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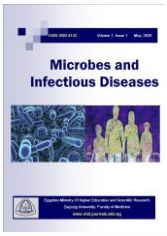

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Original article

Antimicrobial resistance pattern and molecular characterization of hypervirulent *Klebsiella pneumoniae* among hospital-acquired infections in the intensive care unit

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ABSTRACT

Background: A variant of *Klebsiella pneumoniae* has emerged more than twenty years ago and was named "hypervirulent" *K. pneumoniae* (hv*Kp*). This variant can be differentiated from the "classic" *K. pneumoniae* (c*Kp*) by its hypermucoviscosity and being incriminated in invasive infections affecting healthy individuals as well as those with immune suppression. **Aim:** This study aimed to detect the frequency of hv*Kp* in intensive care units among hospital acquired *K. pneumoniae* infections, and to analyze the differences in antimicrobial resistance patterns and molecular characteristics between hv*Kp* and c*Kp*. **Methods** : A total of 127 *K. pneumoniae* isolates were recovered from different hospital acquired infections. These isolates were subjected to string test for detection of hypermucovuscosity, antimicrobial susceptibility testing, biofilm formation, and detection of virulence-associated genes (iuc_A, rmp_A, rmp_{A2}, mag_A) using polymerase chain reaction (PCR). **Results:** Out of 127 *K. pneumoniae* isolates, 57 (44.9%) isolates showed the hypermucoviscous phenotype and 41.7% were hv*Kp*. The rmp_A, rmp_{A2} and mag_A genes were significantly associated with hv*Kp*. All *K. pneumoniae* isolates were multi-drug resistant (MDR) exhibited high resistance rates for most of the tested antibiotics. Biofilm production was detected in 46/53 (86.8%) of hv*Kp* and in 48 out of 74 (64.9%) c*Kp* isolates. **Conclusion:** Hv*Kp* was frequently isolated from hospital acquired infection specifically ICU patients with a warning rate of antimicrobial resistance and high rate of biofilm formation. String test remains an easy and simple phenotypic test however, genotypic detection of hv*Kp* virulence genes remains the gold standard confirmatory tool for identification of these strains.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is an opportunistic, Gram-negative enteric bacillus. It is a pathogen of great importance worldwide that can cause community acquired infections as well as health care associated infections [1].

There are two different pathotypes of *K. pneumoniae*: classical *K. pneumoniae* (c*Kp*) and hypervirulent *K. pneumoniae* (hv*Kp*). The c*Kp* is the most common subtype of *K. pneumoniae* strains, which has been known for its high probability to develop multidrug resistance and being responsible

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for most health care associated infections, in immunocompromised patients [2].

The variant hv*Kp* was first identified in 1986 in Taiwan [3]. Subsequently, there have been numerous reports of hv*Kp* across Asia, particularly in China, South Korea, and Japan. Furthermore, hv*Kp* has gained a global presence [4]. Contrary to c*Kp* infections, the hv*Kp* strains are known for causing community acquired metastatic infections in individuals not known to have any degree of immune suppression. One of the most popular infections in which hv*Kp* is incriminated is pyogenic liver abscesses, as well as other serious disseminated infections such as meningitis and endophthalmitis [2,5].

The hv*Kp* and c*Kp* strains can be identified by combination of phenotypic as well as genotypic criteria. Hypermucoviscosity is a typical criterion of hv*Kp* that is considered a reliable method for hv*Kp* identification in previous studies. Hypermucoviscous phenotype is determined by applying string test on cultivated strain on agar plates [6,7]. However, not all hv*Kp* strains are hypermucoviscous and some c*Kp* strains possess this characteristic as reported by different studies [8-10]; thus, the string test cannot be used alone to identify the hv*Kp* strains.

Hv*Kp* has acquired a number of virulence factors encoded genes, as iron acquisition genes (aerobactin, iuc_A), the regulator of mucoid phenotype (rmp_A, rmp_{A2}), mucoviscosity-associated gene A (mag_A) and additional virulence factors include biofilm formation. These genes are present on large virulence plasmids and within integrated chromosomal elements that confers its hypervirulent phenotype [11].

The siderophore named aerobactin plays a significant role in virulence as studies have demonstrated its importance in regulation of iron acquisition by bacteria enhancing their growth, replication, and virulence. Currently, it has been shown to have a high diagnostic accuracy for identifying hv*Kp* and the strains were defined as hv*Kp* based on its detection [12].

