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Detection of *MagA* Gene Among Clinical Isolates Of *Klebsiella Pneumoniae* Collected From Hospitals Of Qazvin, Iran

Laleh Alimohammadi[1], Amir Peymani[2*], Narges Habibollah-Pourzereshki[2]

[1] Department of Microbiology, Zanjan Branch, Islamic Azad University, Zanjan, Iran
[2] Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
*Corresponding author: Amir Peymani, Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran. Tel/Fax: +98-2813324971, E-mail: a.peymani@gmail.com
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ABSTRACT *Klebsiella pneumoniae* is an opportunistic bacterium that causes serious hospital-acquired infections. Among the different virulence factors of the bacteria, capsule is the most important one and plays an important role in its mucoviscosity. The current cross-sectional study was conducted on 149 *K. pneumoniae* species isolated from different clinical samples obtained from patients hospitalized in teaching hospitals of Qazvin, Iran, from 2014 to 2015. All isolates were identified by laboratory standard methods. First, the isolates were evaluated for harboring the gene *ureD*, as an internal control, and the *magA* gene by polymerase chain reaction (PCR) and sequencing methods. All isolates were positive for the presence of *ureD* gene, the specific gene of *K. pneumoniae*. Eleven isolates (7.4%) harbored the *magA* gene which were mostly collected from trachea (4 isolates, 36.3%) and blood (3 isolates, 27.3%) specimens and from the patients admitted in intensive care units (9 isolates, 81.8%) and infectious diseases wards (2 isolates, 18.2%). Results of the current study showed the high prevalence of *magA* gene in *K. pneumoniae* isolated from hospitals in Qazvin city, Iran, which because of its engagement in capsule production, it is necessary to consider these organisms in prevention and treatment of the infection.

Keywords: *Klebsiella pneumoniae*, polysaccharide capsule, *magA*

INTRODUCTION

Klebsiella pneumoniae is a Gram-negative bacteria which belong to the Enterobacteriaceae family (Falade A.G & Ayede A. I., 2011). This organisms is considered as opportunistic pathogens, which cause serious infections in hospitalized patients and cause a wide range of clinical infections such as septicemia, pneumonia, urinary tract infections, meningitis and purulent abscesses in various

organs, especially liver (Livermore D. M., 2012; Peymani A. et al., 2015). The polysaccharide capsule of these bacteria is considered as the most important virulence factor. The mucoviscosity factor related to *magA* gene plays an important role in the production of polysaccharide capsule and O-antigen ligase forming in *K. pneumoniae*, which is known as the main cause of liver abscesses in the recent years (Lin T .L. et al., 2012). The presence of this gene in clinically isolated *K. pneumoniae* species is very important. The role of *magA* gene in *K. pneumoniae* isolates regarding the incidence of liver diseases, meningitis, bacteremia and septicemia is revealed through different studies in the recent decade. This gene can be used as a marker to diagnose invasive infections of *K. pneumoniae. magA* in *K. pneumoniae* is usually identified by the high viscosity. This gene can act as a pathogenicity island due to its genetic situation and increase in the virulence of bacteria; in such a way that without antibiotic treatment can cause death (Struve C. et al., 2005). The *ureD* gene is a segment of *K. pneumoniae* specific gene which produces urease. Urease is an enzyme containing nickel which produces ammonia and carbon dioxide by urea hydrolyses. The increase of pH following the production of ammonia by this enzyme can play an important role in the incidence of liver diseases; in such a way that following the activation of urease and production of ammonia, blood urea increases and causes metabolic alkalosis. Bacterial urease is directly associated with stone-forming in urinary tract and it also increases the pathogenicity of these bacteria through playing a role in the incidence of diseases such as pyelonephritis, ammonia encephalopathy, gastric ulcer, liver infection and infection in patients with invasive tools (Mobley H. L. et al., 1995). The conducted studies showed that urease enzyme is coded by urease gene cluster including *ureDABCEFG* genes. Four proteins as ureG, ureD, ureE and ureF are the main proteins to form an active site to add metal-cofactor in UreABC apoenzyme. Finally UreD, as a metallochaperone, engages in the transfer of Ni^{2+} to the active site (Liu Q. & Bender R.A., 2007; Carter E.L. & Hausinger R. P., 2010). Several studies of bacterial pathogenesis have documented the potential role of *Klebsiella magA* genes in pyogenic liver abscesses, however little is known about the role of this virulence factor in serious infections at other sites. The current study aimed to evaluate the prevalence of *magA* and *ureD* genes in

