Al-Kufa University Journal for Biology / VOL.10 / NO.1 / Year: 2018

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Detection of some virulence factors with Biochemical investigation of *Listeria monocytogenes* associated with human infections.

Dr .Adil Abead Hassoni Dr. Alaa Abbas Fadhel

Assit .Proff. Biology/ Microbiology Lecturer / Biochemistry

Technical collage/Al- Musayib, Al-Furat Al-Awsat Technical University.

ABSTRACT

This study was aimed to detect different virulence genes that might be present in the *Listeria monocytogenes*. 50 clinical samples were gathered from patients with different sites like (stool, urine and blood). Though, only (22) isolates were contained *L.monocytogenes* using conventional and traditional techniques. Strains were isolated with the following approach 8 (36%), 6 (27%), and 8 (36%) from human intestine (stool), urine, and blood samples respectively.

Virulence genes were detected by using specific PCR primers which include (*iap*, *hly* and*inl* A). The results indicated that four isolates of *L.* monocytogenes possessed (*hly*) gene, two from urine strains 2:6 (33%), one from stool 1:8 (13%) and one from blood 1:8 (13%). In addition to that, only three isolates of inlAgave positive amplification where1:8(13%) from stool 1:6(17) from urine and 1:8 (13%) from blood.

Moreover, six strains gave positive amplification for *jap*gene where 3:8(38%) from stool and 2:6 (25%) from urine,and one fromblood1:8(13%).

Introduction

Listeria monocytogenes are gram positive type, rod in shape, facultative anaerobic bacteria which do not generate endospores. Its, considered as the most important human pathogen, and act as causal agent of the moderately bacterial disease listeriois. The consumption of contaminated food with the bacteria caused a severe infection [1].

The infection might be effect pregnant women, adults with weakened immune systems as well as newborns. Listeriosis considered as serious disease for humans with case fatality rate around 20%.Clinical manifestations divided to sepsis and meningitis. Usually, meningitis is convoluted by encephalitis, when it is recognized as meningoencephalitis apathology that is uncommon for bacterial infections. The incubation period can vary between three and seventy days [2].

Many symptoms associated with listeriosis like fever, muscle aching, diarrhea or nausea. When the disease caused by listeriosisand extended to the nervous system, symptom such as headache, stiff neck, disorder, overcome of balance, or contraction can take place[3] .

While the symptoms of infected pregnant women, appeared like only a mild, flu-like sickness. In contrast, healthy individual who is not pregnant usually does not need any treatment. Symptoms will usually disappearwithin a few weeks. Antibiotic can avoid infection with listeriosisof the fetus or newborn for pregnant women.

Babies who infected with listeriosis could be given the same antibiotics as adults, although a combination of antibiotics isfrequently used for treatment until your doctor persuaded the reason is listeriosis [4].

However the expression of three different *L. monocytogenes* virulence genes such as(*iap*, *hly* and*inl* A) genes , were assessed in separate PCR assays.Virulence gene cluster that is

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

focusing in function is necessary to intracellular endurance, and considered as are cognized and characterized hemolysin gene (*hly*).

Moreover, hemolysin gene act as the key role for run off break inside phagosomes which required for intracellular bacterial proliferation[5]. Hemolysin not only became the original *Listeria* virulence thing to have its own gene characterize, but also the earliest gene of bacteria creation to which a function essential to the endurance of a parasite insidethe host of eukaryote that recognized[6].

The listerial surface protein Internalin A (*inlA*), revealed to be mandatory for invasion of endothelial cells in vitro, the specific bacterial molecules that signifying are actively concerned in the interaction with the *L. monocytogenes* and proficiently multiplicate for long period of time within microvascular brain cells without causing any damage, and create seriously infected emphases from which bacteria extended to adjacent cells by motility [7].

On the other hand, the Invasion-associated protein (*iap*)gene of *Listeria monocytogenes* encrypts the extracellular protein, which possessed a murein hydrolase enzyme that required for septum separation action [8].

