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Original article

Characterization of virulence genetic profile and resistance patterns of clinical *Klebsiella pneumoniae* isolates: Classic versus hypermucoviscous phenotypes

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ABSTRACT

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is one of the most clinically important opportunistic pathogens involved in both community-(CAIs) and hospital-acquired infections (HAIs). The hypervirulent *K. pneumoniae* (hv*Kp*) responsible for disseminated infections in healthy and immunosuppressed individuals has emerged with considerable ability to get antibiotic resistance as well. We aimed to characterize the virulence genetic profile and resistance phenotypes of the clinical *K. pneumoniae* isolates at Menoufia University Hospitals (MUHs) by phenotypic and molecular methods. **Methods:** 84 *K. pneumoniae* isolates were collected and classified as classic (*cKp*) or hypermucoviscous (*hmvKp*) phenotypes by string test. Antimicrobial resistance patterns were determined phenotypically and multiplex PCR verified the existence of some of the suspected virulence genes. **Results:** Out of 84 *K. pneumoniae* isolates, 27 (32.1%) had a positive string test and identified as *hmvKp*. The remaining 57 isolates (67.9%) were string negative and reported as *cKp*. Higher resistance rates associated with ES β L, AmpC and carbapenemase production were observed in *cKp* compared to *hmvKp* particularly those of hospital origin with a significant statistical difference ($p < 0.05$). *RmpA* and *iutA* genes were strongly associated with *hmvKp* than *cKp*. The prevalence of *blaKPC-2* gene was significantly higher in *cKp* (33.3%) than *hmvKp* (7.7%). 80.8% of the isolated *hmvKp* isolates proved to be hv*Kp* (positive for both *rmpA* and *iutA* genes). **Conclusions:** *HmvKp* strains are isolated from patients with increasing frequency and constitute a significant proportion of clinical *K. pneumoniae* isolates. The emergence of *blaKPC*-producing hv*Kp* strains in the hospital settings confirms the importance of epidemiologic surveillance and clinical awareness of this pathogen.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is considered as one of the most clinically relevant species in immunocompromised individuals involved in both CAIs and HAIs [1].

Hypervirulent *K. pneumoniae* (hv*Kp*), generally associated with the hypermucoviscosity (HM) phenotype, has emerged as a clinically significant pathogen since the mid-1980s. Hypervirulent *K. pneumoniae* is commonly

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encountered in pyogenic liver abscesses, osteomyelitis and other invasive disseminated infections, in a generally younger and apparently healthy population. Infections caused by hv*Kp* were primarily found in East Asia and now are increasingly being reported worldwide [2,3].

Over the past years, HM has been regarded as an important *in vitro* parameter for hv*Kp* identification, but several controversies have been declared [4]. The large virulence plasmid pLVPK carrying capsular polysaccharides regulator genes (*rmpA* and *rmpA2*) and other siderophores gene clusters were identified as important contributors for hv*Kp* virulence [5]. A strong correlation between carriage of pLVPK-derived virulence plasmid and pyogenic infection has been reported [6].

Klebsiella pneumoniae can secrete various siderophores regulated by various genes e.g. *iutA* which are aerobactin-encoding genes that acquire iron in iron-depleted environments like in a human host. The hv*Kp* strains have a 6- to 10-fold increased siderophores activity compared with c*Kp* strains [6].

Other genes that are involved in *Kp* virulence include fimbrial and non-fimbrial adherence genes such as *mrkD* gene. *MrkD* is believed to act as the type 3 fimbrial adhesin essential for biofilm formation by *Kp* isolates [7,8].

Although most hv*Kp* isolates are antibiotic-susceptible, some strains with both virulence and resistance, such as the carbapenem-resistant hv*Kp* isolates, are increasingly being detected. Difficulties in managing carbapenem-resistant hv*Kp* infections could make this strain the next worldwide “superbug” in waiting. *K. pneumoniae* carbapenemases (KPCs) have spread globally leading to the emergence of strains capable of infecting healthy persons [9].

Epidemiological analysis of recent clinical isolates warns the global dissemination of hv*Kp* strains with more antibiotic resistance in the near future. Therefore, an immediate response to recognize the global dissemination of this hypervirulent strain with resistance determinants is an urgent priority [2].

The objectives of this work were to assess the antimicrobial susceptibility patterns of the isolated classic and hypermucoviscous *Kp* and determine the frequency of hypervirulent clinical isolates at Menoufia University Hospitals. The biofilm-forming ability was also evaluated and some of the suspected virulence genes were verified by the multiplex PCR assay.

