Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: www.ijmronline.org

Original Research Article

Genetic analysis of virulence factors of MDR *Klebsiella pneumoniae spp. pneumoniae* isolated from clinical specimens - study from a tertiary care hospital

Bhavita Thakorbhai Prajapati[1], Anant Prabhakar Marathe[1]*, Asmabanu Mahammed Hanif Shaikh[1], Shital Sangani[2]

[1]Dept. of Microbiology, Parul Institute of Medical Sciences and Research, Faculty of Medicine, Parul University, Vadodara, Gujarat, India
[2]Faculty of Medicine, Parul University, Vadodara, Gujarat, India

Abstract

Background: *Klebsiella pneumoniae* is a predominant nosocomial pathogen owing to its potent virulence factors, viz, capsule, fimbriae, siderophores, efflux pump, regulatory secretions, and secretory system. As most nosocomial *K. pneumoniae* infections are MDR/XDR, treatment remains limited. The understanding of the different virulence mechanisms and their genetic control becomes imperative.
Aims and Objective: To determine the prevalence and distribution of virulence genes among *Klebsiella pneumoniae* isolates.
Materials and Methods: In the present study, virulence genes were analysed with the help of whole-genome sequencing. A total of 41 *K. pneumoniae* isolates from various nosocomial infections were collected, identified by VITEK 2 and MALDI-TOF-MS, and confirmed by whole genome sequencing. Virulence genes were studied using the Virulence Finder database, a web-based bioinformatics tool that identifies virulence genes in bacterial isolates.
Results: The genes for Type I fimbriae were found in all clinical specimens. Variation was observed in type III fimbriae isolated from bloodstream Infections. Besides the virulence factors seen in Enterobacterales, the *K. pneumoniae* also possessed virulence factors like enterobactin, aerobactin, salmochelin, and yersiniabactin siderophores. Additional secretory system Types 1 and 3 were detected in our strains, and variation was observed in Type 2.
Conclusion: Our study provides insights into the virulence factors of *K. pneumoniae*, revealing regional diversity and identifying predominant genes associated with hypervirulent strains. We found type 3 fimbriae genes in most isolates, linked to biofilm formation, and detected MDR nosocomial hypervirulent strains with K2 and K64 capsular types. Notably, the ST395 strain, prevalent in our region, exhibited multiple virulence factors, making it a highly virulent strain.

Keywords: *Klebsiella pneumoniae*, Virulence factors, Whole genome sequencing.

Received: 01-08-2025; **Accepted:** 10-09-2025; **Available Online**: 20-09-2025

1. Introduction

K. pneumoniae is an encapsulated, gram-negative, non-motile bacterium found in the environment.[1] It is also a colonizer in the mucosal lining of the human Gastrointestinal and oropharynx.[1] *K. pneumoniae* is a common cause of community-acquired pneumonia (CAP).[2] MDR *K. pneumoniae* is a huge problem worldwide as a nosocomial pathogen.[2] The World Health Organization (WHO) classified *K. pneumoniae* as a critical priority healthcare-associated pathogen.[3]

A pooled analysis conducted by Nur Ain Mohd Asri and coworkers revealed a global prevalence of 32.8% nosocomial MDR *K. pneumoniae*.[4] However, a study done by Lodhi, Lakshminarayana S A, and Aaftab G.P indicates 34.37% prevalence in India.[5]

Klebsiella pneumoniae is a leading cause of various nosocomial infections, including bloodstream infections, respiratory tract infections, urinary tract infections, surgical site infections, and meningitis.[4] Neonates, the elderly, and immunocompromised patients are the most vulnerable

*Corresponding author: Anant Prabhakar Marathe
Email: bhavita.prajapati12@gmail.com

https://doi.org/10.18231/j.ijmr.12671.1937092237

groups due to undeveloped bodies, weak immune defence systems, and suffering from underlying chronic illness, respectively.[4]

K. pneumoniae's virulence factors include capsule production, fimbriae, siderophores, and allantoin metabolism, which enable the bacterium to adhere to host cells, acquire essential nutrients like iron, evade the immune system, and persist in the host, thereby contributing to its pathogenicity.[6] This complex array of virulence factors allows *K. pneumoniae* to cause a range of infections, from urinary tract infections to severe pneumonia and sepsis.[6]

Virulence genes in *Klebsiella pneumoniae* vary across different geographic regions and disease statuses.[7] However, research on virulence factors of multidrug-resistant (MDR) *K. pneumoniae* using whole-genome sequencing (WGS) is limited in India.

The objective of a study is to investigate the genetic characteristics of multidrug-resistant *Klebsiella pneumoniae* isolates from our tertiary care hospital, with a primary focus on genetic analysis of various virulence factors, which makes it a predominant nosocomial pathogen. The Secondary Objective is to investigate the pathogenic role of virulence factors in biofilm formation and identify genes associated with hypervirulent strains.