The rmp_A regulates the synthesis of extracellular polysaccharide capsule causing increase in its virulence and the phenotype of hypermucoviscosity. It renders the bacteria resistant to engulfment by phagocytes or to serum bactericidal factors. It also has a role in limiting the bactericidal effects of antimicrobial peptides [13]. The chromosomal mag_A gene has an essential role in *Klebsiella* infections. It is associated with the formation of a muscoviscous string that confers resistance to phagocytosis and makes the organism more virulent [14].

Numerous studies highlight the rising prevalence of multidrug-resistant hv*Kp*, particularly those producing extended-spectrum beta-lactamases and carbapenemases. Some researches indicate that c*Kp* strains have obtained mobilizable hv*Kp* virulence plasmids through horizontal gene transfer, leading to the emergence of multidrug-resistant hv*Kp* strains [15].

This study aimed to detect the frequency of hv*Kp* among hospital acquired *K. pneumoniae* infections in the intensive care units (ICUs) and to analyze the differences in antimicrobial resistance patterns and molecular characteristics between hv*Kp* and c*Kp*.

Material and methods

Study design and setting

This cross-sectional study was carried out during the period from June 2023 to February 2024 in the ICUs of Zagazig University Hospitals and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt.

The study was approved by the Institutional Review Board of Faculty of Medicine, Zagazig University (approval #10689-2-5-2023) and carried out in accordance with the Declaration of Helsinki. Informed written consents were obtained from patients' relatives.

Bacterial isolation and identification

One hundred and twenty-seven *K. pneumoniae* isolates were collected from various clinical samples including endotracheal aspirate, urine, blood and wound swabs.

Collected specimens were cultured on MacConkey agar (Oxoid, UK) then incubated at 37°C for 48 hours. All isolates were identified by conventional microbiological methods; Gram stain, identifying lactose fermenting mucoid colonies on MacConkey agar and by biochemical reactions [16]. *K. pneumoniae* isolates were further confirmed by using an automated VITEK 2 compact system (bioM'erieux, France).

Phenotypic detection of hypermucoviscosity by string test

Bacterial colonies were cultivated overnight on a MacConkey agar plate at 37°C. Bacteriological loop was used to stretch the grown colonies, the test was considered positive when the mucoid string is stretched > 5 mm in length, and the isolate was identified as hypermucoviscous **(Figure 1)** [17].

Molecular detection of *iuc*$_A$ and other virulence-associated genes

DNA extraction was performed using G-spinTM Genomic DNA Extraction Kit (iNtRON Biotechnology, Inc., Korea) as per the manufacturer's instructions. Each PCR reaction mixture was prepared in a total volume of 20µl including: 4µl of template DNA, 10 µl of PCR master mix and 1 µl of each primer then the volume completed with nuclease free water up to 20µl. The following thermal cycling conditions were used for amplification of *rmp*$_A$, *rmp*$_{A2}$ and *mag*$_A$: an initial denaturation step at 95°C for 10 minutes, and 35 cycles of DNA denaturation at 94°C for 30 seconds, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 minute, ending up with 10 minutes at 72°C for the final extension [18].

PCR conditions for amplification of *iuc*$_A$ were set as follows: initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 49°C for 15 min and extension at 72°C for 1 min and final extension at 72°C for 10 min [19]. Gel electrophoresis and visualization of PCR products under UV light was performed **(Figure 2)**. Primers used are listed in **Table 1.** The isolate was identified as hv*Kp* by positivity of *iuc*$_A$ gene [20].

Antibiotic susceptibility testing of *K. pneumoniae* isolates

All isolates were tested for antimicrobial susceptibility by disc diffusion method on Muller-Hinton agar (MHA) (Oxoid, UK) for the following antibiotics: ampicillin (Am,10µg), amoxicillin/clavulanic acid (AMC, 20/10µg), ampicillin/sulbactam (SAM, 10/10µg), piperacillin/tazobactam (TPZ, 100/10 µg), ceftriaxone (CRO, 30µg), cefoxitin (FOX, 30 µg), cefazoline (CZ, 30µg), cefepime (FEP,30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM,10 µg), meropenem (MEM,10 µg), gentamicin (CN,10 µg), tobramycin (TOB, 10 µg), amikacin (AK,30 µg), ciprofloxacin (CIP,5 µg), levofloxacin (LEV,5 µg) and trimethoprime-sulfamethoxazole (SXT, 1.25/23.75 µg). For urine specimens, nitrofurantoin (F, 300 µg). All used antibiotics discs were supplied from (Oxoid, UK). *E. coli* ATCC 25922 was used as a quality control strain. The results were interpreted according to CLSI, 2024 [22].

