



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Distribution, characterization and antibiotic resistance of hypervirulent *Klebsiella pneumoniae* (hvKp) strains versus classical strains (CKp) causing healthcare associated infections in Sohag University Hospitals

Nesma A. Mohamed ^{*1}, Ashgan Hussien Badawy Dawood ¹, Mona Fattouh Mohamed ¹, Sherif A. Sayed ², Dina Hamada Mohamed ¹.

1- Medical microbiology and Immunology Department, Faculty of Medicine, Sohag University, Egypt.

2- Clinical pathology department, Sohag University Hospital, Faculty of Medicine, Sohag, Egypt.

ARTICLE INFO

Article history:

Received 26 February 2024

Received in revised form 25 March 2024

Accepted 30 March 2024

Keywords:

HVKP

Risk factors

Antibiotic resistance

ABSTRACT

Background: *Klebsiella pneumoniae* has two different pathotypes: hypervirulent *Klebsiella pneumoniae* (hvKp) and classical *Klebsiella pneumoniae* (ckp), hvKp is more potent than ckp causing vast types of severe disseminated infections. This cross sectional study done to detect frequency of hvKp versus ckp, their antibiotic resistance and risk factors associated with their infections. **Methods:** Samples were collected from admitted patients cultured on MacConkey agar, pink colonies were confirmed to be *Klebsiella pneumoniae* and sensitivity were done by Vitek 2. String test was done for hypermucoviscosity and PCR to detect virulence genes. **Results:** 100 samples were collected from 2020 to 2023, 64 of them revealed hvKp which were isolated from old age with mean± SD of 62.7±20.5, most of isolates were from ICU from pneumonia patients. There was a significant difference in ceftriaxone, cefoxitin, gentamycin and tobramycin resistance between hvKp, ckp with *p* value 0.03, 0.04, 0.01 and 0.02 respectively, 24% of hvKp strains were ESBL producers. Fourteen (14%) were pandrug resistant, 14(14%) were extremely drug resistant and 36(36%) were multidrug resistant. *iucA* was the most frequent virulence gene 62% followed by *fimH* (42%). Diabetes mellitus, hypertension and cardiovascular disease were the most significant risk factors associated with hvKp infection. **Conclusion:** hvKp is a serious pathogen that utilizes a battery of virulence factors for survival and pathogenesis, also has an ability to acquire drug resistance through transfer of genetic material, so we have to shed light on new strategies to improve diagnosis, treatment and prevention of hvKp causing infections.

Introduction

Being an opportunistic pathogen, *Klebsiella pneumoniae* is a causative agent of wide range of infections such as pneumonia, intra-abdominal infection, urinary tract infection and

bacteremia [1] and can be divided into two main types: classical *Klebsiella pneumoniae* (ckp) and hypervirulent *Klebsiella pneumoniae* (hvKp). Classical *Klebsiella pneumoniae* (ckp) is an opportunistic bacterium that causes healthcare associated infections in hosts with associated

comorbidities who are immunocompromised or who have invasive maneuvers (e.g., intravascular devices, endotracheal tube, or surgical wound). Hvkp is best described as a virulent pathogen. Most of reported hvkp infections are acquired in the community, hvkp are capable of infecting healthy individuals of any age, as well as causing multiple infections and/or subsequent dissemination [2]. Hvkp was first discovered in Taiwan in 1986, it has been identified as the main cause of pyogenic liver abscesses. Hvkp can disseminate to different parts of the body, e.g. ocular/pulmonary/meningitis/necrotizing fasciitis being the most common infections [3]. Hvkp infections were mainly in the Asia-Pacific region, which may be attributed to the higher rate of hvkp carriage among the healthy individuals in this area [4] and it was sensitive to all antibiotics except ampicillin, but unfortunately the scope of hvkp infection has gradually increased, its mutation frequency has expanded. Now, some strains of hvkp are resistant to carbapenems, polymyxin, tigecycline, extended-spectrum β -lactamases (ESBLs), etc., have gradually evolved [5].

Efforts to detect this pathogen have been rendered by a lack of a definitive test for its detection. Identification has historically been done by unique clinical manifestations and phenotypic traits, which are not specific nor sensitive for hvkp [6]. Advances in genomic analysis enabled researchers to detect important virulence genes, which are more reliable for identification of hypervirulence. Phenotypic characters in hyper virulent *Klebsiella pneumoniae* are called hypermucoviscosity, it is a typical character of hvkp strain, but it is not specific. Currently, aerobactin has been considered to be an important virulence factor for hvKp, which is often associated with the hypermucoviscous phenotype. Based on this finding, Chinese researchers conducted research including multiple centers first described the molecular detection of hvKp (defined as aerobactin-positive) isolates. The results of other studies revealed that invasive infections, especially pyogenic liver abscess (PLA), hypermucoviscous phenotype and most of the virulence factors such as rmpA, rmpA2 (that regulate of the mucoid phenotype), and magA (mucoviscosity-associated gene) and capsular serotype-specific genes (K1, K2) are strongly associated with aerobactin-positive Kp [7]. Iron acquisition genes and the genes that encode the hypermucoviscous phenotype are located on the

same virulence plasmid, which is present in most hvkp isolates but rarely present in cKp strains [8]. Thus, it may be more appropriate to combine aerobactin positivity and hypermucoviscosity when defining hvkp, so the aim of this study is to determine the frequency of hvkp versus ckp, their antibiotic resistance and risk factors associated with their infections.