2. Material and Methods

This study was carried out on culture specimens received from ICUs in the central clinical laboratory of a tertiary care hospital in Vadodara, Gujarat, India. The isolates in this study were obtained from clinical samples of patients with confirmed HAI (Hospital Acquired Infection) as defined by standard CDC guidelines for infection prevention and control. *K. pneumoniae* isolates were classified as multidrug-resistant (MDR) based on WHOnet software analysis.

2.1. DNA extraction

Extraction of DNA of MDR *K. pneumoniae* was carried out from culture (confirmed by VITEK 2 and MALDI-TOF-MS) by the manual cetyltrimethylammonium bromide (CTEB) method,[8] and the quality of DNA was checked through gel electrophoresis.[8]

The library was prepared according to the protocol of the Nextera XT DNA Library Prep Kit, and using the QIAxcel Advanced System, a purity check was fulfilled.[8] The prepared libray was sequenced in the Illumina Novaseq6000 machine.[8] Genome assembly and annotation were performed using online software, BV-BRC.[9]

2.2. Tool used for genomic data analysis

1. PubMLST (https://pubmlst.org/) is used for Genomic identification of bacterial species.[10]
2. Virulence gene detection in Virulence Finder Database (VFDB) (http://www.mgc.ac.cn/Vfs/).[11]
3. Kaptive (http://kaptive.holtlab.net/) is used for capsular typing.[12]
4. Sequence typing (ST) - Center for Genomic Epidemiology, Multi Locus Sequence Typing (MLST) 2.0 used. (https://www.genomicepidemiology.org/services/).[10]

3. Result

A total of 41 *K. pneumoniae* phenotypically confirmed (by VITEK 2 Compact System and MALDI-TOF MS) isolates were also confirmed with whole-genome sequencing (WGS). Out of 41, 40 were *K. pneumoniae* spp *pneumoniae* while 1 was identified as *K. quasipneumoniae*.

Klebsiella pneumoniae spp. pneumoniae (*K. pneumoniae*) isolates were recovered from blood samples (49%, 20/41), respiratory samples (37%, 15/41), urine (5%, 2/41), and other sources (9%, 4/41).

3.1. The genomic study of virulence factors

In the present study, we investigated all virulence factors of *K. pneumoniae* (according to genomic analyses, the most prevalent *K. pneumoniae* strain in our hospital is ST 147, followed by ST 395) to determine their role in various nosocomial infections. The sequence types of isolates are shown in **Figure 1**.

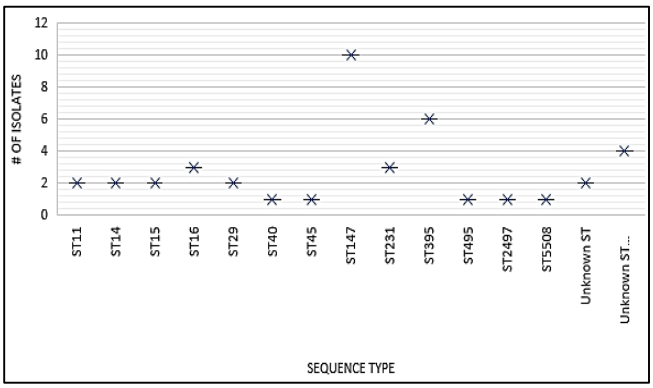


Figure 1: Sequence types (STs) among all MDR *K.pneumoniae* isolates according to MLST analysis

3.2. Capsular polysaccharide (CPS): Antiphagocytic

Out of 41 isolates, 40 isolates had a Capsular gene present, while 1 isolate had the Enterococcus capsular gene cpls, shown in **Table 1**.

3.3. Fimbriae: Adherence

All 42 isolates had Type I fimbria genes (*fimA* to *I* and *K*). The fimK was absent in *K. qusipneumoniae* (isolated from Blood Specimens). However, Type III fimbria-related genes (mrkA to J, except E) were present in Respiratory, urine, and other (SSI) isolates. In the case of isolates from blood specimens, Type 3 fimbriae were detected in 75% (mrkD, F, and J), 80% (mrkA and I), 85% (mrkB), 95% (mrkC), and

100% (mrkH). Type IV pili (biosynthesised by Pseudomonas) gene pilU was found in 3 isolates, shown in **Table 2**. Type IV pili (Yersinia) gene pilW was found in all ST 147 (10/10), followed by ST 231 (3/3), ST 14(2/2), ST 29(2/2), and ST 11 (1/2).

3.4. Iron uptake

As tabulated in the **Table 3**, Enterobactin siderophore genes (entABCDEF and S) were present in all isolates. In Aerobactin, the dominant gene was iutA, found in all specimens, except one, from respiratory specimens. While remaining genes like iucA, iucB, iucC, and iucD were present in ST 395: K64 (5/6). In ST 231: K51 (3/3), iucA, iucB, and iucC genes were present, and the iucD gene was absent (3/3). Out of 41, 40 isolates possessed the iroE and iroN genes; however, the remaining 2 isolates, in which one was *K. quasipneumoniae*, had only the iroE gene, and another from *K. pneumoniae* had only the iroN gene. Out of 41, 32 possess Yersinibactin genes (fyuA, irp1, 2, and ybtAEPQSTUX).

