

Serotyping of *Klebsiella pneumoniae* and Its Relation with Capsule-Associated Virulence Genes, Antimicrobial Resistance Pattern, and Clinical Infections: A Descriptive Study in Medical Practice

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Alka Hasani ¹⁻³
Elghar Soltani ^{1,2}
Mohammad Ahangarzadeh
Rezaee ^{1,2}
Tahereh Pirzadeh²
Mahin Ahangar Oskouee ²
Akbar Hasani⁴
Pourya Gholizadeh ²
Arezoo Noie Oskouie²
Ehsan Binesh⁵

¹Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; ²Department of Bacteriology and Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; ³Sina Hospital, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; ⁴Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; ⁵Department of Infectious Disease, School of Medicine, Shahroud University of Medical Science, Shahroud, Iran

Objective: *Klebsiella pneumoniae*, one of the clinical superbugs, causes diverse infections because of its variable capsular antigens. This study focused on *K. pneumoniae* and aimed to assess any correlation between capsular serotype, capsule-associated virulence genes, and evaluate its resistance to conventional antibiotics in order to gain insight into any regional differences.

Materials and Methods: A total of 61 *K. pneumoniae* collected from various clinical specimens were confirmed genotypically. Clinical and demographic data for all patients were reviewed. All isolates were subjected to antimicrobial susceptibility tests. Capsular serotyping and capsule-associated virulence genes were studied using the molecular method.

Results: All typeable isolates were typed into K5, K20, and K54 serotypes, and among them, K54 was observed to be predominant. The most common capsule-associated virulence genes comprised *uge* (93.4%), *ycfM* (91.8%), and *wabG* (88.5%), while *wcaG* (29.5%) and *rmpA* (21.3%) were noted at much lower prevalence rates. The gene *wcaG* was significantly associated with K54 positive isolates ($p = 0.001$), while *rmpA* was associated with K20 positive isolates ($p = 0.01$).

Conclusion: Serotype K54 had a high frequency in isolates collected from patients with pulmonary diseases, while serotype K20 was associated with burn patients. Carbapenems and levofloxacin were the best therapeutic options for the treatment of infections with serotypes K20 and K54.

Keywords: *Klebsiella pneumoniae*, capsular serotype, virulence factor, extended-spectrum beta-lactamase, multidrug-resistance, multiplex-PCR

Introduction

Klebsiella pneumoniae is an upcoming superbug of clinical concern.^{1,2} The organism possesses a pronounced capsule, described as a K type, which provides a mucoid phenotype to the isolate³ and is an important virulence factor. The organism has been discriminated into 79 capsular serotypes.^{4,5} Of these, serotypes K1 and K2 are associated with bacteremia and are reported to be correlated with high mortality rates in Taiwan, Europe, and North America.^{6,7} Capsule-associated genes are a prime cause of pathogenicity in *K. pneumoniae* isolates. Virulence factors such as *wabG* (responsible

Correspondence: Elghar Soltani
Immunology Research Center and
Department of Bacteriology and Virology,
Faculty of Medicine, Tabriz University of
Medical Sciences, Tabriz, Iran
Tel +989366295410
Fax +984133808693
Email elghar_soltani@yahoo.com

for the biosynthesis of core lipopolysaccharide), *uge* (uridine diphosphate galacturonate 4-epimerase), and *ycfM* (the outer membrane lipoprotein) are involved in capsule production and promote infection by resistance to phagocytosis.⁸ The plasmid gene *rmpA* (regulator of mucoid phenotype A) provides a hypermucoviscous phenotype to *K. pneumoniae* by enhancing capsular polysaccharide production.⁹ The virulence gene *wcaG* is also responsible for *K. pneumoniae* capsule biosynthesis. The presence of this gene boosts the ability of bacteria to evade phagocytosis by macrophages.^{3,10} It is well known that bacteria undergo several modifications depending on the geographical region and with respect to the time, leading to changes in their characteristics. This study aimed to investigate the clinical features, capsular types, and any correlation between the presence of capsular serotypes with capsule-associated genes and antibiotic resistance in clinical *K. pneumoniae* isolates.