Patients and methods

This single center cross sectional study was conducted at Sohag University Hospital, Department of Medical Microbiology and Immunology from June 2020 to December 2023, after taking ethical approval from Ethical Committee in our faculty with IRB number: SOH-MED-23-05-04MD.

Inclusion criteria

Patients with healthcare associated infections (HCAIs are infections that first appear 48 hours or more after hospitalization or within 30 days after having received health care) [9] and their samples were positive for *Klebsiella pneumoniae*.

Exclusion criteria

Samples harboring organisms other than *Klebsiella pneumoniae* or mixed infections.

Sample collection and identification

A total of 100 samples were collected from different departments (Internal medicine, Neuropsychiatry and General surgery) and ICU_s (intensive care unit, coronary care unit) in the hospital after obtaining written consent of the participants, samples included (Pus, urine, sputum, tracheal aspirate and blood).

Samples were collected under complete aseptic conditions [10] sent immediately to microbiology lab for further processing. Samples were cultured on MacConkey agar (Himedia), colonies with pink color were confirmed to be *Klebsiella pneumoniae* by VITEK 2 compact (BioMérieux, France) and antibiotic sensitivity was also done by VITEK using AST GN 73 cards. One hundred *Klebsiella pneumoniae* isolates were included in this study. The strains were stored on 20% glycerol at -60 °C until PCR was done.

Phenotypic detection of hypermucoviscosity by string test

Bacterial colonies grown overnight on MacConkey agar plate at 37 °C were stretched by a standard bacteriological loop. If a mucoviscous

string ≥ 5 mm in length was formed, the test was considered positive, and the isolate was identified as hypermucoviscous [11].

Molecular detection of capsular genes and some virulence associated genes by conventional PCR

DNA extraction

DNA was extracted from freshly subcultured bacteria according to the method of [12], extracted DNA was stored at -20 for further use.

Polymerase chain reaction (PCR)

Conventional PCR (biometra, Germany) was done to detect capsular genes (k1, k2), virulence genes (fim H, rmp A1, rmpA2 and iucA), sequences of primers (Invitrogen, Thermo fisher), cycling condition and amplicon size are as mentioned by [13] **Table 1**. Each PCR reaction was adjusted to a total volume of 25 μ l using the following reaction mixture: 12.5 μ l of cosmo red PCR Master Mix [2x] (Willowfort, Uk), 1.25 μ l of forward primer, 1.25 μ l of reverse primer, 3 μ l of template DNA then the reaction was adjusted to 25 μ l with nuclease free water. Negative control tubes were also included with no DNA template. After amplification, 10 μ l of the PCR amplicon was analyzed by gel electrophoresis (2% agarose in Trisacetate-EDTA stained with ethidium bromide). The gene ruler 50 bp DNA ladder (invitrogen, Thermo fisher) was used as a DNA size marker. Visualization of bands was done by DNA documentation system.

Recognition of hypervirulent *Klebsiella pneumoniae* by the presence of iucA and one of rmpA1 or rmpA2 genes [14].

Statistical analysis

The collected data were coded and verified prior to computerized data entry. The collected data was statistically analysed using Statistical Package for the Social Science (SPSS) version 26 program 14 and expressed in tables and graphs. The data were tested for normality by Kolmogorov-Smirnov. Chi-square test was used for qualitative data difference between groups. Mann Whitney for non-parametric data to get the p value between groups in age and length of stay data. In all analyses, $P < 0.05$ indicated statistical significance.

Results

One hundred samples were collected from patients with healthcare associated infections, hvkp was detected by the presence of iucA genes and one of the two genes rmpA1, rmpA2.

The mean age \pm SD of patients with hvkp infections was 62.7 \pm 20.5 years. The range for age was (48:79) years. While the mean age \pm SD of patients with ckp was 63.2 \pm 24.9, The range for age was (48:82) years, males were more frequent than females both in hvkp and ckp with a ratio of 42%, 26% respectively. Different departments were included in the study. ICU was the most frequent department containing both hvkp and ckp with a ratio of 58%, 30% respectively, also hvkp were more prevalent in patients with pneumonia 38%, followed by ventilator associated pneumonia 14% as shown in **Table 2**.

Antimicrobial susceptibility profile of isolated strains by Vitek 2 system

The following antibiotics were used (Ampicillin, Ampicillin/Sulbactam, Piperacillin – Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazole and Nitrofurantoin) and results were interpreted according to CLSI 2017.