3.5. Efflux pump – AcrA and AcrB

40 isolates possessed both acrA and acrB genes, and one isolate had only the acrB gene.

3.6. Regulation of the RcsA, RcsB, and RmpA gene

One isolate from the respiratory secretion specimen has the RmpA gene present, and the rcsA gene was absent. All isolates had the rcsB gene shown in **Table 4**.

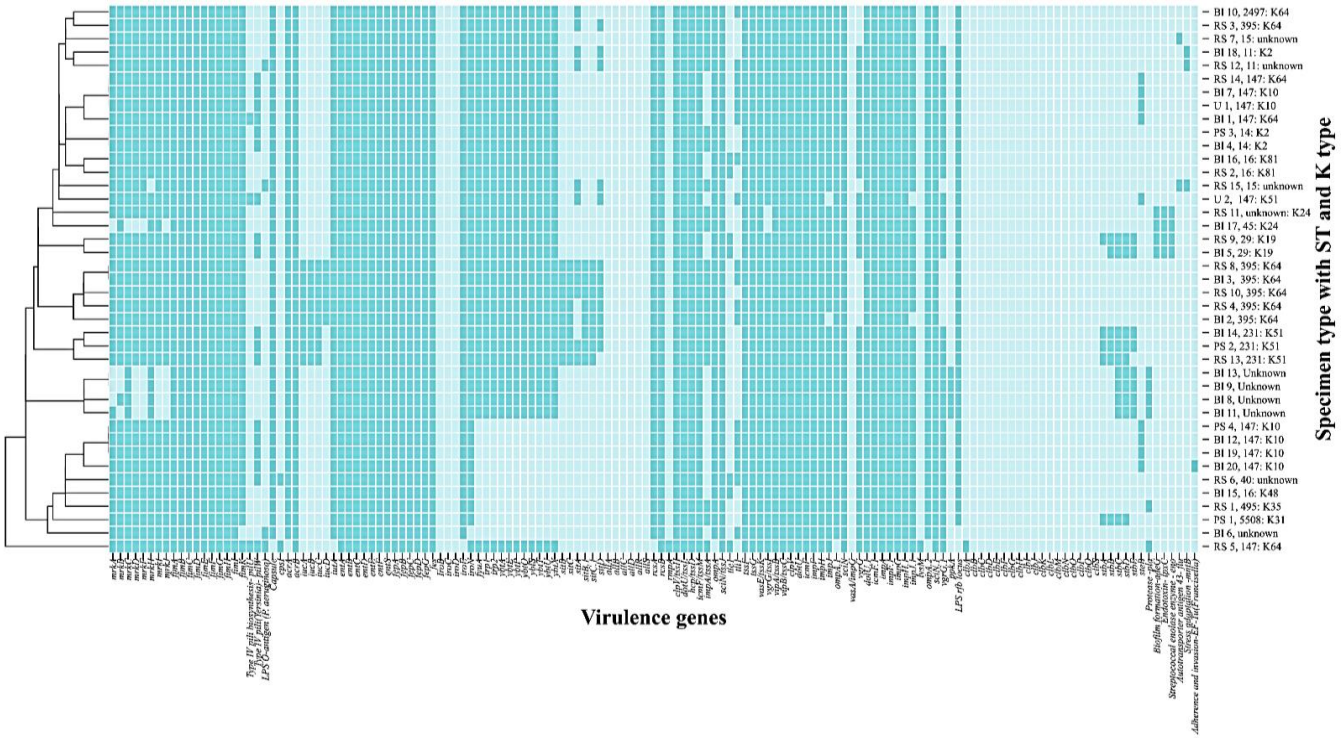
3.7. Type VI secretion system (T6SS)

3 types of Secretory systems, T6SS I, T6SS II and T6SS III. The most predominant were T6SS I and T6SS III. Variations were seen in T6SS II, which was absent in all respiratory specimens, except that the clpV gene was present in all specimens, as shown in **Table 5**. Presence of the *Pseudomonas* Type VI secretion system in an unknown sequence type (3228, 2313) isolated from a blood specimen in the Neonatal Intensive Care Unit (NICU).

3.8. Other factors

The Serum resistance gene was present in 39 isolates. One isolate possesses the colibactin toxin gene (clbA). Only ST 15 (3/3) had K typing unknown (KL112), which has the Antigen 43 gene, flu shown in **Table 6**.

A heatmap (**Figure 2**) created using an online tool (https://www.chiplot.online/) visualizes the distribution of virulence genes across different specimen types and sequence types.



The dark color of the cell represents the presence of the virulence gene, while the light color represents its absence. Heatmap created using online software. (https://www.chiplot.online/).

