

Table 20: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen raw dressed broiler chickens samples collected from station three.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
13	gb	gsb	G+ve	+	+	Motile
1	ng	gsb	G+ve	+	+	Motile
14	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
6	gb	ng	G+ve	-	+	Motile
5	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 21: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen raw dressed broiler chickens samples collected from station four.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
14	gb	gsb	G+ve	+	+	Motile
2	ng	gsb	G+ve	+	+	Motile
16	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
5	gb	ng	G+ve	-	+	Motile
4	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 22: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen raw dressed broiler chickens samples collected from station five.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
13	gb	gsb	G+ve	+	+	Motile
1	ng	gsb	G+ve	+	+	Motile
15	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
4	gb	ng	G+ve	-	+	Motile
3	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

4.2.3 Results of classical biochemical tests and growth on selective media of *Listeria* spp.isolated from broiler chicken ready to eat products.

Morphological studies . Table (23-27).

Growth on selective media . Table (23-27).

Catalase and Oxidase reactions . Table (23-27).

Table 23: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen chicken-burger.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
10	gb	gsb	G+ve	-	+	Motile
11	gbh	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 24: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen chicken-sausage.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
6	gb	gsb	G+ve	-	+	Motile
4	gbh	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 25: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen chicken-balls (Kofta).

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
15	gb	gsb	G+ve	-	+	Motile
10	gbh	gsb	G+ve	-	+	Motile
2	ng	gsb	G+ve	-	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 26: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from RTE chicken-shawerma.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
6	gb	gsb	G+ve	-	+	Motile
2	gbh	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 27: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen chicken-mortadella.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
16	ng	gsb	G+ve	-	+	Motile
2	gbh	gsb	G+ve	-	+	Motile
7	gb	gsb	G+ve	-	+	Motile
1	gb	ng	G+ve	-	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

4.2.4 Results of classical biochemical tests and growth on selective media of *Listeria* spp. isolated from frozen and shock frozen dressed broiler chicken.

Morphological studies . Table (28-30).

Growth on selective media .Table (28-30).

Catalase and Oxidase reactions. Table (28-30).

Table 28: Results of classical biochemical tests and growth on selective media of *Listeria* isolated from fresh dressed broiler chickens.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
15	gb	gsb	G+ve	+	+	Motile
4	Ng	gsb	G+ve	+	+	Motile
12	Gb	gsb	G+ve	-	+	Motile
3	Ng	gsb	G+ve	-	+	Motile
8	Gb	ng	G+ve	-	+	Motile
5	Gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 29: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from frozen dressed broiler chickens.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
13	gb	gsb	G+ve	+	+	Motile
4	Ng	gsb	G+ve	+	+	Motile
11	Gb	gsb	G+ve	-	+	Motile
3	ng	gsb	G+ve	-	+	Motile
7	gb	ng	G+ve	-	+	Motile
5	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 30: Results of classical biochemical tests and growth on selective media of *Listeria* isolates from shock frozen dressed broiler chickens.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
5	gb	gsb	G+ve	+	+	Motile
1	ng	gsb	G+ve	+	+	Motile
7	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
5	gb	ng	G+ve	-	+	Motile
2	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

4.2.5 Results of classical biochemical tests and growth on selective media of the isolates from frozen dressed broiler chickens artificially infected with *Listeria monocytogenes*.

Morphological studies. Table (31&32).

Growth on selective media. Table (31&32).

Catalase and Oxidase reactions. Table (31&32).

Table 31: Results of classical biochemical tests and growth on selective media covered organisms from frozen dressed broiler meat artificially infected.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
120	gbh	gsb	G+ve	-	+	Motile

gbh :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 32: Results of classical biochemical tests and growth on selective media isolates from shock frozen dressed broiler meat artificially infected.

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
120	gbh	gsb	G+ve	-	+	Motile

gbh :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening. **ALOA**: Agar *Listeria* according to Ottaviani and Agosti. **OXA**: Oxford Agar

4.2.6 Results of classical biochemical tests and growth on selective media *Listeria* pp. Isolates from Breeding and Slaughter house (abattoir).

Morphological studies. Table (33&34).

Growth on selective media . Table (33&34).

Catalase and Oxidase reactions. Table (33&34).

Table 33: Results of classical biochemical tests and growth on selective media of *Listeria* spp. isolated from Breeding and Slaughter house (abattoir).

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
7	gb	gsb	G+ve	+	+	Motile
2	ng	gsb	G+ve	+	+	Motile
6	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
3	gb	ng	G+ve	-	+	Motile
2	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

Table 34: Results of classical biochemical tests and growth on selective media of *Listeria* spp. isolated from Slaughter house (abattoir).

Number of isolates	Growth on ALOA Agar	Growth on OXA Agar	Gram Reaction	Oxidase Test	Catalase Test	Motility Test At 25° C
<i>L.monocytogenes</i> ATCC(7644)	gbh	gsb	G+ve	-	+	Motile
9	gb	gsb	G+ve	+	+	Motile
2	ng	gsb	G+ve	+	+	Motile
5	gb	gsb	G+ve	-	+	Motile
1	ng	gsb	G+ve	-	+	Motile
2	gb	ng	G+ve	-	+	Motile
1	gb	ng	G+ve	+	+	Motile

gb :green-blue colony; **gbh** :green-blue colony with halo zone; **gsb** :gray colony, sunken center with blackening; **ng**: no growth. **ALOA**: Agar Listeria according to Ottaviani and Agosti. **OXA**: Oxford Agar.

4. 3. Identification of *L. monocytogenes*

4. 3.1 Rapid biochemical test (Microbact™ Listeria 12L Kit)

4. 3.1.1 Rapid biochemical test (Microbact™ Listeria 12L Kit) used for the identification of *listeria* spp. Isolated from fresh raw dressed broiler chicken.

The tested reference strain *L. monocytogenes* (ATCC 7644) hydrolyzed esculin, utilized arabitol, rhamnase, trehalose, methyl-d-glucose and methyl-d-mannose, and reacted positively with RBCs haemolysis.

Suspected *Listeria* spp. isolates recovered from raw broiler chickens showed variable biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 204 suspected *Listeria* spp. isolates recovered from fresh raw broiler chickens, 68 isolates were confirmed as *L. monocytogenes*. The other isolates were distributed between *L. ivanovii* (99 isolates), *L. grayi* (23 isolates), *L. seeligeri* (5 isolates) and *L. welshimeri* (10 isolate). Table (35-39).

Table 5: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for idintifaction of *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens (station one).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnase	Trehalose	Tagatose	Phos	GmC-I-	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+	
19	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+	
26	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+	
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+	
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+	
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+	

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 36: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens (station two).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
10	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
14	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
4	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
1	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 37: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens (station three).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
14	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
22	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
4	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
3	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 38: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens (station four).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
13	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
23	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 39: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens (station five).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
12	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
14	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
8	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

4. 3.1.2 Rapid biochemical test (Microbact™ Listeria 12L Kit) used for identification of *Listeria* spp. Isolated from frozen raw dressed broiler chicken.

Suspected *Listeria* spp. isolates recovered from frozen raw broiler chickens showed variable biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 195 suspected *Listeria* spp. isolates recovered from frozen raw broiler chickens, 64 isolates were confirmed as *L. monocytogenes*. The other isolates were distributed between *L. ivanovii* (97 isolates), *L. grayi* (20 isolates), *L. seeligeri* (5 isolates) and *L. welshimeri* (9 isolate). Table (41-45).

Table 40: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen raw dressed broiler chickens (station one).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
19	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
26	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 1: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen raw dressed broiler chickens (station two).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
8	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
12	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
1	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 2: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen raw dressed broiler chickens (station three).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
12	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
22	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 43: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on *Listeria* spp. isolates recovered from frozen raw dressed broiler chickens (station four).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
13	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
23	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
3	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 44: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on *Listeria* spp. isolates recovered from frozen raw dressed broiler chickens (station five).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
12	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
14	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
8	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

4.3.1.3 Rapid biochemical test (Microbact™ Listeria 12L Kit) for the *listeria* spp. Isolated from dressed broiler chicken ready to eat (RTE) products.

Suspected *Listeria* spp. isolates recovered from raw broiler chickens and RTE chicken products showed variable biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 95 suspected *Listeria* spp. isolates recovered from raw broiler chickens, 34 isolates were confirmed as *L. monocytogenes*. The other isolates were distributed between *L. ivanovii* (52 isolates), *L. grayi* (4 isolates), *L. seeligeri* (2 isolates) and *L. welshimeri* (3 isolate). Table (45-49).

Table 45: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen chicken-burger.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
8	<i>L. monocytogenes</i>	99.9	+	±	-	+	±	±	+	±	-	+	+	+
14	<i>L. ivanovii</i>	96.3	+	-	-	-	-	-	-	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 46: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen chicken-sausage.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
6	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	±	+	±	-	+	+	+
4	<i>L. ivanovii</i>	99.3	+	-	±	-	±	-	±	-	-	-	-	+
1	<i>L. welshimeri</i>	98.7	+	-	+	+	-	+	+	+	-	+	+	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 47: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen meat chicken-balls (Kofta).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
9	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	±	+	±	-	+	+	+
15	<i>L. ivanovii</i>	99.3	+	-	±	-	±	-	±	-	-	-	-	+
2	<i>L. grayi</i>	59.1	+	+	±	±	+	-	+	±	-	±	±	+
1	<i>L. seeligeri</i>	52.2	+	-	+	-	-	-	-	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 48: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from RTE chicken-shawerma.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
2	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	±	+	±	-	+	+	+
6	<i>L. ivanovii</i>	99.3	+	-	±	-	±	-	±	-	-	-	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 49: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen meat chicken-mortadella.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamonse	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
9	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
13	<i>L. ivanovii</i>	96.3	±	±	-	-	-	-	±	-	-	-	-	+
2	<i>L. grayi</i>	59.1	+	+	±	±	+	-	+	±	-	±	±	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
1	<i>L. seeligeri</i>	52.2	+	-	+	-	-	-	-	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

4.3.1.4 Rapid biochemical test (Microbact™ Listeria 12L Kit) used for identification of *Listeria* spp. Isolated from frozen dressed broiler chicken and shocked frozen dressed broiler chicken.