Methods

This study was conducted at Medical Microbiology and Immunology Department, in collaboration with the Central Laboratory, Faculty of Medicine, Menoufia University during the period from April 2019 to September 2020. Clinical samples were collected from 270 patients admitted to different departments and ICUs of MUHs and Outpatient Clinics. The study protocol was approved by local ethics committee of Faculty of Medicine, Menoufia University. An informed consent was obtained from each patient or their guardians.

• Specimen collection, isolation and identification of *K.pneumoniae*

A total of 340 clinical samples (95 blood, 64 sputum and 12 bronchial aspirate, 53 pus swabs, 36 surgical drain samples, 1 liver abscess drainage, 32 ascetic fluid, 22 urine samples, 17 burn swabs and 8 CSF) were collected, processed, and cultured onto different bacteriological media for isolation of *Klebsiella* spp. *K.pneumoniae* isolates were identified by the automated Vitek- 2 system (bioMerieux, France). The identified isolates were preserved on tryptic soy broth with 16% glycerol and frozen at -80°C.

• Demonstration of hypermucoviscosity (hmv*Kp*) by string test

The hmv*Kp* phenotype was determined by the string test. Briefly, an inoculation loop was used to stretch the bacterial colonies of *Kp* isolates on an agar plate from overnight culture. The formation of viscous string of more than 5mm in length was considered to be positive [10] (Figure 1).

• Antimicrobial susceptibility testing and detection of resistance phenotypes

Antimicrobial susceptibility was performed for all *Kp* isolates by the disk diffusion method on Muller Hinton agar plates (MHA;CM0337, Oxoid, UK) against different antimicrobial agents (Oxoid, UK) and interpreted according to Clinical Laboratory Standard Institute (CLSI/2019) [11] (Table 1) and FDA/2010 break points for tigecycline [12]. All the included *Kp* isolates were investigated for the following:

▪ Phenotypic detection of ESβL production by:

a) *Cephalosporins/clavulanate combination test*
Klebsiella pneumoniae isolates were considered ESβL- producers if the inhibition zone around the

combined ceftazidime/clavulanic acid disk (30/10 μ g) was at least 5 mm larger than that of ceftazidime disk (30 μ g) alone [13].

b) The ES β L NDP (Nordmann-Dortet-Poirel) test

Colorimetric detection of ES β L enzymes was performed by detection of hydrolysis of the lactam ring of cephalosporin (cefotaxime), as it generates a carboxyl group which acidifies the culture media. The change in pH is identified by the color change (from red to yellow/orange) using pH indicator (phenol red). Inhibition of ES β L activity (unchanged red color) is confirmed by adding tazobactam [14].

- **Detection of AmpC β -Lactamases:** by preparing AmpC disk test. The test is based on using Tris-EDTA to permeabilize the bacterial cell and release β -lactamases into the external environment. After incubation, plates were examined for an indentation or a distortion of the zone of inhibition around AmpC disk, indicating an enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no inactivation of cefoxitin (negative result) [15].
- **Phenotypic detection of carbapenemase production:** was performed by the original CarbaNP (carbapenemase Nordmann-Poirel) test. Carbapenemase detection is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, causing change in pH which is detected by phenol red indicator [16] (**Figure 2**).
- **Demonstration of biofilm production by *K.pneumoniae* isolates:** The modified Congo red agar method (MCRA) was applied. Black colored colonies were interpreted as positive biofilm-producing strains in contrast with red colonies which was interpreted as negative biofilm producers [17].
- **Detection of *rmpA*, *mrkD*, *iutA*, and *blaKPC-2* genes by multiplex PCR assay:** Bacterial DNA of 50 *Kp* strains (24 *cKp* and 26 *hmvKp*) was extracted and purified using the gene JET™ genomic DNA purification kit (Thermo Fisher Scientific, UK). The used primers were:

Primer name	Sequence	R
<i>RmpA</i>	F: ACT GGG CTA CCT CTG CTT CA R: CTT GCA TGA GCC ATC TTT CA	18
<i>MrkD</i>	F:AAGCTATCGCTGTACTTCCGGCA R: GCGTTGGCGCTCAGATAGG	19
<i>IutA</i>	F:GGGAAAGGCTTCTCTGCCAT R:TTATTCGCCACCACGCTCTT	20
<i>KPC-2</i>	F:ATCGCCGTCTAGTTCTGCTG R: CCCTCGAGCGCGAGTCTA	21

Amplification was done by: an initial denaturation at (95°C for 15 min), followed by 30 cycles [(DNA denaturation at 94°C for 30 sec), primer annealing (at 60°C for 90 sec), primer extension (72°C for 1 min), and final extension (72°C for 10 min). Electrophoresis was performed with agarose gel 1.5% (Fermentas, Lithuania) stained with ethidium bromide (Sigma, USA) for 20 minutes. The products were visualized by UV trans-illuminator and compared with a 100 bp DNA ladder (535 for *rmpA*, 226 for *mrkD*, 920 bp for *iutA* and 1070 bp for *KPC2* genes) (**Figure 3**).