The highest resistance was to Trimethoprim/Sulfamethoxazole and Nitrofurantoin (100%) followed by Ciprofloxacin (98%), Levofloxacin (92%), Cefepime (82%), Tobramycin (74%) and Amikacin (70 %) (**Table 3**).

When comparing antibiotic resistance pattern in hvkp and ckp, we found a significant difference in ceftriaxone, cefoxitin, gentamycin and tobramycin resistance between hvkp, ckp with p value 0.03, 0.04, 0.01 and 0.02 respectively, 24% of HVKP strains were ESBL producers, also 14(14%) of HVKP were Pandrug resistant (to the tested antibiotics), 14(14%) were extremely drug resistant and 36(36%) were multidrug resistant as in **Table 4**.

MDR: Acquired non-susceptibility (resistant or intermediate) to at least one agent in three or more antibiotic categories. XDR: Non-susceptibility to at least one agent in all but bacterial isolates remain susceptible to only one or two antimicrobial categories. Pandrug-resistant (PDR): Non-susceptibility to all agents in all antibiotic categories [15].

The ESBLs are plasmid mediated β -lactamases that are capable of hydrolysing β -lactams, including penicillins, cephalosporins and monobactams, and they may be inhibited by β -lactamase inhibitors, including clavulanic acid, and avibactam [16].

Phenotypic and genotypic detection of hypermucoviscosity and virulence genes

Regarding phenotypic determination of hypermucoviscosity of *Klebsiella* strains, 28% of HVKP had positive string test while 36 (36%) had negative test, in CKP 18 (18%) had positive string test while 18 (18%) had negative test.

Molecular detection of different genes of virulence in HVKP and CKP

All genes (K1, K2, fimH, iuc A, rmpA1, rmpA2) were present more frequently in hvkp, iuc

A was the most frequent (62%) followed by fimH (42%), K2 and rmpA1 with a ratio of (40%) as in **Table 5**.

Risk factors associated with hvkp

Regarding risk factors associated with HVKP infections there was a significant difference between hvkp and ckp in the following risk factors: Cardiovascular disease, hypertension and diabetes mellitus with p value 0.02 as in **Table 6**.

Table 1. Sequences of the target genes.

Target genes	Primer name	Primer sequence (5'-3')	Amplicon size (bp)	reference
K1	K1-F	GTAGGTATTGCAAGCCATGC	1047	[13]
	K1-R	GCCCAGGTTAATGAATCCGT		
K2	K2-F	GGAGCCATTTGAATTCGGTG	641	
	K2-R	TCCCTAGCACTGGCTTAAGT		
<i>fimH</i>	FimH-F	TGCTGCTGGGCTGGTCGATG	550	
	fimH-R	GGGAGGGTGACGGTGACATC		
<i>iucA</i>	iucA-F	GCTTATTTCTCCCCAACCC	583	
	iucA-R	TCAGCCCTTTAGCGACAAG		
<i>ρrmpA1</i>	rmpA-F	GAGTAGTAATAAATCAATAGCAAT	332	
	rmpA-R	CAGTAGGCATTGCAGCA		
<i>ρrmpA2</i>	rmpA2-F	GTGCAATAAGGATGTTACATTA	430	
	rmpA2-R	GGATGCCCTCCTCCTG		

Table 2. Demographic and clinical characteristics of included patients according to type of *klebsiella*.

Parameters	HVKP (64)	CKP (36)	Total	P value
Age (years) Mean ± SD Range	62.7±20.5 (48.2:79.7)	63.2±24.9 (48:82)	62.9±22	0.5 (NS) by Mann-Whitney
Sex Male Female	42(42%) 22 (22%)	26 (26%) 10(10%)	68 (68%) 32 (32%)	0.5 (NS) By Chi-square test
Department CCU ICU Internal medicine Neuropsychiatry General surgery	2(2%) 58(58%) 0(0%) 2(2%) 2(2%)	4(4%) 30(30%) 2(2%) 0(0%) 0(0%)	6(6%) 88(88%) 2(2%) 2(2%) 2(2%)	0.07 (NS) By Chi-square test
Length of stay Mean ± SD	9.8±3.	9.8±4	9.8±3.5	0.7 (NS) by Mann-Whitney
Type of infection: -Ventilator associated pneumonia. - Pneumonia - sepsis -SSI -UTI	14(14%) 38(38%) 0(0%) 10(10%) 2(2%)	4(4%) 24(24%) 2(2%) 2(2%) 4(4%)	18(18%) 62(62%) 2(2%) 12(12%) 6(6%)	0.1(NS) 0.4 (NS) 0.057(NS) 0.1(NS) 0.1(NS)

CCU: coronary care UNIT

ICU: intensive care unit SSI: surgical site infection

UTI: urinary tract infection

Table 3. Antibiotic profile of isolated *Klebsiella pneumoniae*.