Suspected *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens, frozen broiler chickens & shocked frozen broiler chickens showed variable biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 111 suspected *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens, frozen broiler chickens & shock f frozen broiler chickens, 39 isolates were confirmed as *L. monocytogenes*. The other isolates were distributed between *L. ivanovii* (54 isolates), *L. grayi* (11 isolates), *L. seeligeri* (3 isolates) and *L. welshimeri* (4 isolate). Table (50-52).

Table 00: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from fresh dressed broiler chickens .

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
17	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
23	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
5	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 1: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from frozen dressed broiler chickens .

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
15	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
21	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
4	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
2	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 2: Results of rapid biochemical test using Microbact™ Listeria 12L Kit used for identification of *Listeria* spp. isolates recovered from shock frozen dressed broiler chickens.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
7	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
10	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
2	<i>L. grayi</i>	92.8	+	±	±	±	+	±	+	±	-	±	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
1	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

4.3.1.° Rapid biochemical test (Microbact™ Listeria 12L Kit) used for identification of the *listeria* spp. Isolated from frozen dressed broiler chickens and frozen dressed broiler chickens artificially infected with *L. monocytogenes*.

Listeria spp. isolates recovered from frozen dressed broiler chickens & frozen dressed broiler chickens contaminated in vitro showed same biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 240 *Listeria* spp. isolates recovered from frozen dressed broiler chickens & frozen dressed broiler chickens artificially infected with *L. monocytogenes*, 240 isolates were confirmed as *L. monocytogenes*. Table (53-54).

Table °3: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on frozen dressed broiler chickens artificially infected with *L. monocytogenes*.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
120	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+

(-): negative; (+): positive

Table °4: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on shocked frozen dressed broiler chickens artificially infected with *L. monocytogenes*.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
120	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+

(-): negative; (+): positive

4.3.1.6 Rapid biochemical test (Microbact™ Listeria 12L Kit) for the *listeria* spp. Isolated from broiler chicken Breeding and Slaughter house (abattoir).

Suspected *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens, frozen broiler chickens & shock f frozen broiler chickens showed variable biochemical reactions with the rapid biochemical test (Microbact™ Listeria 12L Kit). Out of 42 suspected *Listeria* spp. isolates recovered from fresh raw dressed broiler chickens, frozen broiler chickens & shock f frozen broiler chickens, 12 isolates were confirmed as *L. monocytogenes*. The other isolates were distributed between *L. ivanovii* (23 isolates), *L. seeligeri* (5 isolates) and *L. welshimeri* (11 isolate). Table (55&56).

Table 55: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on suspected *Listeria* spp. isolates recovered from Breeding house.

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
6	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
11	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
4	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

Table 56: Results of rapid biochemical test using Microbact™ Listeria 12L Kit on suspected *Listeria* spp. isolates recovered from Slaughter house (abattoir).

No. of isolates	Preferred Identification choice	Percent Probability	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	haemolysis
ATCC (7644)	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	+	-	-	+	+	+
6	<i>L. monocytogenes</i>	99.9	+	-	-	+	-	+	-	-	-	+	+	+
12	<i>L. ivanovii</i>	99.8	±	-	±	-	±	±	±	-	-	-	-	+
1	<i>L. welshimeri</i>	85.7	+	-	+	-	-	+	+	+	-	-	-	+
1	<i>L. seeligeri</i>	52.2	+	±	+	-	-	-	±	-	-	-	-	+

± : some of the isolates gave positive reaction while other isolates of the same species gave negative reaction.

4.3.2 CAMP (Christie, Atkins, Munch-Petersen) test.

Out of 205 isolates which were CAMP test positive, 68 (33.17%) isolates were from fresh dressed broiler chicken , 64 (31.21%) isolates from frozen dressed broiler chickens, 34 (16.58%) isolates from chicken products ready to eat and 39 (19.02%) isolates from frozen and shocked frozen dressed broiler chickens . The results are shown in table (57).

Table 57: Results of the isolated *listeria* spp. reaction with CAMP test.

No. of isolates	Sample type	CAMP Test	
		<i>S.aureus</i>	<i>R.equi</i>
<i>L. monocytogenes</i> ATCC (7644)	American Type culture Collection	+	-
<i>L.innocua</i> ATCC (33090)	American Type culture Collection	-	-
<i>L.ivanovii</i> ATCC (19119)	American Type culture Collection	-	+
68	fresh broiler chickens	+	-
99	fresh broiler chickens	-	+
64	frozen dressed broiler chickens	+	-
97	frozen dressed broiler chickens	-	+
34	chicken products ready to eat	+	-
52	chicken products ready to eat	-	+
39	frozen & shock frozen dressed broiler chickens	+	-
54	frozen & shock frozen dressed broiler chickens	-	+
240	Artificially infected broiler meat	+	-
12	Breeding house and Slaughter house	+	-
23	Breeding house and Slaughter house	-	+

+ : > 90% of positive reaction

- : no reaction

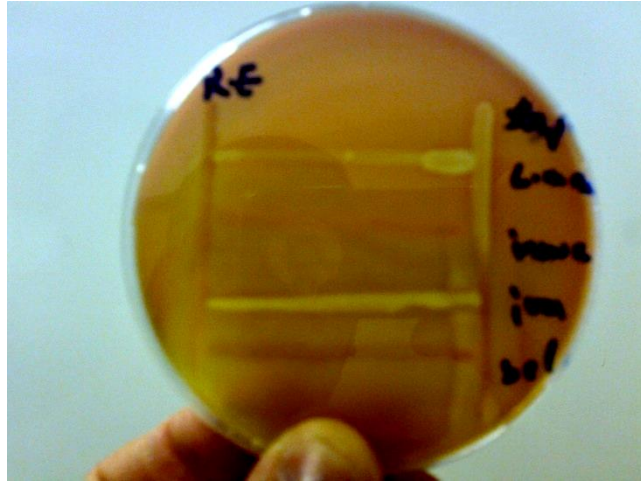


Figure 4: Inoculation of CAMP test plates.

4. 4 Presence of *Listeria* spp. using conventional bacteriological method.

4.4.1 Presence of *Listeria* spp. using conventional bacteriological method for fresh raw dressed broiler chicken.

Among 500 samples in five station chicken abattoir in Khartoum State the isolated listeria ssp. were distributed as follows:- *L. monocytogenes*(13.6%).*Listeria ivanovi* (19.8%).*Listeria grayi* (4.6%).*Listeria seeligeri* (1%), *Listeria welshimeri* (2%) (Table 58) and (Fig 5).

Figurer (6) shows the percentage of each types of listeria from 204 isolated *listeria* spp.:- *L. monocytogenes*(33.33%).*Listeria ivanovi* (48.52.8%).*Listeria grayi* (11.27%).*Listeria seeligeri* (2.45%), *Listeria welshimeri* (4.90%)

Table 58: Presence of *Listeria* spp. in fresh raw dressed broiler in Khartoum- Sudan using conventional methods.

Food Items	No. of samples	<i>Listeria</i> spp. No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Fresh broiler chicken (1)	100	51 (51)	19 (19)	26 (26)	3 (3)	1 (1)	2 (2)
Fresh broiler chicken (2)	100	30 (30)	10 (10)	14 (14)	4 (4)	1 (1)	1 (1)
Fresh broiler chicken (3)	100	44 (44)	14 (14)	22 (22)	4 (4)	1 (1)	3 (3)
Fresh broiler chicken (4)	100	42 (42)	13 (13)	23 (23)	3 (3)	1 (1)	2 (2)
Fresh broiler chicken (5)	100	37 (37)	12 (12)	14 (14)	8 (8)	1 (1)	2 (2)
Total	500	204 (40.8)	68 (13.6)	99 (19.8)	23 (4.6)	5 (1)	10 (2)

(1): Station one, (2): station two, (3): station three, (4): station four, (5): station five.

Figure 5: The isolation percentage of *Listeria* spp. from 500 samples of fresh raw dressed broiler.