K. pneumoniae species isolated from hospitalized patients in different wards of Qazvin teaching hospitals, Iran.

MATERIALS AND METHODS

The current cross-sectional study was conducted on 149 *K. pneumoniae* species isolated from different clinical samples including urine, blood, trachea and wound from 2014 to 2015. The isolates were collected from the patients (one isolate per patient) admitted in five hospitals of Qazvin. The study was approved by the ethics committee of Qazvin University of Medical Sciences (code IR.QUMS.REC.1394.337). Written informed consent was obtained from all subjects enrolled in this study. The bacterial isolates were identified using biochemical and microbiological standard tests such as Gram staining and microscopic evaluations, growth on MacConkey agar, triple sugar iron (TSI) and sulfide indole motility (SIM), oxidase tests, lysine decarboxylase, arginine and ornithine decarboxylase, methyl red and Voges-Proskauer tests (Mahon C.R. et al., 2013). All media used in the current study were purchased from Merck Company (Germany). Bacterial isolates were stored in tryptic soy broth (TSB) supplemented with 20% glycerol at -70°C.

K. pneumoniae isolates were evaluated for harboring the using specific primers by polymerase chain reaction (PCR) method and sequencing (Table 1) (Fang C. T. et al., 2004; Zamani A . et al., 2013). DNAs were extracted using the specific kit following the manufacturer's instruction (Bioneer Co., South Korea). The PCR was performed in the final volume of (25 µl) containing: (12.5 µM) of Master Mix (Ampliqon), (1 µl) of each primer, (8.5 µl) of distilled water and (2 µl) of DNA template. The *magA* and *ureD* genes were amplified separately using the thermocycler apparatus (Applied Biosystems Co., USA) under the following conditions: for *magA* gene, 95ºC for 5min and 35 cycles of 1min at 95ºC, 1min at specific annealing temperature for each primer and 1min at 72ºC. A final extension step of 10 min at 72ºC was performed. To evaluate PCR products, the final products were electrophoresed on 1% gel agarose, stained with ethidium bromide and visualized on gel documentation system (Uvitec, England).

Table 1**.** Primers used for the detection of *magA* and *ureD* genes in *K. pneumoniae* isolates.

Targets	Primer sequence (5'-3')	Annealing temperature (°C)	Product size (bp)
magA	Forward: CGCCGCAAATACGAGAAGTG Reverse: GCAATCGAAGTGAAGAGTGC	56	540
ureD	Forward: CCCGTTTTACCCGGAAGAAG Reverse: GGAAAGAAGATGGCATCCTGC	55	243

Some of the PCR final products of both genes were sent to Macrogen Company (South Korea) for sequencing; then, after confirming the genes, they were used in PCR tests as control samples. Sequence alignment and analysis were performed online using the BLAST program of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis was performed for descriptive statistics including frequencies and cross tabulation of microbiological, clinical, and demographic characteristics using the computer software program Statistical Package for the Social Sciences (SPSS) version 16.

RESULTS

In the current study, 149 species of *K. pneumoniae* were isolated from the following samples: 76 (51%) isolates from urine, 31 (20.8%) isolates from trachea, 23 (15.4%) isolates from blood, and 19 (12.7%) isolates from wound specimens. The isolates were collected from 68 (45.6%) hospitalized patients admitted in the intensive care units, 44 (29.5%) from internal medicine, and 37 (24.8%) from infectious diseases wards, respectively. Totally, 93 species (63.1%) were isolated from females and 56 (37.6%) from males. The age of the patients ranged from 19 to 91 years, with the mean of 52±17 years. According to the PCR results, the *ureD* gene, as an internal control, was carried by all of the isolates and 11 species (7.4%) harbored the *magA* gene (Figure 1).

The species harboring *magA* were mostly isolated from the patients hospitalized in the ICU (9 isolates, 81.8%) and infectious diseases (2 isolates, 18.2%). Also, the highest frequency of the *magA* gene was observed in trachea isolates with 4 (36.3%) isolates and blood with 3 (27.3%) isolates (Table 2).