Figure 2: Virulence Gene: Specimen and sequence type (ST) insights

Table 1: VF class – Antiphagocytic

Virulence factors	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
Capsule	20 (100%)	14 (93.7%)	2 (100%)	4 (100%)
Capsule (Enterococcus) cpls	0 (0%)	2 (12.5%)	0 (0%)	0 (0%)

Table 2: VF class – Adherence

Virulence factors	Related genes	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
Type III Fimbriae	mrkA	16 (80%)	15 (100%)	2 (100%)	4 (100%)
	mrkB	17 (85%)	15 (100%)	2 (100%)	4 (100%)
	mrkC	19 (95%)	15 (100%)	2 (100%)	4 (100%)
	mrkD	15 (75%)	15 (100%)	2 (100%)	4 (100%)
	mrkF	15 (75%)	15 (100%)	2 (100%)	4 (100%)
	mrkH	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	mrkI	16 (80%)	15 (100%)	2 (100%)	4 (100%)
	mrkJ	15 (75%)	15 (100%)	2 (100%)	4 (100%)
Type I Fimbriae	fimA	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimB	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimC	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimD	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimE	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimF	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimG	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimH	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimI	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fimK	19 (95%)	15 (100%)	2 (100%)	4 (100%)
Type IV pili biosynthesis (Pseudomonas)	pilU	1 (5%)	1 (6%)	1 (50%)	0 (0%)
Type IV pili (Yersinia)	pilW	9 (45%)	5 (33%)	2 (100%)	2 (50%)

Table 3: VF Class – iron uptake

Virulence factors	Related genes	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
Aerobactin	iucA	3 (15%)	5 (31 %)	0 (0 %)	0 (0 %)
	iucB	3 (15%)	5 (31 %)	0 (0 %)	0 (0 %)
	iucC	3 (15%)	5 (31 %)	0 (0 %)	0 (0 %)
	iucD	1 (5%)	4 (25%)	0 (0 %)	0 (0 %)
	iutA	20 (100%)	14 (94%)	2 (100%)	4 (100%)
Enterobactin	entA	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entB	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entC	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entD	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entE	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entF	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	entS	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fepA	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fepB	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fepC	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fepD	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	fepG	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	Fes	20 (100%)	15 (100%)	2 (100%)	4 (100%)

Table 3 Continued….					
Salmochelin	iroE	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	iroN	19 (95%)	15 (100%)	2 (100%)	4 (100%)
Yersiniabactin	fyuA	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	irp1	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	irp2	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtA	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtE	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtP	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtQ	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtS	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtT	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtU	15 (75%)	13 (87%)	2 (100%)	2 (50%)
	ybtX	15 (75%)	13 (87%)	2 (100%)	2 (50%)
Ferrous iron transport (Shigella)	sitB	0 (0 %)	2 (13%)	0 (0 %)	0 (0 %)
	sitC	5 (25%)	6 (40%)	1 (50%)	1 (25%)
Iron/manganese transport (Escherichia)	sitA	3 (15%)	6 (40%)	1 (50%)	1 (25%)
	sitB	3 (15%)	5 (31 %)	0 (0 %)	0 (0 %)
	sitC	3 (15%)	5 (31 %)	0 (0 %)	0 (0 %)
	sitD	4 (20%)	6 (40%)	1 (50%)	1 (25%)

Table 4: VF class – Efflux pump (acrA, acrB) and regulation

Virulence factors	Related genes	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
AcrAB	acrA	20 (100%)	14 (93%)	2 (100%)	4 (100%)
	acrB	20 (100%)	15 (100%)	2(100%)	4 (100%)
RcsAB	rcsA	20 (100%)	14 (93%)	2 (100%)	4 (100%)
	rcsB	20 (100%)	15 (100%)	2 (100%)	4 (100%)
RmpA	rmpA	0 (0%)	1 (6%)	0 (0%)	0 (0%)

Table 5: VF class – secretion system

Virulence factors	Related genes	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
T6SS-I	clpV/tssH	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	dotU/tssL	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	hcp/tssD	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	icmF/tssM	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impA/tssA	7 (35%)	12 (80%)	0 (0%)	3 (75%)
	ompA	20 (100%)	14 (93%)	2 (100%)	4 (100%)
	sciN/tssJ	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	tle1	3 (15%)	4 (26%)	0 (0%)	0 (0%)
	tli1	9 (45%)	6 (40%)	2 (100%)	1 (25%)
	tssF	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	tssG	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	vasE/tssK	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	vgrG/tssI	19 (95%)	14 (93%)	2 (100%)	4 (100%)
	vipA/tssB	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	vipB/tssC	20 (100%)	15 (100%)	2 (100%)	4 (100%)