Materials and Methods

Bacterial Isolates

A total of 61 *K. pneumoniae* were isolated from various clinical specimens and the pertinent information on any underlying disease and other demographic data were collected from the medical records. Bacterial isolation and identification were carried out following standard cultural and biochemical techniques such as reactions in TSI (triple sugar iron), MR-VP test, SIM (sulfate/indole/motility) and citrate agar.¹ The hypervirulent phenotype was defined when a colony touched with the loop and lifted vertically from the surface of the agar plate, produced a string-like growth between the loop and the surface of the plate.¹¹ The *K. pneumoniae* was later confirmed genotypically using primers as depicted in [Table S1](#).⁹ The identified isolates were stored in trypticase soy broth containing 20% glycerol at -80°C for further use.

Definition

Hospital-acquired strains or nosocomial-associated bacteria refers to any bacteria contracted by a patient in a hospital at least 72 hours after being admitted, and if the infection was not obviously associated with the clinical conditions of the patient at the time of admission. Otherwise, infection was considered to be community-acquired.¹²

Antibiotic Susceptibility Pattern

Disk diffusion testing was performed and analyzed as per Clinical Laboratory Standards Institute (CLSI) guidelines.¹³ The resistance pattern of *K. pneumoniae* isolates was

analyzed and is presented in [Table 1](#). For confirmation of Extended-Spectrum Beta-Lactamase (ESBL) production, a double-disk test method utilizing cefotaxime and ceftazidime with and without clavulanic acid disks was used.¹³ *Escherichia coli* ATCC 25922 was used as quality control for antibiotic susceptibility testing. The isolates that were resistant to at least one antimicrobial agent in three or more of the categories were considered as MDR (multidrug resistance).¹⁴

DNA Extraction and Multiplex PCR

DNA was extracted from *K. pneumoniae* isolates using the commercial DNA extraction kit (Stratec Biomedical systems, Birkenfeld, Germany). Briefly, 1mL of bacterial suspension matched equivalent to 0.5 McFarland was prepared from an overnight culture and then centrifuged. DNA was extracted as per the instructions provided in the kit from the pellet and finally resolved in 100 μL TE buffer. [Table S1](#) depicts the primers for serotyping (K1, K2, K5, K20, K54, and K57),⁹ confirmation of *K. pneumoniae* (*K. pneumoniae* 16S–23S ITS)⁹ and capsule-associated virulence genes (*rmpA*, *wcaG*, *uge*, *ycfM*, and *wabG*).¹⁰ Amplification of the respective genes was carried out as a multiplex PCR performed as a 25 μL reaction mixture with 5pmol of each primer and 2 μL of DNA added to the Master PCR mixture (Yekta Tajhiz Azma[®], Iran). Two separate Multiplex PCRs were carried out with the same thermal cycling conditions for detecting the capsular serotypes and capsule-associated genes as described previously.^{9,10} The amplified products

Table 1 Antibiotic Pattern of *K. pneumoniae* Isolates

No. of Profiles	Resistance Profiles	No. of AB	No. of Isolates ^a
I	PTZ, NI	2	2
II	SXT, NI	2	2
III	CP, SXT, CAZ, CTX, NI	5	3
IV	SXT, CAZ, CTX, NI, LEV	5	2
V	CP, SXT, CAZ, CTX, PTZ, NI	6	2
VI	GM, CP, SXT, CAZ, CTX, PTZ, NI	7	2
VII	GM, CP, SXT, CAZ, CTX, PTZ, NI	7	2
VIII	GM, CP, AN, SXT, CAZ, CTX, PTZ, NI	8	5
IX	GM, CP, AN, IMI, MEM, SXT, CAZ, CTX, PTZ, NI, LEV	11	2

Note: ^aProfile of two or more similar isolates was included.

Abbreviations: GM, gentamicin; CP, ciprofloxacin; AN, amikacin; IMI, imipenem; MEM, meropenem; SXT, co-trimoxazole; CAZ, ceftazidime; CTX, cefotaxime; PTZ, piperacillin-tazobactam; NI, nitrofurantoin; LEV, levofloxacin; AB, antibiotic disks.

were electrophoresed on 1% agarose gel (Yekta Tajhiz Azma[®], Iran) and stained with Cyber safe stain (Yekta Tajhiz Azma[®], Iran).

Data Analysis

The logistic regression model was performed for the multivariate analysis to identify risk factors for mortality. We used the X^2 test and the Fisher exact test (if necessary) to find the relationship between serotypes and other variables. P-value <0.05 was considered significant statistically. The data were analyzed with SPSS statistics (Version 20) program (IBM Corporation).