Figure 1. String test. A positive string test is defined as the formation of viscous strings of > 5mm in length from colonies on an agar plate.

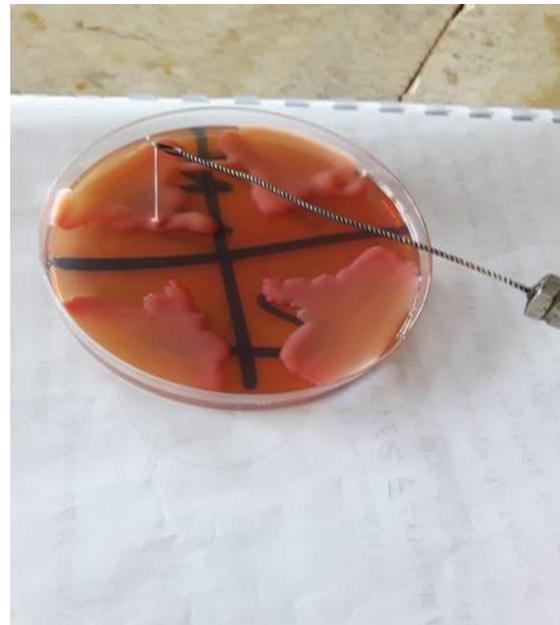


Figure 2. Carpa NP test. Red colour represents negative result while yellow colour represents positive result.

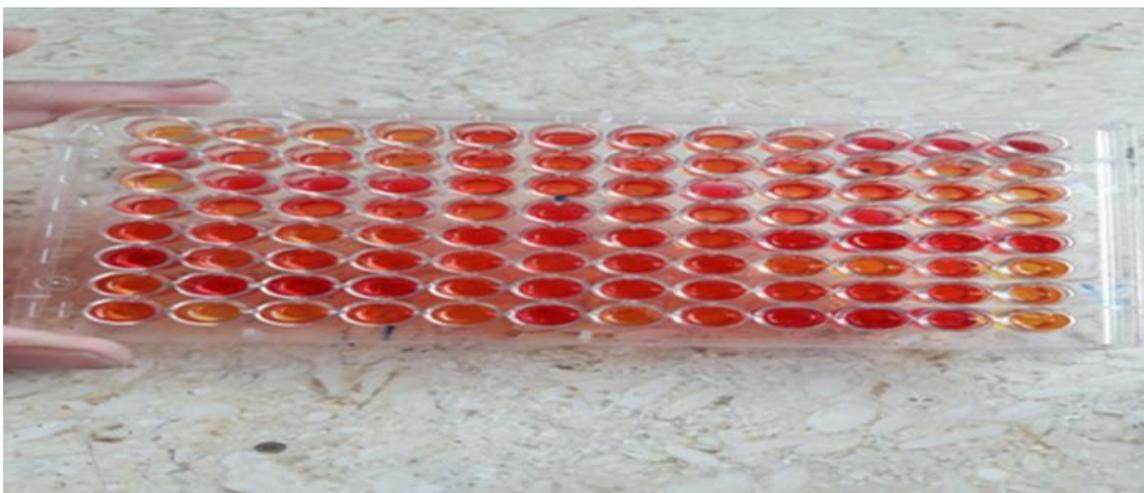
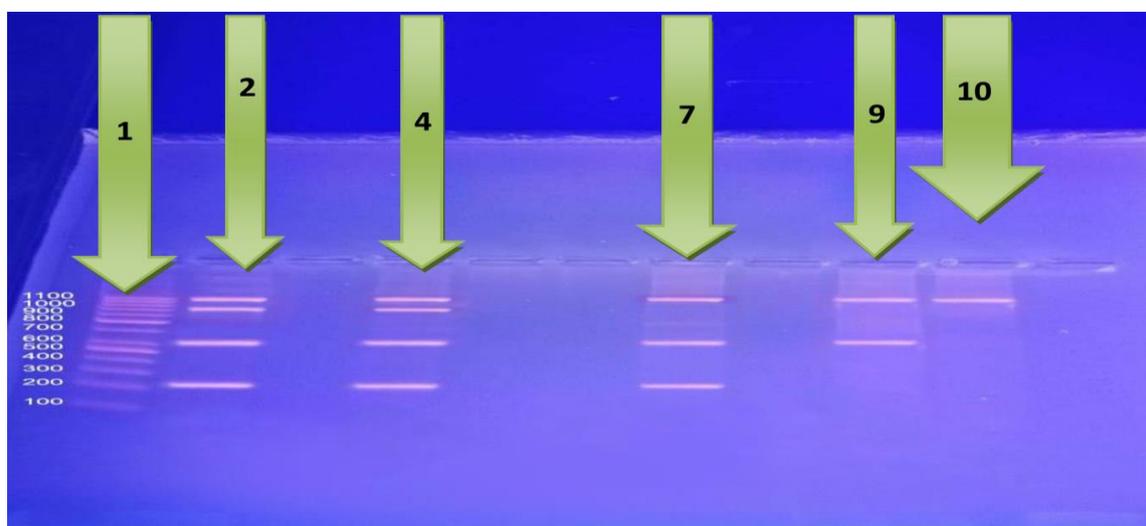