Assessment of biofilm formation

A Microtiter plate was used for detection of biofilm formation in all *K. pneumoniae* isolates. Fresh *K. pneumoniae* colonies were inoculated in trypticase soya broth (TSB) (Oxoid, UK) with 1% glucose, then incubated at 37°C for 18–24 hours. Then, A total volume of 200 µL of inoculated TSB of each isolate was inoculated in sterile microtiter plates (96 well plate) and a well for negative control was done by adding 200 µL of non -inoculated TSB. Incubation was done at 37 °C for 24 hours. After incubation, the plate was gently tapped to remove any free-flowing bacteria and 200 µL of phosphate-buffered saline (PBS) was used to wash wells gently for about three times then the wells were allowed to dry in an inverted position. Crystal violet 0.1% was used for 10 min to stain the formed biofilm. Distilled water was used to wash the plates after staining 3 times and left to dry in an inverted position. After air- drying, 95% ethanol was used to re-solubilize the dye bound to the cells, which was then incubated at room temperature for 15 minutes.

Measurement of optical density (OD) was done using a microplate reader at 570 nm. Each well was tested in triplicate then, the mean OD was calculated. Optical density cut-off (ODc) was calculated as an average OD of negative controls + (3× standard deviation (SD) of negative controls).

If isolate in well showed OD ≤ ODc, it was reported as non-biofilm producer. While the isolate was reported as biofilm producer either weak biofilm producer if ODc < OD ≤ 2 × ODc; moderate biofilm if 2 × ODc < OD ≤4 × ODc; and strong biofilm producer if OD > 4 × ODc [23].

Statistical analysis

Data were statistically analyzed using statistical package for social science (SPSS) software version 25 was used. Qualitative data were expressed as frequency and percentages. The association between the qualitative data was assessed by chi-squared ($\chi 2$) test, and Fisher's exact test. *P* value < 0.05 was considered significant.

Results

A total of 127 isolates of *K. pneumoniae* were isolated from patients with hospital acquired infections in the ICUs of Zagazig University Hospitals. Most of them were isolated from endotracheal aspirate specimens 33.1% (52/127) followed by urine 27.6% (35/127) then blood and pus specimens 22.8% (29/127) and 16.5% (11/127) respectively. **Table 2** shows the distribution of the two variants of *K. pneumoniae* isolates among different clinical specimens.

Hypermucoviscous phenotype was identified by string test. Out of 127 *K. pneumoniae* isolates, 57 (44.9%) isolates showed the hypermucoviscous phenotype confirmed by positive string test, while the remaining 70 (55.1%) tested negative **(Figure 3A)**.

Regarding the distribution of virulence-associated genes, the positivity of *iuc*$_A$ gene was used as a criterion of identification of hv*Kp*, the gene was detectable in 53 out of 127 isolates (41.7%) which were identified as hv*Kp*. The remaining 74 *iuc*$_A$ negative isolates (58.2%) were identified as c*Kp* **(Figure 3B)**.

The other three studied genes were variably detectable among *K. pneumoniae* isolates. It was found that *rmp*$_A$ was significantly associated with hv*Kp*; 49/53 (92.5%) compared with 25/74 (33.8%) in c*Kp*. The *rmp*$_{A2}$ and *mag*$_A$ genes also showed a highly statistically significant difference in their distribution among hv*Kp* and c*Kp* isolates **(Table 3)**.

K. pneumoniae isolates exhibited high resistance rates for most of the tested antibiotics with no statistically significant difference between hv*Kp* and c*Kp* regarding the antimicrobial resistance pattern. The results of antimicrobial susceptibility testing for hv*Kp* and cK*p* were summarized in **Table 4**.

Biofilm formation was evaluated for all *K. pneumoniae* isolates. As regards hv*Kp* isolates, it was found that 46/53 (86.8%) were biofilm producers. The strong biofilm producers were 54.3%, the moderate biofilm producers were 45.7%, and none of them were weak biofilm producers. While biofilm formation was observed in 48 out of 74 (64.9%) of the c*Kp* isolates; 41.7% of them were strong biofilm producers, 37.5% were moderate biofilm producers, and 20.8% were weak biofilm producers **(Figure 4&5)**. There was a high statistically significant difference regarding biofilm formation in both hv*Kp* and c*Kp* isolates **(Table 5)**.

