Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Hemolysin gene detection in some isolates of *Klebsiella pneumonia* by PCR

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(Received 5/8/2017, Accepted 18/11/2017)

Abstract

Hemolysin protein is exotoxin produce by organisms that cause lysis of blood cells. This study was conducted to screen the presence of hemolysin gene from 20 isolates of Klebsiella pneumonia based 16S rRNA gene by using specific primer. This gene potent the pathogenesis of Klebsiella pneumonia. The primer was designed in this study by NCBI-GenBank and primer3 plus. (Bioneer Company provided the primers. Korea). Molecular detection of isolates, which give away specific PCR products of 505bp for hly gene, hemolysin gene, was detected in 70% (14/20).

Keywords: *K. pneumoniae*, Hemolysin gene, PCR, 16S rRNA

Introduction

Hemolysin is cytolyic toxin found in microorganisms, which possess these virulence features of lysis of erythrocytes that associated with pathogenesis of their microorganisms (1). Hemolysins are consider as an important causes of damage to facilitating the dissemination of bacteria, extra intestinal diseases also liberation of host nutrient, and may as well alter pathways of the host by affecting on various pathways, inclusive host cell survival ,inflammatory response, cytoskeletal dynamics(2). *Kle.* Spp are opportunistic bacteria found in environment and in gastrointestinal tracts of a wide domain of animals (3). *Klebsiella* bacteria is facultative anaerobic, opportunistic, encapsulated and lactose fermenting found as normal inhabitants its most member of Enterobacteriaceae (4).The presence of virulence genes in *Klebsiella pneumonia* promote the pathogenicity to evading the immune of the body (5). Many sequined virulence genes have been detection in *Klebsiella* One of them is (*hly*) (6).*Klebsiella* species Although is described as non-hemolytic, the detection of the hemolytic effects for isolate as reported in (7).gene hemolysin production by -negative bacteria is indicative of another virulence and enter toxigenic factors(8).Within 16S rRNA gene analysis and Sequencing of regions can consider effective and speedy ways for pathogen and identification to estimate variety of bacteria.(9).This paper aimed to identify the hemolysin gene in *Klebsiella pneumonia* isolates which obtained from Laboratory of zoonotic diseases unit in the veterinary medicine collage university of Al-Qadisiyah by PCR tech.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 431

This study was done in the veterinary medicine collage university of Al-Qadisiyah.

Samples:

The isolates of *Klebsiella pneumonia*, which tested previously, cultured on

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MacConkey's agar and blood agar plates, and incubated for 24 hours at 37c. Then the isolates were activated by in inoculated in Brain Heart Infusion Broth media and incubated at 37C° for overnight. Identification of isolates based on morphology of colonies, subculturing of isolates onto MacConkey and incubated for 24 hours at 37c, pink, mucoid, lactose-fermented colonies were considered Klebsiella spp.

DNA extraction:

Bacterial DNA of *Klebsiella pneumoniae* solates extracted according to (Geneaid, USA).

PCR Amplification:

PCR assay was carry out for confirmative recognition of *Klebsiella pneumoniae* based 16S rRNA gene and for determination hemolysin gene and by use specified primers that prepared by using NCBI-GenBank, Submitted by (Bioneer) in Korea as in table1

Table 1. PCR primers and their sequence and GenBank codes

Primer	Sequence	Amplicon	GenBank
16S Rrna	CGCGAAGAACCTTACCTGGT	352bp	Y17669.1
	AGTTGCAGACTCCAATCCGG		
Hly	CCGGAGCGTTTTTCGATTGG	505bp	AF293352.1
	AGCATCCGGGTAAAAAGGGG		

Results

Table 2. The virulence genes distribution with the numbers of the isolates

Gene	No .of tested isolates	Positive
16S rRNA genes	20	20
Hly	20	14

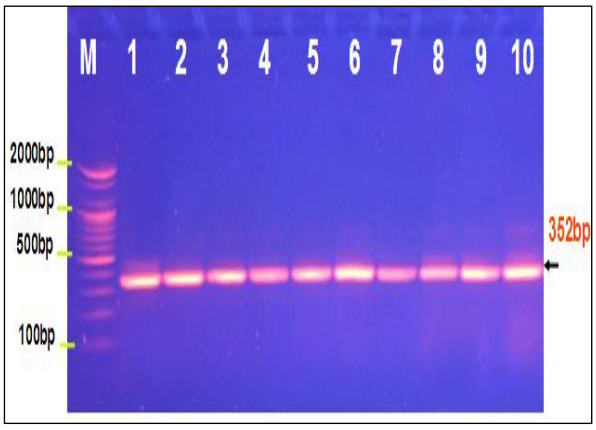

Figure (1): Agarose gel electrophoresis image that show the PCR product analysis of 16S rRNA gene in *Klebsiella pneumoniae* positive isolates. Where M: marker (2000-100bp), lane (1-10) positive at (352bp) 16S rRNA gene PCR product.