Figure 3. Multiplex PCR amplified products of *rmpA* (535bp), *iutA* (920 bp), *mrkD* (226 bp) and *blaKPC-2* (1070 bp) genes from *hmvKp* and *cKp* isolates:



Lane 1: DNA molecular size marker (100-1000 bp). Lane 2,4: Positive for *rmpA*, *iutA*, *mrkD* and *KPC-2* genes. Lane 7: Positive for *rmpA*, *mrkD* and *KPC-2* genes. Lane 9: Positive for *rmpA* and *KPC-2* genes. Lane 10: Positive for *KPC-2* gene.

Statistical analysis

Data were collected, tabulated and analyzed by statistical package for the social sciences (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) software. Chi-square test (χ^2) was done at 5% level of significance. Accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy.

Results

A total of 84 *Kp* isolates were isolated from 270 patients suffering from either CAIs or HAIs. Based on the results of string test, the hypermucoviscous phenotype was detected in 27

isolates (32.1%) and identified as *hmvKp* while the remaining 57 isolates (67.9%) were string test negative and designated as *cKp*.

Both *cKp* and *hmvKp* phenotypes were more frequently isolated from blood specimens (19/57; 33.35% and 10/27; 37%, respectively) followed by respiratory secretions (16/57; 28.1% and 9/27; 33.3%). About 15.8% (9/57) of *cKp* isolates was obtained from ascetic fluid specimens vs. none of *hmvKp* isolates. The least isolation rate was from CSF specimens representing 3.5% (2/57) for *cKp* and surgical drains for *hmvKp* (1/27; 3.7%).

Notably, the highest rate of isolation of both *hmvKp* and *cKp* was from patients within age group of 18-60 years (40.75 for *hmvKp* and 45.6%

for *cKp*) followed by age group below 18 years (33.3% for each phenotype). Both *hmvKp* and *cKp* phenotypes were isolated from males more frequently (63% & 68.4%, respectively) than females (37% & 31.6%, respectively). Neither age nor sex was significantly associated with *hmvKp* infections ($p > 0.05$). Among *cKp*, 52.6% (30/57) were obtained from HAIs. While, CAIs were significantly more likely to be *hmvKp* (19/27; 70.4%) ($p < 0.05$).

In contrast to patients with *cKp* infections, patients with *hmvKp* infections were more likely to have diabetes mellitus with a significant statistical difference ($p < 0.05$). Exposure to invasive procedure was documented as a significant risk factor for acquiring *cKp* infections (70.2%) but not for *hmvKp* (37%) ($p < 0.05$). History of antimicrobial administration proved no significant difference between both *cKp* and *hmvKp* phenotypes ($p > 0.05$).

Both *cKp* and *hmvKp* strains exhibited high antimicrobial-resistance rates. However, *hmvKp* showed significantly lower resistance rates for almost all the tested antimicrobial drugs as compared to *cKp* with a statistically significant difference ($p < 0.05$). All *hmvKp* isolates were susceptible to fosfomycin. The results of antimicrobial susceptibility testing for *hmvKp* and *cKp* are summarized in **table (1)**. Importantly, the hospital-acquired *hmvKp* strains exhibited higher resistance rates than community-acquired isolates with a significant statistical difference for all the tested antibiotics ($p < 0.05$).

As for resistance phenotypes, the frequencies of ES β L production among *hmvKp* were significantly lower than that for *cKp* (14.8% vs. 59.6%) with a highly significant statistical difference ($p < 0.001$). Similarly, both AmpC and carbapenemase production were significantly more

prevalent among *cKp* than *hmvKp* isolates (26.3% vs. 7.4% and 35% vs. 11.1% respectively) ($p < 0.05$) (**Table 2**). Regarding biofilm formation, 100% (27/27) of *hmvKp* isolates and 91.2% (52/57) of *cKp* isolates were potential biofilm producers with no statistically significant difference ($p > 0.05$).

