Infect Epidemiol Med. 2017 Spring; Volume 3, Issue 2: 46-50 DOI: 10.18869/modares.iem.3.2.46

Original article

Association between ESBL Production and the Presence of *magA* Gene among the Clinical Isolates of *Klebsiella pneumonia*e

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Submitted: February 16, 2017; **Revised:** April 06, 2017; **Accepted:** April 18, 2017

Abstract

Background: *Klebsiella pneumonia*e (*K. pneumoniae*) causes a wide range of nosocomial and community-acquired infections. In recent decades, *K. pneumoniae* has been known as the agent of community-acquired primary pyogenic liver abscess. In attempts to find the causes of this disease, researchers found a new virulence gene called *magA* (mucoviscosity-associated gene A). The present study was performed to determine the prevalence rate of *magA* gene among the extended-spectrum beta lactamase (ESBL)-positive and ESBL-negative *K. pneumoniae* strains.

Materials and Methods: The current cross-sectional study was conducted on 130 *K. pneumoniae* isolates collected from patients in Imam Reza hospital and its associated clinics in Mashhad city (Iran) from May 2011 to July 2012. The presence of *K. pneumoniae* species was confirmed by conventional microbiological methods. Samples were tested for the production of ESBLs by the double disk diffusion (DDS) test. PCR was performed to detect *magA* gene. The hypermucoviscosity (HV) phenotype of *Klebsiella* isolates was characterized by the string test.

Results: *magA* gene was detected in 11(8.5%) out of 130 isolates of *K. pneumoniae*. Of 11 isolates with positive result for *magA* gene, three cases were HV^+, and 8 cases were HV^- phenotype. Of 130 *K. pneumoniae* isolates, 56 isolates were ESBL-positive, and 74 isolates were ESBL-negative. The *magA* gene was detected in 4 out of 56 (7.14%) ESBL-positive, and 7 out of 74 (9.46%) ESBL-negative samples.

Conclusion: In the present study, no correlation was observed between the presence of *magA* gene and the production of ESBL in *K. pneumoniae* strains isolated from different clinical samples in Mashhad.

Keywords: Klebsiella pneumoniae; Extended-spectrum beta-lactamase (ESBL), *magA* gene

1. Background

Klebsiella pneumoniae is an encapsulated Gram-negative enteric bacillus belonging to the family *Enterobacteriaceae*, which is capable of causing a wide range of nosocomial and community-acquired infections such as urinary tract, pneumonia, septicemia, meningitis, and wound infections (1). In recent decades, *K. pneumoniae* has been regarded as the cause of community-acquired primary pyogenic liver abscess, first reported from Taiwan, followed by the United States, Europe, Japan, and Australia (2-5). The invasive *K. pneumoniae* disease, if accompanied with sepsis, causes severe complications such as septic metastatic meningitis and endophthalmitis (6, 7). These complications occur more frequently among people with diabetes mellitus (8). The presence of these complications increases the mortality rate from 10 to 30-40% in primary liver abscess caused by *K. pneumoniae* strains susceptible to all cephalosporins and aminoglycosides (9, 10). In attempts to find the underlying mechanisms of this disease, researchers identified a novel virulence gene called mucoviscosity-associated gene A (*magA*). This gene, along with its flanking regions, synthesizes a protective exopolysaccharide web in invasive *K. pneumoniae* strains. The exopolysaccharide web is responsible for high resistance of this bacterium to serum complement system and phagocytosis through the alteration of the physicochemical properties of the bacterial surface. *magA* is responsible for the hypermucoviscosity (HV) phenotype. This property is detected in a majority of invasive strains (9). Cephalosporins are therapeutic targets for pyogenic liver abscess treatment (11). Recent reports have shown a rise in plasmid-mediated extended-spectrum β-lactamases (ESBLs) produced by *K. pneumoniae* strains isolated from inpatients and outpatients (12, 13). ESBLs cause resistance to cephalosporins. The presence of ESBL-carrying plasmids in strains responsible for primary pyogenic liver abscess increases the probability of the disease incidence, the severity of its complications, and mortality rate (11).

