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ORIGINAL ARTICLE

# Detection of *rmpA and magA* genes in Hypervirulent *Klebsiella pneumoniae* Isolates from Tertiary Care Hospitals

**[1]Raghda Hager, [2]Mona A. Khattab***
[1]Medical Microbiology & Immunology Faculty of Medicine, Misr University for Science and Technology, Giza, Egypt
[2]Medical Microbiology & Immunology Faculty of Medicine Ain Shams University, Cairo, Egypt

***Key words:***
***Hypervirulent k. pneumonia (hvKP), string test, mucoviscosity-associated gene A (magA) and regulator of mucoid phenotype A (rmpA)***

***Corresponding Author:***
Mona Adel Khattab
Department of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, Cairo.
Tel: 0100605512
monaadelkhattab@yahoo.com

## ABSTRACT

***Background****: Hypervirulent Klebsiella pneumoniae (hvKp) cause multiple dangerous infections. HvKp can be distinguished from classical K. pneumoniae (cKp) by hyper-mucoviscosity. Presence of multiple genes contribute to hypervirulent phenotype of K. pneumoniae as mucoviscosity-associated gene A (magA) and regulator of mucoid phenotype A (rmpA).* ***Objectives:*** *to detect hvKp frequency among strains of k.pneumoniae by using string test phenotypically, to use multiplex polymerase chain reaction for rmpA and magA genes detection.* ***Methodology****: Fifty isolates of k.pneumoniae, were obtained from hospitalized patients in El-zohour Hospital. Conventional identification and susceptibility to antimicrobials were performed.* ***Results:*** *Most strains were isolated from samples of urine. Thirty strains out of fifty (60%) were hvKp by using string test. magA and rmpA were present in 14 isolates (28%) and 29 isolates (58.0%) respectively among k.pneumoniae isolates. Out of thirty hvKp strains, magA and rmpA genes were present in 14 stains (46.7%) and 25 strains (83.3%) respectively and 12 strains (40%) were coharboring both genes and were string test positive.* ***Conclusions:*** *high frequency of hvKp among clinical samples. Prompt detection of hvKp in different clinical settings is needed through establishing effective diagnostic tools. String test is a sensitive tool for rmpA gene detection. Establishment of both proper preventive measures and antimicrobial stewardship programs may be helpful to overcome spreading of multidrug resistant hvKp.*

## INTRODUCTION

*Klebseilla pneumoniae* (*K.pneumoniae)* is a pathogen of great importance worldwide that causes health care associated infections[1]. Also, *K.pneumoniae* is a leading cause of bacteremia and septicemia in intensive care units (ICU) admitted patients[2]. A new variant of *K.pneumoniae* called hypervirulent *Klebsiella pneumoniae* (hvKp) cause multiple dangerous infections as endophthalmitis and meningitis in healthy and young persons[3]. The hvKp can be differentiated from the classic *K. pneumoniae (*cKp) isolates by hyper-mucoviscosity (HMV)[3]. HMV can be determined phenotypically by using a string test that is considered positive if a mucoviscous string >5 mm in length is produced when bacterial colonies are stretched on agar plates[4]. Several virulence genes contribute to hypervirulent phenotype of *K. pneumoniae* as mucoviscosity-associated gene A (magA) and regulator of mucoid phenotype A (rmpA)[5]. A strong relation between hypervirulence and rmpA had been reported by many studies[6]. rmpA activates production of capsule causing increase in its virulence and the phenotype of HMV[7]. There are three rmpA genes that were detected in hvKp: a chromosomal gene and the other two genes were present in a plasmid[7]. MagA is a chromosomal gene that plays a vital role in Klebsiella infections and is associated with production of a muscoviscous string that makes Klebseilla resistant to phagocytosis[8]. Thus, magA is an island of pathogenicity that increases *K.pneumoniae* virulence[9]. The rate of resistance to antimicrobials in hvKp strains is rare compared to cKp strains[10]. However, it had been reported that there is an increase in resistance of hvKp to antibiotics worldwide[10].

The aim of the present work is to estimate rmpA and mag A genes prevalence by multiplex PCR among *K.pneumoniae* isolates. Additionally, to detect hvKp by using string test and finally to compare the antibiotic sensitivity of cKp and hvKp strains

## METHODOLOGY

**Bacterial isolates:**

Fifty (50) *K. pneumniae* strains isolated from different samples (pus aspirates, vaginal swabs, sputum and urine,) were obtained from hospitalized patients in El-Zohour private Hospital in Giza from August 2021to January 2022. All specimens except urine samples were inoculated on blood agar and MacConkey agar with

0.15% bile salts, crystal violet and NaCl. Urine samples were inoculated on cysteine lactose electrolytes deficient (CLED) agar with Bromo thymol blue. The bacterial strains were identified by (Gram staining, colonial morphology and biochemical reactions tests)[11].

