RESEARCH ARTICLE

Detection of Virulent genes Khe, iuc, rmp, magA in *Klebsiella pneumoniae* Isolated from Urinary Tract Infection

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ABSTRACT

Background: The epidemiology of virulence genes of *Klebsiella pneumoniae* is developing rapidly and shows noted geographic differences in genotyping distribution of genes. *K. pneumoniae* have more virulence factors according to encoding for many virulent genes.

Aim of the Study: Detection of virulent genes Khe, iuc, rmp and magA in isolates of *K. pneumoniae* that caused recurrent urinary tract infection.

Patients and Methods: Samples were cultured on MacConky agar, blood agar, nutrient agar and Chrom agar orientation for isolation and identification of bacterial isolates based on standard bacteriological methods, biochemical tests. And Vitek -2- was used to identify the bacterial isolates, se. Genomic deoxyribonucleic acid (DNA) was extracted using promega™ gDNA Bacteria Kit. Polymerase chain reaction (PCR amplification for detection virulence genes of *K. pneumoniae* Khe, iuc, rmp and magA**.**

Results: The results of PCR reaction for Khe genes showed 17 (56.66%) of *K. pneumoniae* contains this gene. Hemolysin considers proteins to work as exotoxin secreted by bacteria that destroy blood cells. Hemolysin gene are virulence factor for *K. pneumonia* to evade the immune system of the body and its break down red blood cell. The aerobactin synthase genes (iucC) is detected in 17 (56.66%%) among 30 isolates of *K. pneumoniae***.** Moreover, results of PCR reaction don't show any isolate of *K. pneumoniae* containing rmpA and magA genes.

Conclusion: Molecular assay is a suitable technology helpful in estimating the virulence factors such as hemolysin gene in isolates of *K. pneumoniae* that help diagnose disease caused by pathogenic bacteria.

Keyword: *Klebsiella pneumoniae,* iuc, Khe, magA, rmp, Urinary tract infection, Virulence factors.

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INTRODUCTION

Klebsiella pneumoniae is considered opportunistic bacteria that are important causes for urinary tract and catheter-associated urinary-tract infections in patients who stay in hospital and suffer immune compromise.[1] Infection of medical instruments such as catheters are a major site of infections with *K. pneumoniae* and have played a role in forming biofilms on tissue surfaces.[2] Over the last years, there has been an increase in researches activity designed to detect the pathogenesis and virulence of *K. pneumoniae* in the urinary tract infection. These detections have begun to define the virulence factors of bacteria that contribute to growth and formation of biofilm.[3] Many virulence factors have been detected to mediate *K. pneumoniae* infection, adhesion factors, capsular forming, lipopolysaccharide layer, and siderophore.[4] Both *in-vitro* and *in-vivo* methods of infection will lead to further determinants of the molecular pathogenesis of *K. pneumoniae*. As for most opportunistic bacteria. The role of host factors and bacterial traits are crucial in determining the infection.[5] Multidrug-resistant strains (MDR) bacteria have become a complex problem in treating infections with *Klebsiella* spp. Strategies to prevent and inhibit bacterial growth that causes infections need to be more developed.[6] Morbidity associated with *K. pneumoniae* that caused urinary tract infections have increased. Because these bacteria become sources of resistance extended spectrum of antibiotics and represent a challenging problem in treating individuals.[7] *K. pneumoniae* is an opportunistic pathogen and responsible for nosocomial

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infections. Infection with this bacteria infects every part of the body. The urinary and respiratory tract infections are most commonly infected.[8] *K. pneumoniae* has several virulence factors, which facilitate the microbe's ability to spread and evade body immune system cause disease of human hosts. Include capsular polysaccharide, lipopolysaccharide, fimbriae and siderosphores.[9] *K. pneumoniae* have many strategies to grow in the body and protect itself from the host immunity. Four major types of virulence factors have been characterized in *K. pneumoniae*, including the forming of hyper capsule in hyper virulence strains; lipopolysaccharide (LPS), siderophores, and fimbriae.[10] Several other factors were identified as virulence factors for *K. pneumoniae*. Virulence factors include outer membrane proteins (OMPs), porins, efflux pumps, and systems for iron transport. Some genes, responsible for the synthesis of capsule *K. pneumonia***,** although other virulence factors such as fimbriae, siderophores, and O antigen are present.[11] Chrosomal gene magA (Mucoviscosity–associated gene A) is responsible for capsule production in (K1) serotype.[12] As for the lost magA but contain *rmp*A (Regulator of mucoid phenotype gene) responsible for hyper-muco viscosity of pathogenic strains. Presence other genes that contribute with production capsule, *rmp*A2, *rmp*A effect with iron availability.[13]

