous variants can coexist within closely related lineages, demonstrating that the phenotype may change without necessarily reflecting a completely different bacterial lineage [31].

The *rmpA* gene was detected in 31.2% of the isolates. This finding demonstrates that a substantial subset of the urinary isolates possessed a recognized virulence-associated regulatory determinant. The prevalence is higher than the 6.4% *rmpA* frequency reported among hospitalized patients in Poland [32], but lower than frequencies

reported in some studies of hypervirulent or invasive *K. pneumoniae* populations. Such variation is expected because *rmpA* prevalence depends strongly on geographical region, clinical specimen, bacterial lineage, and the criteria used to define hypervirulence.

The detection of *rmpA* in nearly one-third of urinary isolates is nevertheless noteworthy. *RmpA* promotes capsule expression and has been mechanistically linked to the hypermucoviscous phenotype [33]. Experimental studies have demonstrated that *rmpA* can enhance capsular polysaccharide production by regulating promoters within the capsule biosynthesis locus [33]. Therefore, the presence of *rmpA* in a subset of urinary isolates may contribute to the enhanced capsular phenotype observed in those strains.

The present result should, however, not be interpreted as indicating that all ***rmpA*-positive isolates are definitively hypervirulent**. Current literature recommends a broader molecular definition incorporating multiple virulence-associated loci and, where appropriate, genomic background [12,13,30]. In particular, genes encoding siderophores such as aerobactin and salmochelin, together with *rmpA/rmpA2* and other markers, can provide stronger evidence of hypervirulent lineages than *rmpA* alone [28,30].

A statistically significant association was observed between *rmpA* carriage and capsule production. Capsule production occurred in 92.3% of ***rmpA*-positive** isolates compared with 76.2% of ***rmpA*-negative** isolates ($p=0.002$). This is biologically coherent with the known regulatory function of *RmpA*. Previous experimental research has demonstrated that *RmpA* positively regulates capsule production, providing a mechanistic explanation for the greater frequency of capsule-positive isolates among ***rmpA*-positive** strains [33].

The current observation is therefore consistent with molecular evidence showing that *rmpA* contributes to increased capsule production. Recent literature on capsule regulation also indicates that the relationship is more complex than a simple one-gene/one-phenotype model because capsule expression can be influenced by additional regulatory pathways and capsular biosynthesis genes [33,34]. Thus, the present association supports the biological relevance of *rmpA*, while also indicating that other determinants probably contribute to capsule expression in *rmpA*-negative isolates.

The strongest virulence association was observed between *rmpA* and the string-test phenotype. Hypermucoviscosity was present in 70.5% of *rmpA*-positive isolates compared with only 20.9% of *rmpA*-negative isolates ($p<0.001$). This finding strongly agrees with the established role of *RmpA* in regulating capsule-associated mucoid phenotypes. Earlier clinical investigations demonstrated a strong association between *rmpA/rmpA2* carriage and hypermucoviscosity, while more recent molecular studies continue to identify *rmpA* as an important regulator of capsule-associated phenotypes [33,35].

The finding is also compatible with contemporary studies showing that hypermucoviscosity frequently occurs together with other virulence-associated characteristics. However, the 29.5% of *rmpA*-positive isolates that were string-test negative is particularly informative. It indicates that *rmpA* carriage does not guarantee expression of a hypermucoviscous phenotype. Conversely, 20.9% of *rmpA*-negative isolates were string-test positive, demonstrating that hypermucoviscosity can occur in the absence of detectable *rmpA*. These observations are consistent with current understanding that capsule regulation involves multiple genetic pathways and that the string test alone has limited specificity for defining hypervirulence [13,33,34].

The study observed numerically higher resistance among *rmpA*-positive isolates for most antimicrobial agents. For example, resistance to cefotaxime was 79.5% among *rmpA*-positive isolates compared with 71.5% among *rmpA*-negative isolates, while ciprofloxacin resistance was 73.1% versus 65.7%, respectively. Similar trends were observed for amoxicillin-clavulanate, ceftazidime, gentamicin, imipenem, nitrofurantoin, fosfomycin, and polymyxin B. However, none of these associations reached statistical significance ($p>0.05$).