**Antibiotics sensitivity**

Antibiotics susceptibility of all isolates was done by the disc- diffusion method on Muller-Hinton agar plate by Kirby Baur method according to CLSI guidelines[12]. Susceptibilities of ampicillin (Am,10µg), ampicillin sulbactam (SAM,10/10µg), Amoxicillin/Clavulanic acid (AMC,20/10 µg), piperacillin/tazobactam (TPZ,100/10 µg), ceftriaxone (CRO,30µg), cefazoline (CZ,30µg), cefotaxime (CTX,30 µg), Ceftazidime (CAZ,30µg), cefepime (FEP,30 µg), meropenem (MEM,10 µg), imipenem (IPM,10 µg), gentamicin (CN,10 µg), amikacin (AK,30 µg), ciprofloxacin (CIP,5 µg), levofloxacin (LEV,5 µg) and aztreonam (ATM) (30 µg) were tested. We used *E.coli* ATCC 25922 as a quality control strain.

**Detection of hyper-mucoviscosity (HMV) phenotypically:**

A string test was done in which single colonies were tested for their potential to stretch a string which was viscous. It was considered HMV phenotype when the formed string stretched >5 mm in length [13]

**Genotypic detection of magA and rmpA genes:**

DNA extraction was done as per manufacturer's instructions using QIAGEN DNA extraction Kit® (DNeasy® Blood & Tissue Kit) (QIAGEN, USA), for purification of DNA from the bacterial isolates. Then, amplification of magA and rmpA were done by using primers supplied from Invitrogen as shown in table 1.

Amplification was performed by, an initial denaturation at (95°C for 5 min), then 30 cycles of (DNA denaturation at 94°C for 1 min), annealing of primer (58°C for 1 min), extension of primer (72°C for 1 min), and final extension (72°C for 10 min) according to Mohammed and Flayyih [14]. Products of PCR were run on 1.5% agarose gel, stained with ethidium bromide and seen under UV light and photographed.

**Table 1. Sequences of used primers of magA and rmpA genes**

| Target | Sequences (5' → 3' | Amplification product size (bp) |
|---|---|---|
| *magA*_forwrad | GGTGCTCTTTACATCATTGC | 1283 |
| *magA* reverse | GCAATGGCCATTTGCGTTAG | Yu et al., 2006 |
| *rmpA* forward | ACTGGGCTACCTCTGCTTCA | 516 |
| *rmpA* reverse | CTTGCATGAGCCATCTTTCA | Yu et al., 2006 |

**Statistical analysis:**

Data was gathered, tabulated and analyzed by statistical package for the social sciences (SPSS, version 23). Chi-square ($\chi 2$) test was done to correlate genes with string test results. Descriptive statistics, percentages and frequencies were calculated. PCR is known to be the gold standard method. Specificity, sensitivity, positive predictive values (PPV), negative predictive values (NPV) were calculated. Statistical significance level (P-value) was set to 0.05.

## RESULTS

**Bacterial strains**

This study included 50 clinical isolates of *K. pneumoniae*. Most strains of *k. pneumoniae* were recovered from Urine; 41 isolates (82%) (Figure 1)

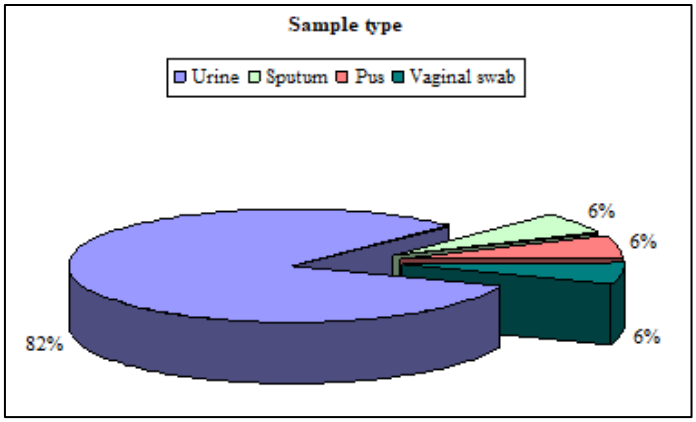


**Fig. 1.** Distribution of *K. pneumoniae* among different samples

**Antibiotics susceptibility testing**

Most of *K. pneumoniae* strains (80-100%) showed resistance to all beta-lactam/beta-lactamase inhibitor combinations, 3rd generation cephalosporine and cefepime. Forty isolates (80%) were susceptible to meropenam, forty-two isolates (84%) were susceptible to imipenem and 36% were susceptible to levofloxacin. Sixteen strains were susceptible to ofloxacin and amikacin. Fourteen strains (28%) were susceptible to norfloxacin and gentamicin (table 2).

