

***Listeria monocytogenes* (PCR)**

/ /

(2014/5/5 2013/ 9 /30)

/ *L.monocytogenes* (14) PCR /

13 *hlyA* API *Listeria*
iap PCR
 (11) (14) *inlA*

Tetracycline : *L.monocytogenes*
 PCR %57.1 %35.7 %78.6 Erythromycin Trimethoprim
tet(S) *L.monocytogenes* 14

Trimethoprim *dfrD* Tetracycline
 Erythromycin *ermB* 3

.*ermB dfrD tet(S) inlA iap hlyA* PCR *L.monocytogenes* :

Using of Polymerase Chain Reaction (PCR) to Confirm the Diagnosis of *Listeria monocytogenes* and Determination its Antimicrobial Resistance

Hiba Kh. Mahmud

Adeeba Y. Shareef

Department of Biology/ College of Science/ University of Mosul

ABSTRACT

PCR technique was used for the identification of (14) strains of *L.monocytogenes* obtained from department of Biology/college of Science/university of Mosul isolated from soil animal barns and grazing areas in Nineveh governorate, that had been identified previously by traditional methods and API *Listeria*. The presence of the first identification gene *hlyA* had been detected, (13) strains exhibited

positive result, but when PCR used for detection of second identification gene *iap*, all strains showed positive result. The third identification gene that have been detected was *inlA*, (11) strains were clear positive, while (3) strains have weak positive results, so (14) strains were positive.

The resistance of *L.monocytogenes* strains was determined to a number of antibiotics: Tetracycline, Trimethoprim and Erythromycin, it was 78.6%, 35.7% and 57.1% respectively. PCR technique was used to detect antibiotic resistance genes like *tet(S)* for Tetracycline resistance, (2) strains gave positive result, the gene *dfpD* for Trimethoprim resistance has also detected and (3) strains showed positive result. The presence of *ermB* for Erythromycin resistance was studied, (2) strains showed positive result.

Keywords: *L.monocytogenes*, PCR, *hlyA*, *iap*, *inlA*, *tet(S)*, *dfpD*, *ermB*.

Listeriosis

Listeria monocytogenes

.(Borucki *et al.*, 2004)

(Fenlon, 1999)

.(Nightingale *et al.*, 2004)

inlA

InlA

L.monocytogenes

E-cadherin

InlA

.Endocytic machinery

(LLO)ListeriolysinO

Cytolysin

Phagosome

Phospholipase

hlyA

P60

L.monocytogenes

.(Pizarro- Cerda and Cossart, 2006; Schnupf and Portnoy, 2007)*iap*

.(Liu *et al.*, 2011; Cytryn, 2013)

L.monocytogenes

.(Conter *et al.*, 2009)

15

Tetracycline

3

Energy-dependent efflux

.(Hansen *et al.*, 1993)

tet(S)

..... (PCR)

Tetrahydrofolic Dihydrofolate Dihydrofolatereductase (DHFR) Tetrahydrofolate Dihydrofolate

Trimethoprim

dfrA S1 Trimethoprim DHFRs

Trimethoprim .(Dale *et al.*, 1995)*dfrD* DHFR S2

.(Sekiguchi *et al.*, 2005)S3 DHFR 165 *dfrG*

Dihydrofolatereductase *dfrK* Trimethoprim

.(Kadlec and Schwarz, 2009)

rRNAmethylase Erythromycin

50S 23S rRNA Peptidyltransferase

30 Erythromycin ribosome methylation (*erm*) rRNAmethylase

.(Weisblum, 1995) *erm*

:

Listeria API *L.monocytogenes*

L.monocytogenes

/ / *L.monocytogenes* 14

(2013) API *Listeria*

: **DNA** -1

(2004) Moore

%10 SDS -2 (TE Buffer) TE -1

Proteinase k -4 5 NaCl -3

CTAB/NaCl -6 0.7 NaCl -5

20

:PCR -2

(1) :Primers -1

Promega :Green Master Mix -2

Biolabs :DNA Ladder 100 bp -3

:1

	5→3	()
<i>hlyA</i>	F: CATTAGTGGAAAGATGGAATG R: GTATCCTCCAGAGTGATCGA	730
<i>iap</i>	F: GCAACTATCGCGGCTACAGC R: CCAAGTTGCGCTAACAGATT	250
<i>inlA</i>	F: ACTACTAGTAACACGATTAGTGA R: CAAATTTGTTAAAACCCAAGTGG	250
<i>tetS</i>	F: ATTGCAGAACTTGAAAAGGA AATCCCGTCATATTTCTTACC	589
<i>dfrD</i>	F: AGAGTAATCGGCAAGGATAAGG R: AATGGGCAATTTACATCC	199
<i>ermB</i>	F: GAAAAGGTACTCAACCAAATA R: AGTAACGGTATTAATTGTTTAC	636