The pesent study was aime to detect different virulence genes that might be present in the *L. monocytogenes* making it resistance to different antimicrobial agents

Materials and Methods

Collection of Specimens

The study was conducted at Teaching General Hilla Hospital in Babylon province. 50 samples collected from different sources(urine, blood and blood) during the period from (November , 2017 to February , 2018) .

Bacterial Identification

L. monocytogenes were cultured on Brain Heart Infusion broth (BHI) media incubated for 24 hours at 37°C. Next, pelleted and diluted in phosphate buffer saline (PBS). specimens were treated with selective media for the isolation and culture of *L. monocytogenes* from samples that contain bacterial flora.

On the other hand, Polymyxin acriflavine lithium chloride ceftazidime esculin mannitol (PALCAM), Listeria selective agar were used for differential diagnosis, isolation, and detection of *L. monocytogenes* which were incubated after the enrichment media. The inoculums was plated on PALCAM agar (HiMedia) and incubated for 48 h at 37°C.

Standard biochemical methods were used for catalase test, oxidase test, Nitrate, Rhamnose,Xylose, α-Methyl-d-mannoside, Mannitol , Methyl red, Voges Proskauer were done to confirm identification of bacteria .

DNA extraction

DNA extraction was assayed using genomic DNA purification kit supplemented by(Geneaid, UK) company.

Virulence Gene detection by PCR technique .

PCR technique was used for amplification (*iap*, *hly* and *inl* A) primers to amplify genes encoding factors (Table 1). 2.5 µl of each upstream and downstream primers were added to each 25 µl of PCR reaction. Next, 2.5µl of free nuclease water, 5µl of extracted DNA, and

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

12.5µl of master were mixed. After that, the amplification products of PCR were visualized using electrophoresis on 1% Agarose gels for 45min at 70 volt. Determination of the amplicons size were done by comparison to the 100-1000bp ladder (Promega, USA).

Table (1):- Primers sequences and PCR condition

Genes	Primer sequence (5′-3′)	Size of product bp	PCR condition	Reference
inl A	AGATCTAGACCAAGTTACAACGCTTC AG TAA TAT CAT TTG CTG TTT TAT CTG TC	255 bp	94ºC 3min 1x 94ºC 30sec 55ºC 30sec 30x 72ºC 1min 72ºC 7min 1x	(9)
Hly	CCT AAG ACG CCA ATC GAA AAG CGC TTG CAA GTC CTC	702bp	94ºC 3min 1x 94ºC 30sec 55ºC 30sec 30x 72ºC 1min 72ºC 7min 1x	(10)
Iap	ACA AGC TGC ACC TGT TGC AG TGA CAG CGT TGT TAG TAG C	131bp	94ºC 30sec 55ºC 30sec 30x 72ºC 1min 72ºC 1min 72ºC 7min 1x	(11)

Results and Discussion

Bacterial diagnosis

The color of colonies were gray-green and surrounded by black zone on PALCAM agar were elected and purified with Tryptone Soya Yeast Extract agar (TSYEA).Furthermore, colonies with pinpoint shape were exposed to recognition that include Gram's staining followed by a examination with microscopic,as well as catalase and oxidase tests. After that, Sub-cultured in Brain heart infusion (BHI) broth for 12 -18 hours at 25°C. The results showed of diagnosed bacteriawas Gram-positive, coccobacillary or short rod-shaped organisms. The oxidase test was negative and catalase test was positive.

The cultures viewing distinctive tumbling motility were consider as indication for listeria isolates, biochemical tests which were exposed toVoges-Proskauer, methyl red, sugar

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

fermentation tests and nitrate, withrhamnose ,xylose ,mannitol and methyl D-mannoside as shown in Table (2). Only 22 isolates of *L. monocytogenes* were obtained from patients with gastro enteritis, urinary tract infection and blood by standard bacteriological methods. Since, (8 isolates were collected from stool samples, 6 from urine, and 8 from blood samples).