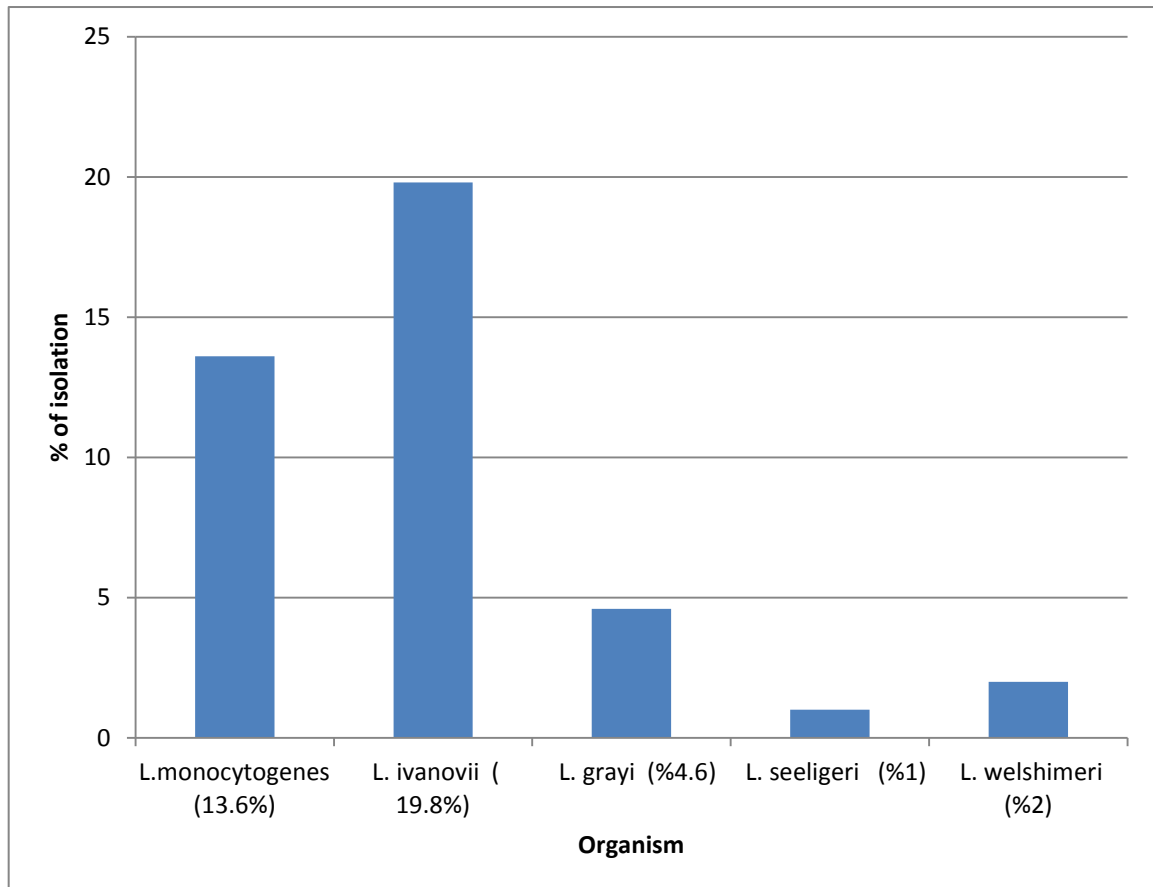
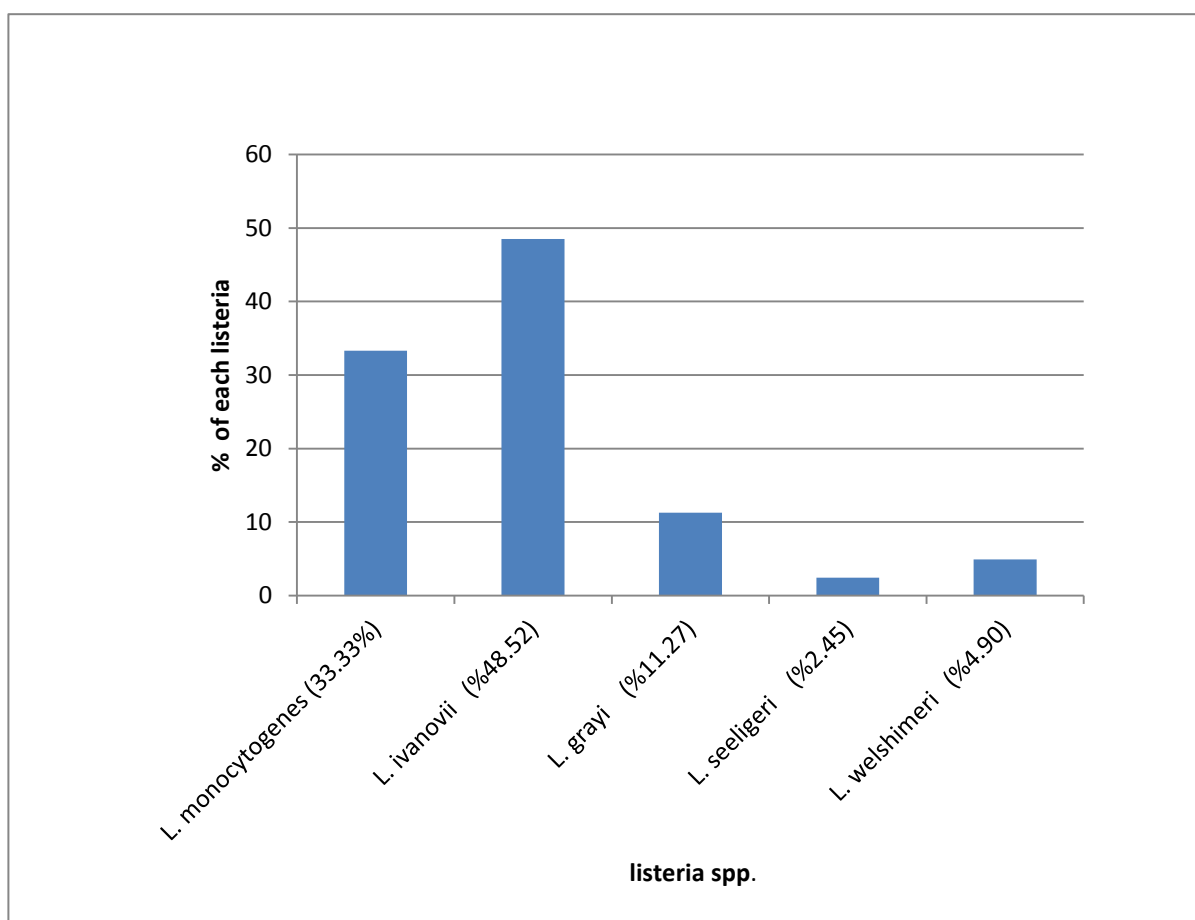


Figure 6: The isolation percentage of different types of *Listeria* spp. from 204 isolates of *Listeria* spp. from fresh raw dressed broiler.



4.4.2 Presence of *Listeria* spp. using conventional bacteriological method in frozen dressed broiler chicken.

Among 500 samples in five station chicken abattoir in Khartoum State the isolated *listeria* spp. were distributed as follows:- *L. monocytogenes*(12.8%).*Listeria ivanovi* (19.4%).*Listeria grayi* (4%).*Listeria seeligeri* (1%), *Listeria welshimeri* (1.8%) (Table 59) and (Fig 7).

Figurer (8) shows the percentage of each types of listeria from 195 isolated *listeria* spp.:- *L. monocytogenes*(32.82%).*Listeria ivanovi* (49.74%).*Listeria grayi* (10.25%).*Listeria seeligeri* (2.56%), *Listeria welshimeri* (4.61%)

Table 59: Presence of *Listeria* spp. in frozen raw dressed broiler chickens in Khartoum- Sudan using conventional methods.

Food Items	No. of samples	<i>Listeria</i> spp. No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Frozen broiler chicken (1)	100	51 (51)	19 (19)	26 (26)	3 (3)	1 (1)	2 (2)
Frozen broiler chicken (2)	100	25 (25)	8 (8)	12 (12)	3 (3)	1 (1)	1 (1)
Frozen broiler chicken (3)	100	40 (40)	12 (12)	22 (22)	3 (3)	1 (1)	2 (2)
Frozen broiler chicken (4)	100	42 (42)	13 (13)	23 (24)	3 (3)	1 (1)	2 (2)
Frozen broiler chicken (5)	100	37 (37)	12 (12)	14 (14)	8 (8)	1 (1)	2 (2)
Total	500	195 (39)	64 (12.8)	97(19.4)	20 (4)	5 (1)	9 (1.8)

(1): Station one, (2): station two, (3): station three, (4): station four, (5): station five.

Figure 7: The isolation percentage of *Listeria* spp. from 500 samples of in frozen raw dressed broiler.

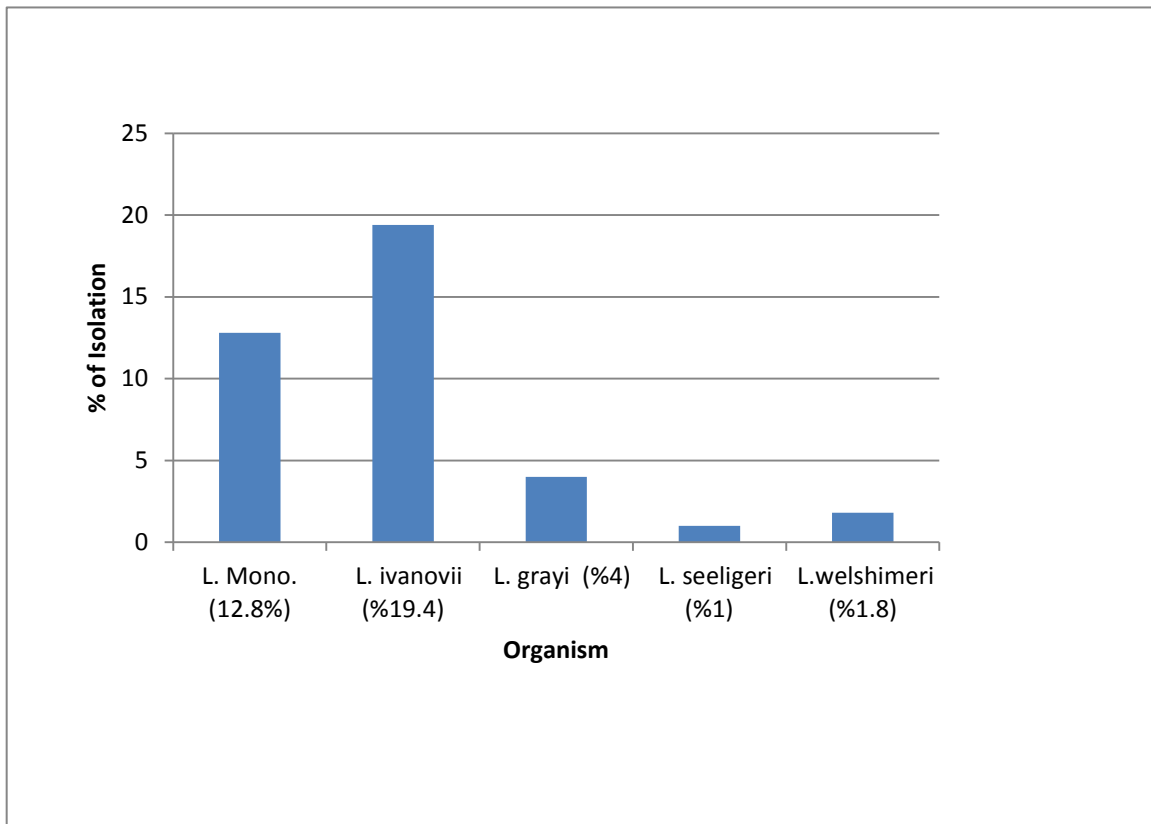
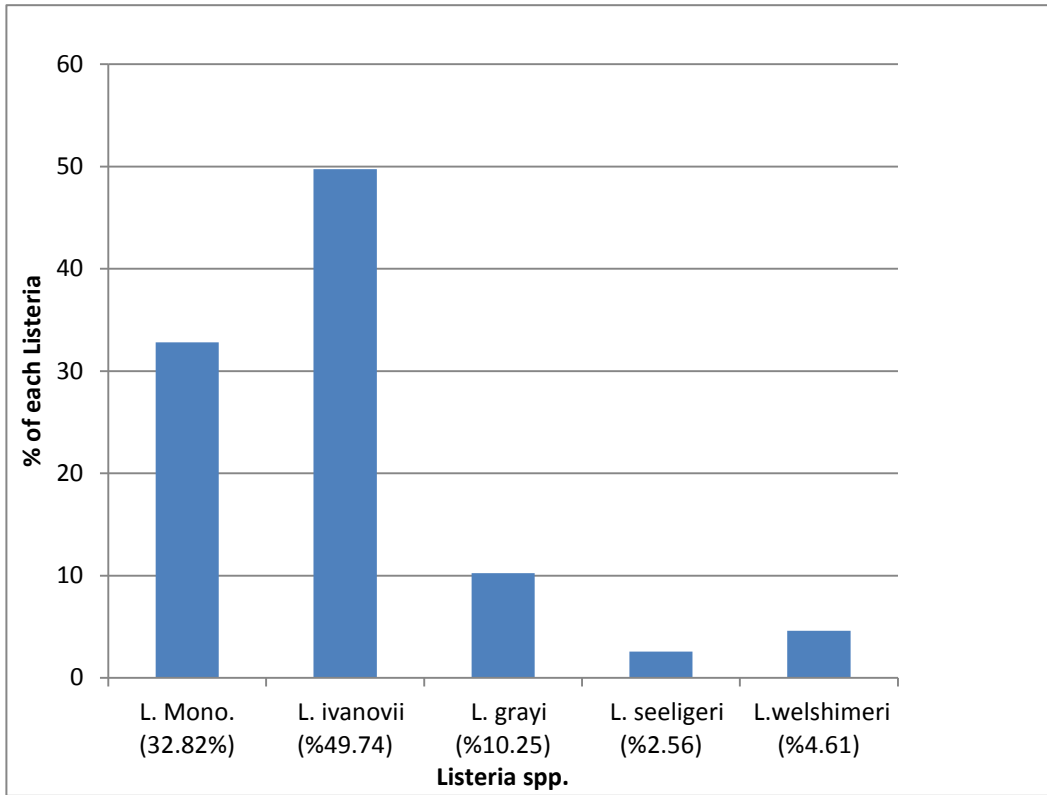