Ethical Approval

This study was approved by the Regional Ethics Committee of Tabriz (Tabriz University of Medical Sciences, Tabriz, Iran, No. IR.TBZMED.REC.1397.058).

Results

Among 468 bacterial isolates obtained from various clinical specimens, 61 (13.03%) isolates were identified as *K. pneumoniae* by traditional biochemical tests. Urine was the most common [n=31; (50.8%)] clinical specimen from which *K. pneumoniae* was isolated, followed by wound [n=15; (24.6%)], blood [n=8; (13.1%)], endotracheal aspirate [n=4; (6.6%)], and other body fluids [n=3; (4.9%)]. All 61 isolates were positive for the internal

transcribed spacer region (*K. pneumoniae* 16S–23S) and were confirmed at the molecular level as *K. pneumoniae*.

Serotyping

Of the 61 *K. pneumoniae* isolates, 36 (59%) were found to be typeable with K5, K20, and K54 primers, while 25 (41%) were non-typeable. Serotype K54 was the most prevalent [n=18; 29.5%] followed by K20 and K5, which accounted for 13 (21.3%) and 5 (8.1%) isolates, respectively (Table 2).

Clinical Wards

Considering the source, 43 (70.5%) isolates were obtained from in-patients admitted to five different intensive care units (ICU) and six other wards (Table 2). *K. pneumoniae* was the causative agent of nosocomial infection in ICU patients with a high prevalence [n= 22; 36.2%], and the most common isolates were obtained from the burn ICU [n=7; 11.5%]. The hospital lengths of stay of patients from whom *K. pneumoniae* were isolated ranged from 7 to 60 days. One patient admitted to the infectious ICU had the longest duration of hospitalization, equaling approximately 205 days. The distribution of capsular serotypes, and capsule-associated virulence genes among the various clinical wards of the hospital is depicted in Table 2. The K20 serotype and *rmpA* gene were prevalent in patients admitted to the burn ICU and burn wards ($p < 0.05$, Table 2).

Table 2 Relation of K-Serotypes with Capsule-Associated Virulence Factors in Various Clinical Wards

Wards	K. pneumoniae N (%)	K-Serotypes			Virulence Factors				
		K54 (n=18)	K20 (n=13)	K5 (n=5)	wcaG (n=18)	rmpA (n=13)	uge (n=57)	ycfM (n=56)	wabG (n=54)
		Burn ICU	7 (11.5)	2	5*	0	2	4*	7
General ICU	6 (9.8)	1	0	0	1	0	6	6	6
Infectious ICU	4 (6.6)	4	1	0	4	0	3	4	4
Internal ICU	3 (4.9)	0	0	1	0	0	2	2	2
Surgery ICU	2 (3.3)	1	0	1	1	0	2	2	2
Internal	8 (13.1)	2	0	2	2	0	8	7	7
Burn	6 (9.8)	1	4*	0	1	5*	6	5	5
Urology	6 (9.8)	2	0	0	2	1	5	5	4
Surgery	2 (3.3)	2	0	0	2	2	2	2	2
Emergency	2 (3.3)	0	0	0	0	0	2	2	2
Infectious	4 (6.6)	1	2	0	1	0	4	4	3
Out-patients	11 (18)	2	1	1	2	1	10	10	10

Note: *P-value<0.05, calculated by Chi-square test or Fisher exact test.

Abbreviations: ICU, intensive care unit; K, capsular polysaccharide (K antigen).

Table 3 Demographic and Clinical Data of 61 *K. pneumoniae*-Infected Patients and Relation with Serotypes

Variables	Number (%)	Statistically Significant Association with Capsular Serotypes
Gender		
Male	28 (45.9)	-
Female	33 (54.1)	K20*
Infection setting		
Hospital-acquired	34 (55.6)	K5, K20, K54*
Community-acquired	27 (44.4)	-
Age (mean ± SD)	56.70 ± 23.42 years	-
Accommodation		
Urban	43 (70.5)	-
Rural	18 (29.5)	-
Underlying medical conditions		
ICU stay	22 (36.2)	-
Bed-ridden	43 (70.5)	-
Radiography	34 (55.7)	-
Ultrasonography	21 (34.4)	-
CT scan	18 (29.5)	-
Tracheostomy	5 (8.2)	-
Bronchoscopy	4 (6.6)	-
Cystoscopy	4 (6.6)	-
Skin graft	11 (18)	K20*
Mechanical ventilation	9 (14.8)	-
Pneumonia	7 (11.5)	-
Surgery procedure	18 (29.5)	-
Prior antibiotic usage	43 (70.5)	-
Diseases		
Pulmonary	11 (18)	K54*
Renal diseases and UTI	20 (32.7)	-
Gastrointestinal	7 (11.4)	-
Infectious disease	7 (11.4)	-
Ulcer and abscess	6 (9.7)	-
Burn	10 (16.4)	K20*
Diabetes mellitus	5 (8.2)	-
DJ procedure	5 (8.2)	-
Bacteremia	3 (4.9)	-
Cardiovascular	2 (3.3)	-
Hyperplasia of prostate	2 (3.3)	-
Syndromes		
Kimmel-Wilson syndrome	4 (6.6)	-
Respiratory distress syndrome	2 (3.3)	-