The association between biofilm formation and the distribution of virulence-associated genes revealed that there was no statistically significant difference in the distribution of these genes among hv*Kp* isolates and biofilm producing capacity **(Table 6)**.

Considering PCR as the gold standard, on correlating string test results with *iuc*$_A$ gene results, the sensitivity and the specificity of string test in our study were 96.2% and 91.9% respectively **(Table 7)**.

Table 1. Specific primers used for various virulence genes of *K. pneumoniae*.

Gene	Primers Sequences	Amplicon size	Reference
***rmp*$_A$**	Forward 5′-ACTGGGCTACCTCTGCTTCA-3′ Reverse 5′-CTTGCATGAGCCATCTTTCA-3′	516 bp	[18]
rmp$_{A2}$	Forward 5′-CTTTATGTGCAATAAG-GATGTT-3′ Reverse 5′-CCTCCTGGAGAGTAAGCATT-3′	450 bp	[21]
***mag*$_A$**	Forward 5′-GGTGCTCTTTACATCATTGC-3′ Reverse 5′-GCAATGGCCATTTGCGTTAG-3′	1282 bp	[18]
***iuc*$_A$**	Forward 5′-GCATAGGCGGATACGAACAT-3′ Reverse 5′-CACAGGGCAATTGCTTACCT-3′	556 bp	[9]

Table 2. Distribution of the two variants of *K. pneumoniae* among different clinical samples.

Specimen	hv*Kp* (n= 53)	c*Kp* (n= 74)	Test of significance	p-value
	No (%)	No (%)		
Endotracheal aspirate (52/127)	32 (61.5)	20 (38.5)	χ^2 =14.21	<0.001**
Urine (35/127)	17 (48.6)	18 (51.4)	χ^2 =0.93	0.34 (NS)
Blood (27/127)	12 (44.4)	15 (55.6)	χ^2 =0.10	0.75 (NS)
Pus (11/127)	4 (36.4)	7 (63.6)	Fisher's exact	0.97 (NS)

**statistically highly significant difference ($p < 0.05$), χ^2 = Chi square test. NS: non-significant.

Table 3. Distribution of virulence-associated genes among hv*Kp* and c*Kp* isolates.

Genes	hv*Kp* (n=53)		c*Kp* (n=74)		Test of significance	p-value
	Positive No (%)	Negative No (%)	Positive No (%)	Negative No (%)		
***rmp*A**	49 (92.5%)	4 (7.5%)	25 (33.8%)	49 (66.2%)	χ^2=43.71	**<0.001****
***rmp*A2**	40 (75.5%)	13(24.5%)	19 (25.7%)	55 (74.3%)	χ^2=30.79	**<0.001****
***mag*A**	23 (43.4%)	30 (56.6%)	5 (6.8%)	69 (93.2%)	χ^2=24.12	**<0.001****

**Statistically highly significant difference ($p < 0.05$), χ^2 = Chi square test.

Table 4. Antimicrobial resistance pattern of hv*Kp* and c*Kp* clinical isolates.

Antibiotic	hv*Kp* (n=53)		c*Kp* (n=74)		Test of significance	p-value
	No	%	No	%		
Ampicillin	53	100%	74	100%	----	----
Amoxicillin/clavulanic acid	53	100%	74	100%	----	----
Ampicillin/sulbactam	53	100%	74	100%	----	----
Piperacillin/tazobactam	48	90.6%	64	86.5%	χ^2=0.49	0.48 (NS)
Ceftriaxone	47	88.7%	69	93.2%	Fisher's exact	0.56 (NS)
Cefoxitin	48	90.6%	64	86.5%	χ^2=0.49	0.48 (NS)
Cefazoline	46	86.8%	65	87.8%	χ^2=0.03	0.86 (NS)
Cefepime	45	84.9%	62	83.8%	χ^2=0.03	0.86 (NS)
Ceftazidime	50	94.3%	69	93.2%	Fisher's exact	>0.99 (NS)
Imipenem	17	32.1%	14	18.9%	χ^2=2.90	0.09 (NS)
Meropenem	19	35.8%	18	24.3%	χ^2=1.99	0.16 (NS)
Gentamicin	41	77.4%	48	64.9%	χ^2=2.30	0.13 (NS)
Tobramycin	39	73.6%	44	59.5%	χ^2=2.72	0.10 (NS)
Amikacin	38	71.7%	43	58.1%	χ^2=2.47	0.12 (NS)
Ciprofloxacin	37	69.8%	48	64.9%	χ^2=0.34	0.56 (NS)
Levofloxacin	36	67.9%	46	62.1%	χ^2=0.45	0.50 (NS)
Trimethoprim- sulfamethoxazole	43	81.1%	66	89.2%	χ^2=1.65	0.20 (NS)
Nitrofurantoin (for urine samples)	14/17	82.4%	13/18	72.2%	Fisher's exact	0.76 (NS)

