Mucoviscosity Determination and Detection of magA and rmpA Genes in Clinical Isolates of Klebsiella pneumoniae in Northern Iran

Maryam Nahavandinejad¹, Leila Asadpour¹*

Abstract
Objective: Klebsiella pneumoniae is an opportunistic pathogen with several pathogenic factors including capsular type and the presence of magA and rmpA genes. The present study aimed to investigate the frequency of magA and rmpA genes and mucoviscosity among clinical isolates of K. pneumoniae, to determine the virulence of local strains of this bacterium.

Materials and Methods: In this cross-sectional study, during 2015, a total of 65 capsulated K. pneumoniae were isolated and identified from urinary tract infections (UTIs) in Rasht, using microbiological test and CPS gene amplification in polymer chain reaction (PCR). Mucoviscosity of test bacteria measured by string test and the presence of magA and rmpA genes detected in PCR using specific primers.

Results: All of 65 isolates of K. pneumoniae recognized as CPS positive in PCR assay. Out of them, 22 (33.48%) strains showed an HV-positive phenotype. The presence of magA gene was confirmed in 2 (3.07%) isolates and 10 (15.38%) isolates were positive for the presence of rmpA gene. Also, 8 of the rmpA-positive and the 2 magA-positive isolates showed hypermucoviscous phenotypes.

Conclusion: Presence of virulence genes magA and rmpA and relatively high prevalence of hypermucoviscosity (HV) in local K. pneumoniae strains, clarifies the importance of rapid diagnosis and suitable treatment of infections caused by this bacterium in the prevention of complicated clinical infections.

Keywords: Klebsiella pneumoniae, magA, rmpA, Virulence factor

Introduction
Klebsiella pneumoniae is an opportunistic pathogen that causes various infections such as pneumonia, septicemia, diarrhea, endophthalmitis, meningitis, urinary tract infections (UTIs), and bacteremia (1,2). In recent decades, an invasive type of K. pneumoniae has been isolated that causes metastatic liver abscess, particularly in Asia (3-5). Several pathogenic factors have been identified in this invasive type, including K1 and K2 capsular type and the presence of magA and rmpA genes and aerobactin (6,7). The magA gene is an important virulence gene in the invasive K. pneumoniae, with a length of 1.2 kb and encoding an enzyme protein known as wzy, which acts as a polymerase in capsule synthesis (8,9). Therefore, the presence of magA in K. pneumoniae strains confers resistance to serum and phagocytosis. Serum complement factors cannot easily reach the bacterial cell membrane, thus making phagocytosis difficult. Consequently, magA impairment causes the complete loss of resistance to serum and phagocytosis so acts as an important virulence determinant in K. pneumoniae K1-induced metastatic infections (10). Although the presence of other virulence factors such as rmpA has been attributed to pathogenesis of K. pneumoniae (7,11). rmpA is a regulator of capsular antigen expression in bacteria and controls the mucoviscosity phenotype of K. pneumoniae (11). The presence of this gene leads to more thickness in capsular polysaccharide and plays a role in invasive clinical infections caused by K. pneumoniae (7), but compared with magA, it has less importance in pathogenesis.

Klebsiella pneumoniae is one of the most common species causing UTIs but the virulence genes of this bacterium related to UTI are poorly understood (12). Although some studies indicated the significant role of hypermucoviscosity (HV) phenotype and rmpA gene in UTI pathogenesis (7,12). This study was designed to investigate the mucoviscosity phenotype and presence of magA and rmpA genes among UTI isolates of K. pneumoniae to determine the virulence of local strains of this bacterium.

Materials and Methods
Study Design and Test Bacteria
This descriptive cross-sectional study has been done during 2015. A total of 65 K. pneumoniae isolates were collected from patients with UTIs, which were obtained...
from clinical laboratories in Rasht city, using biochemical tests (13). The bacterial genomic DNA was extracted using kit for the isolation of DNA from gram-negative bacteria (Cinnagen, Iran). All the isolates were tested for the presence of *K. pneumoniae* capsular antigens by polymerase chain reaction (PCR), using capsule (CPS) gene specific primers (14). The nucleotide sequences of the primers used are shown in Table 1.

**Mucoviscosity of the Isolates**
Each of the *K. pneumoniae* isolates were streaked (plated) onto blood agar medium (containing 5% sheep blood) and then incubated at 37°C for 24 hours. Then, they were analyzed for mucoviscosity by the string test, in which a loop is passed through a bacterial colony and viscous strings are measured in millimeters. Samples that exhibit viscous strings >5 mm are considered to possess HV (15).

**Detection of magA and rmpA**
The presence of *magA* and *rmpA* genes was assessed by conventional (PCR) using bacterial-specific primers (6). The sequences of oligonucleotide primers used in this study are shown in Table 1.

The extracted nucleic acid was used as the template DNA for PCR. PCR was performed in a total volume of 25 μL containing 0.5 μLdNTPS (10 mM), 5 μL enzyme buffer (10×), 3 μL of forward and reverse primers (10 pmole), 2 μL template DNA (2 μg), 0.5 μL enzyme (2.5 U), and 14 μL deionized water.

The thermocycler program consisted of initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 60 seconds, annealing at 45°C for 60 seconds, and elongation at 72°C for 75 seconds. Then, a final extension step was included for 10 minutes, and the PCR products were detected by electrophoresis on 1% agarose gel and the results were recorded.

**Results**

**Test Bacteria Identification**
In this study, 65 isolates of gram-negative, non-motile, rod-shaped bacteria, which were lactose fermenters and had mucoid colonies when grown on MacConkey agar (MAC), methyl-red (MR) negative, Voges–Proskauer (VP) positive, indole negative, catalase positive, urease positive, hydrogen sulphide negative (SH₁) were recognized as *K. pneumoniae*. In all of 65 isolates tested by PCR using *K. pneumoniae* capsular gene specific primers, a fragment with an approximate length of 418 bp was produced and the presence of capsulated *K. pneumoniae* was confirmed (Figure 1).