Antibiotic Resistance	Total (100)		
	R	I	S
Ampicillin	58(58%)	0(0%)	42(42%)
Ampicillin/Sulbactam	44(44%)	2(2%)	54(34%)
Piperacillin -Tazobactam	52 (52%)	2(2%)	46 (46%)
Cefazolin	48(48%)	6(6%)	46 (46%)
Cefoxitin	52 (52%)	6 (6%)	42(42%)
Ceftazidime	46 (46%)	6 (6%)	48 (48%)
Ceftriaxone	44 (44%)	8 (8%)	48 (48%)
Cefepime	82 (82%)	2(2%)	16 (16%)
Meropenem	62 (62%)	2(2%)	36(36%)
Amikacin	70 (70%)	0 (0%)	30 (30%)
Gentamycin	56 (56%)	0 (0%)	44 (44%)
Tobramycin	74 (74%)	0 (0%)	26 (26%)
Ciprofloxacin	98 (98%)	0 (0%)	2 (2%)
Levofloxacin	92 (92%)	4 (4%)	4 (4%)
Trimethoprim/Sulfamethoxazole	100 (100%)	0 (0%)	0 (0%)
Nitrofurantoin	100 (100%)	0 (0%)	0 (0%)

Table 4. Antibiotic resistance according to type of *Klebsiella*.

Antibiotic resistance	HVKP (64)	CKP (36)	P value
	R	R	
Ampicillin	38(38%)	20(20%)	0.7 (NS)
Ampicillin/ Sulbactam	28(28%)	16(16%)	0.5(NS)
Piperacillin- Tazobactam	36(36%)	16 (16%)	0.1(NS)
Cefazolin	30 (30%)	18 (18%)	0.1(NS)
Cefoxitin	36 (36%)	16 (16%)	0.04*
Ceftazidime	34 (34%)	12(12%)	0.1(NS)
Ceftriaxone	32 (32%)	12(12%)	0.03*
Cefepime	54(54%)	28(28%)	0.2(NS)
Meropenem	40(40%)	22(22%)	0.15(NS)
Amikacin	44 (44%)	26(26%)	0.7(NS)
Gentamycin	42 (42%)	14(14%)	0.01*
Tobramycin	52(52%)	22(22%)	0.02*
Ciprofloxacin	64 (64%)	34(34%)	0.05(NS)
Levofloxacin	56 (56%)	36(36%)	0.08(NS)
Trimethoprim Sulfamethoxazole	64 (64%)	36(36%)	---
Nitrofurantoin	64 (64%)	36 (36%)	---
ESBL Positive Negative	24(24%) 40 (40%)	8(8%) 28 (28%)	0.11 (NS) By Chi-square test
Antibiotic resistance: Pan drug resistance	14(14%)	8(8%)	0.9(NS)
Extreme drug resistance	14(14%)	4(4%)	0.1(NS)
Multidrug resistance	36(36%)	24 (24%)	0.3(NS)

Table 5. Virulence genes and string test according to type of *Klebsiella*.

Virulence genes	HVKP (64)			CKP (36)		P value
	Positive	Negative		Positive	Negative	
K1 gene	10(10%)	54(54%)		8(8%)	28(28%)	0.4 (NS)
K2 gene	40(40%)	24(24%)		14(14%)	22(22%)	0.02*
fimH	42 (42%)	22 (22%)		20 (20%)	16 (16%)	0.3(NS)
iucA gene	62 (62%)	2 (2%)		0 (0%)	36 (36%)	<0.001***
rmpA1 gene	40 (40%)	24 (24%)		20(20%)	16 (16%)	0.5 (NS)
rmpA2 gene	28 (28%)	36 (36%)		22 (12%)	14 (14%)	0.09 (NS)
String test	28(28%)	36 (36%)		18 (18%)	18 (18%)	0.5 (NS)