Table (2) biochemical tests for *L.monocytogenes*

Test	Catalase	Oxidase	Nitrate	Rhamnose	Xylose	Methyl-d-mannoside	Mannitol	Methyl red	VogesProskauer
result	+	--	--	+	--	+	--	+	+

Moreover, Specific PCR primers were used for the recognition of(*iap*, *hly* and*inl* A) genes. The results showed that only four of investigated isolates contained the (*hly*) gene. two from urine strains 2:6 (33%), one from stool 1:8 (13%) and one from blood 1:8 (13%)as shown in figure (1-1).

Also the results revealed that only six strains gave positive results for *jap*gene where 3:8(38%) from stool and 2:6 (25%) from urine(33%),and one from blood1:8 (13%) as revealed in figure(1-2). Besides only three isolates from stool 1:8(13%), 1:6 (17%)from urine and1;8(13%) from blood gave positive amplicon for (*inl* A) gene and other did not gave amplification for this gene as revealed in figure(1-3).

This result is correlated with the results done by [12] who investigated the high prevalence of Erythromycin resistance ***L.monocytogenes*** in clinical isolates than commensal isolates. Other study investigated that the high prevalence of Erythromycin resistance ***L.monocytogenes*** among clinical strain sowed this resistance due to the present ***L.monocytogenes*** of (*iap*) and (*hly*) genes describing this a new putative efflux genes play a role in bacterial resistance to antimicrobial agents [13].

The appearance of virulence genes, involving *hly*, have been revealed to give significantly to *L. monocytogenes* virulence and formation of biofilm [14]. biofilm formation by contributing of ActA together with the moderate impacts on *hly* inactivation detected here, due to the contribution of much more distinct biofilm reductions exhibit by the *hly* gene.

The Investigations of *iap* region may possibly donate epidemiological and molecular information about this important human pathogen [15].InlA gene is a part of large family of proteins, the internalin family, composed of 25 proteins sharing a common construction that includes amino acid leucine rich repeat at asignal peptide as able to be different from other.For the InlI., LRR repeating unit are ordinary to a large number of proteins and involved in protein to protein interactions.

Downstream of the LRR, internalins showed numerous other regions that are less conserved within the family and can confer specific functions to different members. An LPXTG pattern for the anchor of InlA at the cell wall ofbacteria is present at the carboxyl terminal followed by a sorting peptide [16].