The multiplex PCR assay revealed that, the prevalence of both *rmpA* and *iutA* genes was higher among *hmvKp* as compared to *cKp* isolates with a highly significant difference ($p < 0.001$). Nevertheless, the genetic determinant of carbapenem resistance *blaKPC-2*, was highly detectable among *cKp* strains than *hmvKp* ($P < 0.05$). However, no significant difference was found regarding the distribution of *mrkD* gene ($P > 0.05$). According to PCR results, the incidence of *hmvKp* reached 80.8% (21/26) among the collected *hmvKp*. Such strains proved to carry the large virulence plasmid pLVPK by the multiplex PCR assay with a highly significant statistical difference ($p < 0.001$) (**Table 3**).

According to the source of infection, the most frequently detected gene was *mrkD* for both community and hospital-acquired *Kp* isolates either *cKp* or *hmvKp* with no significant statistical difference ($p > 0.05$). However, the positive rates of *rmpA* and *iutA* genes were significantly higher among community-acquired *hmvKp* isolates than hospital-acquired ones ($p < 0.001$ & $p < 0.05$ respectively). On the contrary, *blaKPC-2* gene was more detectable in hospital- than community-acquired *hmvKp* with a significant difference ($p < 0.05$) (**Figure 4**).

Considering PCR as the gold standard, the sensitivity, specificity, PPV, NPV and accuracy of the string test in detecting *hmvKp* isolates respectively were 95.2 %, 79.3%, 76.9%, 95.8% and 86%.

Table 1.Antimicrobial resistance pattern of *cKp* and *hmvKp* clinical isolates.

Antibiotics	Abbreviation- Disk content (μ g)	<i>cKp</i> (n = 57)		<i>hmvKp</i> (n=27)		χ^2	p value
		No.	(%)	No.	(%)		
Piperacillin	Prp (100)	41	(71.9)	15	(55.6)	2.21	>0.05
Piperacillin/ tazobactam	TPZ (100/10)	35	(61.4)	13	(48.1)	1.31	>0.05
Cefoxitin	FOX (30)	37	(64.9)	10	(37)	5.77	<0.05*
Ceftriaxone*	CRO (30)	46	(80.7)	12	(44.4)	11.26	<0.001**
Ceftazidime	CZC (30)	42	(73.7)	14	(51.9)	3.92	<0.05*
Cefepime	FEP (30)	38	(66.7)	9	(33.3)	8.26	<0.05*
Aztreonam	ATM (30)	46	(80.7)	11	(40.7)	13.41	<0.001**
Imipenem	IPM (10)	33	(57.9)	12	(44.4)	1.33	>0.05
Meropenem	MEM (10)	35	(61.4)	14	(51.9)	0.68	>0.05
Ertapenem	ETP (10)	40	(70.2)	15	(55.6)	1.73	>0.05
Amikacin	AK (30)	24	(42.1)	10	(37)	1.35	>0.05
Gentamycin*	CN (10)	38	(66.7)	11	(40.7)	5.06	<0.05*
Tobramycin	TOB (10)	32	(56.1)	10	(37.0)	2.67	>0.05
Ciprofloxacin*	CIP (5)	42	(73.7)	13	(48.1)	5.28	<0.05*
Levofloxacin	LEV(5)	39	(68.4)	13	(48.1)	3.19	>0.05
Tigecycline	TGC (30)	22	(38.6)	4	(14.8)	4.84	<0.05*
Fosfomycin	FOS (200)	7	(12.3)	0	(0.0)	3.61	>0.05
MDR		37	(64.9)	6	(22.2)	14.50	<0.05*
XDR		15	(26.3)	2	(7.4)	4.05	<0.05*
PDR		5	(8.8)	0	0	2.60	>0.05

** : Highly Significant statistical difference, MDR: Multidrug resistant, XDR: Extremely drug resistant, PDR: Pandrug resistant