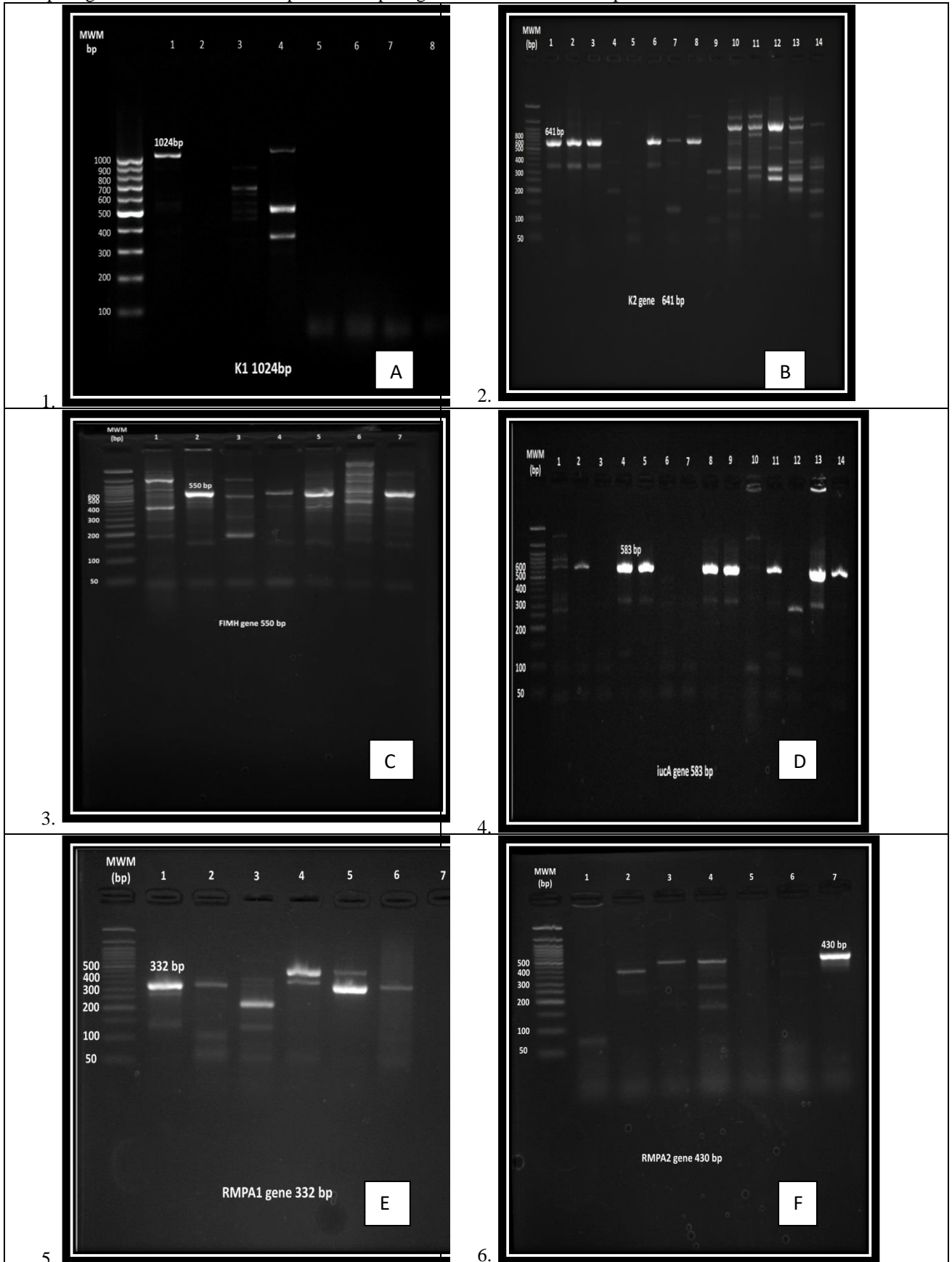
Table 6. Risk factors associated with hvkp.

Risk factors	HVKP (64)	CKP (36)	Total	P value	P value
Cancer	2 (2%)	0(0%)	2 (2%)	0.2(NS)	0.004** By chi-square test
Cardiovascular disease	24(24%)	6(6%)	30(30%)	0.02*	
Cerebral stroke	2(2%)	0(0%)	0(0%)	0.2(NS)	
Chronic renal failure	0(0%)	2(2%)	2(2%)	0.057(NS)	
Sepsis	0(0%)	2(2%)	2(2%)	0.057(NS)	
Diabetes mellitus	10(10%)	12(12%)	22(22%)	0.04*	
Diabetes mellitus, hypertension	8(8%)	0(0%)	0(0%)	0.02*	
DM, HTN, IHD and ESRD	2(2%)	0(0%)	0(0%)	0.2(NS)	
Epilepsy	0(0%)	2(2%)	2(2%)	0.057(NS)	
Hypertension	12(12%)	10(10%)	22(22%)	0.2(NS)	
Liver cirrhosis	2(2%)	2(2%)	4(4%)	0.5(NS)	
Lymphadenopathy	2(2%)	0(0%)	2(2%)	0.2(NS)	

DM: Diabetes mellitus HTN: Hypertension IHD: Ischemic heart disease
ESRD: End stage renal disease.

Figure 1. Gel electrophoresis showing bands of different genes:

- A: k1 gene with band size 1024 bp. B: K2 gene with band size 641 bp.
C: fimH gene with band size 550 bp. D: iucA gene with band size 583 bp.
E: rmpA1 gene with band size 332 bp. F: rmpA2 gene with band size 430 bp.



Discussion

Hypervirulent *Klebsiella pneumoniae* is an increasingly mentioned pathotype of *K. pneumoniae* characterized clinically by its ability to cause life-threatening infections in immunocompetent and even healthy individuals.

We collected 100 *Klebsiella pneumoniae* from different samples; hvkp was detected in 64% of the isolated bacteria. This finding is higher than previous study of [11] who reported 15.8% hvkp rate.