χ^2 = Chi square test. NS: non-significant.

Table 5. Biofilm formation capacity of *K. pneumoniae* clinical isolates.

Characteristics	hv*Kp* (n=53)	c*Kp* (n=74)	Test of significance	p-value
Biofilm producers	46(86.8%)	48 (64.9%)		
Non-biofilm producers	7(13.2%)	26(35.1%)	χ^2=**7.72**	**0.005***

*Statistically significant difference ($p < 0.05$), χ^2 = Chi square test.

Table 6. Association between biofilm formation and virulence-associated genes among hv*Kp* isolates.

Genes	hv*Kp* (n=53)				Test of significance	p-value
	Biofilm producers hv*Kp* (n=46)		Non-biofilm producers hv*Kp* (n=7)			
	Positive No (%)	Negative No (%)	Positive No (%)	Negative No (%)		
***rmp*A**	44(95.7%)	2(4.3%)	5(71.4%)	2(28.6%)	Fisher's exact	0.16 (NS)
***rmp*A2**	37(80.4%)	9 (19.6%)	3 (42.9%)	4 (57.1%)	Fisher's exact	0.10 (NS)
***mag*A**	21(45.7%)	25(54.3%)	2 (28.6%)	5(71.4%)	Fisher's exact	0.67 (NS)

NS: non-significant.

Table 7. Diagnostic performance of string test in relation to PCR results of *iuc*$_A$ gene.

String test			***iuc*$_A$ gene**	
			Positive (n=53)	**Negative (n=74)**
Positive (n=57)			51 (96.2%)	6 (8%)
Negative (n=70)			2 (3.8%)	68 (92%)
Sensitivity	**Specificity**	**Accuracy**	**Positive predictive value**	**Negative predictive value**
96.2%	91.9%	93.7%	89.4%	97.1%

Figure 1. Hypermucoviscosity by string test.

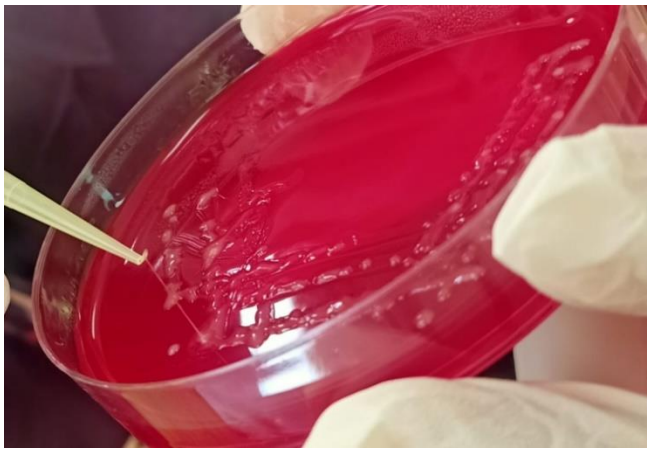


Figure 2. Agarose gel electrophoresis of different genes amplicons.
A: Lanes (1&3): positive for *rmp*$_A$ gene (516 bp); lanes (5-7): positive for rmpA2 gene (450 bp).
B: Lanes (1&2): positive for *mag*$_A$ gene (1282 bp); lanes (4): DNA ladder 100 bp (Thermofisher, USA); Lanes (5&6): positive for *iuc*$_A$ gene (556 bp).