: DNA -3

.1989 Sambrook

X10 Loading buffer -2 TAE 1X -1

Ethidium bromide (10 mg/ml) -3

.(Bauer *et al.*, 1966) Kirby–Bauer : -1

.15 µgErythromycin Trimethoprim10 µg,Tetracycline30µg

Genomic DNA extraction DNA -2

(2004) Moore

:PCR -3

(Promega)

(2) Thermocycler

..... (PCR)

PCR :2

<i>hlyA</i>	80C° for 10 min, 94 C° for 3 min, 30 cycles of 94 C° for 30s, 55 C° for 30s, 72 C° for 30s, 72 C° for 2 min	(Gouws and Liedemann, 2005)
<i>iap</i>	5 min at 94 C°, 30 cycles of 1 min at 94 C°, 1 min at 72 C°, 5min at 72 C°	(deMello <i>et al.</i> , 2008)
<i>inlA</i>	95 C° for 3 min, 35 cycles of 45s at 95 C°, 52 C° for 45s, 72 C° for 60s, 72 C° for 7 min	(Taban, 2012)
<i>tetS</i>	94 C° for 10 min, 30 cycles of 94 C° for 1min, 55 C° for 1min, 72 C° for 2 min, 72C° for 10 min	(Bertrand <i>et al.</i> , 2005)
<i>dfpD</i>	3 min at 94 C°, 35 cycle of 50s at 94 C°, 50s at 50 C°, 1min 30s at 72 C°	(Charpentier <i>et al.</i> , 1999; Morvan <i>et al.</i> , 2010)
<i>ermB</i>	94 C° for 30s, 50 C° for 30s, 72 C° for 2min, the cycle repeated 35 times	(Morvan <i>et al.</i> , 2010)

: **DNA** -4

Agarose gel

PCR

DNA

electrophoresis

. °55 %1 -1

.Comb Gel tray -2

DNA 10 5 -4

TAE 1X -5

. 90 80 Power Supply -6

U.V -8

DNA-Ladder DNA -9

.(Sambrook and Russel, 2001) . 12

:PCR *Listeria monocytogenes* -1

L. monocytogenes 14 PCR

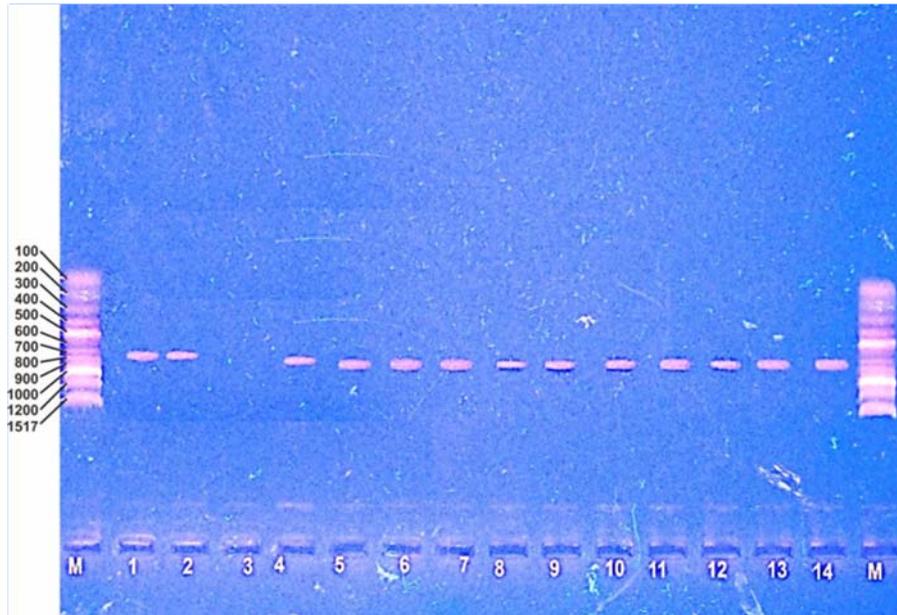
PCR *Listeria*API

L. monocytogenes DNA

hlyA (Fitter *et al.*, 1992)