**Table 2. Antibiotics susceptibility testing:**

| | Resistant | | Intermediate | | Sensitive | |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | NO | % |
| SAM | 44 | 88.0 | 2 | 4.0 | 4 | 8.0 |
| FOX | 40 | 80.0 | 2 | 4.0 | 8 | 16.0 |
| CRO | 43 | 86.0 | 0 | 0.0 | 7 | 14.0 |
| CAZ | 48 | 96.0 | 0 | 0.0 | 2 | 4.0 |
| CTX | 45 | 90.0 | 1 | 2.0 | 4 | 8.0% |
| TPZ | 45 | 90.0 | 1 | 2.0 | 4 | 8.0 |
| FEP | 46 | 92.0 | 1 | 2.0 | 3 | 6.0 |
| IMP | 8 | 16.0 | 0 | 0.0 | 42 | 84.0 |
| MEM | 9 | 18.0 | 1 | 2.0 | 40 | 80.0 |
| CN | 33 | 66.0 | 3 | 6.0 | 14 | 28.0 |
| AK | 31 | 62.0 | 3 | 6.0 | 16 | 32.0 |
| CIP | 34 | 68.0 | 1 | 2.0 | 15 | 30.0 |
| LEV | 32 | 64.0 | 0 | 0.0 | 18 | 36.0 |
| NOR | 36 | 72.0 | 0 | 0.0 | 14 | 28.0 |
| OFX | 33 | 67.3 | 0 | 0.0 | 16 | 32.7 |
| AMC | 50 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Am | 50 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| ATM | 43 | 86.0 | 0 | 0.0 | 7 | 14.0 |
| CZ | 44 | 88.0 | 0 | 0.0 | 6 | 12.0 |
| CPM | 44 | 88.0 | 0 | 0.0 | 6 | 12.0 |
| F | 33 | 76.7 | 0 | 0.0 | 10 | 23.3 |

**String test**

Thirty isolates (60%) were positive for string test and it were considered as hypervirulent strains and twenty isolates (40%) were negative and considered as classical *K.pneumoniae* (figure 2)

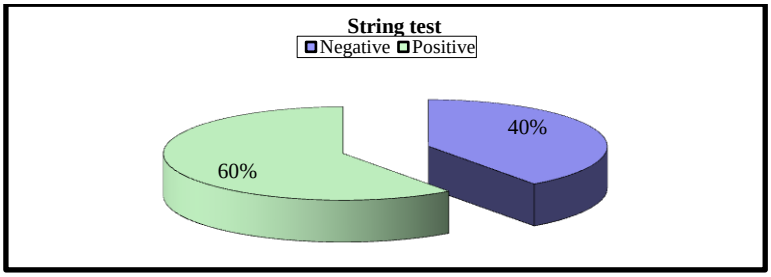


**Fig. 2.** Results of String test among *k.pneumoniae isolates*

Most of *hvKp* were isolated from urine samples. They were present in 27 isolates (66%) (table 3). No significant relation was found between phenotypically detected *hvKp* and resistance to antimicrobials (table 4)

**Table 3. Frequency of hvKp among different clinical samples:**

| Sample type | HvKp (30) NO | % | cKp (20) NO | % |
|---|---|---|---|---|
| Urine | 27 | 90% | 14 | 70% |
| Sputum | 2 | 6.7% | 1 | 5% |
| Pus | 0 | 0 | 3 | 15% |
| Vaginal swab | 1 | 3.3% | 2 | 10% |