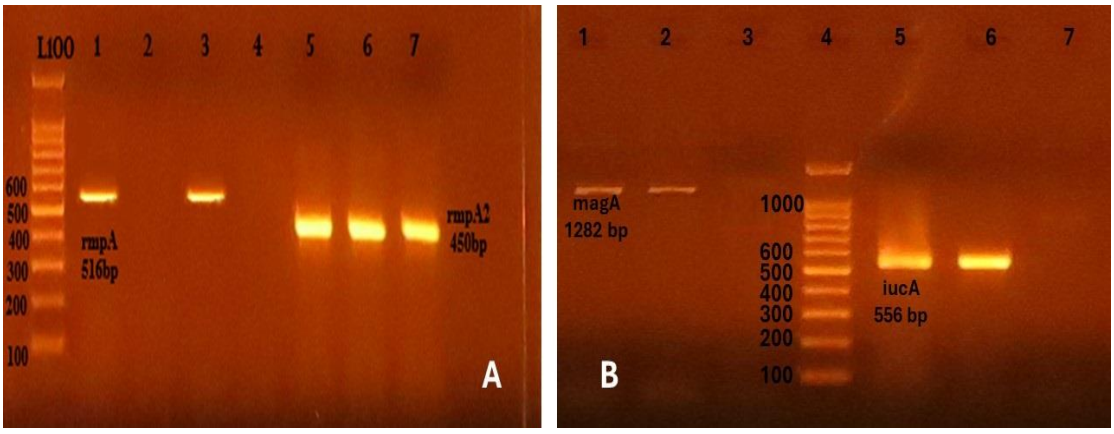


Figure 3A. Frequency of hypermucoviscous phenotype by string test, **3B.** Identification of hv*Kp* by *iuc*$_A$ gene.

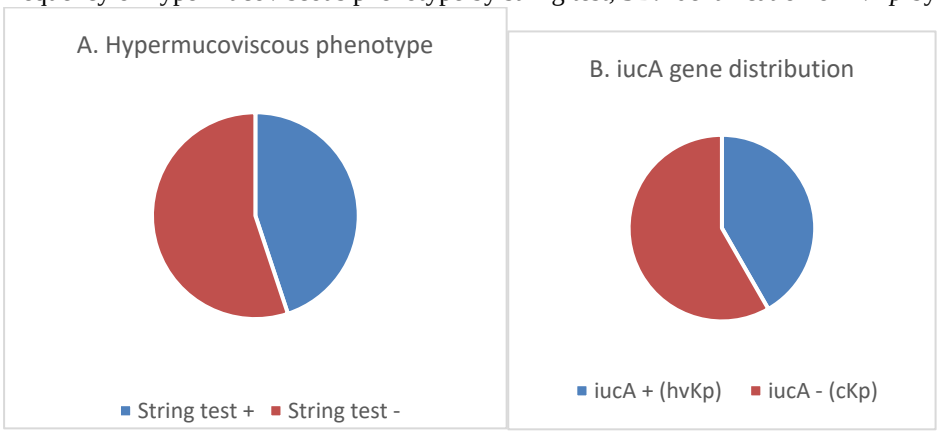


Figure 4. Detection of biofilm production capacity among *K. pneumoniae* isolates by using microtiter plate**.** A- negative control, B- positive control, C-strong biofilm producer, D-weak biofilm producer and E- moderate biofilm producer. All isolates were tested in triplicates.