Table 5 Continued…..					
T6SS-II	clpV	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	dotU	2 (10%)	3 (20%)	0 (0%)	2 (50%)
	icmF	2 (10%)	2 (13%)	0 (0%)	1 (25%)
	impF	2 (10%)	2 (13%)	0 (0%)	2 (50%)
	impH	2 (10%)	2 (13%)	0 (0%)	2 (50%)
	impJ	2 (10%)	2 (13%)	0 (0%)	2 (50%)
	ompA	2 (10%)	2 (13%)	0 (0%)	2 (50%)
	sciN	2 (10%)	2 (13%)	0 (0%)	2 (50%)
	vasA/impG	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	vgrG	1 (5%)	2 (13%)	0 (0%)	1 (25%)
T6SS-III	dotU	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	icmF	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impA	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impF	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impG	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impH	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	impJ	19 (95%)	15 (100%)	2 (100%)	4 (100%)
	lysM	0 (0%)	1 (6%)	0 (0%)	0 (0%)
	ompA	20 (100%)	15 (94%)	2 (100%)	4 (100%)
	sciN	20 (100%)	15 (100%)	2 (100%)	4 (100%)
	vgrG	18 (90%)	9 (60%)	2 (100%)	3 (75%)
Hcp secretion island-1 encoded type VI secretion system (H-T6SS) (Pseudomonas)	ppkA	4 (20%)	0 (0%)	0 (0%)	0 (0%)

Table 6: VF class – Other virulence factors

VF Class	Virulence factors	Related genes	Blood Specimens (20)	Respiratory Specimens (15)	Urine Specimens (2)	Other Specimens (4)
Serum resistance	LPS rfb locus	-	19 (95%)	14 (93%)	2 (100%)	4 (100%)
Toxin	Colibactin	clbA	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Autotransporter	Antigen 43	Flu	0 (0%)	2 (12%)	0 (0%)	1 (25%)

4. Discussion

This study highlights the substantial geographical and sequence type diversity of virulence factors amongst *Klebsiella pneumoniae* isolated from clinical specimens. The isolation rates of *K. pneumoniae* vary significantly across different geographical regions. The proportion of isolation of *K. pneumoniae* from the ICU in the present study is as follows: blood samples (49%, 20/41), respiratory samples (37%, 15/41), urine (5%, 2/41), and other sources (9%, 4/41).

4.1. Capsule production

The extracellular capsular polysaccharides of *Klebsiella pneumoniae* play a critical role in virulence, enabling the bacteria to evade the host's immune system by contributing to its pathogenicity.[13] A cluster of genes wzi, wza, wzb, wzc, wzx, and wzy is responsible for encoding the capsule in *K. pneumoniae.*[14] *K. pneumoniae* has multiple capsule types[14] like Capsular types K1, K2, K5, K20, K54, and K57 are mainly related to invasive infections,[15] and capsular types K1, K2, K16, K28, K57, and K63 were associated with hypervirulent strains,[16] among which K1 and K2 are the most common serotypes in hypervirulent strains of *K. pneumoniae* (hvKP).[15]

In this study, we found capsular type K2 in 7% (3/42), while K1 was absent. Other than these, several capsule type like K64, K10, K51, K81, K48, K19, K24, K35, K31and K24 detected but more predominant capsular type was K64 (24%) followed by K10 (14%), K51(9%). A study conducted by H. Diab *et al*. from Israel, on MDR *K. pneumoniae* reported five different K-types: K64 (50%), K62 (15%), K2 (10%), K71 (5%), and K30 (5%).[17] Another study conducted by Hussain *et al*. in Bangladesh in 2023 reported K20 (9%), K2 (9%), K62 (6%), and K16 (6%), indicating geographical variation in capsular types.[18]

Ngoc T *et al*. reported that capsular type 64 was more commonly associated with cases of liver abscess, invasive disease, and showed hypervirulence.[19] In this study, capsular type 64 was found in bloodstream infections and respiratory tract infections.

4.2. Fimbriae

Type 1 fimbriae attach to urinary bladder cells and form biofilm.[15] The biofilm formation is expressed in the urinary tract but not in the gastrointestinal tract or lungs. [15] Various genes encoding Type I fimbriae (fimABCDEFGHI and K) were detected in all isolates (41/41) irrespective of specimen type; however, variations were observed in genes encoding Type III fimbria genes (mrkA, B, E, F, and J) in the *K. pneumoniae* strains isolated from blood from the NICU. These isolated sequenced types from NICU were mentioned as unknown in MLST, and it was a combination of 3228 - 2313. A PCR-based study done by R. El Fertas-Ais sani on *Klebsiella pneumoniae* also showed 100% presence of the fimH gene and 96.3% the mrkD gene.[20] Also, Maleki NS *et al.* in 2023 noted 91% of isolates had the fimH gene.[21] Our study shows similar findings, although our WGS study detected other genes besides these genes.