**Table 4. Comparison between the antibiotics' sensitivity of hvKp and cKp**

| | | String test | | Test value* | P- value | Sig. |
|---|---|---|---|---|---|---|
| | | Negative cKP | Positive hvKP | | | |
| | | NO.= 20 | NO.= 30 | | | |
| SAM | Resistant | 17 (85.0%) | 27 (90.0%) | 3.409 | 0.182 | NS |
| | Intermediate | 0 (0.0%) | 2 (6.7%) | | | |
| | Sensitive | 3 (15.0%) | 1 (3.3%) | | | |
| FOX | Resistant | 14 (70.0%) | 26 (86.7%) | 5.833 | 0.054 | NS |
| | Intermediate | 0 (0.0%) | 2 (6.7%) | | | |
| | Sensitive | 6 (30.0%) | 2 (6.7%) | | | |
| CRO | Resistant | 17 (85.0%) | 26 (86.7%) | 0.028 | 0.868 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 3 (15.0%) | 4 (13.3%) | | | |
| CAZ | Resistant | 19 (95.0%) | 29 (96.7%) | 0.087 | 0.768 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 1 (5.0%) | 1 (3.3%) | | | |
| CTX | Resistant | 17 (85.0%) | 28 (93.3%) | 2.801 | 0.246 | NS |
| | Intermediate | 0 (0.0%) | 1 (3.3%) | | | |
| | Sensitive | 3 (15.0%) | 1 (3.3%) | | | |
| TPZ | Resistant | 16 (80.0%) | 29 (96.7%) | 3.912 | 0.141 | NS |
| | Intermediate | 1 (5.0%) | 0 (0.0%) | | | |
| | Sensitive | 3 (15.0%) | 1 (3.3%) | | | |
| FEP | Resistant | 17 (85.0%) | 29 (96.7%) | 2.566 | 0.277 | NS |
| | Intermediate | 1 (5.0%) | 0 (0.0%) | | | |
| | Sensitive | 2 (10.0%) | 1 (3.3%) | | | |
| IMP | Resistant | 1 (5.0%) | 7 (23.3%) | 3.001 | 0.083 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 19 (95.0%) | 23 (76.7%) | | | |
| MEM | Resistant | 1 (5.0%) | 8 (26.7%) | 5.046 | 0.080 | NS |
| | Intermediate | 1 (5.0%) | 0 (0.0%) | | | |
| | Sensitive | 18 (90.0%) | 22 (73.3%) | | | |
| CN | Resistant | 11 (55.0%) | 22 (73.3%) | 2.083 | 0.353 | NS |
| | Intermediate | 2 (10.0%) | 1 (3.3%) | | | |
| | Sensitive | 7 (35.0%) | 7 (23.3%) | | | |
| AK | Resistant | 9 (45.0%) | 22 (73.3%) | 4.203 | 0.122 | NS |
| | Intermediate | 2 (10.0%) | 1 (3.3%) | | | |
| | Sensitive | 9 (45.0%) | 7 (23.3%) | | | |
| CIP | Resistant | 13 (65.0%) | 21 (70.0%) | 1.544 | 0.462 | NS |
| | Intermediate | 1 (5.0%) | 0 (0.0%) | | | |
| | Sensitive | 6 (30.0%) | 9 (30.0%) | | | |
| LEV | Resistant | 13 (65.0%) | 19 (63.3%) | 0.014 | 0.904 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 7 (35.0%) | 11 (36.7%) | | | |
| OFX | Resistant | 11 (57.9%) | 22 (73.3%) | 1.261 | 0.261 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 8 (42.1%) | 8 (26.7%) | | | |
| AMC | Resistant | 20 (100.0%) | 30 (100.0%) | – | – | – |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 0 (0.0%) | 0 (0.0%) | | | |
| Am | Resistant | 20 (100.0%) | 30 (100.0%) | – | – | – |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 0 (0.0%) | 0 (0.0%) | | | |
| ATM | Resistant | 17 (85.0%) | 26 (86.7%) | 0.028 | 0.868 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 3 (15.0%) | 4 (13.3%) | | | |
| CZ | Resistant | 18 (90.0%) | 26 (86.7%) | 0.126 | 0.722 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 2 (10.0%) | 4 (13.3%) | | | |
| CPM | Resistant | 16 (80.0%) | 28 (93.3%) | 2.020 | 0.155 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 4 (20.0%) | 2 (6.7%) | | | |
| F | Resistant | 12 (75.0%) | 21 (77.8%) | 0.043 | 0.835 | NS |
| | Intermediate | 0 (0.0%) | 0 (0.0%) | | | |
| | Sensitive | 4 (25.0%) | 6 (22.2%) | | | |

**Molecular detection of magA *and* rmpA genes**

The most detected gene among the isolates was rmpA gene. It was detected in (29/50 isolates, 58%). magA gene was detected in (14/50 isolates, 28%). Twelve isolates (24%) were coharboring both genes (figure 3).