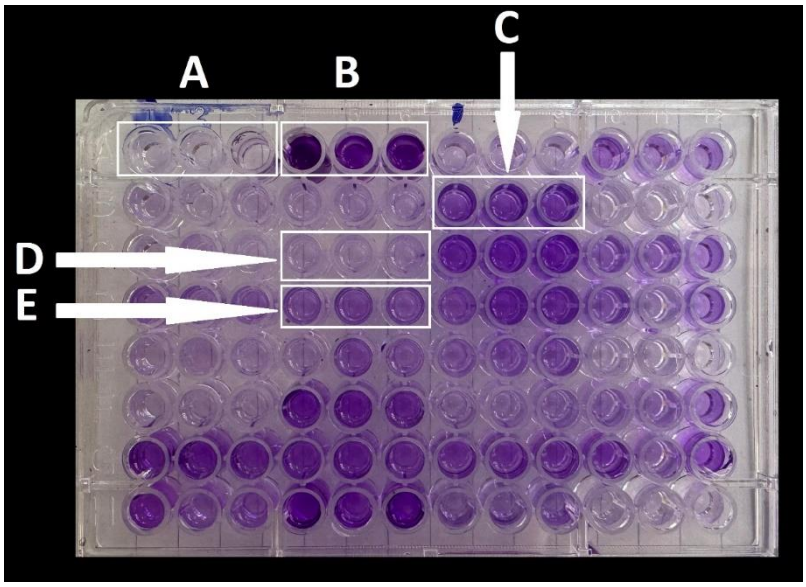
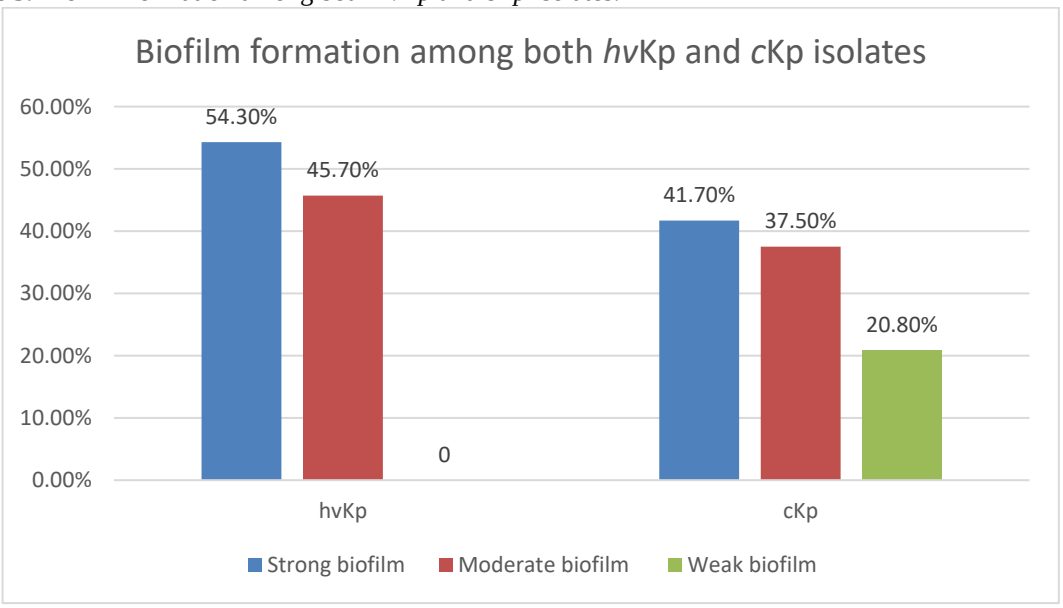


Figure 5. Biofilm formation among both hv*Kp* and c*Kp* isolates.



Discussion

Hv*Kp* is an emerging pathotype of *K. pneumoniae* that is known for its ability to develop multidrug resistance and is responsible for most health care associated infections in immunocompromised patients [2].

The current study included a total of 127 isolates of *K. pneumoniae*. Most of them were isolated from endotracheal aspirate specimens 33.1% (52/127) followed by urine 27.6% (35/127) then blood and pus specimens 22.8% (29/127) and 16.5% (11/127) respectively. This goes in agreement with another Egyptian and non-Egyptian studies that reported the highest isolation rate from respiratory specimens [20, 24-27].

The hypermucoviscous phenotype was detected in 57/127 isolates (44.9%) by positivity of string test. These results were in agreement with results reported by other studies [24-27].

Out of the total 127 *K. pneumoniae* isolates, 53 (41.7%) isolates were identified as hv*Kp* by iuc_A positivity. This ratio was near to that stated by other studies [20,24,26] who reported hv*Kp* isolation rate of 46.25%, 40.7% and 40.6% respectively. Lower hv*Kp* isolation ratio of 6.2%

was reported by an Egyptian study [19]. Considering the progressive and continuing rate of hv*Kp* spread among healthcare settings, it seems justifiable as this study was performed in 2018. Lower hv*Kp* isolation rate of 15.8% was also reported by a Saudi study [26]. Variations in hospital environmental conditions and sticking to infection control protocols may lead to variable colonization and accordingly isolation rates. Contrarily, higher hv*Kp* isolation rate was reported in an Egyptian study with 64% hv*Kp* isolation rate [20]. The different genotypic criteria used to identify hv*Kp* in this study may justify the different isolation rate.