2. Objective

This study was designed to determine the distribution of *magA* gene among ESBL-positive and ESBL-negative clinical isolates of *K. pneumoniae*.

3. Materials and Methods

3.1. Study design

This cross-sectional study was performed on 130 *K. pneumoniae* isolates collected during 15-month period (May 2011 to July 2012) from patients in Imam Reza hospital and related clinics in Mashhad (Iran). Different clinical samples including urine, blood, and wound were included in this study. The present study was approved by the ethics committee of Mashhad University of Medical Sciences.

[DOR: 20.1001.1.25884107.2017.3.2.8.7]

3.2. Organism identification and ESBL detection

After growth on blood agar and Mac-Conky agar media for 48 hours at 37°C, the presence of *K. pneumoniae* species was confirmed by conventional microbiological methods including catalase and oxidase tests and culture on TSI (Triple Sugar Iron Agar), SIM (sulfide-in dole-motility), LIA (Lysine Iron Agar), urea and Simon citrate media (14). Samples were tested for the production of ESBLs using the double disk diffusion (DDS) test (15). The reference strain *K. pneumonia*e ATCC 700603 was used as an ESBL-positive control.

3.3. String test

Hyperviscous phenotype is characterized by the formation of elongated (>5 mm) mucoviscous strings when a loop of colony is picked up from a plate. This phenotype is considered as string test positive (9).

3.4. DNA extraction

Two or three colonies of bacteria were suspended in 500 µL of distilled sterile water. Suspensions were heated at 100°C for 15 minutes and centrifuged at 4696 g for 10 minutes. The supernatant was transferred to a new microtube and stored at -20°C.

3.5. Detection of magA gene by PCR

A fragment of 303bp *magA* gene was amplified by specific primers: forward 5'-GCCGCAAATACGAGAAGTG-3' and reverse 5'- TTCCCACTCCCTCTCCAAG -3'(This study). The PCR mixture with the final volume of 20 µL contained 2µL of 10X PCR buffer, 2.5 mM of $MgCl_2$, 200 µM of each dNTPs, 500 nM of each primers, 1 U *Taq* DNA polymerase (CinnaGen, Iran), and 100 ng of template DNA (nucleic acid quantities were determined by nanodrop instrument).

The thermocycler (Eppendorf 1659, Germany) program for *magA* gene amplification was as follow: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, 30 s at 72°C, and a final extension at 72°C for 10 minutes. Amplification of the desired fragment was detected by electrophoresis on 1.5% agarose gel. Agarose gel was stained with GreenViewer (Pars Tous, Iran) and visualized on 1.5% agarose gel with a UV light transilluminator.

3.6. Statistical analysis

Data were analyzed by the chi-square (Pearson Chi-Square) test. Fisher's exact test was used. SPSS v. 20 (Chicago, IL, USA) was used in the current study, and probability level < .05 was considered as statistically significant.

4. Results

A total of 130 *K. pneumoniae* strains were isolated from 102 hospitalized patients and 28 outpatients in Imam Reza hospital in Mashhad, Iran, during May 2011 to July 2012. The most specimens were urine samples (25 cases from outpatients, 39 cases from hospitalized patients, in total 49.2%), followed by wound samples (3 cases from outpatients, 21 cases from hospitalized patients, in total 21.5%), and blood samples (19 cases from hospitalized patients, 14.6%).

Overall, the patients were 65 males and 65 females. The average age of the patients was 39 years. Hyperviscous phenotype was detected by string test (Fig. 1).

MagA was present in 11 out of 130 isolates (6 isolates from urine, 3 isolates from blood, 1 isolate from wound, and 1 isolate from urethral discharge sample), among which 3 cases were HV^+, and 8 cases were HV^-. Four out of 11 *magA* positive cases were also positive for ESBL (2 isolates from urine and 2 isolates from blood samples) while seven *magA* isolates were negative for ESBL.