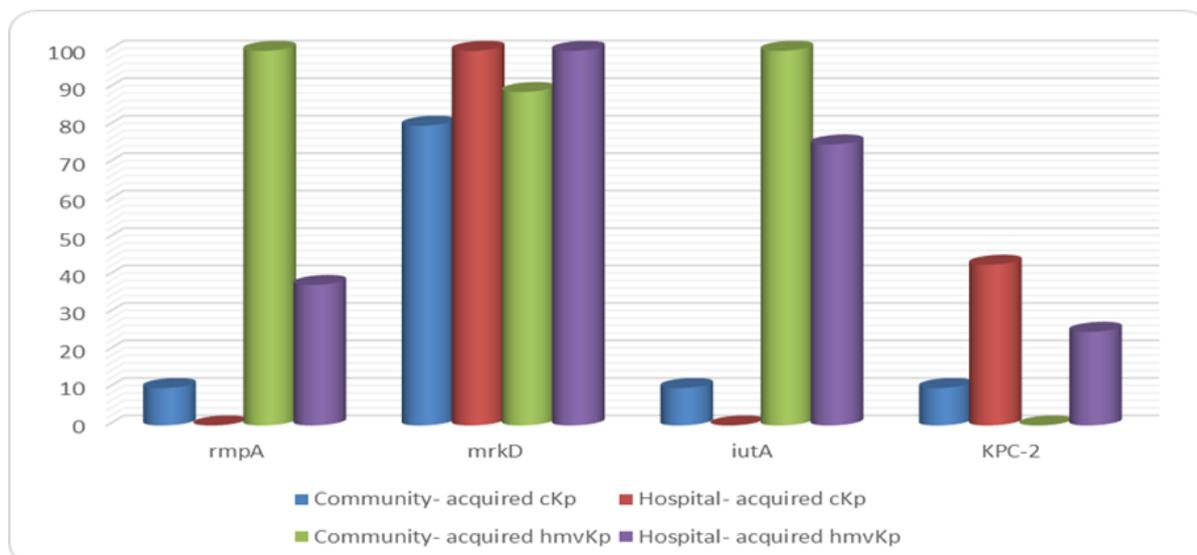
Table 2. Comparison between *cKp* and *hmvKp* isolates regarding ES β L-, AmpC and carbapenemase production.

Beta-lactamase type	<i>cKp</i> (n=57) No. (%)	<i>hmvKp</i> (n=27) No. (%)	χ^2	p value
• ES β L*	34 (59.6)	5 (14.8)	12.46	<0.001
• AmpC	15 (26.3)	2 (7.4)	4.05	<0.05
• Carbapenemase production	20 (35)	3 (11.1)	5.29	<0.05

*Positive isolates for both combined disk and ES β L NDP tests

Table 3. Distribution of *rmpA*, *mrkD*, *iutA* and *KPC2* genes among *K.pneumoniae* phenotypes by multiplex PCR assay.

Virulence genes	<i>cKp</i> (n=24)				<i>hmvKp</i> (n=26)				χ^2	<i>p</i> value
	Positive		Negative		Positive		Negative			
	No.	%	No.	%	No.	%	No.	%		
<i>rmpA</i>	1	4.2	23	95.8	21	80.8	5	19.2	29.72	<0.001
<i>mrkD</i>	22	91.7	2	8.3	24	92.3	2	7.7	0.007	>0.05
<i>iutA</i>	1	4.2	23	95.8	24	92.3	2	7.7	38.78	<0.001
<i>blaKPC-2</i>	8	33.3	16	66.7	2	7.7	24	92.3	5.12	<0.05
Coexistence of <i>rmpA</i> + <i>iutA</i>	0	0	0	0	21	80.8	5	19.2	29.72	<0.001

Figure 4. Distribution of *rmpA*, *mrkD*, *iutA* and *blaKPC2* genes among *K.pneumoniae* phenotypes regarding to the source of infection.

Discussion

K. pneumoniae is involved in a diverse array of HAIs and CAIs and contributes to substantial morbidity and mortality. The emergence of the multi-resistant hypervirulent clones is worrisome; such isolates are capable of producing fatal invasive infections even in younger and healthy individuals [3].

In the present study, out of 84 *Kp* isolates, 27 (32.1%) *hmvKp* were identified by positive string test while, the remaining 57 (67.9%) isolates were string negative and categorized as *cKp*. Regarding *hmvKp*, quite similar results were also reported in other countries (33%, 31.4% and 37.4%) [22-24]. On the other hand, *hmvKp* accounted only for 8.2% and 5.4% among all the isolated *Klebsiella* in other

previous studies [25,26]. In the same field, **Yan et al.** and **Khaertynov et al.** reported higher prevalence rates for *hmvKp* of about 46.6% and 53.6% respectively [27,28].

Compared with *cKp* infection, *hmvKp* infections were mainly community-acquired which demonstrates that *hmvKp* isolates play an important role in community-acquired infections but now emerging also as a hospital-acquired pathogen as proved in this study. Such observation agreed with **Lin et al.** and **Hao et al.** [29,30].

According to current results, the highest rate for isolation of *Kp* was from blood samples (33.3% for *cKp* and 37% for *hmvKp*) followed by respiratory secretions (28.1% and 33.3%, respectively) which coincided with **Li et al.**[20].

Also, **Rafat et al.** [31] recorded the same findings (37.2% for blood, 30% for urine and other specimens totally represented 32.8%). Previous studies showed that liver abscess was positively associated with *hmvKp* infection [32,33]. One *hmvKp* isolate was obtained from community-acquired liver abscess drainage specimen in our study.