Figure 8: The isolation percentage of different types of *Listeria* spp. from 195 isolates of *Listeria* spp. from in frozen raw dressed broiler.



4.4.3 Presence of *Listeria* spp. using conventional bacteriological method in dressed broiler chicken ready to eat products.

Among 250 samples collected from restaurants in Khartoum State as *Listeria* spp. isolated were distributed as follows: *L. monocytogenes* (13.6%), *Listeria ivanovi* (20.8%), *Listeria grayi* (1.6%), *Listeria seeligeri* (0.8%), *Listeria welshimeri* (1.2%) (Table 60) and (Fig 9).

Figure (10) shows the percentage of each type of *Listeria* identified from 95 isolated *Listeria* spp.:- *L. monocytogenes* (35.78%), *Listeria ivanovi* (54.73%), *Listeria grayi* (4.21%), *Listeria seeligeri* (2.10%), *Listeria welshimeri* (3.15%)

Table 60: Presence of *Listeria* spp. in retail broiler chicken ready to eat meat products in Khartoum- Sudan using conventional methods.

Type of food	No. of samples	<i>Listeria</i> spp. No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Frozen chicken burgar	50	22 (44)	8 (16)	14 (28)	0	0	0
frozen chicken sausages	50	11 (22)	6 (12)	4 (8)	0	0	1 (2)
frozen chicken meat balls (kofta)	50	27 (54)	9 (18)	15 (30)	2 (4)	1 (2)	0
Chicken shawerma	50	9 (18)	2 (4)	6 (12)	0	0	1 (2)
chicken mortedella	50	26 (52)	9 (18)	13 (26)	2 (4)	1 (1)	1 (2)
Total	250	95(38)	34 (13.6)	52 (20.8)	4(1.6)	2 (0.8 %)	3 (1.2)

Figure 9: The isolation percentage of *Listeria* spp. from 250 samples of retail broiler chicken ready to eat (RET) meat products.

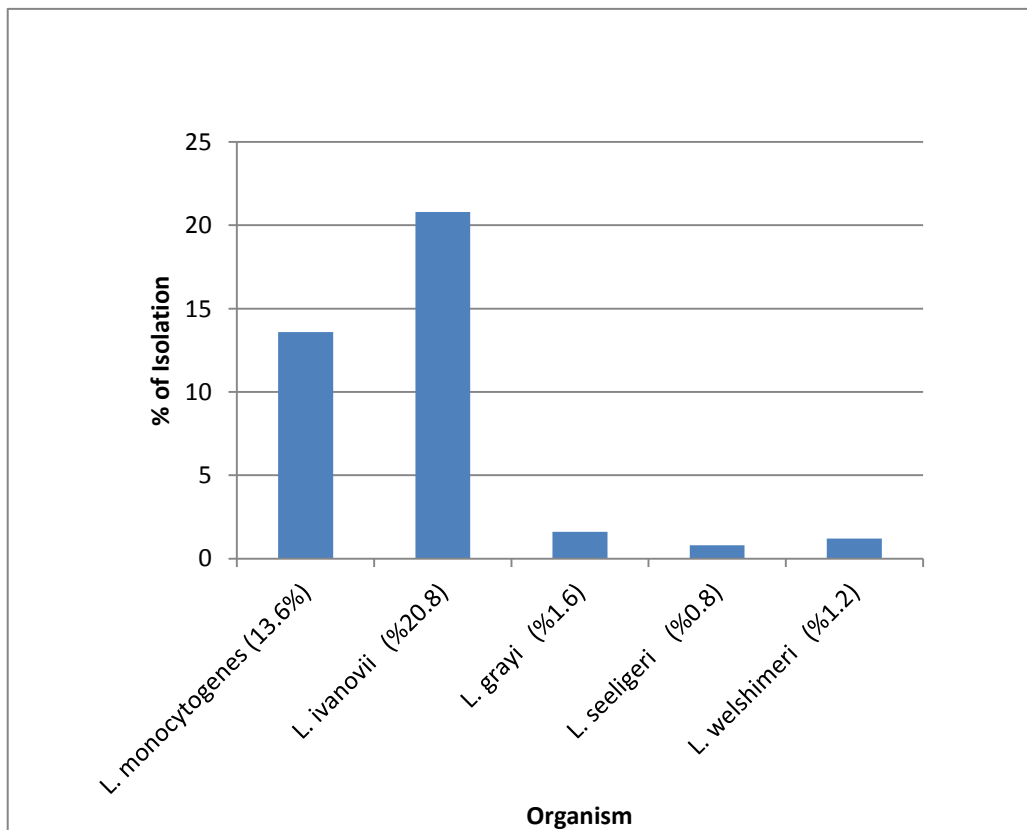
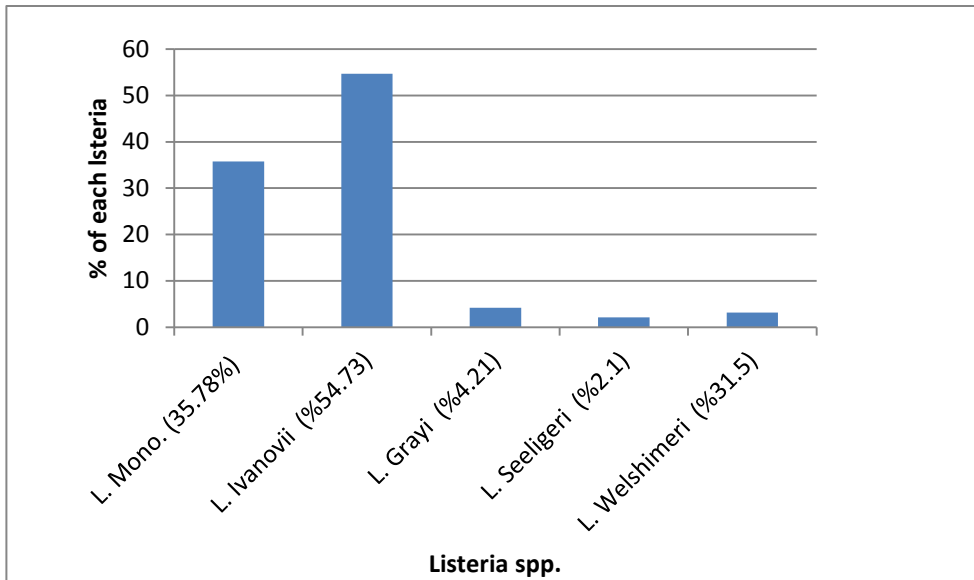


Figure 10: The isolation percentage of different types of *Listeria* spp. from 95 isolates of *Listeria* spp. from retail broiler chicken ready to eat meat products.



4.4.4 Presence of *Listeria* spp. using conventional bacteriological method for of frozen and shocked frozen dressed broiler chicken.

Among 300 samples collected from one station chicken abattoir in Khartoum State, the listeria spp. Isolated were distributed as follows *L. monocytogenes* (13 %).*listeria ivanovi* (18%).*listeria grayi* (3.6%).*listeria seeligeri* (1%), *listeria welshimeri* (1.3%) (Table 61) and (Fig 11).

Figurer (12) shows the percentage of each types of listeria from 111 isolated *listeria* spp.:- *L. monocytogenes*(35.13%).*Listeria ivanovi* (48.64%).*Listeria grayi* (9.90%).*Listeria seeligeri* (2.70%) and *Listeria welshimeri* (3.60%)

Table 61: Presence of *Listeria* spp. in fresh dressed broiler chickens, frozen dressed broiler chickens and shocked frozen dressed broiler chickens in Khartoum- Sudan using conventional methods.

Food Items	No. of samples	<i>Listeria</i> spp. No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Fresh broiler chickens	100	47 (47)	17 (17)	23 (23)	5 (5)	1 (1)	2 (2)
Frozen broiler chickens	100	43 (43)	15 (15)	21 (21)	4 (4)	1 (1)	2 (2)
Shock frozen broiler chickens	100	21 (21)	7 (7)	10 (10)	2 (2)	1 (1)	1 (1)
Total	300	111 (37)	39 (13)	54 (18)	11 (3.6)	3 (1)	4 (1.3)

Figure 11: The isolation percentage of *Listeria* spp. from 300 sampled, fresh dressed broiler chickens, frozen dressed broiler chickens and shocked frozen dressed broiler chickens.

