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

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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 polymerase 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 pnemoniae, 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

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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 (SH_2) 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 *mag*A gene and 10 (15.38%) for *rmpA* gene. The string test showed that 8 of the *rmpA*-

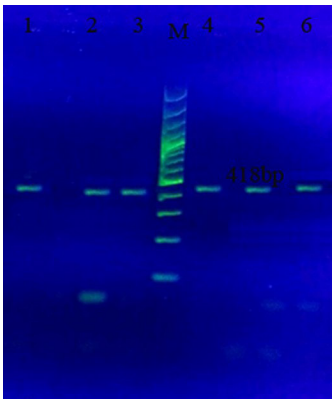

Figure 1. Agarose Gel Electrophoresis of *CPS* Gene PCR Amplicons. Lane M: 100 bp DNA marker, Lane 1: Positive control (418 bp amplicon of CPS confirmed by sequencing), Lanes 2-6: CPS positive strains.

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

Gene	Primers	Amplicon Size (bp)
CPS	F:5' TATTCATCAGAAGCACGAGCTGGGAGAAGCC 3′	418
	R:5' GTCGGTAGCTGTTAAGCCAGGGGCGGTAGCG 3′	
magA	F: 5′ GGT GCT CTT TAC ATC ATT GC 3′	1280
	R: 5′ GCA ATG GCC ATT TGC GTT AG 3′	
rmpA	F: 5′ ACT GGG CTA CCT CTG CTT CA 3′	535
	R: 5′ CTT GCA TGA GCC ATC TTT CA 3′	

Figure 2. Hypermucoviscous phenotype of *Klebsiella pneumoniae*

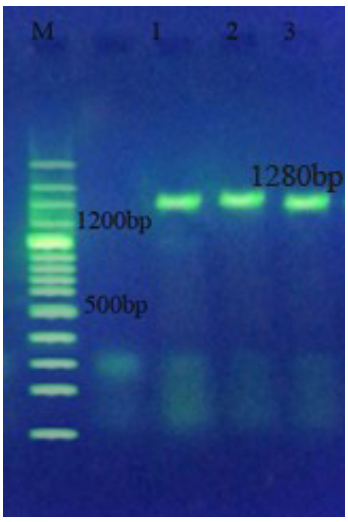

Figure 3. Agarose Gel Electrophoresis of *magA* Gene PCR Amplicon. Lane M: 100 bp DNA marker, Lane 1: positive control (1280 bp amplicon of magA confirmed by sequencing), Lanes 2 and 3: magA positive strains.

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 *rmp*A 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.

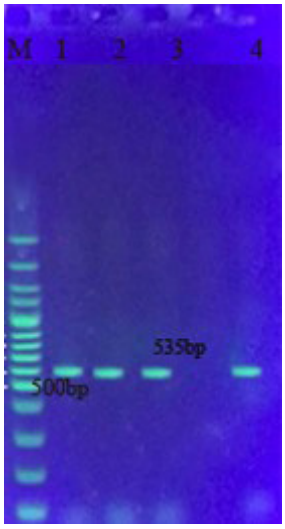

Figure 4. Agarose Gel Electrophoresis of *rmpA* Gene PCR Amplicon. Lane M: 100 bp DNA marker, Lane 1: Positive control (535 bp amplicon of rmpA confirmed by sequencing), Lanes 2- 4: rmpA positive strains.

According to our knowledge this is the first report of presence of *rmpA* gene in clinical isolates of *K. pnemoniae* 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.

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