There may be some overlap between the results of string test as a phenotypic indicator of the hypermucoviscous phenotype and the isolation rate of hv*Kp* recorded by several studies as the definitive identification criteria of hv*Kp* are somehow debatable. Some studies use the string test as the sole identifying criterion and consider hv*Kp* and hypermucoviscous phenotypes to be inseparable. However, many studies report that string test alone is insufficient for hv*Kp* identification and consider *iuc*$_A$ gene positivity either alone or along with other virulence genes more sensitive and specific for hv*Kp* identification [10, 28]. This is evidenced by string test positivity in 6 of the 74 *iuc*$_A$ negative c*Kp* isolates (8.1%) in the current study, string test positivity in c*Kp* is also reported in other studies [19,27].

Along with *iuc*$_A$ gene that was considered the identification criterion of hv*Kp* in this study, the most frequently detected virulence gene was *rmp*$_A$ gene that was detectable in 92.5%, followed by *rmp*$_{A2}$ gene that was detectable in 75.5%, and the least detectable was the *mag*$_A$ gene that was detectable in 43.4% of hv*Kp* isolates.

Molecular characters reported in the current study are comparable to other studies. Regarding *iuc*$_A$ detection rate among hv*Kp*, other studies reported comparable results [19,20,26]. Results of *rmp*$_A$ and *rmp*$_{A2}$ gene in the current study are also comparable to results reported by other studies [19, 20,24,26].

Other studies reported different gene detection rates, with lower detection rate of *iuc*$_A$, *rmp*$_A$ and *mag*$_A$ genes [27] but this study reported the gene detection rate collectively among both hv*Kp* and c*Kp* which makes these results justifiable. Lower total *rmp*$_A$ detection rate of 64% was also reported by an Egyptian study that reported the gene carriage rate in relation to the specimen from which the strain of hv*Kp* was isolated, gene carriage rate was as high as 100% in strains isolated from blood stream infection and as low as 0% in strains isolated from urinary tract infection which justifies the overall lower gene detection rate.

In the current study, there was no statistically significant difference between c*Kp* and hv*Kp* regarding rates and patterns of resistance to different antibiotics. These results come in agreement with results reported in other studies [19,24].

However, significantly lower rates of antibiotic resistance in hv*Kp* when compared to c*Kp* were reported by several studies [25-27]. The lower resistance rate reported by these studies could be explained by the fact that large plasmid carriage in virulent isolates may influence stability of resistance plasmids [29], hypermucoviscous phenotype may act as a barrier against acquisition of drug resistance genes [30] or loss of drug resistance genes upon acquisition of virulence genes [6]. In comparison to these studies, hv*Kp* isolates of the current study show higher levels of resistance, this may be justified by the role of biofilm production and the coexistence of multispecies sharing drug resistance mechanisms.

Contrarily, significantly higher rates of antibiotic resistance were reported by an Egyptian study [20], considering the significantly higher ratio of hv*Kp* isolation in this study, these results appear justifiable.

In the current study biofilm formation was evaluated for all *K. pneumoniae* isolates. There was a statistically significant difference in biofilm production among both study groups. As regards hv*Kp* isolates 46/53 (86.8%) were biofilm producers. With 54.3%, strong biofilm producers and 45.7% moderate biofilm producers. Regarding c*Kp*, 48 out of 74 (64.9%) were biofilm producers. 41.7% of them were strong biofilm producers, 37.5% were moderate biofilm producers, and 20.8% were weak biofilm producers. These results are comparable to those reported by other studies [24-26,31]. Contrarily, an Egyptian study reported non-significant difference in biofilm formation among hv*Kp* when compared to c*Kp* [19] this is probably due to the very low hv*Kp* isolation rate in this study 6.2%.

In this study non-significant difference was found on associating biofilm formation with hv*Kp*

virulence-associated genes. Same results were reported in an Egyptian study [24].

String test is still debatable whether it could be relied upon in hv*Kp* identification or screening as an easy and simple test. In this study, *iuc*$_A$ gene was considered the gold standard test for hv*Kp* identification, and comparably the string test has a sensitivity of 96.2% and specificity of 91.9%. These results are somehow different from results reported by another study with string test sensitivity of 86.11% and a specificity of 86.36% [24]. This difference is attributed to the *rmp*$_A$ gene being considered as the gold standard test in this study. Another study reported a sensitivity of 89% and a specificity of 91% [10].

Conclusion

High frequency of hv*Kp* isolates was detected among ICU patients with high rate of antimicrobial resistance and biofilm formation. Although the validity of the string test in detecting hv*Kp* strains is questionable, it is a simple and easy test that can be done in any laboratory. However, genotypic detection of virulence gene provides helpful and confirmatory tool to diagnose these strains.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

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