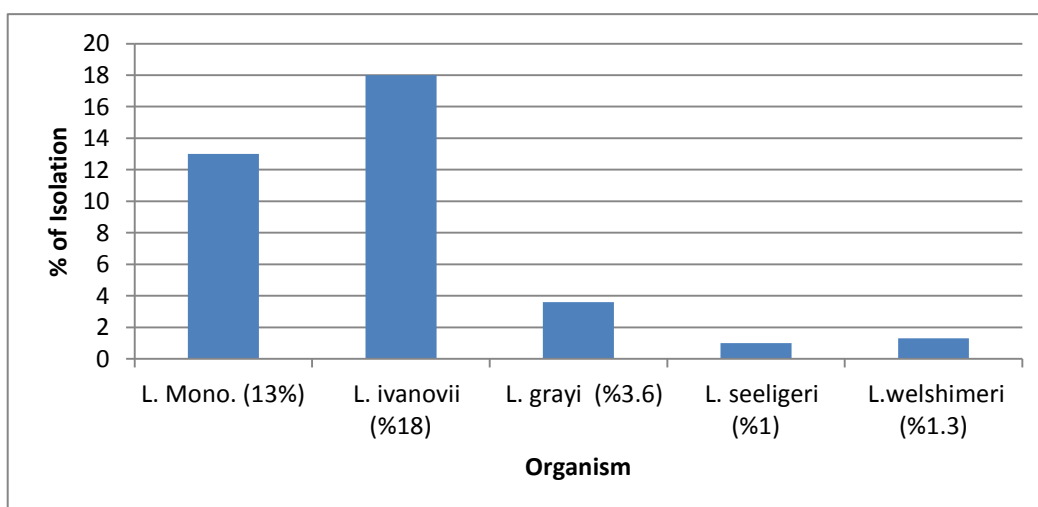
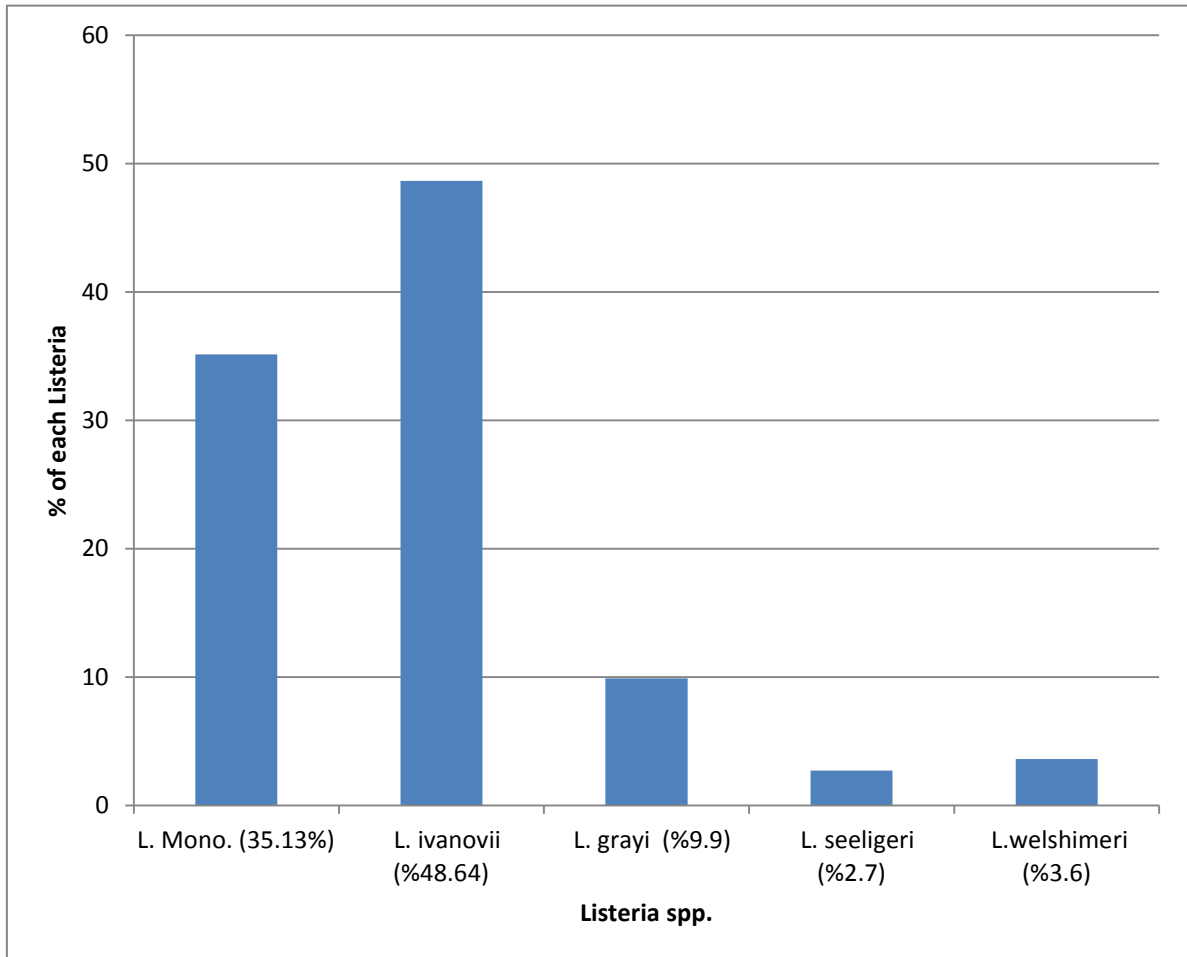


Figure 12: The isolation percentage of different types of *Listeria* spp. from 111 isolates of *Listeria* spp. From fresh dressed broiler chickens, frozen dressed broiler chickens and shocked frozen dressed broiler chickens.



4.4.5 Presence of *Listeria monocytogenes* in frozen dressed broiler chickens and frozen dressed broiler meat artificial infected using conventional bacteriological method.

Among 240 samples from frozen dressed broiler chickens and shocked frozen dressed broiler meat artificially infected. All isolated is (100%) *L. monocytogenes*. (Table 62)

Table 62: Presence of *Listeria monocytogenes* in frozen dressed broiler chickens & shock frozen dressed broiler meat artificial infected.conventional methods.

Food Items	No. of samples	<i>L. monocytogenes</i> No. (%)
Frozen broiler chickens	120	120 (100)
Shock frozen broiler chickens	120	120 (100)
Total	240	240 (100)

4.4.6 Presence of *Listeria* spp. using conventional bacteriological method in breeding house broiler chicken.

Among 100 samples collected from breeding house and Slaughter house (abattoir) chicken abattoir in Khartoum State the (41%) isolated listeria spp. were distributed as follows:- *L. monocytogenes* (12%) .*Listeria ivanovi* (23%). *Listeria seeligeri* (5 %), *Listeria seeligeri* (5%), *Listeria welshimeri* (11%) (Table 65) and (Fig 13).

Figurer (14) shows the percentage of each types of listeria from 41 isolated listeria spp.:- *L. monocytogenes* (29.26%).*Listeria ivanovi* (56.09%). *Listeria seeligeri* (12.19%), *Listeria welshimeri* (26.82%)

Table 63: Presence of *Listeria* spp. in breeding chickens in Khartoum- Sudan using conventional methods.

Breeding house	No. of samples	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Floor	5	0	2 (40)	0	0	0
Roof	5	0	2 (40)	0	0	0
Wall	5	0	2 (40)	0	2 (40)	0
Food line	10	0	5 (50)	0	2 (20)	0
Water line	10	4 (40)	0	0	0	0
Hand of workers	5	2 (40)	0	0	0	0
Total	40	6 (15)	11 (27.5)	0	4 (10)	0

Table 64: Presence of *Listeria* spp. in Slaughter house (abattoir). in Khartoum-Sudan using conventional methods.

Breeding house	No. of samples	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Chicken transport cage	10	0	4 (40)	0	1 (10)	0
Hand of workers	5	3 (60)	0	0	0	0
Slaughter knife	10	0	2 (20)	0	0	1 (10)
Hand of Slaughter worker	5	1 (20)	0	0	0	0
feather	5	0	2 (40)	0	0	0
Chicken head cutting worker hand	5	1 (20)	1 (20)	0	0	0
Neck cutting knife	10	0	2 (20)	0	0	0
Hand of worker cutting neck	10	1 (10)	1 (10)	0	0	0
Total	60	6 (10)	12 (20)	0	1 (1.66)	1 (1.66)

Table 65: Presence of *Listeria* spp. in breeding house and Slaughter house (abattoir) broiler in Khartoum- Sudan using conventional methods.

Food Items	No. of samples	<i>Listeria spp.</i> No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Breeding house	40	21 (52.5)	6 (15)	11 (27.5)	4 (10)	0
Slaughter house (abattoir)	60	20 (33.3)	6 (10)	12 (20)	1 (1.66)	1 (1.66)
Total	100	41 (41)	12 (12)	23 (23)	5 (5)	11 (11)

Figure 13: The isolation percentage of *Listeria* spp. from 100 samples of breeding house and Slaughter house (abattoir).

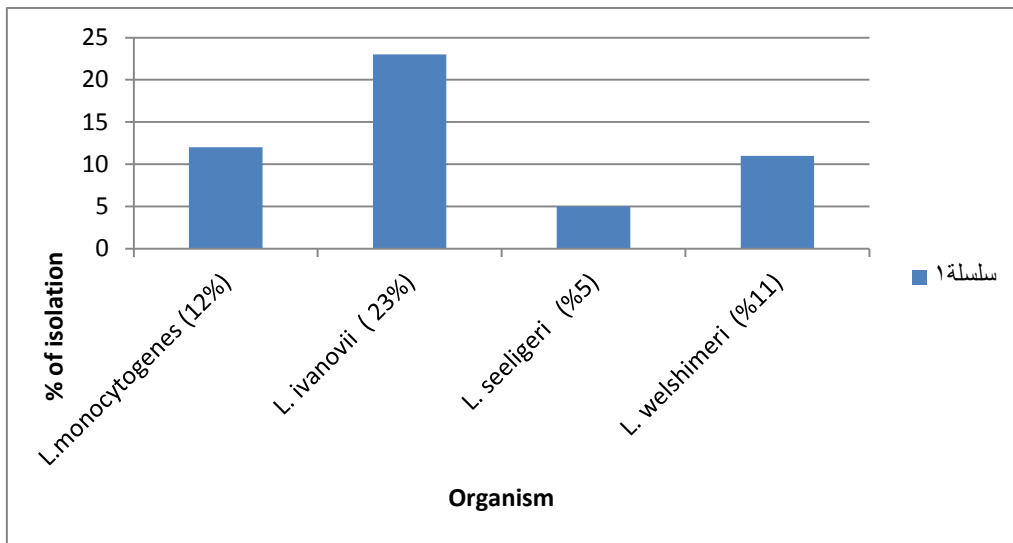


Figure 14: The isolation percentage of different types of *Listeria* spp. from 41 isolates of *Listeria* spp. from breeding house and Slaughter house (abattoir).