Neither age nor sex was significantly associated with *hmvKp* infections ($p > 0.05$) which came in agreement with other studies [22,34,35] ($p > 0.05$). However, **Yang et al.** in China showed that patients infected with *hmvKp* were younger and mostly of male gender than those infected with *cKp* ($p < 0.05$) [32]. Among the studied underlying systemic diseases, only diabetes mellitus was significantly associated with *hmvKp* infections compared to *cKp* (33.3% vs. 8.8%). This finding was in parallel with that of **Li et al.**[34] and **Gu et al.**[35] who declared diabetes mellitus as a significant risk factor for acquiring *hmvKp* infections.

In accordance with previous reports [22, 27, 36], the current study proved that *hmvKp* were less resistant to most antimicrobials than *cKp*. There was also a significant difference between the resistance rates to all antibiotics for HAIs as compared to CAIs which came in agreement with **EL-Mahdy et al.**[37] and **Cubero et al.**[24] who noticed that antibiotic resistance was higher in hospital-acquired *hmvKp* infections. It might be caused by the increased exposure to antimicrobials in the hospital environments that promote the selection pressure for antimicrobial resistance. It may also suggest that more drug-resistant *K. pneumoniae* have spread to the community or the wild-type strains have evolved and became resistant [24].

Virulence and multi-drug resistance have historically been associated with non overlapping populations of *Kp*, with only the occasional and sporadic report of a hypervirulent strain acquiring antimicrobial resistance. Nevertheless, as both entities are transmissible within the population, the convergence of MDR and virulence factors in the same strain is possible and could erode the current boundaries between MDR and hypervirulent clones, exacerbating the world wide threat posed by *Kp* [38]. Interestingly, results in this study showed that 22.2% (6/27) of *hmvKp* isolates were MDR and 7.4% (2/27) were XDR. **Liu and Guo**[25] found that 20% of *hmvKp* isolates were MDR. Recently,

Fu et al.[39] identified an XDR carbapenemase-producing *Kp* strain carrying a hybrid virulent plasmid in Taiwan.

Importantly, 59.6% and 14.8% of *cKp* and *hmvKp* isolates in this study were respectively ES β L producers with a highly significant statistical difference ($p < 0.001$) which came in concordance with **Khaertynov et al.**[28] who found that 56% and 17% of *cKp* and *hmvKp* were respectively ES β L-producing isolates with a highly significant statistical difference. These results agreed with much extent with **Abd-Elmonsef et al.**[36] in Egypt and **Liu et al.**[21] who noticed that the positive rates of ES β L production among *cKp* and *hmvKp* isolates were 55.2% and 8.7% and 50% and 9.1% respectively. Higher rates were documented by others [27, 35, 40].

In our study, 26.3% and 7.4% of *cKp* and *hmvKp* isolates were respectively AmpC producers with a statistically significant difference ($p < 0.05$), a finding that matched with results of **Abd-Elmonsef et al.** [36]. Higher rates were showed by **El-Mahdy et al.**[37] in Egypt who reported 50% of *hmvKp* and 55.7% *cKp* isolates as AmpC positive isolates.

In this study, carbapenemase phenotypic activity was detected in 35% of *cKp* and 11.1% of *hmvKp* isolates by Carba NP test with a statistically significant difference ($p < 0.05$). Similar results were reported by **Xu et al.**[41] who addressed 43.5% and 5.8% respectively of *cKp* and *hmvKp* as carbapenemase producers while **Li et al.**[20] reported higher rates for carbapenemase production among *cKp* (57.3%) and *hmvKp* (59.3%). Carbapenemase production by *hmvKp* strains is a worrisome finding; such strains may result in a deleterious outcome [9].

Formation of biofilm provides superior protection for *Kp* species against the host immune responses, the action of antibiotics and enhances its persistence [35]. According to current results about 91.2% and 100% of *cKp* and *hmvKp* isolates were respectively potential biofilm producers but with no statistically significant difference. This finding was similar to the previous studies of **El-Mahdy et al.**[37], **Yan et al.**[27] and **Cubero et al.**[24]. On the other hand, **Fu et al.** [39] and **Wu et al.** [40] documented that biofilm production was higher in *hmvKp* than *cKp* with a statistically significant difference.

The *hmvKp* and *cKp* strains can be distinguished by a combination of phenotypic and genotypic characteristics. Several virulence genes,

including regulator of mucoid phenotype A (*rmpA* and *rmpA2*) and hypermucoviscosity-associated gene A (*magA*) have been documented to contribute to the hypervirulent phenotype. In addition, aerobactin was a major virulence factor for the enhanced production of siderophores in *hvKp* strains and used for the definition of *hvKp* [3]. In this study, we found that the prevalence of virulence-associated genes *rmpA* and aerobactin (*iutA*) were strongly associated with *hmvKp* than *cKp* strains. These data were consistent with previous reports regarding the virulence genetic profiles of clinical *Kp* isolates [28, 29].