AIM OF THE STUDY

Determination of virulent genes Khe, iuc, rmp, magA in *K. pneumoniae* isolated from urinary tract infection.

PATIENTS AND METHODS

A total of 302 urine samples were collected from patients women suffering from UTI who attend Baquba teaching hospital and Al-Batool teaching hospital from November 2019 to June 2020. The ages of patients women were ranged between 5 to 65 years old. A volume of 10 mL aliquots of mid-stream urine samples was taken from each subject, kept in a sterile, screw-capped tube, and transferred immediately to the laboratory and analyzed within two hours. Samples were cultured on MacConky agar, blood agar, nutrient agar, and Chrom agar Orientation for isolation and identification of bacterial isolates based on standard bacteriological methods and many biochemical tests.[14,15] And VITEK -2- was used to identify the isolates of pathogenic bacteria, antibiotics susceptibility test. Genomic DNA was extracted using promega™ gDNA Bacteria Kit. According to the protocol stated by the kit manufacturer, DNA was extracted from cultures of the pathogenic bacterial isolates. Quantus Fluorometer was used to detect the purity of extracted DNA for downstream steps of applications. After PCR amplification, PCR steps were completed.

Primers Preparation

Lyophilized primers are indicated in Table 1. Dissolve primer in a nuclease free water to give stock solution with final concentration of 100 pmol/µL. A working solution10 pmol/µL was prepared by adding 10 µL of primer stock solution (stored at freezer -20°C) to 90 µL of nuclease free water, as indicated in the following Table 2:

Optimization of PCR Program

Optimum conditions for the implication of each transposable element was described in Table 3 and 4.

RESULTS

Detect the presence of haemolysin genes (Khe) in *K. pneumoniae* and detection of Khe gene in isolates of MDR *K. pneumoniae*. The results showed that 17 (56.66%) of *K. pneumoniae* contains this gene as shown in Figure 1. Hemolysin gene as virulence factor for *K. pneumonia* to evade the immune system of the body and its break down red blood cell.[16] In the present study, the results of PCR reaction for Khe gene showed high rate (56.66%) of *K. pneumoniae* containing this gene. In al-Qadisiyah province, detected hemolysin gene in 70% (14/20) of *K. pneumoniae* as virulence factors by molecular assay.[17] And these results are closely related to findings of some previous researches.[18,19] Molecular technique a benefits method for detection of virulence genes of *K. pneumoniae*.[20] Although 22(73.33%) of *K. pneumoniae* as non-hemolytic in pheno typing detection, 13(43.33%) isolates did not express hemolysin. This difference between phenotyping and molecular detection is possible due to bacterial isolates having other systems to draw and regulate iron in tissues or production of hemolysin in small quantities, the effect of which cannot be noted. PCR is a specific approach as a good tool for the detection of virulence genes of pathogenic bacteria.[21]

Table 1: Oligonucleotide primers

Primer name	*Sequence (5→3)*	*Annealing Temp. (ºC)*	*Amplicon size (bp)*
khe	F: TGATTGCATTCGCCACTGG R:GGTCAACCCAACGATCCTGG	55	428
iucC	F: GTGCTGTCGATGAGCGATGC R: GTGAGCCAGGTTTCAGCGTC	65	944
rmpA	F: ACTGGGCTACCTCTGCTTCA R: CTTGCATGAGCCATCTTTCA	55	535
magA	F: TCTGTCATGGCTTAGACCGAT R: GCAATCGAAGTGAAGAGTGC	55	1137