Though type I fimbriae cannot form biofilm.[22] Most of our strains possess Type III fimbriae that play a critical role in biofilm formation.[22]

4.3. Iron uptake

Iron regulation occurs in bacteria through various iron uptake siderophores by absorbing iron from host cells. It is an important virulence factor that accelerates the infection process by absorbing iron from the host cell and secreting siderophore to provide energy. The different siderophores found in *K. pneumoniae* are Aerobactin, Enterobactin, Salmochelin, and Yersiniabactin.[23] Nahar N *et al.* and Maleki NS *et al.* found Enterobactin (ent) from 66% to 94%, while we found Enterobactin in all our (100%) isolates.[21,24]

Aerobactin and Salmochelin are common among hypervirulent *K. pneumoniae* strains. The Aerobactin-encoded gene iuc is associated with increased mortality in MDR strains in a hospital setting. In the community-acquired pneumoniae and liver abscess-like invasive diseases are associated with Salmochelin and aerobactin.[25] An experimental study performed by R. El Fertas-Aissani detected the iutA gene (encoding for Aerobactin) in 5.5% of isolates.[20] According to a report by Kocsis Bet *et al.* prevalence of the aerobactin gene, like iucABCD, was high in hypervirulent *K. pneumoniae.*[26]

In our study, the Aerobactin (predominant gene - iutA) was detected in 40/41 (98%) isolates.

We also report aerobactin genes, like iucA, iucB, iucC, and iucD, which were present in ST395: K64 (5/6), and iucA, iucB, and iucC genes were detected in ST 231: K51 (3/3).

A study conducted by Ali *et al.* found, Salmochelin gene, iroN, in only a few (6/23) isolates.[27] We found the Salmochelin gene (iroE and iroN) in 95% of isolates.

It has been observed that yersiniabactin or aerobactin may be a key factor in enhancing the virulence of *K. pneumonia.*[28] Peng Lan *et al.* detected 80.5% ybt locus in a hypervirulent *K. pneumoniae* strain.[29] We detected yersinibactin genes (fyuA, irp1, 2, and ybtAEPQSTUX) in 78% (33/41) of isolates.

4.4. Efflux pump

Emerging evidence suggests that the AcrAB efflux pump functions as a critical virulence factor in *Klebsiella pneumoniae* pathogenesis, facilitating resistance to innate immunity in the lungs and thereby promoting the onset of pneumonia.[30] It is also responsible for resistance to various antibiotics. In our study, the AcrB efflux pump is detected in all (100%, 41/41), while the AcrA is detected in 98% of the isolates. (40/41). Abid Fazaa Almiyah detected 100% of AcrA & 100% of AcrB from a clinical specimen (pus samples).[32]

4.5. Regulation of the RcsA, RcsB, and RmpA genes

A study by D. Peng *et al.* revealed that RcsAB significantly influences CPS (Capsular Polysaccharide) secretion and biofilm function, serving as a primary co-activator of CPS in *K. pneumoniae.*[31] In our study, 40 (98%) isolates had RcsA, and 41 (100%) had RcsB.

The absence of the rmpA gene was reported in an experimental study conducted by Nahar and Rashid *et al.*, titled "Phylogenetic Analysis of Antibiotic Resistance Genes and Virulence Genes of *Klebsiella species* in silico," through PCR amplification.[24] The RmpA gene is associated with a hypermucoviscous phenotype.[25] Another study conducted by R. El Fertas-Aissani detected 3.7% rmpA gene.[20] In our study, one isolate from respiratory specimens had the rmpA gene (6.25%, 1/15).

4.6. Type VI secretion system (T6SS)

The Type VI secretory system (T6SS) in *Klebsiella pneumoniae* is understudied.[33] The Type VI Secretion System (T6SS) is a critical virulence factor of *K. pneumoniae*[34] and is involved in microbial antagonism and host-pathogen interactions.[34] In our study, the most predominant secretory system subtypes were T6SS I and T6SS III. T6SS II, in the present study, was found only in ST14, ST15, and ST45 with capsular typing K2, K24, and KL112.

4.7. Other virulence factors

The colibactin gene (clbA) is responsible for siderophore synthesis.[33] In our research, the colibactin responsible gene (clbA) was found only in 1/41 (2%), which also possessed the RmpA gene (responsible for capsular secretion).

The gene for Antigen 43, associated with biofilm formation, is generally detected in *E.coli*.[35] A study conducted by Tambassi M *et al.* reported that 3.7% *K. pneumoniae* had antigen 43.[35] In our study, the antigen 43 gene was detected in 7% (3/41) of isolates, belonging to ST15:KL112 strains.

Hypothetically, downregulation of Virulence gene activity can be used as an adjuvant to antibacterial treatment. Various studies are carried out to reduce (down-regulate) virulence gene activity.[36] One such study done by M. Shafik *et al.* demonstrated that some drugs like secnidazole (anti-amoebic), metformin (anti diabetic), can reduce the capsular and fimbrial expression by downregulating the rmpA, wcaG, fimH-1, mrkD genes.[36]

5. Conclusion

The genetic studies done in the past about virulence factors of *K. pneumoniae* suggest that there exists regional diversity of virulence genes. This variation has significant effect on the nosocomial pathogenesis, drug resistance and clinical outcomes. Our study gives us deep insight about drug resistance and predominant virulence factors associated with virulent and hypervirulent strains. Whole genome sequencing has the advantage over PCR of a more comprehensive analysis of genes related to various virulence factors of *K. pneumoniae* for a better understanding of the pathogenic process and the persistence of this pathogen in the hospital environment.