(Continued)

Table 3 (Continued).

Variables	Number (%)	Statistically Significant Association with Capsular Serotypes
Outcome		
Survived	50 (82)	-
Died	11 (18)	-

Note: *P-value<0.05, calculated by Chi-square test or Fisher exact test.

Abbreviations: SD, standard deviation; ICU, intensive care unit; CT, computed tomography; UTI, urinary tract infection; DJ, double J; K, capsular polysaccharide (K antigen).

Clinical Data

Table 3 shows that 28 (45.9%) and 33 (54.1%) of isolates were isolated from males and females, respectively. Thirty-four isolates (55.6%) were collected as hospital-acquired and 27 (44.4%) were community-acquired. Eighteen (29.5%) patients were residents of rural areas. The ages of patients ranged from 3 to 89 years (mean ± SD 56.7 ± 23.42 yrs.). Almost 50% of infections occurred in elderly patients (≥60 years). When patients were studied for any underlying medical condition or had undergone medical modality, it was found that most of the patients had undergone either radiography [n=34; 55.7%] or ultrasonography [n=21; 34.4%]. Other medical interventions included previous surgery [n=18; 29.5%] and a need for mechanical ventilation [n=9; 14.8%]. Table 3 also shows the patients afflicted with various types of *K. pneumoniae*-associated diseases. K54 was the most common serotype significantly ($p = 0.001$) compared to patients with pulmonary diseases [8 (72.7%) of the 11 patients afflicted with pulmonary disease], while K20 correlated ($p = 0.003$) with burn patients who had undergone skin grafting [7 (70%) of 10 infected burn patients]. When the typeability of *K. pneumoniae* was compared between the two hospital settings, 24 of 34 (71%) hospital-acquired isolates were typeable in comparison to only 12 of 27 (45%) community-acquired isolates ($p = 0.03$). Urinary tract infection (UTI), renal disease, diabetes mellitus, and bacteremia with frequencies of 13 (21.3%), 7 (11.4%), 5 (8.1%), and 3 (4.9%) patients, respectively, were the common diseases observed from community-acquired *K. pneumoniae* infections in this study. One patient suffered from both diabetes mellitus and renal disease. There was no statistical relation between the three common capsular serotypes and community-acquired infections.

Table 4 Distribution of Putative Capsule-Associated Virulence Genes in *K. pneumoniae* in Predominant Capsular Serotypes

		Capsule-Associated Genes ^a				
		<i>rmpA</i> (n=13)	<i>wcaG</i> (n=18)	<i>uge</i> (n=57)	<i>ycfM</i> (n=56)	<i>wabG</i> (n=54)
Hospital-acquired isolates		12*	13*	32	31	31
Capsular serotypes	K5 (n=5)	0	2	5	5	5
	K20 (n=13)	8*	3	12	12	12
	K54 (n=18)	6	18*	18	17	18

Note: ^aAs the capsule-associated genes were common among two serotypes, thus, the numbers cannot add up to numbers mentioned for each of them. *P-value<0.05, calculated by Chi-square test or Fisher exact test.

Abbreviation: K, capsular polysaccharide (K antigen).

Prevalence of Capsule-Associated Genes in Predominant Serotypes

Overall, *uge* was the most commonly detected putative virulence gene [n=57; (93.4%)], followed by *ycfM* [n=56; (91.8%)] and *wabG* [n=54; (88.5%)]. The *wcaG* [n=18; (29.5%)] and *rmpA* [n=13; (21.3%)] genes had lower distributions among various K-serotypes. The presence of *wcaG* was highly associated with K54 positive isolates [18/18 (100%); $p = 0.001$], while *rmpA* had a higher prevalence among K20 positive isolates [8/13 (61.5%); $p = 0.01$]. The source of both *wcaG* and *rmpA* positive isolates was the hospital [$p < 0.05$] (Table 4).