The mean age \pm SD of the patients that had hvkp was 62.7 \pm 20.5 years, 63.2 \pm 24.9 in ckp which is near to [17] who found that mean age \pm SD was of (57 \pm 14), but different from [11] who reported prevalence of hvkp was common in young patients. The highest number of *K. pneumoniae* associated infections was seen in the older age group of 48–81 years with highest number of cases in males, this could be due to their weak immune system and high chances of co morbidities as noted by other studies [18].

The highest percentage of patients were admitted in ICU and suffered from pneumonia like [17] who found that patients with CR-hvKP mainly had pneumonia (77.8%). In our study, most of the isolates were collected from ICU patients, suggesting that the majority of hvKp infection comes from the hospital environment, contaminated surfaces in hospital environments which act as a reservoir for hvkp infections. Several studies have demonstrated that hvkp strains are increasingly detected within hospitals [19].

Our results are different from [20] Who reported that (14 of 18; 77.7%) of hvkp were isolated from cases of septicaemia, pyogenic infections, and UTI.

Regarding antibiotic resistance of all isolated *Klebsiella* including ckp and hvkp, the highest resistance was to Trimethoprim/Sulfamethoxazole and Nitrofurantoin (100%) followed by Quinolone resistance (Ciprofloxacin 98%, Levofloxacin 92%), Cefepime (82%), Tobramycin (74%) and Amikacin (70 %). Our results are similar to [21] who reported that the majority of strains were non-susceptible to Nitrofurantoin (NIT), Ciprofloxacin (CIP), and Trimethoprim-sulfamethoxazole.

Ciprofloxacin resistance in our study (98%) is lower than [21] who reported 100% Ciprofloxacin resistance.

Carbapenem-resistant *K. pneumoniae* is a “serious concern” for the World Health Organization. In the current study, the resistance rate of hvkp Meropenem is 62% which is higher than [22, 21] who reported 48% Meropenem resistance, this difference could be explained by increasing use of this antibiotic in our patients. When comparing antibiotic resistance patterns in hvkp and ckp, we found a significant difference in Ceftriaxone, Cefoxitin, Gentamycin and Tobramycin resistance between hvkp, ckp with p value 0.03, 0.04, 0.01 and 0.02 respectively.

As regards ESBL production in hvkp; 24% of HVKP strains were ESBL producers. Our results differ from [11] who reported the resistance rate to common antibiotics in hvKp strains was significantly lower than in cKp strains and that ESBL production was more frequent in the cKp strains than in the hvKp strains. The present study percentage of ESBL-producing Kp was 38%. Other studies showed different rates of ESBL-producing Kp ranging from 9.6% to 81.1%. The difference in ESBL-producing Kp percentages may be explained by the exposure to beta-lactam antibiotics in this organism or by plasmid transfer that carries the ESBL genes. The growing prevalence of ESBL-producing Kp and carbapenemase-producing Kp in humans and chickens provide health risk and need more judicious use of antibiotics in farms to prevent the dissemination of both ESBL and carbapenem resistance in Kp [23].

Then, we tried to assess the frequency of MDR, XDR, pan drug resistance in both hvkp and ckp. We found higher frequency of MDR, XDR and pan drug resistance in hvkp as follows: 14(14%) of HVKP were Pandrug resistant, 14(14%) were extremely drug resistant and 36(36%) were multidrug resistant while 8(8%) of CKP were Pandrug resistant, 4(4%) were extremely drug resistant and 24(24%) were multidrug resistant.

Our results are dissimilar to [21] who reported a significant and worrying level of multidrug resistant strains, being ten strains classified as MDR (10/14, 71%) and three strains as XDR (3/14, 21%). None of the strains were classified as PDR.

A previous study reported two main mechanisms of MDR-hvKp development: One is the

acquisition of virulence-associated genes in cKp [24]. The other is the acquisition of various resistance genes/plasmids in hvKp [25]. The study of [25] found that ST65 hvkp had acquired both resistance genes and MDR plasmids that produced the MDR-hvkp phenotype, shedding light on the urgent need to enhance surveillance to prevent dissemination.

Regarding the virulence factors and hypermucoviscosity, string test (phenotypic detection of hypermucoviscosity) was positive in 28% of hvkp. These results are near to [27] who found 22% hvkp with positive string test.

The capsule is also considered the major virulence factor in *K. pneumoniae*, there are several types of K-antigens. K1 and K2 are the most important serotypes as they frequently result in severe infections. In our study, k2 was significantly present in hvkp in contrast to [11] who found k1 gene is more predominant in hvkp. The genes responsible for the hypermucoviscous phenotype (rmpA1, rmpA2) are considered another virulence determinants in addition to K1/K2. Our results showed that rmpA1, rmpA2 were not significantly associated with hvkp strains in contrast to [11] who found significant association of rmpA1, rmpA2 and hvkp. This could be explained by although the presence of rmpA⁺/rmpA2⁺ combined with virulence genes is generally a known marker of hvkp, there are other combinations e.g. rmpA⁻/HMV⁺, rmpA⁻/rmpA2⁺/HMV⁺, and rmpA⁺/HMV⁻. This exclusion illustrates that *K. pneumoniae* is capable of hypervirulence with or without presence of ramp genes [28].