The development of carbapenems-resistant Gram-negative pathogens is a major clinical and public health issue. An increasing prevalence of *Kp* carbapenemases (*blaKPCs*) has been observed worldwide which are mostly plasmid-mediated enzymes, and bacteria producing these enzymes are only susceptible to a few antibiotics such as tigecycline and colistin [42].

In the present study, 33.3% of *cKp* and 7.7% of *hmvKp* isolates had *blaKPC-2* gene with a significant statistical difference. Similarly, **Wu et al.** [40] found that 37.5% of *cKp* and 3.6% of *hmvKp* isolates carrying the *blaKPC-2* gene. Also, **Xu et al.**[38] documented 42.5% of *cKp* and 4.4% of *hmvKp* isolates to be *blaKPC2*-positive. However, higher rates were obtained by **Xu et al.**[41] who showed higher percentage of *hvKp* possessing *blaKPC-2* gene (68.2%) while **Kkhalil et al.**[43] found that none of *cKp* or *hmvKp* isolates to harbor the *blaKPC-2* gene. In addition, the prevalence of *blaKPC-2* was significantly higher among hospital-acquired *Kp* isolates with a positive rate of 42.9% for *cKp* and 25% for *hmvKp* isolated from hospital-acquired infection, but only 10% in *cKp* and none of *hmvKp* isolates from community-acquired isolates. In accordance with our results **Li et al.**[34] reported that the positive rate of *blaKPC-2* was 44.2% in isolates from hospital-acquired infection, but only 5.1% in isolates obtained from community-acquired infection.

Higher prevalence of *mrkD* on hospital -acquired pathogens was noticed in this study when compared with community-acquired ones (100% vs.80% for *cKp* and 100% vs. 89% for *hmvKp*). These results agreed with that of **Caneiras et al.**[44] who reported higher prevalence of fimbriae on hospital-acquired *Kp* isolates when compared with community-acquired isolates. According to **Bandeira et al.**[45] biofilms formed on medical

devices promote the onset and spread of healthcare-associated infections and biofilm-forming bacteria are generally more resistant to antibiotics.

Existence of *rmpA* and *iutA* genes is associated with *hvKp* strains. These virulent strains are spreading and have no longer been restricted in the community. The virulent strains in hospitals must be monitored to prevent fatal drawbacks among susceptible patients. The occurrence of virulent strains in HAIs as proved in the present study might indicate the transmission between community and hospital. The active molecular screening of capsular genotypes and *rmpA* in *Kp* isolates in hospitals could be an effective strategy in controlling and preventing the spread of such infections [46].

In agreement with previous studies [24,27] the positive rates of *rmpA* and *iutA* genes in this study were significantly higher among community-acquired *hmvKp* isolates than hospital-acquired ones. Notably, all (100%) community- acquired *hmvKp* strains were positive for both genes while for hospital- acquired strains, only 37.5% and 75% of *hmvKp* respectively harbored the two genes.

Differentiation of *hvKp* from *cKp* strains could ultimately impact patient care and contribute to improved outcomes. Specifically, accurate identification of *hvKp* would allow more rapid consideration of possible unrecognized sites of infection, which often manifest as occult abscesses. In medical literature, the terms hypervirulent and hypermucoviscous are commonly used as synonyms. Additionally, a positive string test has been considered as indicative of hypervirulence [48]. The current study evaluated the performance and accuracy of string test as a simple tool for laboratory diagnosis of *hvKp*. The test had 95.2 % sensitivity, 79.3% specificity, 76.9% PPV, 95.8% NPV and 86% accuracy in relation to PCR results. Our results came in parallel with previous study by **Russo and Gulick** who reported 91% sensitivity, 89% specificity, and 90% accuracy for string test [46]. **Tan et al.**found that string test had 90.5% sensitivity, 63.9% specificity, 97.2% NPV and 32.7% PPV [48].

Conclusion

The proportion of *hvKp* isolates among clinical *Kp* isolates is being increased. The degree of antimicrobial resistance among *hvKp* strains is rising over time. These life-threatening pathogens require further investigation to avoid potential

damage that may be caused in the future. Higher degree of awareness among physicians and microbiologists are urgently needed in order to better control the emergence and spread of drug-resistant hvKp isolates among the hospital settings. The employment of effective and objective diagnostic tools for simple identification of hvKp in the clinical field, implementation of epidemiological surveillance and development of novel antimicrobial agents are paramount.

Conflict of interest : The authors report no conflicts of interest

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