Table 2**.** The Frequency of *magA* gene in *K. pneumoniae* isolates based on the clinical samples and hospital wards.

Hospital Wards	No. (%)
ICU*	9 (81.8)
Infectious diseases	2 (18.2)
Clinical Samples	
Trachea	4 (36.3)
Blood	3 (27.3)
Wound	2 (18.2)
Urine	2 (18.2)

ICU: intensive care unit

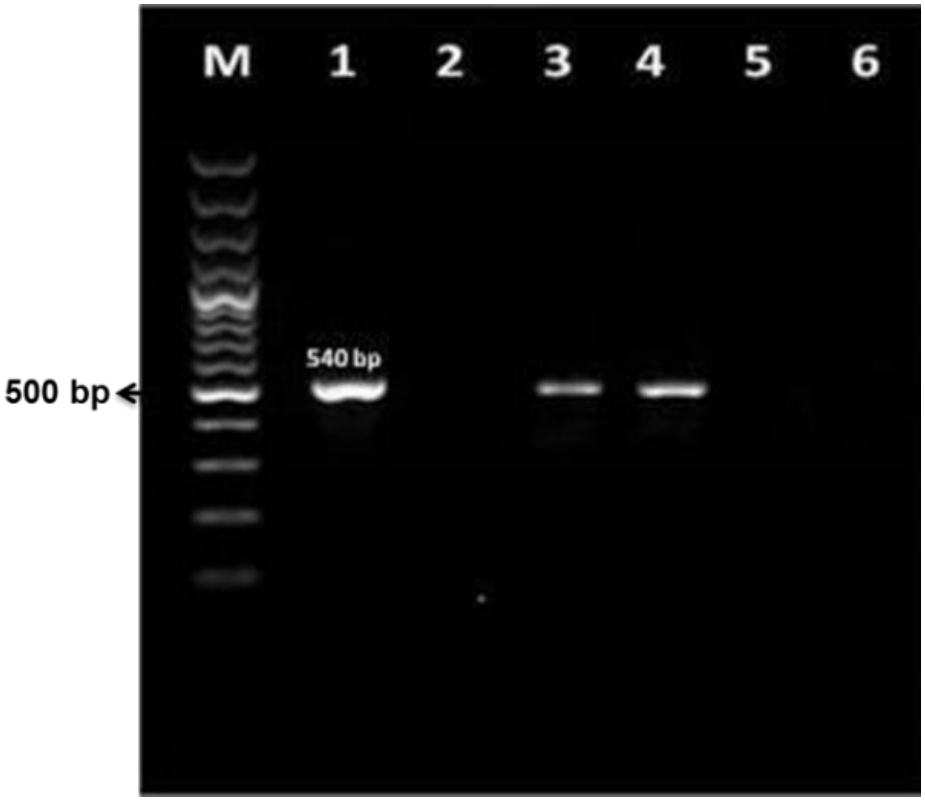

Figure 1. PCR amplification of *magA* gene in *K. pneumoniae* isolates; lane M: DNA marker (100 bp); lane 1: *magA*-positive *K. pneumoniae* (as a control isolates confirmed by sequencing); lane 2: negative control [*Escherichia coli* American Type Culture Collection (ATCC) 25922]; lane 3-4: positive clinical isolates; lane 5: negative clinical isolates; and lane 6: negative control for PCR (with no DNA template).

DISCUSSION

K. pneumoniae plays an important role in the incidence of hospital-acquired infections among the elderly and children (Bagheri P. & Sepand M. R., 2015). The main reason for high morbidity following urinary tract infections, septicemia and pneumonia caused by this organism is because of high resistance of the bacteria against antibiotics routinely administrated in clinics and hospitals (Won S.Y. et al., 2011). According to the studies conducted in Iran, the resistance of *K. pneumoniae* against antibiotics in different infections was reported at different rates; the respiratory tract infection was the most common infection caused by these bacteria (Bagheri P. & Sepand M. R., 2015). The