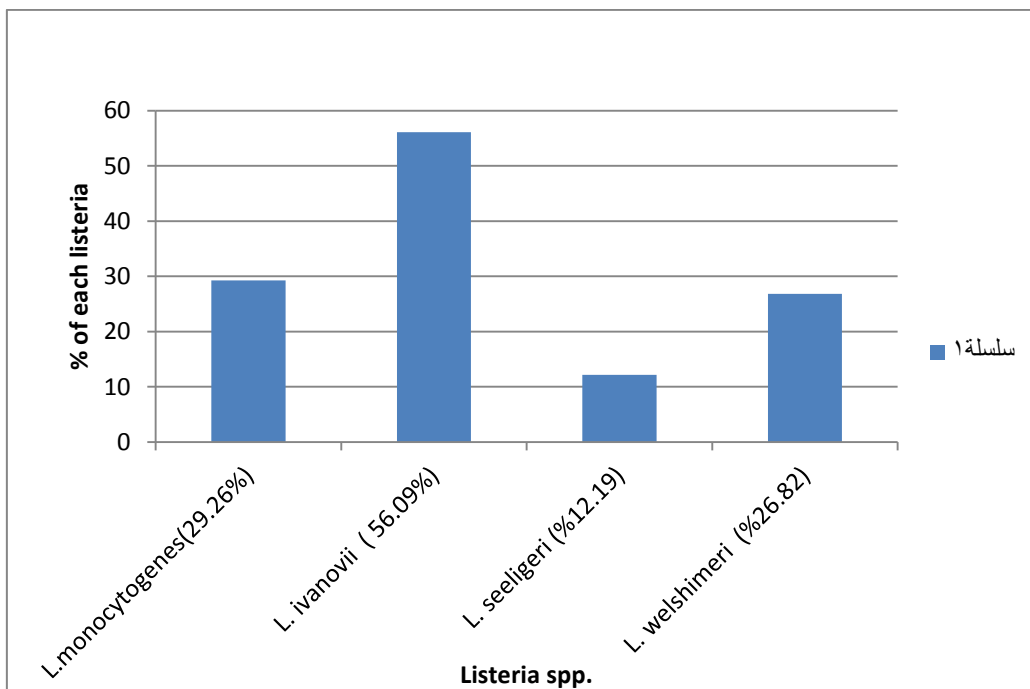


Table 66: Summary of Presence of *Listeria* spp. From fresh, frozen, ready to eat (RED), shocked frozen broiler and breeding-slaughter house. using conventional bacteriological method.

Food Items	No. of samples	<i>Listeria</i> spp. No. (%)	<i>L. monocytogenes</i> No. (%)	<i>L. ivanovii</i> No. (%)	<i>L. grayi</i> No. (%)	<i>L. seeligeri</i> No. (%)	<i>L. welshimeri</i> No. (%)
Fresh broiler chicken	500	204 (40.8)	68 (13.6)	99 (19.8)	23 (4.6)	5 (1)	10 (2)
Frozen broiler chicken	500	195 (39)	64 (12.8)	97(19.4)	20 (4)	5 (1)	9 (1.8)
RTE chicken products	250	95(38)	34 (13.6)	52 (20.8)	4(1.6)	2 (0.8 %)	3 (1.2)
Frozen and shocked frozen broiler	300	111 (37)	39 (13)	54 (18)	11 (3.6)	3 (1)	4 (1.3)
Breeding and slaughter house	100	41 (41)	12 (12)	23 (23)	0	5 (5)	1 (1)
Total	1650	646 (39.15)	217 (13.15)	325 (19.69)	58 (3.51)	20 (1.21)	27 (1.63)

4.5 Confirmations of *L. monocytogenes* using PCR molecular technique.

4.5.1 Amplification of the actA gene (827 bp) target sequence

The 827 bp product amplified by the primer sets for actA gene in all 217 *L. monocytogenes* isolates were detected only in 37 isolates. Twelve isolates were from first experimente, fresh raw dressed broiler chickens (four from station one, one from station two, three from station three, one from station four and three from station five) , twelve isolates were from second experimente, frozen raw dressed broiler chickens. (four from station one, one from station two, three from station three, one from station four and three from station five) ,seven isolates were from thried experimente, broiler chicken ready to eat products (one from frozen chicken-burger, one from frozen chicken-sausages, three from chickens meat balls (Kofta), one from chicken- shawerma and one from chicken- mortadella) and six isolates were from fourth experimente, Effect of Frozen & shock frozen (three from Fresh broiler chickens, two from Frozen broiler chickens and one from Shocked frozen broiler chickens, the results are shown in Figure (15-20).

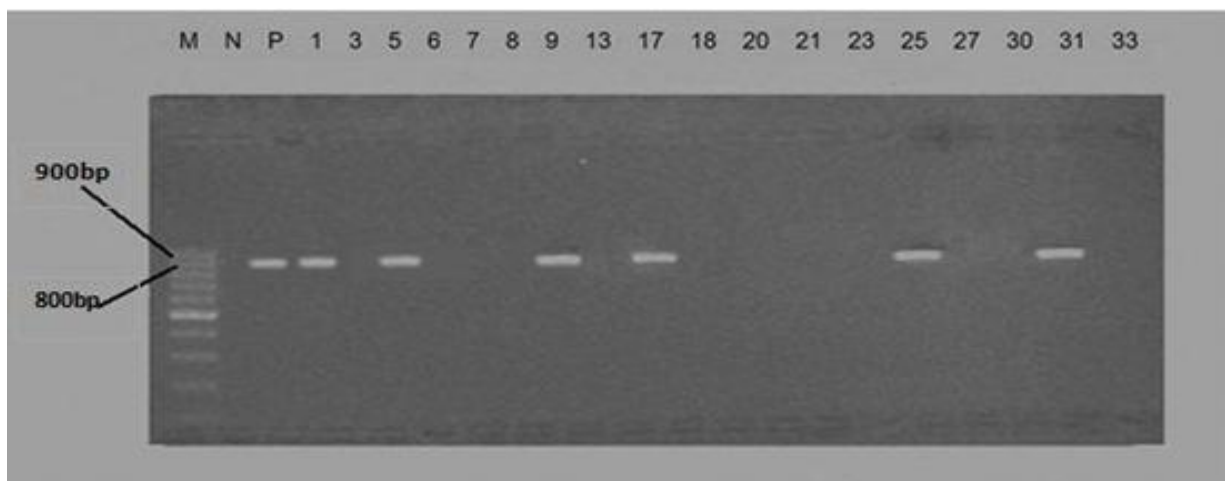


Figure 15: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 1, 5, 9 and 17: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station one, Lane 25: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station two, Lane 31: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three.



Figure 16: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 39 and 43: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three, Lane 50: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station four, Lane 59 and 66: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station five.

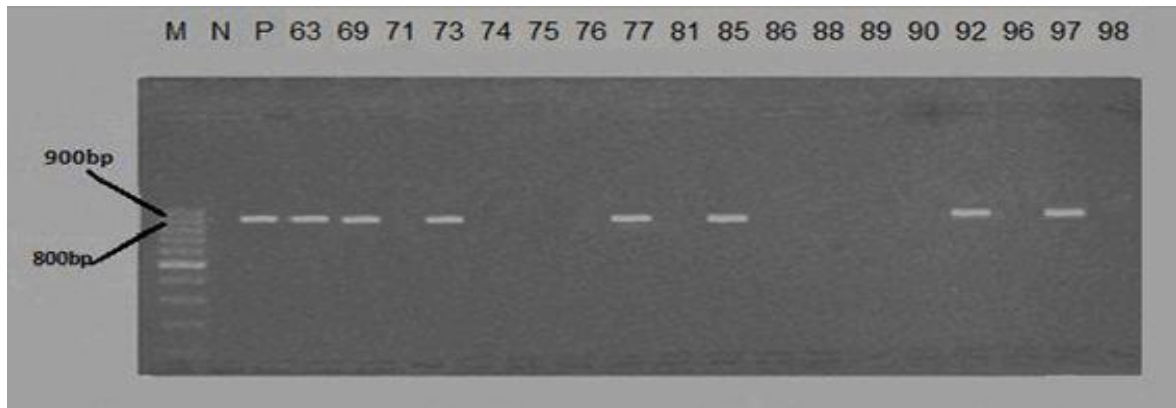


Figure 17: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 68: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station five, Lane 69, 73, 77 and 85: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station one, Lane 92: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station two, Lane 97: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three.



Figure 18: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 103 and 107: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three, Lane 114: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station four, Lane 123, 130 and 132: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station five.

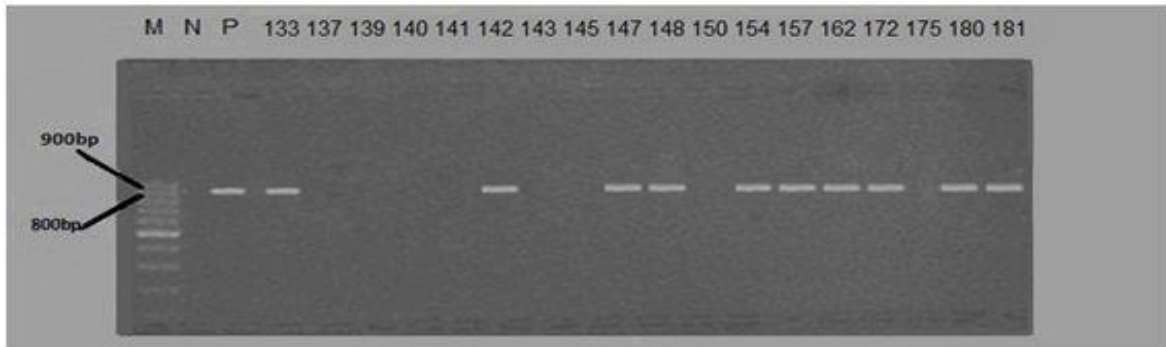


Figure 19: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 133: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken burger, Lane 142: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken sausages, Lane 147, 148 and 154: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken meat balls (Kofta), Lane 157: viewing confirmed *L. monocytogenes* isolated from third experiment, RET chicken shawerma, Lane 162: viewing confirmed *L. monocytogenes* isolated from third experiment, RET chicken mortadella, Lane 172, 180 and 181: viewing confirmed *L. monocytogenes* isolated from four experiment, effect of temperature on fresh meat chicken, fresh raw dressed broiler chickens.