Al-Kufa University Journal for Biology / VOL.10 / NO.1 / Year: 2018

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

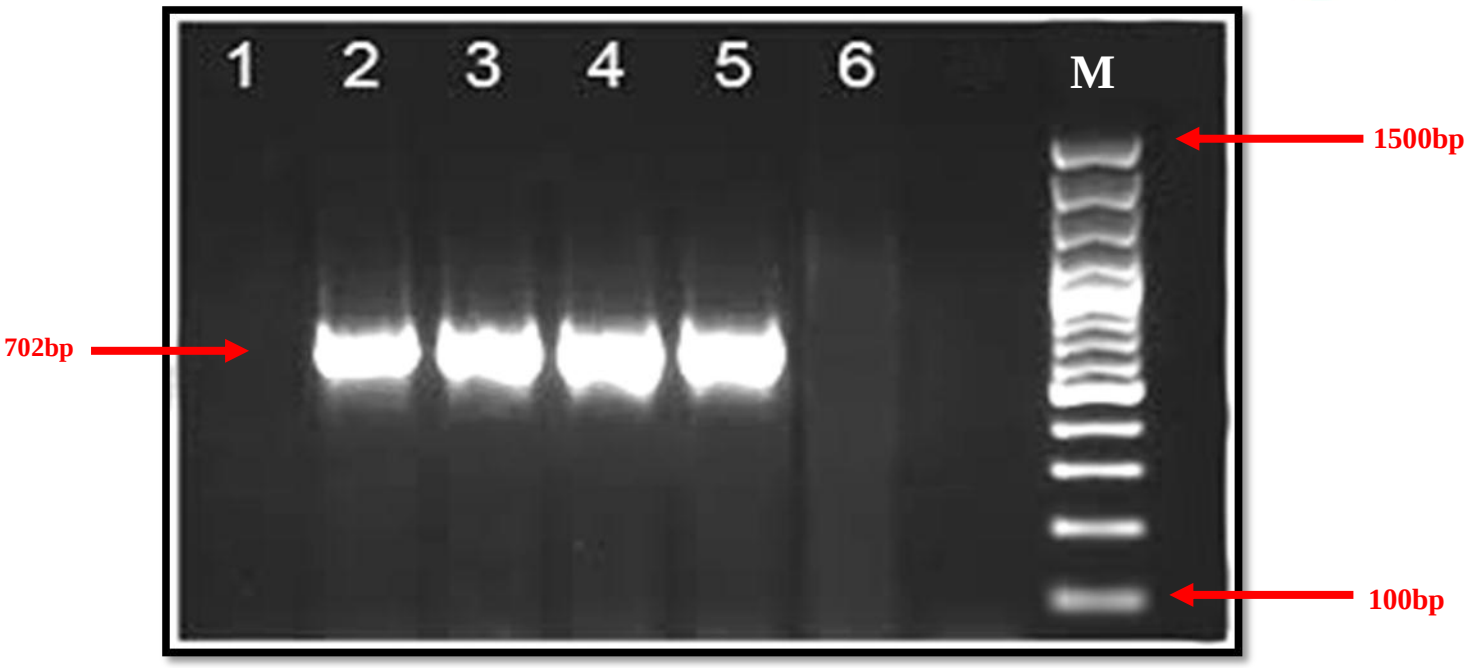

Figure (1-1):Gel electrophoresis of PCR amplified product of *hly* with 702bp on 1% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -1500 bp ladder.Lane (2,3,4,5) No. of clinical isolates of *L. monocytogenes* gave positive amplification for *hly* , (2and 3) isolate from urine ,(4) from stool and (5) from blood.