.(Deneer and Boychuk, 1991) *L. monocytogenes*

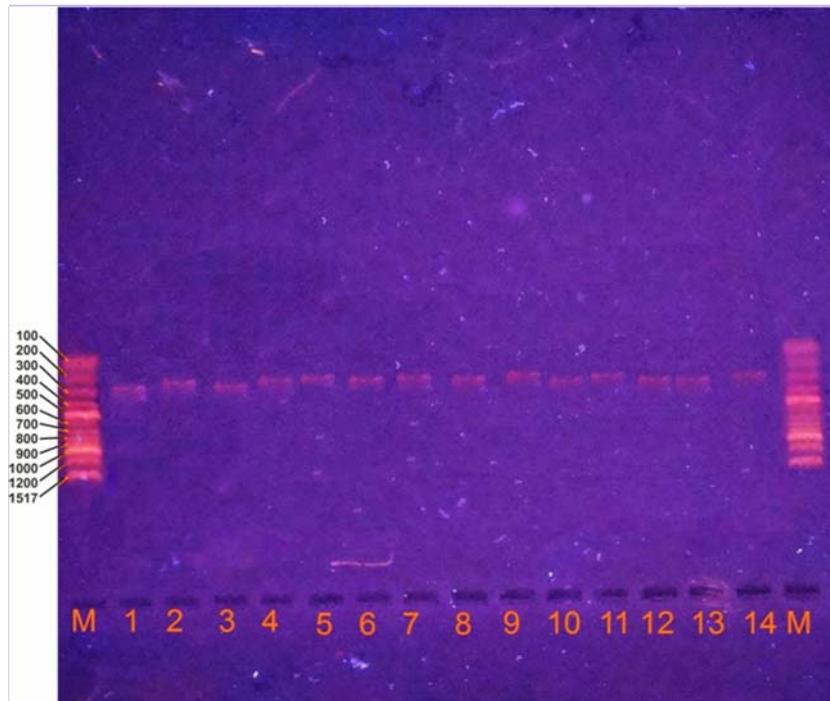
*Listeria*API 14 *hlyA*
hlyA 13 *L. monocytogenes*
 DNA-Laddar
 13 730 (1)
 .(2005) Liedemann Gouws *L.monocytogenes*



hlyA PCR *L. Monocytogenes* DNA :1

L. 3
hlyA monocytogenes
 (1994) Cooray PCR *hlyA*
L. monocytogenes
L.
 (Ritu *et al.*, 2007) *L. Monocytogenes* *iap Monocytogenes*
 DNA- 250
 .(2008) deMello (2) Ladder

..... (PCR)



iap PCR *L. Monocytogenes* DNA :2

iap hlyA L.monocytogenes

L. monocytogenes

Rasmussen *et al.*,) PCR *L.monocytogenes*

inlA L.monocytogenes

.(1995

11

L.monocytogenes

250

(3)