The greatest proportion (119 out of 130 isolates, 91.5%) of the isolates were negative for *magA*, in which 52 isolates were positive for ESBL (22 isolates from urine, 10 isolates from blood, 8 isolates from wound, and 12 isolates from the other samples). Some of the isolates in all age groups possessed *magA* and produced ESBL. Additionally, some of the samples collected from males (3 out of 65 isolates) and females (1 out of 65 isolates) contained both *magA* genes and produced ESBL. Results are summarized in Table1.

Figure 1. HV phenotype. Illustration of a positive string test: formation of viscous strings >5 mm in length.

The statistical analysis showed no significant association between the presence of *magA* gene and ESBL production (Fisher's exact tests; p= .759). There was also no significant relationship between the presence of *magA* and sex, age, and the patient's status (outpatients or inpatients) (p values are listed in Table 1).

5. Discussion

K. pneumoniae is an opportunistic bacterial pathogen associated with nosocomial infections such as UTI, pneumonia, and sepsis. Extended spectrum B-lactamase (ESBL) producing *K. pneumoniae* poses unique challenges to infection control professionals (11).

A new type of community-acquired *K. pneumoniae* associated with pyogenic liver abscess has been reported to occur in Taiwan. The other reports from United States and Asian countries showed that pyogenic liver abscess was caused by *K. pneumoniae.* A new virulence gene called mucoviscosity-associated gene A (*magA*) has been identified in this pathogen. Mucoviscosity-associated gene A is related to hypermucoviscosity (HV), resistance to killing by human serum and phagocytosis, and high virulence in animal models (16).

[DOR: 20.1001.1.25884107.2017.3.2.8.7]

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Table 1. The distribution of *magA* gene in the clinical isolates of ESBL-KP and non ESBL-KP.

		ESBL	*magA*						Fisher's Exact Test P value
			Positive(56)		Negative (74)		Total		
			N	%	N	%	N	%	
Sex	Male	Positive	3	9.1	4	12.5	7	10.8	0.708
		Negative	30	90.9	28	87.5	58	89.2	
	Female	Positive	1	4.3	3	7.3	4	6.3	0.999
		Negative	22	95.7	38	92.7	60	93.8	
Situation	Out patient	Positive	0	0.0	1	4.0	1	3.6	0.999
		Negative	3	100.0	24	96.0	27	96.4	
	In patient	Positive	4	7.5	6	12.2	10	9.8	0.551
		Negative	49	92.5	43	87.8	92	90.2	
Age	Child	Positive	2	11.8	0	0.0	2	6.5	0.488
		Negative	15	88.2	14	100	29	93.5	
	Middle aged	Positive	1	3.1	2	5.4	3	4.3	0.999
		Negative	31	96.9	35	94.6	66	95.7	
	Old	Positive	1	14.3	5	21.7	6	20.0	0.999
		Negative	6	85.7	18	78.3	24	80.0	
Specimen	Urine	Positive	2	8.3	4	10.0	6	9.4	0.999
		Negative	22	91.7	36	90.0	58	90.6	
	Blood	Positive	2	16.7	1	14.3	3	15.8	0.999
		Negative	10	83.3	6	85.7	16	84.2	
	Wound	Positive	0	0.0	1	6.2	1	4.2	0.999
		Negative	8	100.0	15	93.8	23	95.8	
	Others	Positive	0	0.0	1	9.1	1	4.3	0.478
		Negative	12	100.0	10	90.9	22	95.7	