mucoviscosity factor related to *magA* is one of the most important factors in the virulent of *K. pneumoniae*. The *magA* gene plays an important role in the production of polysaccharide capsule with lipid base in *Klebsiella* spp., which makes bacteria resistant against macrophages (Lin J. C. et al., 2004). According to the conducted studies, the secondary acquired infections such as eye infection, bacteremia, sepsis and meningitis are *magA* related diseases (Lederman E. R. & Crum N. F., 2005; Yeh K. M et al., 2007). In the current study, 7.4% of the isolates were positive for the presence of *magA* gene. There are few studies conducted in Iran on the prevalence of *magA* gene in clinically important bacteria. Amrai et al. in 2014 evaluated 173 *K. pneumoniae* species isolated from different clinical samples in Shahre-Kurd, Iran; their results showed the prevalence of *magA* as 2.31%. There was no significant difference between the presence of *magA* and the variables such as age and gender of the patients, the hospital-acquired infection, type of clinical sample and an underlying disease (Amraie H. et al., 2014). In another study performed by Zamani A et al. (2013) on the detection of *magA* virulence factor in 150 *Klebsiella* spp. isolated from different clinical samples in Hamadan, 96.2% of the isolates belonged to *K. pneumoniae* and 3.8% belonged to *Klebsiella oxytoca*. Results of the current study indicated that the presence of *magA* only in *K. pneumoniae* isolates highlights its virulence in these bacteria (Zamani A. et al., 2013). It seems that the lack of an appropriate infection control strategies and a local antimicrobial resistance surveillance system are the most important predisposing factors that could eventually lead to appearance and spread of these virulent pathogens in our hospital settings. Lin et al. conducted a study in Taiwan on 26 *K. pneumoniae* species isolated from patients with liver abscess; their results showed that the prevalence of *magA* among the patients was 100% (Lin Y.T et al., 2010). In another study conducted by Fang et al., the prevalence of *magA* in *K. pneumoniae* species isolated from invasive and non-invasive infections were 98% and 29%, respectively (Fang C.T. et al., 2010). Fang et al. in another study showed that 98% of *K. pneumonia* species isolated from liver abscesses carried the gene *magA* and recognized this gene as a virulence factor which can be considered as a diagnostic index in the invasive diseases caused by these bacteria (Fang C. T .et al., 2004).

Yu et al. in Taiwan conducted a study on 151 samples collected from patients with

bacteremia and liver abscesses caused by *K. pneumoniae*, capsule forming and mucoid phenotype were induced by *rmp* and *magA* genes. Hypermucoviscosity phenotype, *rmp* and *magA* genes were identified in 38%, 48% and 17% of the isolates, respectively (Yu W.L. et al., 2006). Lee et al. in a silimar study in Taiwan showed that 20% of the patients infected with these bacteria had invasive infections and 58% of the patients had hospital-acquired infections. Results of the study showed that 32% of *K. pneumoniae* species were positive for *magA* gene (Lee C.H. et al., 2010).

Based on the studies conducted on clinical isolates in East and South Asian countries, it seems that the relationship among geographical situation, genetic predisposition and susceptibility to the infections caused by *magA* can be considered as one of the main reasons for the prevalence of these bacteria (Rahimian J. et al., 2004). Struve et al. in Denmark conducted a study on *Klebsiella* spp. isolated from patients with liver abscesses; results of the study showed that all of the isolates which carried *magA,* also had capsule K1 serotype (Struve C. et al., 2005). In the current study, most of the species containing *magA* gene were isolated from patients in ICUs, and also trachea and blood samples. It seems that long-time hospitalization in this unit and using medical invasive tools, such as trachea, are among the main reasons for the presence of these bacteria in patients. Evaluation of *magA* can be considered as a suitable index for rapid molecular identification and diagnosis of these bacteria and can help with the timely treatment of infections caused by *K. pneumoniae*.

CONCLUSION

Findings of this study highlight an important role of *magA* gene in *K. pneumoniae* strains isolated from different clinical specimens. *MagA* gene as genetic marker can be used for the rapid diagnosis and for tracing the source of invasive *K. pneumoniae* infections. Considering the presence of *magA* gene among clinical isolates of *K. pneumoniae*, especially in ICUs, it is necessary to use suitable infection controlling tools and suitable therapeutic approaches to prevent further spread of this virulent organism in our hospital settings.

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