14

250

12 8 7

DNA-Laddar

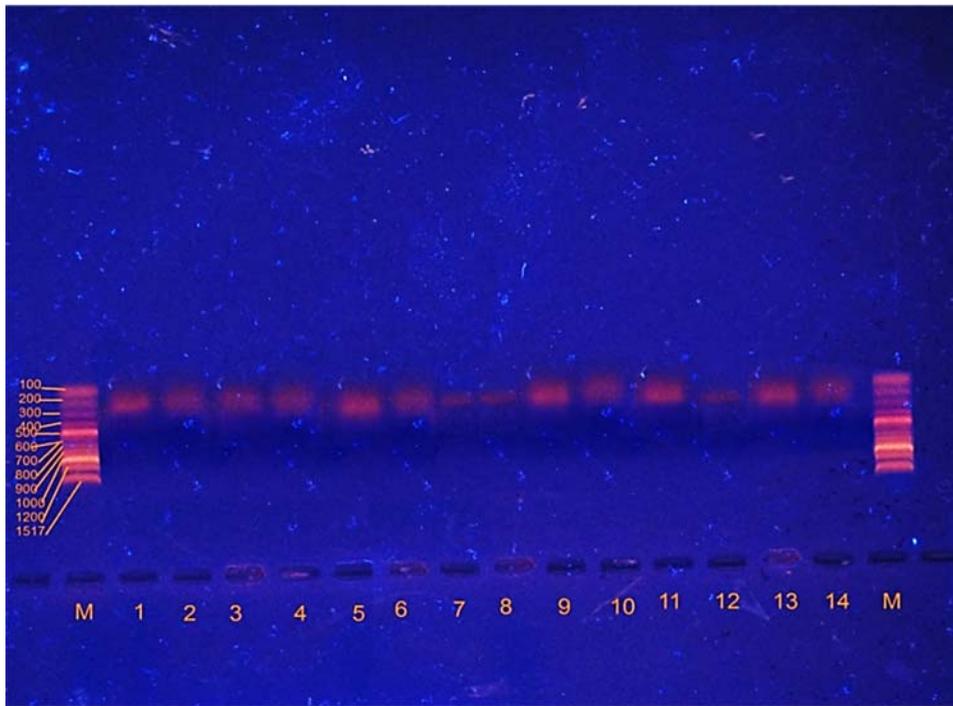
L. monocytogenes

(2004)

Jacquet

Full-length Functional inlA

inlA



inlA PCR *L. Monocytogenes* DNA :3
L. monocytogenes inlA (2000)Almeida Almeida

L.monocytogenes

inlA

inlA

L.monocytogenes

L.monocytogenes

WeakerSignals

L.monocytogenes

-2

Morvan

% 78.6

Tetracycline

L.monocytogenes

L.monocytogenes

(2010)

L.monocytogenes

.tet

L.monocytogenes

%35.7

Trimethoprim

dfrD

1995

Trimethoprim

L.monocytogenes

.(Charpentier and Courvalin, 1997) Dihydrofolatereductase

..... (PCR)

(1990) MacGowan %57.1 Erythromycin
 Courvalin Charpentier Erythromycin *L.monocytogenes*
 Erythromycin *ermA* (1999)
 Conjugative *L.monocytogenes*
 Transposons

:3

14 / <i>L. monocytogenes</i>						
%		%		%		
78.6	11	7.1	1	14.3	2	Tetracycline
35.7	5	7.1	1	57.1	8	Trimethoprim
57.1	8	-	0	42.9	6	Erythromycin

:PCR *L. Monocytogenes*

-3

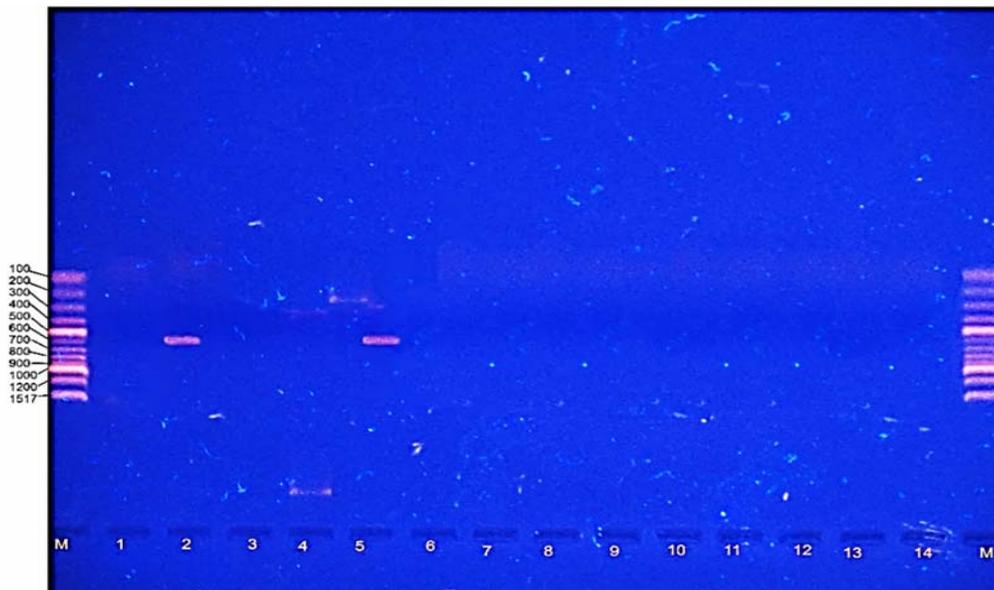
tet(S)