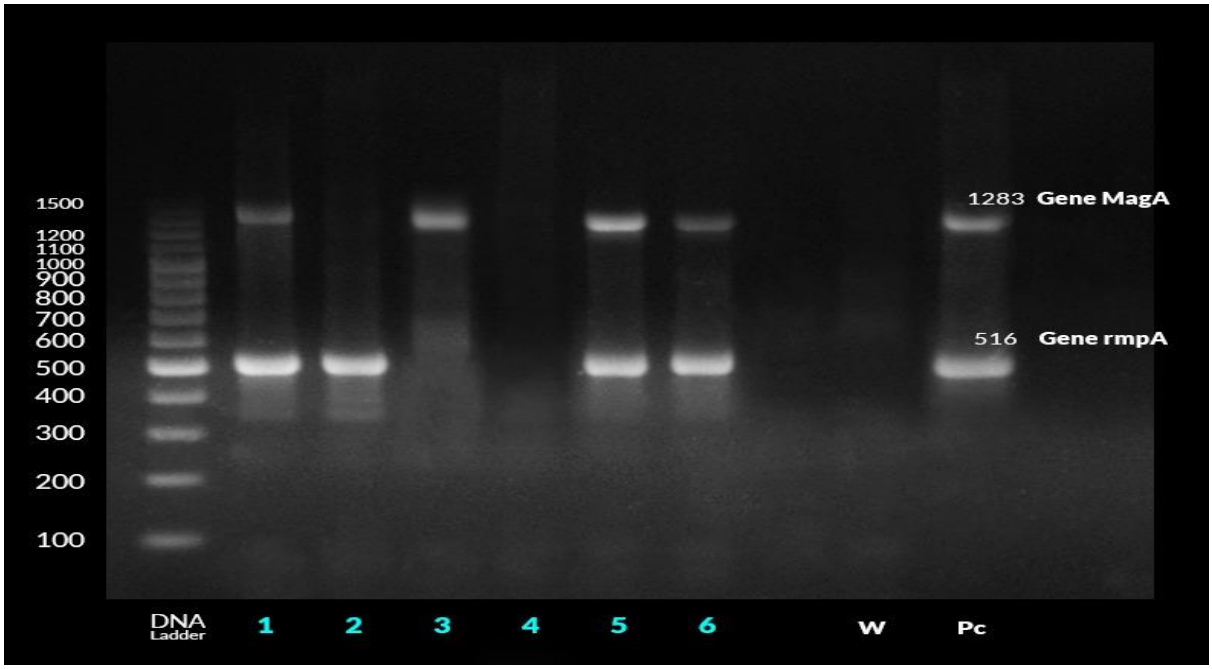


**Fig. 3:** Positive detection of magA and rmp A genes in lane 1,5 and 6

**Correlation between magA and rmpA genes and string test**

Out of thirty hvKp isolates magA and rmpA genes were detected in 14 isolates (46.7%) and 25 isolates (83.3%) respectively and 12 (40%) isolates carry both genes. The specificity, sensitivity, NPV and PPV of the string test were shown in table 5.

**Table 5. String test result in comparison to PCR.**

| | | String test | | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|
| | | Negative | Positive | | | | |
| | | No.= 20 | No.= 30 | | | | |
| magA (1283) | Negative | 20 (100.0%) | 16 (53.3%) | 46.7% | 100% | 46.7% | 100% |
| | Positive | 0 (0.0%) | 14 (46.7%) | | | | |
| rmpA (516) | Negative | 16 (80.0%) | 5 (16.7%) | 83.3% | 80% | 83.3 | 80% |
| | Positive | 4 (20.0%) | 25 (83.3%) | | | | |

## DISCUSSION

Strains of *K. pneumoniae* were obtained mainly from urine samples (41 isolates ,82%). This was in agreement with Amraie et al.[15] who reported that *K. pneumoniae* was detected in 93 urine sample (94.89%). Seifi et al.[16] collected different samples, they showed that 61.7%. *K. pneumoniae* samples were mainly obtained from urine. Different results were reported by Nirwati et al. [17] and Wang et al.[18] who stated that the main infection was the respiratory tract.