Prevalence of Serotypes and Capsule-Associated Genes in Both Genders

Figure 1 displays the distribution of capsular serotypes with capsule-associated genes in *K. pneumoniae*. K20 serotype had a higher prevalence in clinical specimens collected from females than males ($P = 0.02$). In contrast, the distribution of K5, and K54 serotypes with five capsule-associated virulence factors was not different between genders.

Mucoid Phenotype

The mucoid phenotype detected by the string test was observed in 16 (26.22%) isolates, and the sources of these isolates comprised 7 (43.7%) from urine, 5 (31.2%) from wounds, 2 (12.5%) from blood, and 2 (12.5%) from other body fluid.

Table 5 depicts that of the 16 mucoid phenotype isolates, 14 were typeable [87.5%; $p=0.01$]. Six of the 18 K54 positive isolates had a mucoid phenotype ($p = 0.304$), while such correlation was observed in 10 of the 13 K20 positive isolates ($p < 0.001$) and in only 1 of the K5

positive isolates ($p = 0.606$). Among the 13 *rmpA* positive *K. pneumoniae* isolates, 10 (76.9%; $p = 0.003$) had mucoid phenotype.

Among 36 ESBL-positive isolates, only 10 were found to be associated with mucoid phenotypes ($p = 0.490$). In two isolates (with both serotype K5 and ESBL positivity), no mucoid phenotype was detected, while among nine isolates with both serotype K20 and ESBL positivity, 7 were observed to be associated with mucoid phenotypes ($p < 0.001$). Similarly, among 12 isolates with serotype K54 and ESBL producers, 5 were found to be associated with mucoid phenotypes. In 12 isolates with both *rmpA* and ESBL positivity, 7 were observed to be associated with mucoid phenotypes ($p < 0.001$). Comparatively, among 25 non-ESBL isolates, only 6 were found to have the mucoid phenotype. Among the *K. pneumoniae* isolates obtained from in-patients, 9 (75%) had mucoid phenotype. The one isolate obtained from an out-patient also had the same morphology.

Antibiotic Resistance

The outcome of the antibiotic resistance pattern is depicted in Table 1. All *K. pneumoniae* isolates could be typed into nine different antibiotypes, and among these resistance patterns, profiles number VIII (with 5 isolates) and III (with 3 isolates) had the highest frequencies.

Resistance to the tested antibiotics was as follows: cefotaxime 78.8%, ceftazidime 75.4%, ciprofloxacin 68.9%, nitrofurantoin 68.9%, co-trimoxazole 67.2%, piperacillin-tazobactam 57.4%, gentamicin 45.9%, amikacin 39.3%, imipenem 24.6%, meropenem 24.6%, and levofloxacin 24.6%. Among the *K. pneumoniae* isolates studied, 36 (59%) of them were found to be ESBL producers, while 47 (77%) produced MDR. Twenty-four of these ESBL-positive isolates were related to hospital-acquired infections ($p = 0.036$). Serotype K20 was significantly associated with resistance to amikacin and gentamicin [n=9 (69%), $p = 0.01$], while serotype K54