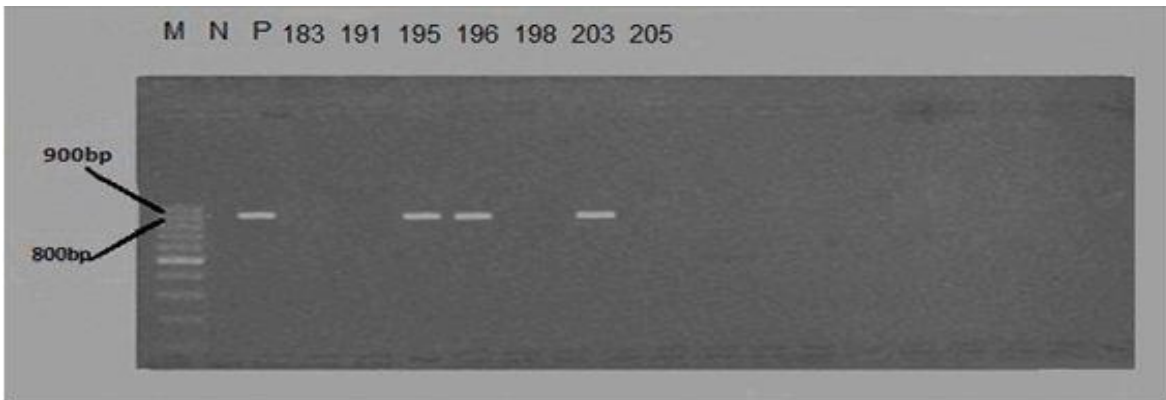


Figure 20: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 195 and 196: viewing confirmed *L. monocytogenes* isolated From four experiments, effect of temperature on fresh meat chicken, frozen raw dressed broiler chickens, lane 203: viewing confirmed *L. monocytogenes* isolated from four experiments, effect of temperature on fresh meat chicken, shock frozen dressed broiler chickens.

4.5. 2 Amplification of the hlyA gene (417 bp) target sequence.

The 417 bp product amplified by the primer sets for hlyA gene in all 217 *L. monocytogenes* isolates were detected only in 32 isolates. Twelve isolates were from first experimente, fresh raw dressed broiler chickens (three from station one, two from station two, three from station three, one from station four and three from station five) , eleven isolates were from second experimete, frozen raw dressed broiler chickens. (three from station one, one from station two, three from station three, one from station four and three from station five) ,four isolates were from thried experimental, broiler chicken ready to eat products (two from frozen chicken-burger, one from frozen chicken-sausages and three from chickens meat balls (Kofta)) and five isolates were from fourth experimete, effect of frozen & shock frozen (two from fresh broiler chickens, two from frozen broiler chickens and one from shock frozen broiler chickens, the results are shown in figure (21-26).

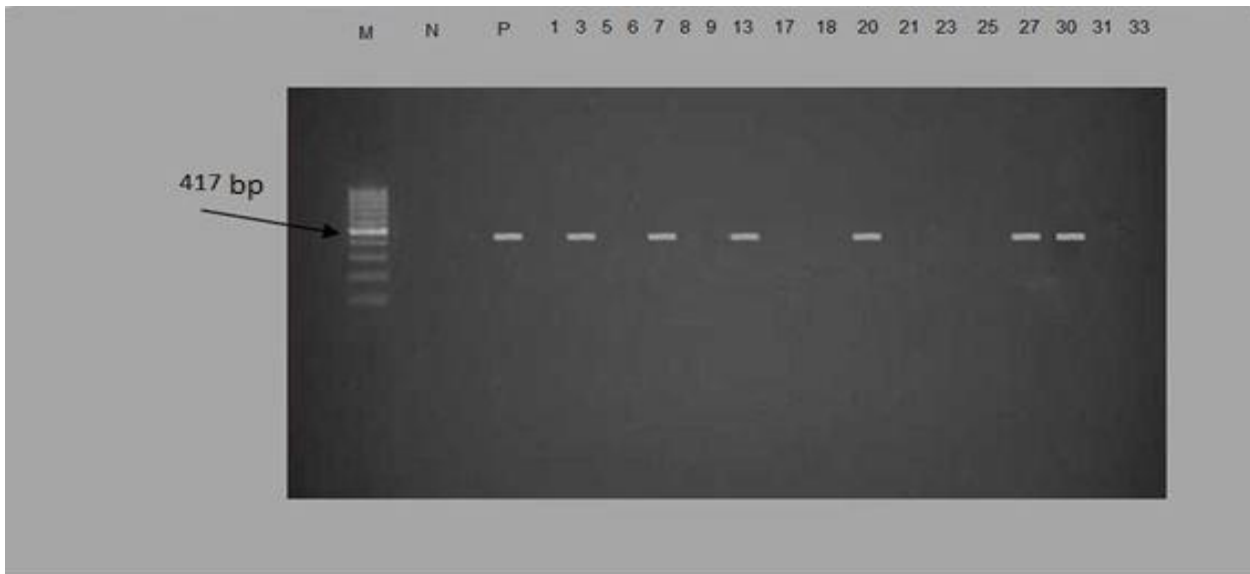


Figure 21: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 3, 7 and 13: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station one, Lane 20 and 27: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station two, Lane 30: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three.

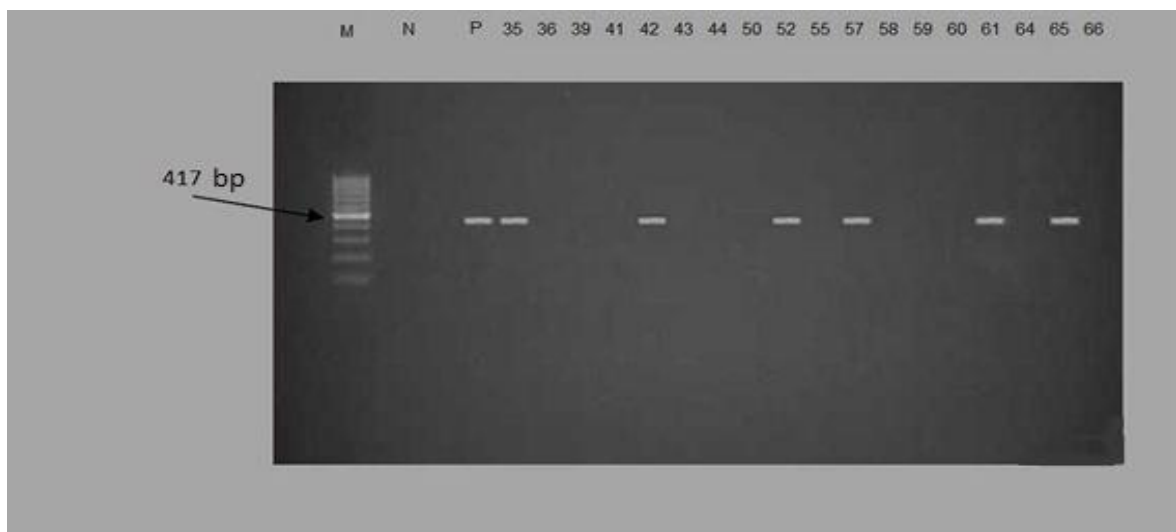


Figure 22: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 35 and 42: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three, Lane 52: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station four, Lane 57, 61 and 65: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station five.

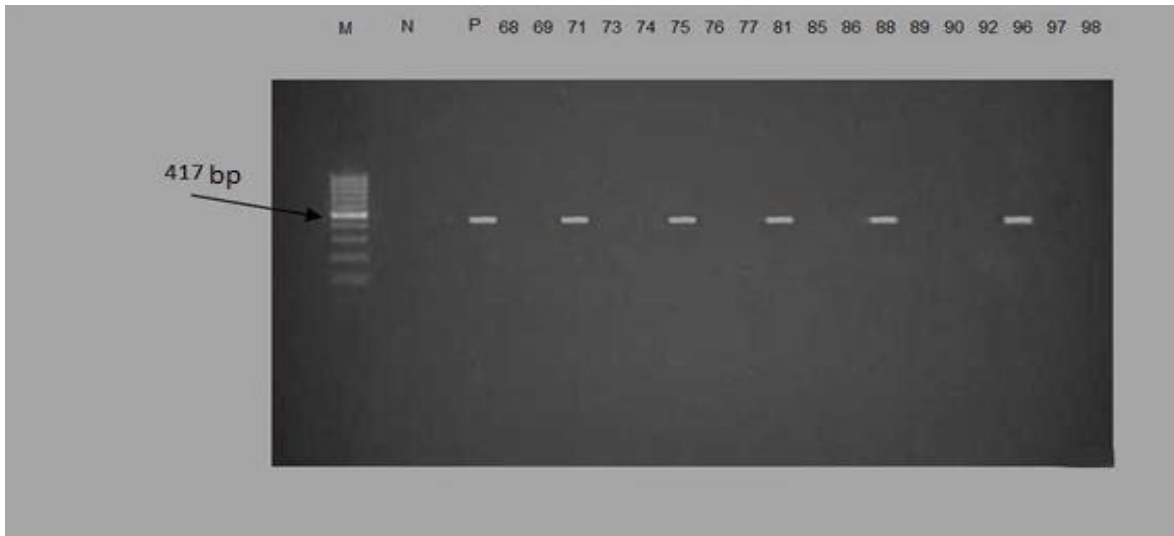


Figure 23: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 71,75 and 81: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station one, Lane 88: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station two , Lane 96: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three.