5 2 DNA-Ladder

(4) *tet(S)*

tet(S)

.tetM tetL tetK tet(S)



tet(S) PCR *L.monocytogenes* DNA :4

(2011) Granier

.tet(M)

L.monocytogenes

E.faecalis

tet(S)

(1990)

Poyart-Salmeron

Enterococci

BM4210 *L.monocytogenes*

pIP811

L.monocytogenes L017

E.faecalis JH 2-2

tet

tet(S)

E.faecalis

.(Charpentier *et al.*, 1994)

(*dfrD*) Trimethoprim

13 7 2

PCR

L.monocytogenes

11

Charpentier

199

.Trimethoprim

dfrD

(1997) Courvalin

Charpentier

.(5)

(1999)

Staphylococcus aureus

dfrD

L.monocytogenes BM4293

Trimethoprim

L.monocytogenes BM4293

Trimethoprim

MUR313

.*Staphylococci*



dfrD

PCR

L.monocytogenes

DNA

:5

Trimethoprim

Trimethoprim

dfrD

dfrD

dfrD

Trimethoprim

dfrG

..... (PCR)

L.monocytogenes Tn6198

.(Bertschet *al.*, 2013) inter-genus

intra-species

14 Erythromycin

ermB

10 4

L.monocytogenes

636

(6)

Erythromycin

3

(2010)

Morvan

DNA-Ladder

23s rRNA

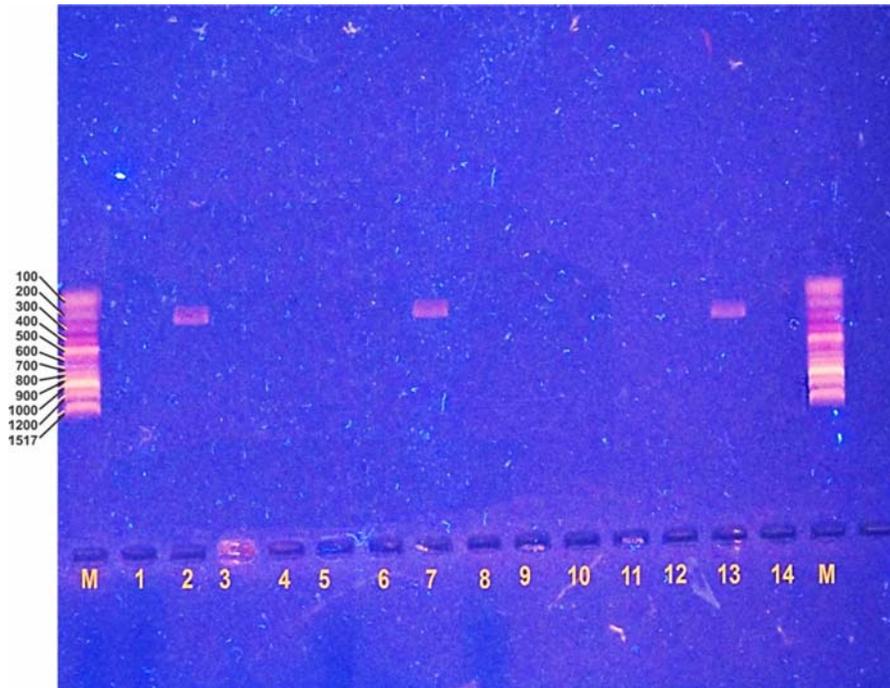
ermB

Erythromycin

L.monocytogenes

.Macrolide-lincosamide-streptograminB (MLS_B)

Methyl transferase



ermB

PCR

L.monocytogenes

DNA

:6

ermB

(1993)

Hadorn

ermB

L.monocytogenes

Doucet-Populaire *et al.*,)

L.monocytogenes

Tn1545

.(1996

L.monocytogenes

PCR

ermB

ermB

Erythromycin

ermB

Listeria (2013)

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