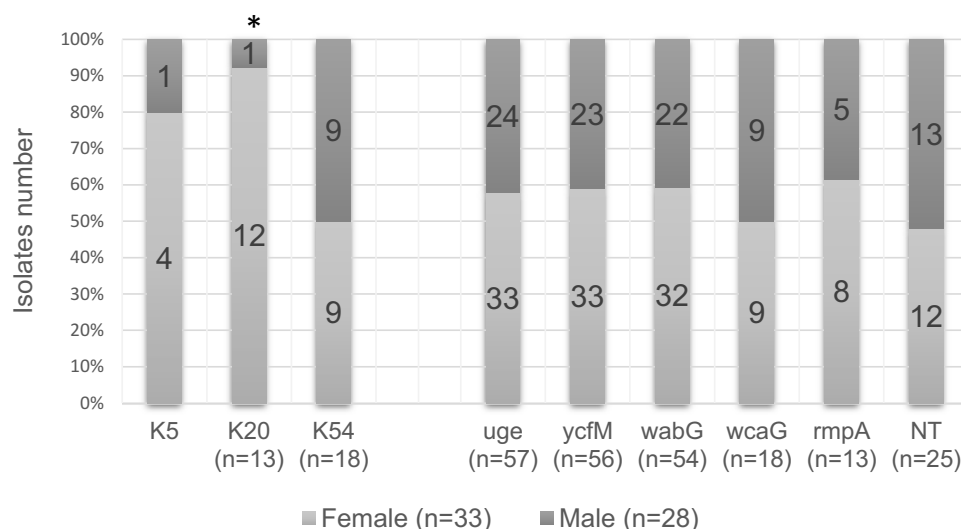


Figure 1 Distribution of capsular serotypes and capsule-associated virulence genes among the isolates collected from male and female specimens.

Note: *Higher prevalence of K20 serotype was found in the clinical specimens collected from females in comparison to males (p -value = 0.02). NT: the isolates that did not belong to the K1, K2, K5, K20, K54, or K57 serotype.

Abbreviations: K, capsular polysaccharide (K antigen); NT, non-typeable.

was found to be significantly associated with resistance to ciprofloxacin [16 of 18 [88%], $p = 0.02$]. Serotype K54 was a prevalent serotype with MDR [17 of 18 (94.4%), $p = 0.02$]. Among the capsule-associated virulence genes, *rmpA* had a high frequency in ESBL-producing isolates [12 of 13 (92.3%), $p = 0.01$], while *wcaG* had a high prevalence in MDR-producing isolates [17 of 18 (94.4%), $p = 0.02$] and was also related to ciprofloxacin resistance [16 of 18 (88.8%), $p = 0.03$]. Other capsule-associated virulence factors, *uge*, *ycfM*, and *wabG*, had high prevalence rates and were similarly distributed among all antibiotic-resistant isolates. Interestingly, resistance to imipenem, meropenem, and levofloxacin was low and was found to be the best choices for treatment of these serotypes.

When hospital-acquired ESBL *K. pneumoniae* strains were compared for capsule-associated genes, 11 of the 13 *rmpA* positive isolates had a significant ($p < 0.001$) relation with hospital-acquired ESBL strains. Conversely, 6 of 18 *wcaG* positive, 21 of 53 *wabG* positive, 21 of 55 *ycfM* positive, and 21 of 56 *uge* positive isolates were not related to hospital-acquired ESBL strains [$p = 0.500$), ($p = 0.451$), ($p = 0.642$), and ($p = 0.498$), respectively].

Risk Factor for Mortality

In the current study, the crude mortality rate was 18%. By multivariate logistic regression analysis, some variables, like old age [≥ 60 years] ($p=0.02$), infection with MDR ($p=0.04$), long-term usage of amikacin ($p=0.01$), infectious

diseases [septicemia, tetanus, peritonitis, and wound infections] ($p=0.03$), and lung disease ($p=0.01$), were associated with a significantly higher mortality rate. Among the three predominant serotypes in this investigation, the K54 serotype was associated with higher mortality and variables such as old age ($p=0.04$), infection with MDR ($p=0.03$), and pulmonary diseases ($p=0.02$).

Discussion

In the present investigation, six capsular serotypes and capsule-associated virulence factors were assessed in 61 *K. pneumoniae* isolates obtained from various clinical sources. The rate of hospital-acquired *K. pneumoniae* infections was 55.7%, which is higher than other hospital-acquired reports in Vietnam (29.5%)¹⁵ and in ICUs in Southern Europe, Turkey, and Iran (23.5%),¹⁶ which may be due to the type of infections seen in medical practices at various places.

The mortality rate of *K. pneumoniae*-associated bacteremia varies from 20% to 70% depending on the treatment regimen and disease severity.^{17–19} In two studies which dealt with bloodstream infections (BSI) caused by *K. pneumoniae*, the overall mortality ranged from 35.6% to 36.7%.^{20,21} By multivariate analysis, some variables were recognized as predictors of mortality associated with *K. pneumoniae* infections such as isolates being MDR, resistance to amikacin, presence of infectious diseases, and involvement of the pulmonary system. The total

Table 5 Correlation of Mucoïd Phenotype with K-Serotypes, Capsule-Associated Virulence Genes, and ESBL Among *K. pneumoniae* Isolates