Table 2: Primers preparation

Primer Name	*Volume of nuclease free water (µL)*	*Concentrations (pmol/µl)*
khe-Forward	300	100
khe- Revesrward	300	100
iucC- Forward	300	100
iucC- Revesrward	300	100
rmpA- Forward	300	100
rmpA- Revesrward	300	100
magA- Forward	300	100
magA- Revesrward	300	100

Table 3: PCR program for amplification of 12, APHA1, ERMB1, int, xis transposable elements

Step	*Temperature (°C)*	*Time m:s*	*No. of cycles*
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

Table 4: PCR program for amplification of TETM2 transposable elements

Step	*Temperature (°C)*	*Time (m:s)*	*No. of cycles*
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

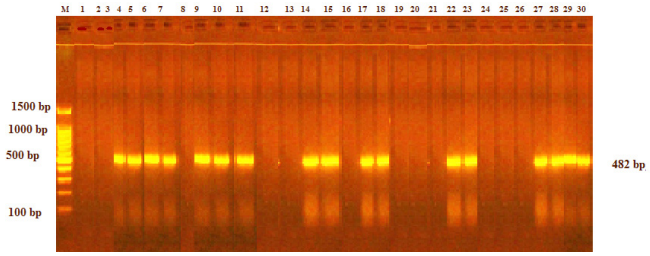

Figure 1: Amplification product of *K. pneumoniae* Khe gene after electrophoresis on 1.5% agarose gel for 90 min at 70 volt/ cm. (M): DNA ladder marker; Lanes (1–30): Bacterial isolate number.

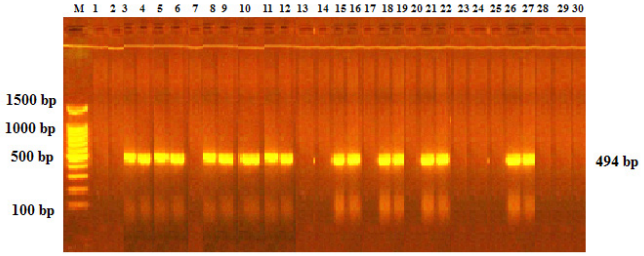

Figure 2: Amplification product of *K. pneumoniae* iucC gene after electrophoresis on 1.5% agarose gel for 90 min at 70 volt/cm. (M): DNA ladder marker; Lanes (1-30): Bacterial isolate number. Molecular detection of regulator of mucoid genes (rmpA) and Mucoviscosity –associated gene(*magA*) in *K .pneumoniae*.

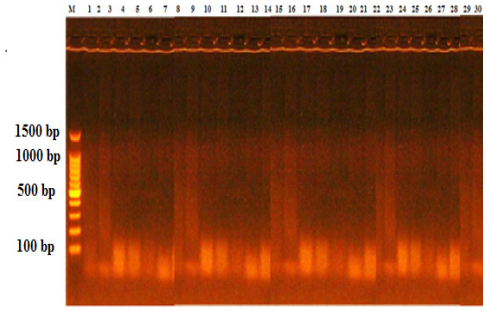

Figure 3: Amplification product of *K. pneumoniae* rmpA gene after electrophoresis on 1.5% agarose gel for 90 minutes at 70 volt/cm. (M): DNA ladder marker; Lanes (1-30): Bacterial isolate number.

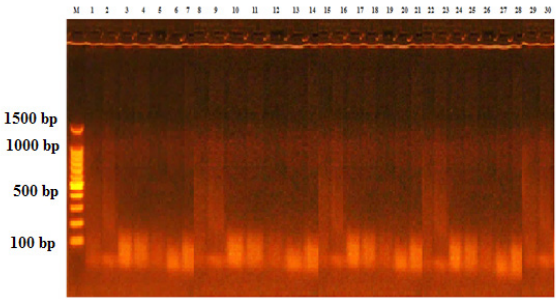

Figure 4: Amplification product of *K. pneumoniae* magA gene after electrophoresis on 1.5% agarose gel for 90 minutes at 70 volt/cm. (M): DNA ladder marker; Lanes (1–30): Bacterial isolate number.