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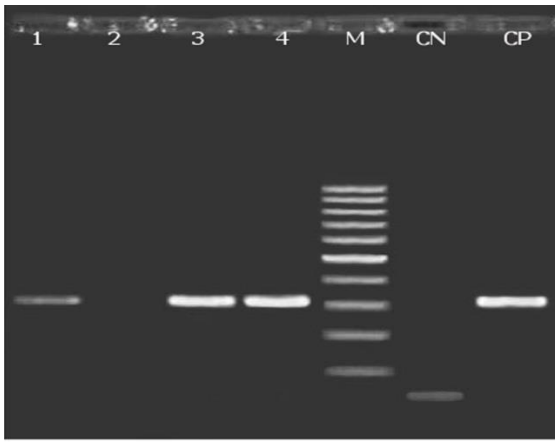

Figure 2. PCR results for *magA* gene. Lane numbers 1, 3, 4 show a 303 bp fragment of *magA* gene. Lane numbers 2 was negative for *magA* gene. Lane M represents the 100bp DNA size marker. CN: negative control. CP: positive control.

The present study demonstrated the prevalence rate of *magA* gene among *K. pneumoniae* isolates collected from the Imam Reza hospital in Mashhad, Iran. In our study, all *K. pneumoniae* strains were isolated from patients without pyogenic liver abscess (PLA) syndrome. About 11 strains (8.5%) were positive for *magA* gene, and 3 out of 11 isolates were positive for HV phenotype.

In a study conducted in Hamadan, Iran, 4 out of 105 (3.8%) *K. pneumoniae* isolates were recognized as *magA* gene-positive. Among these 4 isolates, 3 isolates were collected from blood samples and one isolate from abscess. Furthermore, more than 60% of the isolates were positive for HV phenotype (17). Similar to our study, their findings showed that 2 isolates were positive for both *magA* and HV phenotype.

In Fang's study conducted in Taiwan, 98% (52 of 53) of the isolates collected from patients with pyogenic liver abscess (PLA) were positive for *magA* gene. Based on the Fang's study, *magA* gene was regarded as the new chromosomal virulence factor in *Klebsiella* isolates responsible for PLA, which could be used as a diagnostic tool. Also, they showed that *magA* is the most significant factor contributing to HV phenotype, which is only found in invasive strains causing PLA (9).

The presence of *magA* gene in non-invasive strains was previously reported in other studies (16, 18). Unlike to Fang's report, in present study, 3 out of 11 isolates were positive for HV phenotype. According to the results of other studies in Iran and other countries such as South Korea, Singapore, and North America, *magA* gene-positive isolates could be identified from other cases like sepsis, meningitis, and bacterimia, but in contrast to Fang's studies, these samples included HV^+ and HV^- phenotypes.

Therefore, containing HV^+ phenotype is not a certain reason for the presence of *magA* gene since the HV^- phenotype may have *magA* gene too (19-22).

The statistical analysis showed no significant association between the presence of *magA* gene and the production of ESBL. There was also no significant relationship between the presence of *magA* gene and sex, age, and patients' status (outpatients or inpatients).

6. Conclusion

Based on the results of this study, there was no correlation between the presence of *magA* gene and the production of ESBL in *K. pneumoniae* strains isolated from different clinical samples of Imam Reza hospital in Mashhad.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

This study was supported by the Student Research Committee (SRC), Mashhad University of Medical Sciences, Mashhad, IR Iran.

Authors' Contribution

Nafiseh Izadi, Elnaz Harifi Mood and Mastoureh Momen Heravi: Assistance with performing laboratory tests; ahboubeh Naderi Nasab: conception and design of the study, guarantor of integrity of the entire study; Zahra Meshkat: Obtaining funding for the study, conception and design of the study, guarantor of integrity of the entire study.

Funding/support

This study was supported by Mashhad University of Medical Sciences, Mashhad, Iran (grant No. 910406).

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[DOR: 20.1001.1.25884107.2017.3.2.8.7]

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[DOR: 20.1001.1.25884107.2017.3.2.8.7]

How to cite this article: Izadi N., Naderi Nasab M., Harifi Mood E., Momen Heravi M. Meshkat Z. Association between ESBL production and presence of magA gene among clinical isolates of *Klebsiella pneumoniae*. Infection, Epidemiology and Medicine. 2017; 3(2): 46-50.