**Mucoviscosity Test Results**
Out of the 65 *K. pneumoniae* isolates, 22 (33.48%) strains showed an HV-positive phenotype (Figure 2).

**Detection of magA and rmpA**
The presence of *magA* gene was confirmed in 2 (3.07%) isolates via PCR using *magA* gene-specific primers that resulted in bands with a length of approximately 1280 bp (Figure 3). The presence of *rmpA* gene was confirmed in 10 (15.38%) isolates using specific primers that resulted in bands with a length of approximately 535 bp (Figure 4). Out of the 22 HV-positive *K. pneumoniae* isolates, 2 of the *magA*- and 8 of *rmpA*-harboring isolates were detected and 2 of the *rmpA*-positive isolates showed HV-negative phenotype.

**Discussion**
This study investigated mucoid phenotype and prevalence of *magA* and *rmpA* genes among capsulated clinical isolates of *K. pneumoniae* as some virulence factors of these bacterium. Out of the 65 *K. pneumoniae* isolates, 2 (3.07%) were positive for *magA* gene and 10 (15.38%) for *rmpA* gene. The string test showed that 8 of the *rmpA*-positive isolates were HV-negative phenotype and 2 of the *magA*-positive isolates showed HV-positive phenotype.

---

**Table 1. Oligonucleotide Primers Used for Amplification of Particular Sequences of *Klebsiella pneumoniae* CPS, *magA* and *rmpA* Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS</td>
<td>F: 5‘ TTTCATCAGAAGCAGGAGCTGGGAGAAGCC 3’</td>
<td>418</td>
</tr>
<tr>
<td></td>
<td>R: 5‘ GTCCGTAGCTGTGTAAGCGAGGGCGGTAGCG 3’</td>
<td></td>
</tr>
<tr>
<td>magA</td>
<td>F: 5‘ GGT GCT CCT TTG TAC ATC ATT GC 3’</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>R: 5‘ GCA ATG GCC ATT TGC GTT AG 3’</td>
<td></td>
</tr>
<tr>
<td>rmpA</td>
<td>F: 5‘ ACT GGG CTA CCT CTG CCT CA 3’</td>
<td>535</td>
</tr>
<tr>
<td></td>
<td>R: 5‘ CCT GCA TGA GCC ATC TTT CA 3’</td>
<td></td>
</tr>
</tbody>
</table>

---

Figure 1. Agarose Gel Electrophoresis of CPS Gene PCR Amplicons. Lane M: 100 bp DNA marker, Lane 1: Positive control (418 bp amplicon of CPS confirmed by sequencing), Lanes 2-6: CPS positive strains.
positive and the 2 magA-positive isolates showed HV and 14 strains with HV phenotype were negative for the presence of both genes.

Fang et al in 2004 first identified magA gene as a primary pathogenic factor of K. pneumoniae liver abscess (16), and rmpA gene was first identified by Yu et al in 2006 as a causative agent displaying invasive K. pneumoniae clinical infections (7). The first case of liver abscess outside of Asia in Argentina was reported by Vila et al, in which K. pneumoniae isolated from the patients contained K1 capsule and HV+ phenotype and carried the rmpA gene (17). Yu et al reported the prevalence rates of HV and rmpA and magA genes to be 38%, 48%, and 17%, respectively, in 151 K. pneumoniae strains isolated in southern Taiwan (7). In a study conducted by Lin et al in Taiwan, the HV phenotype and rmpA gene were more often found in K. pneumoniae isolates from UTIs, compared with Klebsiella isolated from healthy adults but no significant difference in the frequency of magA gene was found (12). Most of these studies investigated the presence of magA and rmpA genes in invasive infections caused by K. pneumoniae. Maybe this is the reason of less frequency of these genes in the present study.

According to our knowledge this is the first report of presence of rmpA gene in clinical isolates of K. pneumoniae in Iran. But similar studies on the frequency of magA gene in this bacterium has been done previously. Zamani et al reported 105 Klebsiella spp. isolated from clinical samples, of which 4 (3.8%) were positive for magA gene but none of the 40 urine isolated strains were detected as magA positive. Two of these magA isolates were also positive for the HV phenotype and another 2 were negative for the HV phenotype (18). In accordance with the present study, the presence of HV phenotype was not restricted to the presence of magA gene. In addition, Amraie et al investigated the presence of magA gene in 173 K. pneumoniae strains isolated from clinical samples and found 4 (2.3%) isolates carrying this gene (19).

**Conclusion**

According to these results difference in the prevalence of virulence factors in K. pneumoniae strains is concluded. Also the present study demonstrated the presence of virulence genes magA and rmpA in local urinary infection isolates of K. pneumoniae, which could more rapidly progress from a lower urinary tract to an upper urinary tract and complicate the clinical consequences of infections caused by this bacterium. These findings can be used for the development of preventative and novel therapeutic measures and further investigations to identify the source and environmentally virulent strains of K. pneumoniae, including the identification of appropriate treatment, can help prevent a major health problem.

**Conflict of Interests**

The authors have no conflict of interest in this study.

**Ethical Issues**

Non to be declared.
Financial Support
Funding for this project was provided by Islamic Azad University, Rasht Branch.

Acknowledgments
This manuscript is prepared from MSc thesis of first author at Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch for supporting the study.

References

Copyright © 2017 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Crescent Journal of Medical and Biological Sciences, Vol. 4, No. 3, July 2017 | 107