Number (%) of		Capsular serotypes			NT ^a isolate (n=25)	Capsule-associated virulence factors						Extended-spectrum beta-lactamase					
Mucoïd phenotype	Typeable isolate (n=36)	K5 (n=5)	K20 (n=13)	K54 (n=18)	NT ^a isolate (n=25)	rmpA (n=13)	wcaG (n=18)	uge (n=57)	ycfM (n=56)	wabG (n=54)	ESBL isolates (n=36)	K5, ESBL ^b (n=2)	K20, ESBL ^b (n=9)	K54, ESBL ^b (n=12)	rmpA, ESBL ^c (n=12)	Non-ESBL isolates (n=25)	
																	Total=16 (26.22%)
					2 (12.5)	10 (62.5)*	3 (18.7)	15 (93.7)	15 (93.7)	15 (93.7)	10 (62.5)	0	7 (43.7)*	3 (18.7)	7 (43.7)*	6 (37.5)	

Note: ^aNT, the isolates that were not found to be typeable with K1, K2, K5, K20, K54, and K57 primers. ^bIsolates with both K5/K20 serotype and ESBL positivity. ^cIsolates with both rmpA gene and ESBL positivity (the only virulence factor with a significant association with mucoïd phenotype). *P-value<0.05, calculated by Chi-square test or Fisher exact test.

Abbreviations: NT, non-typeable; ESBL, extended-spectrum beta-lactamase; K, capsular polysaccharide (K antigen).

mortality rate in the current study was 18%, which is lower than the mortality rates reported earlier.²² Because all kinds of infections caused by *Klebsiella pneumoniae* were assessed in this study, the mortality rate was low compared with specifically mentioned infections such as bacteremia.^{19,20} Concerning community-acquired infections, two available studies from India²³ and New York²⁴ reported that *K. pneumoniae* infections are often associated with pyogenic liver abscess. In contrast, research data from the present investigation showed a higher prevalence of hospital-acquired infections and the absence of serotypes K1, K2, and K57. Moreover, this study did not include any patients with liver abscess.

ESBL production and MDR in *K. pneumoniae* are major problems in patient care globally.²⁰ According to the current results, MDR isolates were more related to mortality outcomes, perhaps because of delays at the beginning of the appropriate therapy. Similar to the study by Tsay Ren-Wen et al,¹² high mortality was correlated to the presence of lung infection.

Of the 61 *K. pneumoniae* isolates, 36 (59%) were typeable, distributed in the three serotypes K5, K20, and K54. Though research studies performed elsewhere have shown more than 85% of isolates as being typeable with vast distributions,^{25,26} other investigations with high numbers of isolates could not wholly type the isolates.^{12,27,28} Most of the serological studies done on *K. pneumoniae* have been related to pyogenic liver abscess syndrome and its relationship with capsular serotypes. *K. pneumoniae* isolates from liver abscess specimens have similar characteristics, like the prevalence of serotype K1 or K2 and genomic heterogeneity.^{28,29}

A recent study reported from Iran³⁰ found K54 as the most frequent (68%) capsular serotype while K1 (8%) was the lowest frequency. However, contrary to our findings, a higher frequency of serotype K5 (60%) was reported.³⁰ Prevalence of K5, K20, and K54 serotypes was significantly lower in Taiwan and Europe^{25,31} in comparison to our study. In a study similar to the present study by Turton et al⁹ from the UK in 2010, reported a higher incidence of serotype K54 compared to other capsular types. However, contrary to the results of the present study, the K2 serotype had the highest frequency. The differences in the frequency of capsular serotypes may be due to differences in the types of samples collected in other studies. Only serotyping was performed in this investigation; thus, we cannot confirm an outbreak. Secondly, as the isolates were obtained from different wards (though same hospital sources), it can be an outbreak

of three predominant serotypes. Nevertheless, precise typing molecular methods are required to confirm this.