This pattern is important because it illustrates that coexistence of virulence and antimicrobial resistance does not necessarily mean that a specific virulence gene directly causes resistance. Recent research has demonstrated that resistance and virulence determinants may be located on separate plasmids, chromosomes, or mobile genetic elements and may therefore occur independently in individual bacterial populations [15,16,24]. Ragheb and Osei Sekyere similarly found no significant association between resistance and virulence genes in hypermucoviscous carbapenem-resistant *K. pneumoniae* isolates from an Egyptian hospital [16]. This provides a particularly relevant comparison because their investigation also involved urinary isolates.

The absence of statistically significant associations in the present analysis is therefore biologically plausible. At the same time, the numerical tendency toward greater resistance among ***rmpA*-positive** isolates deserves surveillance because the convergence of resistance and virulence is increasingly recognized in contemporary *K. pneumoniae*. Recent genomic studies from South and Southeast Asia have identified virulence-associated loci in resistant lineages and have demonstrated the importance of mobile genetic elements in the regional dissemination of both resistance and virulence traits [18]. Similarly, global genomic analyses have identified increasing overlap between antimicrobial-resistant populations and acquisition of virulence loci [25].

A similar pattern was observed when antimicrobial resistance was compared according to the string-test phenotype. Resistance was numerically higher among hypermucoviscous isolates for all tested antibiotics, but none of the differences was statistically significant. This finding again supports the concept that hypermucoviscosity and antimicrobial resistance are related biological characteristics but are not necessarily linked in a simple statistical relationship within every local population.

Recent literature provides evidence for both convergence and independence between these characteristics. A 2024 study of carbapenem-resistant hypermucoviscous *K. pneumoniae* reported heterogeneous resistance and virulence profiles and no significant association between resistance and virulence genes [16]. Conversely, studies of MDR-

hypervirulent strains have documented the simultaneous presence of resistance and virulence determinants, particularly in strains carrying plasmids or other mobile genetic elements [15,24].

The present findings therefore suggest that hypermucoviscosity should not be used as a surrogate for antimicrobial resistance. Similarly, antimicrobial resistance alone cannot establish whether an isolate possesses a hypervirulent phenotype. The two characteristics should be evaluated independently and, where clinically relevant, complemented by molecular detection of multiple virulence and resistance determinants.

When the major virulence characteristics were considered together, capsule production was the most common characteristic (81.2%), followed by hypermucoviscosity (36.4%) and *rmpA* carriage (31.2%). The combination of *rmpA* positivity and hypermucoviscosity occurred in 22.0% of isolates, while *rmpA* positivity with capsule production occurred in 28.8%. The coexistence of these characteristics is noteworthy because strains carrying multiple virulence-associated determinants may have enhanced capacity for persistence, immune evasion, and host colonization.

Recent systematic evidence suggests that hypervirulent *K. pneumoniae* is no longer restricted to a small group of community-associated, antimicrobial-susceptible strains. The prevalence of hypervirulent *K. pneumoniae* has increased over time, while hospital-associated and carbapenem-resistant hypervirulent strains are being increasingly recognized [25]. This epidemiological transition makes local surveillance particularly important.

The findings are also relevant to the regional situation. Recent work from Khyber Pakhtunkhwa identified hypermucoviscous and biofilm-producing *K. pneumoniae* together with substantial antimicrobial resistance, while recent Peshawar research documented a high prevalence of virulence-associated genes among UTI isolates [19,28]. The present study therefore adds institution-specific evidence from Mardan and supports the need for continued surveillance of both antimicrobial resistance and virulence characteristics in urinary *K. pneumoniae*.

Importantly, the coexistence of capsule production, hypermucoviscosity, *rmpA*, and antimicrobial resistance should not automatically be equated with clinically confirmed hypervirulent *K. pneumoniae*. Definitive classification would ideally require additional molecular markers, such as *iucA/iutA*, *iroB/iroN*, *rmpA2*, *peg-344*, capsular typing, and genomic characterization, together with appropriate clinical correlation [12,13,30]. This distinction is increasingly important because contemporary studies demonstrate that hypervirulence is a complex, multifactorial phenotype rather than a single-gene or single-test characteristic.