In our study, genes for type 3 fimbria, responsible for biofilm formation, were found in the majority of isolates. Hyper-virulent, less antimicrobial-resistant strains of *K. pneumoniae* responsible for community- acquired pneumonia usually bear K2 capsular type. In our study, we encountered MDR *K. pneumoniae* nosocomial strains with K2 capsular type. Some of them also possessed K64 capsular type. The ST147 and ST395 strains are common in our region. ST395 has both Type I and Type III Fimbriae, and all four Siderophores (aerobactin, enterobactin, salmochelin, and yersiniabactin), which makes it a super virulent strain.

6. Data Availability

All data generated and analysed are included within this research article. The genetic data for *Klebsiella pneumoniae* were uploaded to the NCBI database under Bio project number PRJNA1288171.

7. Ethical Approval Statement

Approving body: Parul University Institutional Ethics Committee for Human Research (PU/;.-IECHR). Approval number: PUIECHR/PIMSR/00/081734/5813. No direct experimentation was done on Humans or Animals.

8. Informed Consent Statement

No direct experimentation was done on Humans or Animals.

9. Source of Funding

None.

10. Conflict of Interest

None.

11. Acknowledgment

We are thankful to the Gujarat Biotechnology Research Centre (GBRC) in Gandhinagar for their valuable assistance and support.

References

1. Ashurst JV, Dawson A. *Klebsiella pneumoniae*. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2025
2. Chen IR, Lin SN, Wu XN, Chou SH, Wang FD, Lin YT. Clinical and microbiological characteristics of bacteremic pneumonia caused by *Klebsiella pneumoniae*. *Front Cell Infect Microbiol*. 2022;12:903682.
3. Huang X, Yao X, Hou Y, Zhang D, Xie R, Shi C, et al. Global trends of antimicrobial resistance and virulence of *Klebsiella pneumoniae* from different host sources. *Commun Med (Lond)*. 2025;5(1):383.
4. Asri NAM, Ahmad S, Mohamud R, Hanafi NM, Zaidi NFM, Irekeola AA, et al. Global prevalence of nosocomial multidrug-resistant *Klebsiella pneumoniae*: a systematic review and meta-analysis. *Antibiotics (Basel)*. 2021;10(12):1508.
5. Lodhi M, Lakshminarayana GP. Occurrence of nosocomial multi-drug resistant *Klebsiella pneumoniae* in India: a systemic review and meta-analysis. *IP Int J Med Microbiol Trop Dis*. 2024;10(1):17–23.
6. Monteiro ASS, Cordeiro SM, Reis JN. Virulence factors in *Klebsiella pneumoniae*: a literature review. *Indian J Microbiol*. 2024;64(2):389–401.
7. Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Hosseini MS. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic *Escherichia coli* isolates from Iranian patients. *BMC Infect Dis*. 2018;18:572.
8. Marathe AP, Shaikh AMH, Prajapati BT, Sangani S. Species identification of lactose non-fermenting isolates from ventilator-associated pneumonia using VITEK 2 and matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a comparative study with next-generation sequencing. *J Appl Biol Biotech*. 2025;13(5):146–50.
9. Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, et al. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res*. 2023;51(D1):D678–89.
10. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res*. 2018;3:124.
11. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res*. 2005;33(Database issue):D325–8.
12. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive Web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. *J Clin Microbiol*. 2018;56(6):e00197-18.
13. Rendueles O. Deciphering the role of the capsule of *Klebsiella pneumoniae* during pathogenesis: A cautionary tale. *Mol Microbiol*. 2020;113(5):883–8.
14. Chen J, Zhang H, Liao X. Hypervirulent *Klebsiella pneumoniae*. *Infect Drug Resist*. 2023;16:5243–9.