Aerobactin is considered an important virulence determinant of hvkp [29]. Our results showed that aerobactin was significantly associated with hvKp as showed by [11]. Therefore, our results showed that k2, iucA virulence factors are more strongly associated with hvkp than with ckp.

FimH is present in 42% of hvkp less than [29] who detected fimH in 63% of the isolates. FimH, which encodes type 1 fimbrial adhesins, which mediate binding to epithelial cells and promote biofilm development which add apoint to virulence [29].

Our results revealed that diabetes mellitus and hypertension and cardiovascular disease were statistically significant risk factors associated with hvkp infections and that there were no significant

differences between hvkp and ckp strains regarding other underlying conditions of participants, but [11] reported that diabetes was the only significant risk factor in his study, our results could be explained that our patients are in old age and cardiovascular disease, diabetes and hypertension are common in this age group.

Conclusion

The frequency of hvkp is high in the healthcare setting and become a dominant healthcare associated pathogen with high rates of antibiotic resistance, the infection of hvkp is great in elderly ICU patients. Awareness of detection and management is a must.

Recommendations

Our study is an alarm to the presence of hvkp in our hospital with high percentage and with high resistance rates to the most commonly used antibiotics, which need strict awareness to infection control measures and wise use of antibiotics according to the antibiotic sensitivity testing.

Conflict of interest

The authors declared no conflict of interest.

Fund

No fund was applied to this work.

References

- 1- Paczosa MK, and Meccas J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. *Microbiol Mol Biol Rev* 2016 Jun 15; 80(3):629-61.
- 2- Zhang QB, Zhu P, Zhang S, Rong YJ, Huang ZA, Sun LW, et al. Hypervirulent Klebsiella pneumoniae detection methods: a minireview. *Arch Microbiol* 2023 Sep 6;205(10):326.
- 3- De Francesco MA, Tiecco G, Scaltriti E, Piccinelli G, Corbellini S, Gurrieri F, et al. First Italian report of a liver abscess and metastatic endogenous endophthalmitis caused by ST-23 hypervirulent Klebsiella pneumoniae in an immunocompetent individual. *Infection* 2023 Feb;51(1):271-276.
- 4- Chung DR, Lee H, Park MH, Jung SI, Chang HH, Kim YS, et al. Fecal carriage of serotype K1 Klebsiella pneumoniae ST23 strains closely related to liver abscess isolates in

- Koreans living in Korea. *Eur J Clin Microbiol Infect Dis* 2012 Apr;31(4):481-6.
- 5- Huang YH, Chou SH, Liang SW, Ni CE, Lin YT, Huang YW, et al. Emergence of an XDR and carbapenemase-producing hypervirulent *Klebsiella pneumoniae* strain in Taiwan. *J Antimicrob Chemother* 2018 Aug 1;73(8):2039-2046.
- 6- Parrott AM, Shi J, Aaron J, Green DA, Whittier S, Wu F. Detection of multiple hypervirulent *Klebsiella pneumoniae* strains in a New York City hospital through screening of virulence genes. *Clin Microbiol Infect* 2021 Apr;27(4):583-589.
- 7- Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Wang H, et al. High Prevalence of Hypervirulent *Klebsiella pneumoniae* Infection in China: Geographic Distribution, Clinical Characteristics, and Antimicrobial Resistance. *Antimicrob Agents Chemother* 2016 Sep 23;60(10):6115-20.
- 8- Struve C, Roe CC, Stegger M, Stahlhut SG, Hansen DS, Engelthaler DM, et al. Mapping the Evolution of Hypervirulent *Klebsiella pneumoniae*. *mBio* 2015 Jul 21;6(4): e00630.
- 9- Haque M, Sartelli M, McKimm J, Abu Bakar M. Health care-associated infections - an overview. *Infect Drug Resist* 2018 Nov 15; 11:2321-2333.
- 10- BAILEY and SCOTT'S MICROBIOLOGY LAB MANUAL FOR DIAGNOSTIC MICROBIOLOGY. Mosby; Publishing, Ltd:1998. 9th.
- 11- Alharbi MT, Almuhayawi MS, Nagshabandi MK, Tarabulsi MK, Alruhaili MH, Gattan HS, et al. Antimicrobial Resistance Pattern, Pathogenicity and Molecular Properties of Hypervirulent *Klebsiella pneumoniae* (hvKp) among Hospital-Acquired Infections in the Intensive Care Unit (ICU). *Microorganisms* 2023; 11(3):661.
- 12- Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. *Int J Infect Dis* 2013;17(6):e450-e453.
- 13- Tang Y, Liu H, Zhao J, Yi M, Yuan Y, Xia Y. Clinical and Microbiological Prognostic Factors of in-Hospital Mortality Caused by Hypervirulent *Klebsiella pneumoniae* Infections: A Retrospective Study in a Tertiary Hospital in Southwestern China. *Infect Drug Resist* 2020;13: 3739-3749.
- 14- Russo TA, and Marr CM. Hypervirulent *Klebsiella pneumoniae*. *Clin Microbiol Rev* 2019;32(3):10.1128/cmr.00001-19.
- 15- Zhang X, Li X X, Song J W, Zhang X C, Zhen C, Bi J, et al. Clinical features, microbial spectrum, and antibiotic susceptibility patterns of spontaneous bacterial peritonitis in cirrhotic patients. *Digestive and Liver Disease* 2023; 55(11): 1554–1561.
- 16- McDonald K L, Garland S, Carson CA, Gibbens K, Parmley EJ, Finley R, et al. Measures used to assess the burden of ESBL-producing *Escherichia coli* infections in humans: a scoping review. *JAC-antimicrobial resistance* 2021;3(1): 104.
- 17- Li L, Li S, Wei X, Lu Z, Qin X, Li M. Infection with Carbapenem-resistant Hypervirulent *Klebsiella pneumoniae*: clinical, virulence and molecular epidemiological characteristics. *Antimicrob Resist Infect Control* 2023;12(1):124.
- 18- Vandhana V, Saralaya KV, Bhat S, Shenoy Mulki S, & Bhat AK. (Characterization of Hypervirulent *Klebsiella pneumoniae* (Hv-Kp): Correlation of Virulence with