In our study most strains of *K. pneumoniae* showed resistance to all beta lactam/beta-lactamase inhibitor combinations, 3rd generation cephalosporine and cefepime. Similarly, Nirwati et al.[17] stated that strains of *K. pneumoniae* showed resistance to different antibiotics. Meropenem and imipenem were the most effective antibiotics and these results were consistent with Borges et al.[19] who have identified meropenem as

an effective antibiotic against *K. pneumoniae*. In our study lowest resistance was related to imipenem (16%). Furthermore, Mehr et al.[20] found that imipenem resistance showed the least resistance rate 5.5%.

In the current study, thirty isolates (60%) were hvKp by using string test. Similarly, Zamani et al.[21] reported that 64 out of 105 (60.95%) were hvKp. Lower percentage of hvKp was stated by many studies as Jung et al.[22] who showed that 14 out of 33 (42.2%) were positive HMV phenotype.

The hvKp prevalence rate was 5.4% in different European countries[23]. Thus, the hvKp frequency was relatively low in European countries, in comparison to Asia and Africa.

In our study, strains of hvKp showed more resistance to antibiotics than cKP although this difference was not significant. Different results were stated by Ghonaim et al.[24] they reported that the resistance rate in hvKp was lower than cKp as hvKps do not take up DNA from other resistant bacteria due to an increase expression of capsule-related genes large capsule size [25].

rmpA gene is a regulator of synthesis of polysaccharide capsule that results in HMV phenotype [26]. This study showed that the most detected gene among strains of *K. pneumoniae* was rmpA gene, where (29/50 isolates, 58%) were rmpA gene positive. These results were consistent with Abdul-Razzaq et al.[27] who showed that 21 isolates out of 43(49%) harbored rmpA.

As regards magA gene, it is involved in the production of a muscoviscous string[8]. This study reported that (14/50 isolates, 28%) were magA gene positive. Similarly, Abdul-Razzaq et al.[27] reported that 11 strains out of 43 (26%) harbored magA genes. Moreover, Fang et al. [28] showed that magA gene frequency was more in invasive *K. pneumoniae* strains. Thus, magA is a useful diagnostic marker for invasive *K. pneumoniae* infections[15].

For the genotypic characterization of hmvKp, 25 isolates out of 30 (83.3%) were rmpA gene positive. Similarly, Guo et al[29], Ghonaim et al.[24]showed that 12 out of 14 (85.7%), 17 out of 19 (89.5%) hmvKp strains carried rmpA gene respectively. Moreover, Lin et al.[30] showed that 100 out of 124 (80.65%) of strains of hvKp harbored rmpA gene. They, also compared rmpA expression levels between strains of hvKp and cKp, they found that cKp strains express lower level of rmpA than hvKp strains, then absence of hypervirulent phenotype in strains of cKp is contributed to low rmpA gene expression.

The agreement degree between rmpA, magA and detection of hmvKp by string test phenotypically showed that 14 isolates (46.7%) and 25 strains (83.3%) carried magA gene and rmpA gene respectively and were string test positive, 12 isolates (40%) were coharboring both genes and were string test positive and 3 strains (10%) did not carry both genes and were string test positive. Moreover, Hartman et al.[31] reported that 64 out of 117 (55%) of *K. pneumoniae* isolates were positive HMV phenotypically, 42 out of 64 isolates (66%) and 15 out of 64 isolates (23%) carried rmpA and magA respectively and 7 isolates (11%) did not carry both genes. This may be due to the presence of other regulator genes that are associated with HMV phenotype [31].

Our study showed that the specificity and sensitivity of string test versus magA was 100.0%, 46.7% respectively while the specificity and sensitivity of string test versus rmpA was 80%,83% respectively. Thus, string test showed high sensitivity when compared to PCR in rmpA gene detection. On the other hand, Russo et al.[32] showed that string test results were suboptimal as its specificity and sensitivity were lower in comparison to other plasmid biomarkers as its specificity and sensitivity were 0.89, and 0.91 respectively. They concluded that in low-prevalence areas, string test should not be used as a definitive method for hvKp detection. Moreover, Parrot et al.[33] reported that only two-thirds of rmpA gene-positive isolates (12/18) were string test positive. Meanwhile, string test sensitivity, specificity and negative predictive value (NPV) were 66.7%, 95.2%,98.6% respectively so they concluded that in low prevalence areas, string test would better serve as a negative predictive value test.

## CONCLUSIONS

The majority of *K. pneumoniae* strains were multidrug resistant and these need development of novel antibiotics and implementation of infection control measures and antimicrobial stewardship programs to limit its spread. Also, hvKp frequency is considered high with presence of virulent genes that make *K. pneumoniae* more dangerous pathogen.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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