The present results demonstrated the presence of *uge* (in 93.4% of isolates), *ycfM* (91.8%), and *wabG* (88.5%) capsule-associated genes among the *K. pneumoniae* isolates encoding capsule lipoprotein, external membrane protein, and capsule, respectively. Two other studies from Turkey and Iraq^{10,32} reported the prevalence of these genes in more than 80% of their *K. pneumoniae* isolates. These genes are also involved in lipopolysaccharide biosynthesis and promote infection by resistance to phagocytosis.⁸ Therefore, because *uge*, *ycfM*, and *wabG* were commonly found in *K. pneumoniae* isolates, they seem to be at the basis of the pathogenicity of *K. pneumoniae*. Earlier *uge* and *wabG* genes have been identified from patients with invasive and serious infections.³³

According to previous studies,^{34,35} the plasmid-borne gene *rmpA* is related to capsule production. It has also been associated with 6 PLA (pyogenic liver abscess)-related capsular types (K1, K2, K5, K54, K57, and KN1).^{34,35} In the current investigation, the absence of a liver abscess specimen may be the main reason for the absence of K1 and K2 capsular serotypes. The results showed that *rmpA* had a high frequency with K20 serotype ($p < 0.05$). Moreover, 61.53% of *rmpA* genes and 76.9% of K20 serotypes were associated with the mucoid phenotype. Two other research studies previously performed in Taiwan showed that a 180-kilobase plasmid holding *rmpA* (regulator of mucoid phenotypes) was associated with the mucoid phenotype.^{36,37} Another putative virulence factor which may encode capsular fucose synthesis in *K. pneumoniae* and assist the bacterial evasion from the phagocytosis is the presence of *wcaG*,^{34,35} a gene involved in capsule production.³⁸ Turton et al demonstrated that the presence of *wcaG* in their isolates was correlated with K1, K16, K54, and K58 capsular serotypes.⁹ The current results showed that *wcaG* was associated with the K54 serotype ($p < 0.05$). Earlier, *K. pneumoniae* isolates possessing *wcaG* were isolated from patients with serious and invasive diseases.⁹ The outcomes of two researches indicated *wcaG* had a high effect on *K. pneumoniae* virulence.^{9,39} Of the five capsule-associated genes studied in the present investigation, *wcaG* was correlated with serotype K54, while *rmpA* was correlated with K20. According to the present findings, *rmpA* had a high prevalence in ESBL-producing isolates, and *wcaG* was found to be the predominant capsule-associated gene in MDR isolates. Serotype K20 and *rmpA* positive isolates were associated with a mucoid phenotype. Moreover, isolates with resistance to gentamicin

and amikacin were correlated with serotype K20, while ciprofloxacin-resistant isolates were concomitantly related to serotype K54. These antibiotic consequences are significant in therapeutic spheres, where physicians can prescribe either carbapenems or medications from other drug groups apart from aminoglycosides or fluoroquinolones for *K. pneumoniae* infections in our region; however, this necessitates the confirmation of antibiotic susceptibility towards carbapenems at the molecular level.

In this study, it was found that K5 and K54 had higher prevalence rates in females compared with males. As the sample size was small, no further conclusions cannot be made on such type of associations. Not many publications are available on the relationship of K-serotypes with gender or age. However, Liu and Guo (2019) in their investigation on bacteremic patients collected 175 *K. pneumoniae* isolates but found no significant differences in gender or age between the two groups in terms of serotype prevalence.⁴⁰ Similarly, Lee et al (2010) studied 91 *K. pneumoniae* from various clinical disorders and found K1/K2 serotypes; however, none of them were correlated with the male gender.⁴¹

The distribution of prevalent capsular serotypes in various medical wards was also assessed, and serotype K20 was found to be associated with infections in the burn ICU and burn wards, especially among patients who developed skin infections after grafting. Serotype K54 was found to be associated with pulmonary diseases. Other studies have assessed the association of serotypes K1 and K2 with diseases only. For example, Chi-Tai Fang et al from Taiwan found serotype K1 to be an emerging pathogen capable of causing central nervous system complications and catastrophic septic ocular.³¹ The current study is the first of its kind to focus on any association between capsular serotypes, various medical wards, and antibiotic resistance and to show the relationship of serotypes K20 and K54 with the above-mentioned diseases.

Conclusion

The current study found that diverse capsular serotypes are involved in *K. pneumoniae*-associated infections. Serotype K54 had a high frequency in pulmonary diseases, while serotype K20 was associated with burn infections. Of the five capsule-associated genes studied, *wcaG* was correlated with serotype K54, while *rmpA* was correlated with K20. Serotype K20 and *rmpA* positive isolates were associated with a mucoid phenotype. Carbapenems and levofloxacin could be recommended for the treatment of

infections with serotypes K20 and K54. Information about the distribution of capsular serotypes in specific diseases and medical wards with a special pattern of antibiotic resistance could aid physicians in prescribing appropriate treatments.

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Disclosure

The authors report no conflicts of interest in this work.

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