Molecular Detection of Aerobactin genes (iucC) in *K. pneumoniae*

The aerobactin synthase genes (iucC) is detected in 17(56.66%%) among 30 isolates of *K. pneumoniae*. Figure 2 showed iucC gene in MDR *K. pneumoniae*. Many studies showed that aerobactin synthesis is a more important virulence factor in isolates of *K. pneumonia*.[22] Genotyping studies of *K. pneumoniae* reveal the iuc plasmid associated with carbapenemase-producing isolates with 100% mortality.[23] The biosynthesis of siderophores aerobactin (*iuc*) is common among MDR *K. pneumoniae* that cause chronic community-associated infections such as UTI.[24] *Iuc* gene plays the most critical role in bacterial virulence. These plasmids also carry additional virulence genes, rmpA, and magA genes that upregulate the capsular production of MDR strains.[25]

The study detects the presence of regulator of mucoid gene rmpA and Mucoviscosity-associated gene magA in *K .pneumoniae* and prevalence gene in therty isolates of *K. pneumoniae*. PCR results don't appear any isolate of *K. pneumoniae* contains these genes as appear in Figure 3 and 4. Found magA gene among 173 of *K. pneumoniae* isolates were 4 (2.31%) positive and 169 (97.68%) were negative.[26] Prevalence of magA in Taiwan was 17% in isolates of *K. pneumoniae* . The magA gene in isolates of *K. pneumoniae* macke high viscosity and more pathogen.[25] PCR analysis for detection *K. pneumoniae* strain K1 and K2 to prevalence magA in *K. pneumoniae* strain K1 isolated from invasive pyogenic infections in rate 52.3%. prevalence isolates coding rmpA gene was related to the MDR phenotype in invasive infections.[278]

CONCLUSION

The results of PCR reaction for Khe gene showed high rate (56.66%) of *K. pneumoniae* containing this gene. The molecular assay is a suitable technology helpful in the detection of Khe gene. Iuc gene plays the most critical role in bacterial virulence

in MDR isolates. The results of the PCR reaction don't show any isolate of *K. pneumoniae* containing these genes.