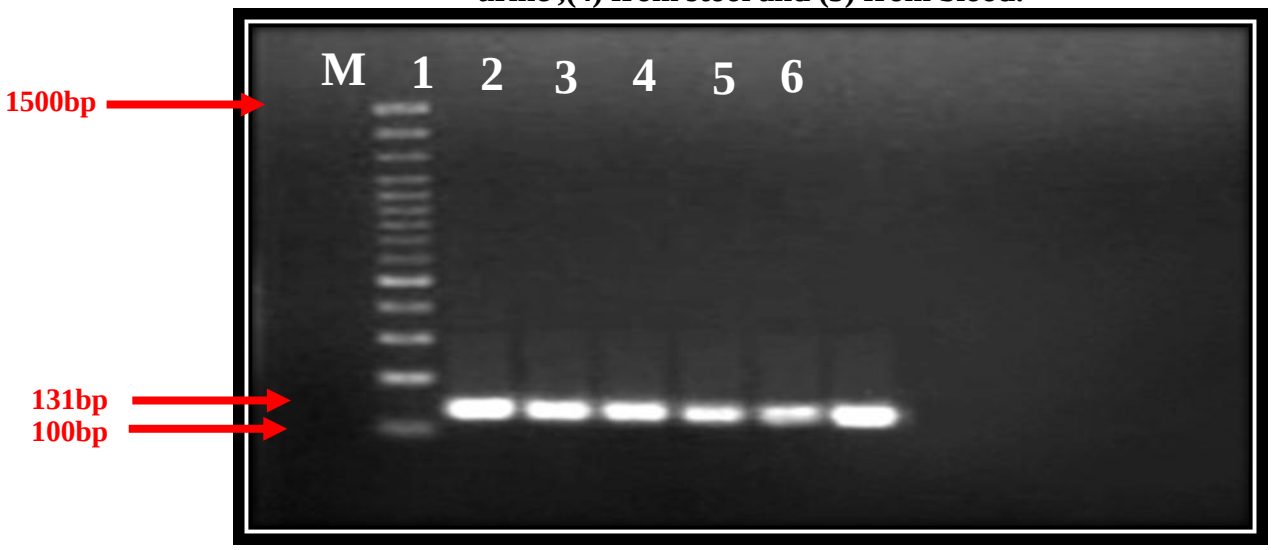

Figure (1-2):Gel electrophoresis of PCR amplified product of *jap* with 131bp on 1% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -1500 bp ladder.Lane (1,2,3,4,5.6) No. of clinical isolates of *L. monocytogenes* gave positive amplification for *hly* , (2and 3) isolate from urine ,(1,4,6) from stool and (5) from blood.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Figure (1-3):Gel electrophoresis of PCR amplified product of *inlA*with 255 bp on 1% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -1500 bp ladder.Lane (2,5,6) No. of clinical isolates of*L. monocytogenes* gave positive amplification for *inlA* , (2) isolate from urine ,(6) from stool and (5) from blood.

References.

1- Jeyaletchumi P, Tunung R, Margaret S.(2010). Detection of *Listeria monocytogenes* in foods. Int. Food. Res. J. ;17:1–11.

2- Perrin M, Bremer M, Delamare C. (2003). Fatal cases of *Listeria innocua* bacteria. J. Clin. Microbiol. ;41:5308–5309.

3- Liu D.(2006). Identification, subtyping and virulence determination of *Listeria monocytogenes* an important foodborne pathogen. J. Med. Microbiol. ;55:645–659.

4- Ramaswamy V; Cresence VM; Rejitha JS; Lekshmi MU. (2007). Listeria review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect*. 40 (1): 4–13.

5- Bortolussi, R, (2008). Listeriosis: A Primer. CANADIAN MEDICAL ASSOCIAION JOURNAL, Vol. 179, No. 8, pp. 795-7.

6- Schmid M. W;Lampidis R.; Emmerth M.; Walcher M.; Kreft J.; Goebel . (2005). Evolutionary history of the genus *Listeria* and its virulence genes. *Systematic and Applied Microbiology*. 28 (1): 1–18.

7- Todar, K. (2008). "Listeria monocytogenes". Todar's Online Textbook of Bacteriology.

8- Ward, T. J.; Gorski, L.; Borucki, M. K.; Mandrell, R. E.; Hutchins, J.; Pupedis, K. (2004). Intraspecific Phylogeny and Lineage Group Identification Based on the prfA Virulence Gene Cluster of Listeria monocytogenes". *Journal of Bacteriology*. **186** (15): 4994–5002.

9- Joseph B., Goebel W.(2007). Life of *Listeria monocytogenes* in the host cells' cytosol. *Microbes and Infection*. 2007;9(10):1188–1195

10- Témoin S., Roche S. M., GrépinetO., Fardini Y., Velge P. (2008). Multiple point mutations in virulence genes explain the low virulence of *Listeria monocytogenes* field strains. *Microbiology*. 154(3):939–948.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

11- Jallewar P. K., Kalorey D. R., Kurkure N. V., Pande V. V., Barbuddhe S. B. (2007). Genotypic characterization of *Listeria* spp. isolated from fresh water fish. *International Journal of Food Microbiology*. ;114(1):120–123.

12- Todar, K. (2008). Listeria monocytogenes. Todar's Online Textbook of Bacteriology.

13- Travier, L.; Guadagnini, S.; Gouin, E.; Dufour, A.; Chenal-Francisque,V.(2013). ActA promotes *Listeria monocytogenes* aggregation, intestinal colonization and carriage. *PLoSPathog.* , *9*.

14- Hamon M, Bierne H, CossartP.(2006). *Listeria monocytogenes*: A multifaceted model. Nat Rev Microbiol4:423–434.

15 -Veiga E, Guttman JA, Bonazzi M, Boucrot E, Toledo-Arana A, Lin AE, Enninga J, Pizarro-Cerda J.(2007). Invasive and adherent bacterial pathogens co-opt host clathrin for infection. Cell Host Microbe 2:340–351.

16- Bierne H, Sabet C, Personnic N, Cossart(2007). Internalins: A complex family of leucine-rich repeat-containing proteins in *Listeria monocytogenes*. Microbes Infect 9:1156–1166.