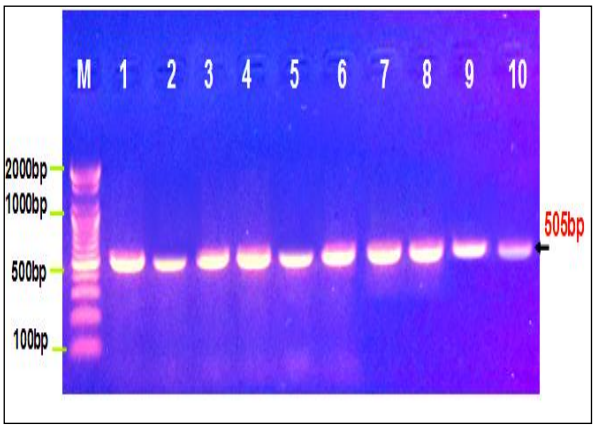

Figure (2): Agarose gel electrophoresis image that show the PCR product analysis of *hly* gene in Klebsiella pneumoniae isolates. Where M: marker (2000-100bp), lane (1-10) positive isolates at (505bp) PCR product.

Discussion

Hemolysin is the factor be responsible for cells segregation in vitro (10**).** Detection of these genes may indicate the virulence potential of *Klebsiella* isolates. In present study hemolysin gene of *Klebsiella pneumonia* isolates detected in 70% (14/20). These results closely related to findings of (11) and (12). The production of hemolysin among gram-negative bacteria is indicative of other virulence and enterotoxigenic factors

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(13).The oxygen-labile hemolysin has been detected in Klebsiella *pneumoniae.*It has characteristics similar to other thiolactivated Lysins new source of this type of hemolysin and its adsorption to erythrocytes and factors that may affect this process are of interest (7**).** A study (14) detected the presence of the virulence factors gene in feces of cattle; while this gene was not detected in (15) revealed the results. In conclusion. PCR is a specific approach as good tool for detection hemolysin toxin gene of pathogens.

Conclusion

(1) Molecular assay a suitable technology helpful in diagnostics of *K. pneumoniae* (2) Detection of the hemolysin gene in *K. pneumoniae* as virulence factors will be aid in detection of the disease caused by this bacteria (3) *K. pneumoniae* hemolysin requires more investigations to compare it with other bacterial hemolysin.

References

1-Brauna and focareta. Hemolysin gene expressions. (1999).

2-Schmidt H, Beutin L, Karch H. Molecular Analysis of the Plasmid-Encoded Hemolysin of Escherichia coli O157:H7 Strain EDL 933. Vol.63, No. 3. (1995); p.1055-1061.

3-Podschun R, U Ullmann. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev. (1998); 11:589-603.

4-Shon As, Bajwa RPC, Russo TA. Hyper virulent (hypermucoviscous) *Klebsiella pneumonia* Anew and dangerous breed .virulence (2013); 4:107-118.

5-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.

6-Hartman LJ, Selby EB, Whitehouse CA, Coyne SR, Jaissle JG, Twenhafel NA, Burke RL, Kulesh DA (2009) Rapid Real-Time PCR assays for detection of *Klebsiella pneumoniae* with the rmpA or magA genes associated with the hypermucoviscosity phenotype. *J Mol Diagn* 11: 464-471. http://dx.doi.org/10.2353/jmoldx.2009.080136.

7-Albesa IL, I Barberis, MC Pajaro, AJ Eraso. A thiolactivated hemolysin in gram-negative bacteria. Can. J. Microbiol. (1985); 31:297-300.

8-Gundogan N, U Yakar. Siderophore production, serum resistance, hemolytic activity and extended spectrum beta lactamase–producing Klebsiella species isolated from milk and milk products'. Food Saf. (2007); 3:251-260.

9-Kolbert CP, Persing DH. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. Curr Opin Microbiol (1999); 2(3): 299-305

10-Peter K Fagan, Michael A Hornitzky, Karl ABettelheim, Steven P Djordjevic (1999). Detection of Shiga-Like Toxin (stx1 andstx2), Intimin (eaeA), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (EHEC hlyA) Genes in Animal Feces by Multiplex PC.

11-El-Sukhon SN. Identification and characterization of klebsiellae isolated from milk and milk products in Jordan. Food Microbiol. (2003); 20:225-230.

12-Gundogan N, Citak S, Yalcin E. Virulence Properties of Extended Spectrum b-Lactamase-Producing Klebsiella Species in Meat Samples. Journal of Food Protection, Vol. 74, No. 4, Pages 559–564J. Food Saf. (2010); 3:251-260.

13-Baret TJ, PA Blake. Epidemiological usefulness of changes in hemolytic activity of Vibrio cholerae biotype El Tor during the seventh pandemic. J. Clin. Microbiol. (1981); 13:126-129.

14-Law D, Kelly J. Use of haem and haemoglobin by Escherichia coli O157 and other Shiga-like-toxin-producing E. coli serogroups. Infect Immun. (1995); 63:700-712.

15-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. http://dx.doi.org/10.1016/j.patbio.2012.10.004.

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