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REFERENCES

1. Berrazeg M, Diene M, Drissi M, Kempf M, Richet H, Landraud L, Rolaiin M. Biotyping of Multidrug-Resistant *Klebsiella pneumoniae* Clinical Isolates from France and Algeria Using MALDI-TOF MS. P L O S ONE. 2013; 8(2):234-238.
2. Fodah RA, Scott JB, Tam HH, Yan P, Pfeffer TL, Bundschuh R, Warawa JM. Correlation of *Klebsiella pneumoniae* comparative genetic analyses with virulence profiles in a murine respiratory disease model. P L O S ONE. 2014;9(4):107394.
3. Vuotto C, Longo F, Balice M, Donelli G, Varaldo P. Antibiotic Resistance Related to Biofilm Formation in *Klebsiella pneumoniae*. Pathogens. 2014;3:743-758.
4. Sharma N, Gupta A, Walia G, Bakhshi R. A retrospective study of the changing trends of antimicrobial resistance of Klebsiella *pneumoniae* isolated from urine samples. J Nat Sci Biol Med. 2016;7:39-42.
5. Ranjbar R, Memariani R, Sorouri R. Molecular epidemiology of extendedspectrum beta-lactamase-Producing *Klebsiella pneumoniae* strains isolated from children with urinary tract infections, Arch. Pediatr. Infect. 2017;5(2):39000.
6. Tenney J, Hudson N, Alnifaidy H, Li J, Fung K. Risk factors for aquiring multidrug-resistant organisms in urinary tract infections: A systematic literature review. Saudi Pharm J. 2018;26:678-684.
7. Eulalia C A, Caio M L, Anderson C F, Maria C P. High Prevalence of Multidrug-Resistant *Klebsiella pneumoniae* Harboring Several Virulence and β-Lactamase Encoding Genes in a Brazilian Intensive Care Unit.Front. Microbiol. 2019;(9):3198-3213.
8. Curiel M, Blackburn D, Saldaña Z, Gayosso-Vázquez C, Iovine N, De la Cruz M. Multi-functional analysis of *Klebsiella pneumoniae* fimbrial types in adherence and biofilm formation. Virulence J. 2013;4(2):129-138.
9 Fang H, Tzu-lung L, Feng-lung Y, Meng-chuan W, Yi-jiun P, Shih-hsiung W, Jin-town W. Lipopolysaccharide O1 Antigen Contributes to the Virulence in *Klebsiella pneumoniae* Causing Pyogenic Liver Abscess. PLOS ONE. 2012;7.
10. Pan YJ, Lin TL, Chen CT, Chen YY, Hsich PE. Genetic analysis of Capsular Polysaccharide synthesis gene clusters in 79 capsular types of Klebsiella spp. Sci . Rep . 2015;5:15573-78.
11. Stahlhut S G, Struve C, Krogfelt K A. Klebsiella pneumoniae type 3 fimbriae agglutinate yeast in a mannose-resistant manner. J. Med. Microbiol. 2012;61:317-322.
12. March C, Cano V, Moranta D, Llobet E, Perez-Gutierrez C, Tomas JM, Suarez T, Garmendia J, Bengoechea JA. Role of bacterial surface structures on the interaction of Klebsiella pneumoniae with phagocytes. PLoS One. 2013; 8: 56847.
13. Kidd TJ, Mills G, Sa-Pessoa J, Dumigan A, Frank CG, Insua JL, Ingram R, Hobley L, Bengoechea JA. A *Klebsiella pneumoniae* antibiotic resistance mechanism that subdues host defences and promotes virulence. EMBO Mol Med. 2017;9:430-447.
14. Baron EJ, FinegoldSM, Peterson, L.R. Bailey and scotts Diagnostic Microbiology.9th ed. Mosby Company Missouri, U.S.A. 1994.
15. Collee JG, Fraser AG, Marmion BP, simmons A. Mackie and McCartney practical Medical Microbiology . 14th ed . Churchill Livingston . 1996;173-174.
16. Elliott SJ, Wainwright LA, McDaniel TK, Jarvis KG, Deng YK, Lai LC, McNamara BP, Donnenberg MS, Kaper JB. The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic Escherichia coli E2348/69. Mol Microbiol. 1998; 28:1-4.
17. El-Sukhon SN. Identification and characterization of klebsiellae isolated from milk and milk products in Jordan. Food Microbiol. 2003;20:225-230.
18. Gundogan N, Citak S, Yalcin E. Virulence Properties of Extended Spectrum b-Lactamase-Producing Klebsiella Species in Meat Samples. J. Food Prot, 2010;74(4):559-564J. Food Saf. 2010;3:251-260.
19. Schmidt H, Beutin L, Karch H. Molecular Analysis of the Plasmid-Encoded Hemolysin of *Escherichia coli* O157:H7 Strain EDL 933.1995; 63(3):1055-1061.
20. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathol Biol. 2013;61:209-216.
21. Jameela RE, Jenan N S. Hemolysin gene detection in some isolates of *Klebsiella pneumoniae* by PCR. QJVMS. 2018; 17 (2): 6th.
22. Russo, TA, Olson R, Macdonald U, Metzger D, Maltese LM, Drake EJ. Aerobactin Mediates Virulence and Accounts for Increased Siderophore Production under Iron-Limiting Conditions by Hypervirulent (Hypermucoviscous) *Klebsiella pneumoniae*. Infect Immun. 2014; 82(6):2356-2367.
23. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis. 2018;(17):30489-30489.
24. Turton JF, Payne Z, Coward A, Hopkins K, Turton J, Doumith M. Virulence genes in isolates of *Klebsiella pneumoniae* from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and "non-hypervirulent" types ST147, ST15 and ST383. J Med Microbiol. 2018;67(1):118-128.
25. Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC. Association between rmpA and magA genes and clinical syndromes cused by *Klebsiella pneumoniae* in Taiwan. Clin Infect Dis. 2006; 42:1351-1358.
26. Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP, Lin JC. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiella pneumoniae liver abscess in Singapore and Taiwan. J Clin Microbiol. 2007;45:466-471.
27. Hadis A, Pegah S, Samaneh R, Neda B, Behnam Z. Prevalence assessment of magA gene and antimicrobial susceptibility of Klebsiella pneumoniae isolated from clinical specimens in Shahrekord, Iran. Iran. J. Microbiol. 2014;6(5): 311-316.