- Antimicrobial Susceptibility. International journal of microbiology 2022; 2022: 4532707.
- 19-Behera B, Swain PP, Rout B, Panigrahy R, & Sahoo RK. Genotypic characterization of hypervirulent *Klebsiella pneumoniae* (hvKp) in a tertiary care Indian hospital. Int Microbiol 2024.
- 20-Nakamura-Silva R, Oliveira-Silva M, Furlan, JPR, Stehling EG, Miranda CES, & Pitondo-Silva A. Characterization of multidrug-resistant and virulent *Klebsiella pneumoniae* strains belonging to the high-risk clonal group 258 (CG258) isolated from inpatients in northeastern Brazil. Archives of microbiology 2021; 203(7):4351–4359.
- 21-Osama DM, Zaki BM, Khalaf WS, Mohamed MYA, Tawfick MM, Amin HM. Occurrence and Molecular Study of Hypermucoviscous/Hypervirulence Trait in Gut Commensal *K. pneumoniae* from Healthy Subjects. Microorganisms 2023;11(3):704.
- 22-Elmonir W, Abd El-Aziz NK, Tartor YH, Moustafa SM, Abo Remela EM, Eissa R, et al. Emergence of Colistin and Carbapenem Resistance in Extended-Spectrum β -Lactamase Producing *Klebsiella pneumoniae* Isolated from Chickens and Humans in Egypt. Biology 2021; 10(5): 373.
- 23-Liu C, Du P, Xiao N, Ji F, Russo TA, & Guo J. Hypervirulent *Klebsiella pneumoniae* is emerging as an increasingly prevalent *K. pneumoniae* pathotype responsible for nosocomial and healthcare-associated infections in Beijing, China. Virulence 2020;11(1):1215–1224.
- 24-Du P, Liu C, Fan S, Baker S, Guo J. The Role of Plasmid and Resistance Gene Acquisition in the Emergence of ST23 Multi-Drug Resistant, Hypervirulent *Klebsiella pneumoniae*. Microbiol Spectr 2022;10(2): e 0192921.
- 25-Liu C, Du P, Yang P, Yi J, Lu M, Shen N. Emergence of Extensively Drug-Resistant and Hypervirulent KL2-ST65 *Klebsiella pneumoniae* Harboring blaKPC-3 in Beijing, China. Microbiol Spectr 2022;10(6): e0304422.
- 26-Imtiaz W, Syed Z, Razaque Z, Andrews SC, Dasti JI. Analysis of Antibiotic Resistance and Virulence Traits (Genetic and Phenotypic) in *Klebsiella pneumoniae* Clinical Isolates from Pakistan: Identification of Significant Levels of Carbapenem and Colistin Resistance. Infect Drug Resist 2021;14: 227-236.
- 27-Tutelyan AV, Shlykova DS, Voskanyan SL, Gaponov AM, Pisarev VM. Molecular Epidemiology of Hypervirulent *K. pneumoniae* and Problems of Health-Care Associated Infections. Bull Exp Biol Med 2022;172(5):507-522
- 28-Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Triplicane Dwarakanathan H, Murugan D, Umashankar Y, Monk PN, et al. The Influence of Biofilms on Carbapenem Susceptibility and Patient Outcome in Device Associated *K. pneumoniae* Infections: Insights Into Phenotype vs Genome-Wide Analysis and Correlation. Frontiers in microbiology 2020; 11:591679
- 29-Caneiras C, Lito L, Melo-Cristino J, Duarte A. Community- and Hospital-Acquired *Klebsiella pneumoniae* Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance. Microorganisms 2019;7(5):138.