Original article

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

Asmaa M. Elbrolosy, Naira A. Eissa, Nahed A. Al-Rajhy, Esraa El-Sayed A. El-Mahdy, Rasha G. Mostafa*

Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt

ABSTRACT

Background: Klebsiella pneumoniae (K. pneumoniae) is one of the most clinically important opportunistic pathogens involved in both community-acquired infections (CAIs) and hospital-acquired infections (HAIs). The hypervirulent K. pneumoniae (hvKp) 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 ESβL, 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 hvKp (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 hvKp 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 (hvKp), generally associated with the hypermucoviscosity (HM) phenotype, has emerged as a clinically significant pathogen since the mid-1980s. Hypervirulent K. pneumoniae is commonly
encountered in pyogenic liver abscesses, osteomyelitis and other invasive disseminated infections, in a generally younger and apparently healthy population. Infections caused by hvKp 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 hvKp 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 hvKp 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 hvKp strains have a 6- to 10-fold increased siderophores activity compared with cKp 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 hvKp isolates are antibiotic-susceptible, some strains with both virulence and resistance, such as the carbapenem-resistant hvKp isolates, are increasingly being detected. Difficulties in managing carbapenem-resistant hvKp 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 hvKp 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 hyperviscous *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 hyperviscosity (hmvKp) by string test***

  The hmvKp 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 5 mm 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 (30/10μg) was at least 5 mm larger than that of ceftazidime disk (30μg) alone [13].

b) The ESβL NDP (Nordmann-Dortet-Poirel) test
Colorimetric detection of ESβL 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 ESβL 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:

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>RmpA</td>
<td>F: ACT GGG CTA CCT CTF CTT CA</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>R: CTT GCA TGA GCC ATC TTT CA</td>
<td></td>
</tr>
<tr>
<td>MrkD</td>
<td>F: AAAGCTATCGCTGTACTTCCGCAAC</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>R: GGCATGGCCGCTCGAATTG</td>
<td></td>
</tr>
<tr>
<td>IutA</td>
<td>F: GGGAAAGGCTTTCTCGCAT</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>R: TTATTCGCCACACCACCCTT</td>
<td></td>
</tr>
<tr>
<td>KPC-2</td>
<td>F: ATCGCCGTCTAGTCTGTCG</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>R: CCCCCAGCGCGAGTCTA</td>
<td></td>
</tr>
</tbody>
</table>

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 transilluminator 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 hmv*Kp* and c*Kp* 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 ($\chi^2$) 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 hmv*Kp* while the remaining 57 isolates (67.9%) were string test negative and designated as c*Kp*.

Both c*Kp* and hmv*Kp* phenotypes were more frequently isolated from blood specimens (19/57; 33.55% and 10/27; 37%, respectively) followed by respiratory secretions (16/57; 28.1% and 9/27; 33.3%). About 15.8% (9/57) of c*Kp* isolates was obtained from ascetic fluid specimens vs. none of hmv*Kp* isolates. The least isolation rate was from CSF specimens representing 3.5% (2/57) for c*Kp* and surgical drains for hmv*Kp* (1/27; 3.7%).