15. Liao Y, Gong J, Yuan X, Wang X, Huang Y, Chen X. Virulence factors and carbapenem-resistance mechanisms in hypervirulent *Klebsiella pneumoniae*. *Infect Drug Resist.* 2024;17:1551–9.
16. Huang X, Li X, An H, Wang J, Ding M, Wang L, et al. Capsule type defines the capability of *Klebsiella pneumoniae* in evading Kupffer cell capture in the liver. *PLoS Pathog*. 2022;18(8):e1010693.
17. Diab H, Rahy K, Jisr T, El Chaar M, Abboud E, Tokajian S. Phenotypic and molecular characterization of multi-drug resistant *Klebsiella spp.* isolates recovered from clinical settings. *Infect Genet Evol*. 2024;119(105583):105583.
18. Hussain A, Mazumder R, Ahmed A, Saima U, Phelan JE, Campino S, et al. Genome dynamics of high-risk resistant and hypervirulent *Klebsiella pneumoniae* clones in Dhaka, Bangladesh. *Front Microbiol*. 2023;14:1184196.
19. Ngoc BVT, Brisse S, Tuyet TD, Viet DVT, Holt KE, Vu TN, et al. *Klebsiella pneumoniae* with capsule type K64 is overrepresented among invasive disease in Vietnam. *F1000Res*. 2025;10:454.
20. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. *Pathol Biol (Paris).* 2013;61(5):209–16.
21. Maleki NS, Babazadeh F, Arzanlou M, Teimourpour R, Dogaheh HP. Serotyping and molecular profiles of virulence-associated genes among *Klebsiella pneumoniae* isolates from teaching hospitals of Ardabil, Iran: a cross-sectional study. *Health Sci Rep*. 2023;6(9):e1557.
22. Di Martino P, Cafferini N, Joly B, Darfeuille-Michaud A. *Klebsiella pneumoniae* type 3 pili facilitate adherence and biofilm formation on abiotic surfaces. *Res Microbiol*. 2003;154(1):9–16.
23. Hider RC, Kong X. Chemistry and biology of siderophores. *Nat Prod Rep*. 2010;27(5):637–57.
24. Nahar N, Rashid RB. Phylogenetic analysis of antibiotic resistance genes and virulence genes of *Klebsiella species* in silico. *Dhaka Univ J Pharm Sci.* 2017;16(1):119–27.
25. Lam MMC, Wyres KL, Judd LM, Wick RR, Jenney A, Brisse S, et al. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. *Genome Med*. 2018;10(1):77.
26. Kocsis B. Hypervirulent *Klebsiella pneumoniae*: An update on epidemiology, detection and antibiotic resistance. *Acta Microbiol Immunol Hung.* 2023;70(4):278–87.
27. Ali MR, Yang Y, Dai Y, Lu H, He Z, Li Y, et al. Prevalence of multidrug-resistant hypervirulent *Klebsiella pneumoniae* without defined hypervirulent biomarkers in Anhui, China: a new dimension of hypervirulence. *Front Microbiol*. 2023;14:1247091.
28. Lawlor MS, O'connor C, Miller VL. Yersiniabactin is a virulence factor for *Klebsiella pneumoniae* during pulmonary infection. *Infect Immun*. 2007;75(3):1463–72.
29. Lan P, Yan R, Lu Y, Zhao D, Shi Q, Jiang Y, et al. Genetic diversity of siderophores and hypermucoviscosity phenotype in *Klebsiella pneumoniae*. *Microb Pathog*. 2021;158(105014):105014.
30. Bachman MA, Oyler JE, Burns SH, Caza M, Lépine F, Dozois CM, et al. *Klebsiella pneumoniae* yersiniabactin promotes respiratory tract infection through evasion of lipocalin 2. *Infect Immun.* 2011;79(8):3309–16.
31. Padilla E, Llobet E, Doménech-Sánchez A, Martínez-Martínez L, Bengoechea JA, Albertí S. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. *Antimicrob Agents Chemother.* 2010;54(1):177–83.
32. ALmiyah SAF. Detection of AcrA and AcrB efflux pumps in multidrug-resistant *Klebsiella pneumonia* that isolated from wounds infection patients in Al-Diwaniyah province. *Arch Razi Inst.* 2023;78(1):269–76.
33. Liu W, Li M, Cao S, Ishaq HM, Zhao H, Yang F, et al. The biological and regulatory role of type VI secretion system of *Klebsiella pneumoniae*. *Infect Drug Resist.* 2023;16:6911–22.
34. Storey D, McNally A, Åstrand M, Santos J, Rodriguez-Escudero I, Elmore B, et al. *Klebsiella pneumoniae* type VI secretion system-mediated microbial competition is PhoPQ controlled and reactive oxygen species dependent. *PLoS Pathog*. 2020;16(3):e1007969.
35. Tambassi M, Passarini E, Menozzi I, Berni M, Bracchi C, Dodi A, et al. *Klebsiella pneumoniae* carrying multiple alleles of antigen 43-encoding gene of *Escherichia coli* associated with biofilm formation. *Eur J Clin Microbiol Infect Dis*. 2023;42(3):371–7.
36. Shafik SM, Abbas HA, Yousef N, Saleh MM. Crippling of *Klebsiella pneumoniae* virulence by metformin, N-acetylcysteine and secnidazole. *BMC Microbiol*. 2023;23(1):229.

Cite this article: Prajapati BT, Marathe AP, Shaikh AMH, Sangani S. Genetic analysis of virulence factors of MDR Klebsiella pneumoniae spp. pneumoniae isolated from clinical specimens - study from a tertiary care hospital. *Indian J Microbiol Res*. 2025;12(3):410–418.