The study also demonstrates the value of combining phenotypic and molecular characterization. The significant association between *rmpA*, capsule production, and hypermucoviscosity supports a biological relationship between these characteristics, whereas the absence of significant associations between virulence markers and antimicrobial resistance highlights the complexity of their genetic relationship. Similar findings have been reported in recent international investigations, reinforcing the importance of monitoring both resistance and virulence rather than using either characteristic as a surrogate for the other [16,18,25].

CONCLUSION

The present study demonstrated a substantial burden of antimicrobial resistance among urinary *Klebsiella pneumoniae* isolates recovered at Mardan Medical Complex. Very high resistance was observed against ampicillin, while considerable resistance was also detected against cefotaxime, ceftazidime, ciprofloxacin, amoxicillin-clavulanic acid, and gentamicin. In contrast, comparatively higher susceptibility was observed to imipenem and fosfomycin. Regarding virulence-associated characteristics, capsule production was highly prevalent, while hypermucoviscosity and *rmpA* carriage were detected in a substantial proportion of isolates. The significant associations of *rmpA* with capsule production and hypermucoviscosity support its contribution to capsule-associated virulence phenotypes. However, antimicrobial resistance was not significantly associated with *rmpA* carriage or hypermucoviscosity. Overall, the findings highlight the coexistence of antimicrobial resistance and important virulence-associated characteristics among urinary *K. pneumoniae* isolates and emphasize the need for continuous local surveillance, appropriate antimicrobial stewardship, and integrated monitoring of resistance and virulence profiles.

Recommendations

1. Routine antimicrobial susceptibility testing should be performed for urinary *K. pneumoniae* isolates to support laboratory-guided antimicrobial therapy and reduce inappropriate empirical antibiotic use.
2. Antimicrobial stewardship programs should be strengthened at Mardan Medical Complex, particularly regarding the use of third-generation cephalosporins, fluoroquinolones, and other agents showing high levels of resistance.
3. Regular surveillance of antimicrobial resistance should be conducted to identify changes in resistance patterns and guide periodic updates of local empirical treatment protocols.
4. Virulence-associated characteristics should be monitored, particularly capsule production, hypermucoviscosity, and *rmpA*, because their coexistence may identify isolates requiring increased epidemiological attention.
5. Future studies should include additional molecular virulence markers, such as *rmpA2*, *iucA/iutA*, *iroB/iroN*, and other relevant siderophore-associated genes, to provide a more comprehensive assessment of hypervirulence.
6. Molecular characterization of major antimicrobial-resistance determinants, including ESBL and carbapenemase genes, should be incorporated into future investigations to determine the genetic basis of the observed resistance.

7. Infection-prevention and control measures should be reinforced, particularly hand hygiene, appropriate catheter care, environmental cleaning, and screening of important healthcare-associated transmission pathways.
8. Larger multicentre studies involving hospitals from different districts of Khyber Pakhtunkhwa should be conducted to determine the broader regional distribution of antimicrobial resistance and virulence-associated characteristics.
9. Future investigations should incorporate genomic or molecular typing approaches to determine whether resistant and virulent isolates belong to particular sequence types or circulating clones.

Limitations

This study has several limitations.

1. First, it was conducted at a single healthcare facility, Mardan Medical Complex, which may limit the generalizability of the findings to other hospitals or regions.
2. Second, non-probability consecutive sampling was used, and therefore selection bias cannot be completely excluded.
3. Third, the study evaluated selected phenotypic and genotypic virulence characteristics rather than the complete virulence repertoire of *K. pneumoniae*. In particular, detection of *rmpA* alone cannot definitively classify an isolate as hypervirulent.
4. Fourth, additional important virulence determinants, including siderophore-associated and other hypervirulence-associated genes, were not investigated.
5. Fifth, the molecular analysis was limited to *rmpA*, while the genetic mechanisms responsible for antimicrobial resistance were not characterized.

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