Notably, the highest rate of isolation of both hmv*Kp* and c*Kp* was from patients within age group of 18-60 years (40.75 for hmv*Kp* and 45.6% for c*Kp*).
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 ESβL 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 hvKp 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 hvKp isolates respectively were 95.2%, 79.3%, 76.9%, 95.8% and 86%.
Table 1. Antimicrobial resistance pattern of cKp and hmvKp clinical isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Abbreviation-Disk content (μg)</th>
<th>cKp (n = 57)</th>
<th>hmvKp (n = 27)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>Prp (100)</td>
<td>41 (71.9)</td>
<td>15 (55.6)</td>
<td>2.21</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Piperacillin/ tazobactam</td>
<td>TPZ (100/10)</td>
<td>35 (61.4)</td>
<td>13 (48.1)</td>
<td>1.31</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>FOX (30)</td>
<td>37 (64.9)</td>
<td>10 (37)</td>
<td>5.77</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td>CRO (30)</td>
<td>46 (80.7)</td>
<td>12 (44.4)</td>
<td>11.26</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CZC (30)</td>
<td>42 (73.7)</td>
<td>14 (51.9)</td>
<td>3.92</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Cefepime</td>
<td>FEP (30)</td>
<td>38 (66.7)</td>
<td>9 (33.3)</td>
<td>8.26</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>ATM (30)</td>
<td>46 (80.7)</td>
<td>11 (40.7)</td>
<td>13.41</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Imipenem</td>
<td>IPM (10)</td>
<td>33 (57.9)</td>
<td>12 (44.4)</td>
<td>1.33</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Meropenem</td>
<td>MEM (10)</td>
<td>35 (61.4)</td>
<td>14 (51.9)</td>
<td>0.68</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>ETP (10)</td>
<td>40 (70.2)</td>
<td>15 (55.6)</td>
<td>1.73</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AK (30)</td>
<td>24 (42.1)</td>
<td>10 (37)</td>
<td>1.35</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gentamycin*</td>
<td>CN (10)</td>
<td>38 (66.7)</td>
<td>11 (40.7)</td>
<td>5.06</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>TOB (10)</td>
<td>32 (56.1)</td>
<td>10 (37.0)</td>
<td>2.67</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>CIP (5)</td>
<td>42 (73.7)</td>
<td>13 (48.1)</td>
<td>5.28</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>LEV (5)</td>
<td>39 (68.4)</td>
<td>13 (48.1)</td>
<td>3.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>TGC (30)</td>
<td>22 (38.6)</td>
<td>4 (14.8)</td>
<td>4.84</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>FOS (200)</td>
<td>7 (12.3)</td>
<td>0 (0.0)</td>
<td>3.61</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MDR</td>
<td></td>
<td>37 (64.9)</td>
<td>6 (22.2)</td>
<td>14.50</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>XDR</td>
<td></td>
<td>15 (26.3)</td>
<td>2 (7.4)</td>
<td>4.05</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>PDR</td>
<td></td>
<td>5 (8.8)</td>
<td>0 (0)</td>
<td>2.60</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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

Table 2. Comparison between cKp and hmvKp isolates regarding ESβL-, AmpC and carbapenemase production.

<table>
<thead>
<tr>
<th>Beta-lactamase type</th>
<th>cKp (n=57)</th>
<th>hmvKp (n=27)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ESβL*</td>
<td>34 (59.6)</td>
<td>5 (14.8)</td>
<td>12.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>• AmpC</td>
<td>15 (26.3)</td>
<td>2 (7.4)</td>
<td>4.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>• Carbapenemase production</td>
<td>20 (35)</td>
<td>3 (11.1)</td>
<td>5.29</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Positive isolates for both combined disk and ESβL NDP tests
Table 3. Distribution of rmpA, mrkD, iutA and KPC2 genes among K. pneumoniae phenotypes by multiplex PCR assay.

<table>
<thead>
<tr>
<th>Virulence genes</th>
<th>cKp (n=24)</th>
<th>hmvKp (n=26)</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>rmpA</td>
<td>1</td>
<td>4.2%</td>
<td>21</td>
<td>80.8%</td>
</tr>
<tr>
<td>mrkD</td>
<td>22</td>
<td>91.7%</td>
<td>24</td>
<td>92.3%</td>
</tr>
<tr>
<td>iutA</td>
<td>1</td>
<td>4.2%</td>
<td>24</td>
<td>92.3%</td>
</tr>
<tr>
<td>blaKPC-2</td>
<td>8</td>
<td>33.3%</td>
<td>2</td>
<td>7.7%</td>
</tr>
<tr>
<td>Coexistence of rmpA+ iutA</td>
<td>0</td>
<td>0%</td>
<td>21</td>
<td>80.8%</td>
</tr>
</tbody>
</table>

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 Kharytnov 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 ESβL 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 ESβL-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 ESβL 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 hvKp 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 Khallil 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 HAs 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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