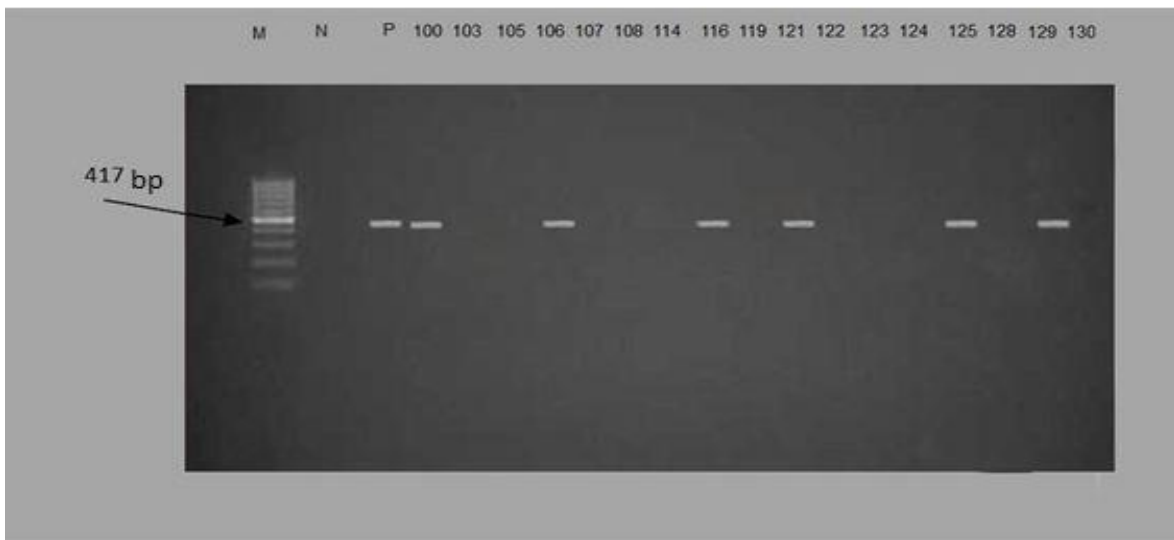


Figure 24: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 100 and 106: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three, Lane 116: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station four, Lane 121, 125 and 129: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station five.

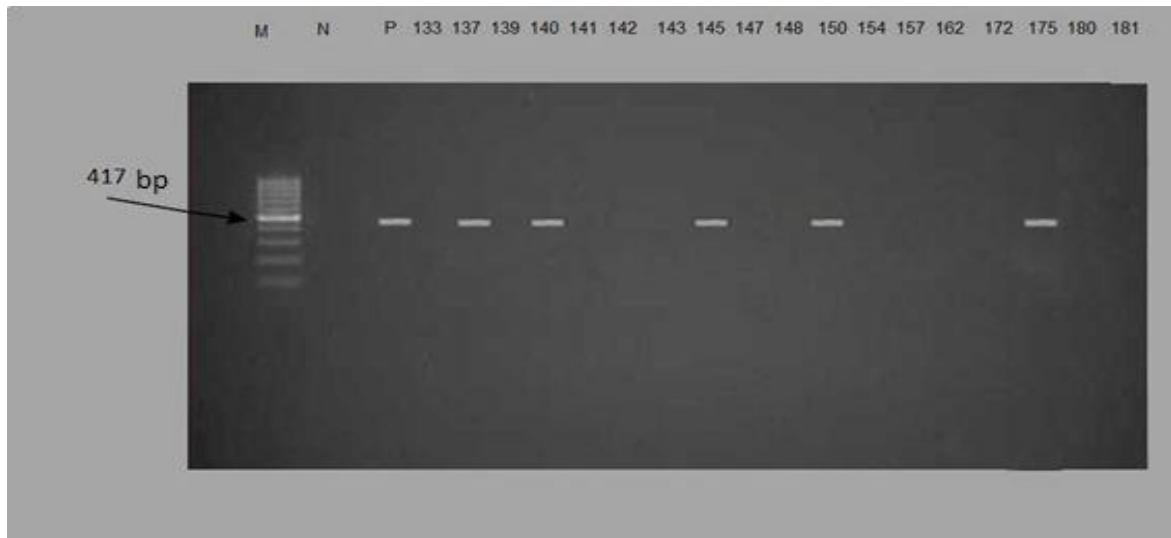


Figure 25: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 137 and 140: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken burger, Lane 145: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken sausages, Lane 150: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken meat balls (Kofta), Lane 175: viewing confirmed *L. monocytogenes* isolated four experiment, effect of temperature on fresh meat chicken, fresh raw dressed broiler chickens.



Figure 26: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 183: viewing confirmed *L. monocytogenes* isolated four experiment, effect of temperature on fresh meat chicken, fresh raw dressed broiler chickens Lane 191 and 198: viewing confirmed *L. monocytogenes* isolated From four experiments, effect of temperature on fresh meat chicken, frozen raw dressed broiler chickens, lane 205: viewing confirmed *L. monocytogenes* isolated from four experiments, effect of temperature on fresh meat chicken, shock frozen dressed broiler chickens.

4.5. 3 Amplification of the iap gene (131 bp) target sequence.

The 131 bp product amplified by the primer sets for iap gene in all 217 *L. monocytogenes* isolates were detected only in 28 isolates. thirteen isolates were from first experimentee, fresh raw dressed broiler chickens (three from station one, two from station two, three from station three, two from station four and three from station five) , thirteen isolates were from second experimente, frozen raw dressed broiler chickens. (three from station one, two from station two, three from station three, two from station four and three from station five) and three isolates were from third experimente, broiler chicken ready to eat products (one from frozen chicken-burger and two from frozen chicken, as it appeared in Figrue (27-31).

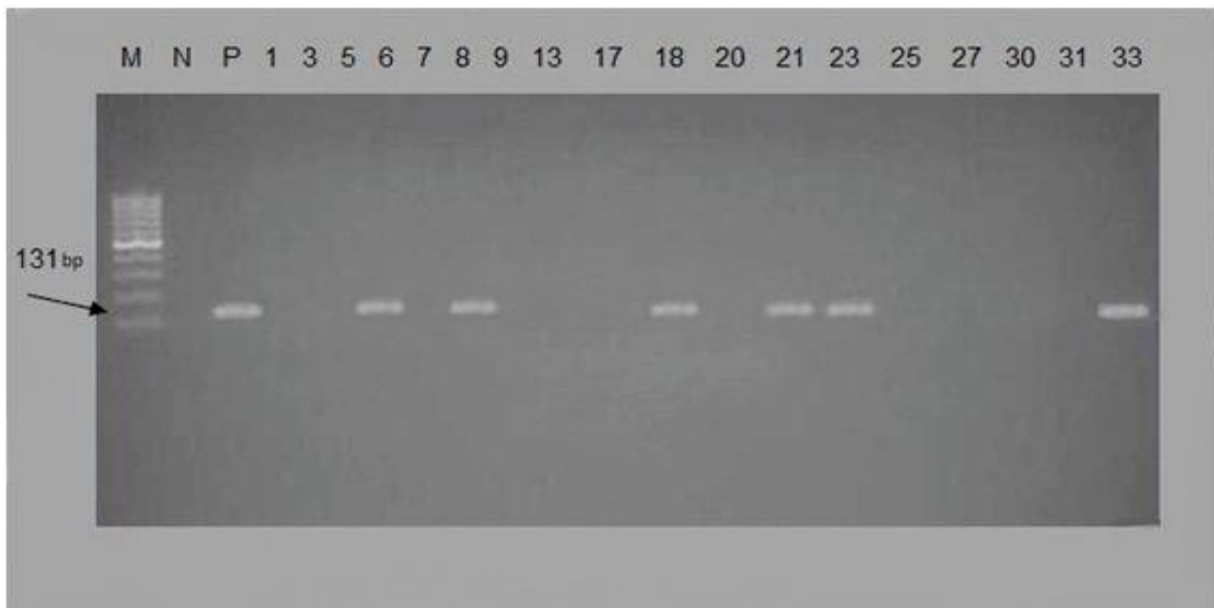


Figure 27: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 6, 8 and 18: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station one, Lane 21 and 23: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station two, Lane 33: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three.

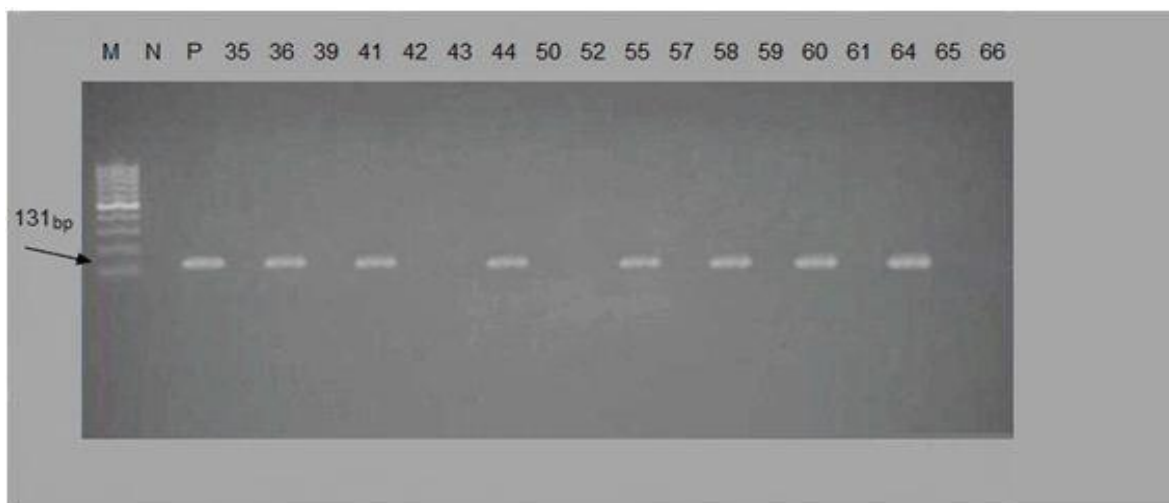


Figure 28: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 36 and 41: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station three, Lane 44 and 55: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station four, Lane 58, 60 and 64: viewing confirmed *L. monocytogenes* isolated from first experiment, fresh raw dressed broiler chickens, station five.

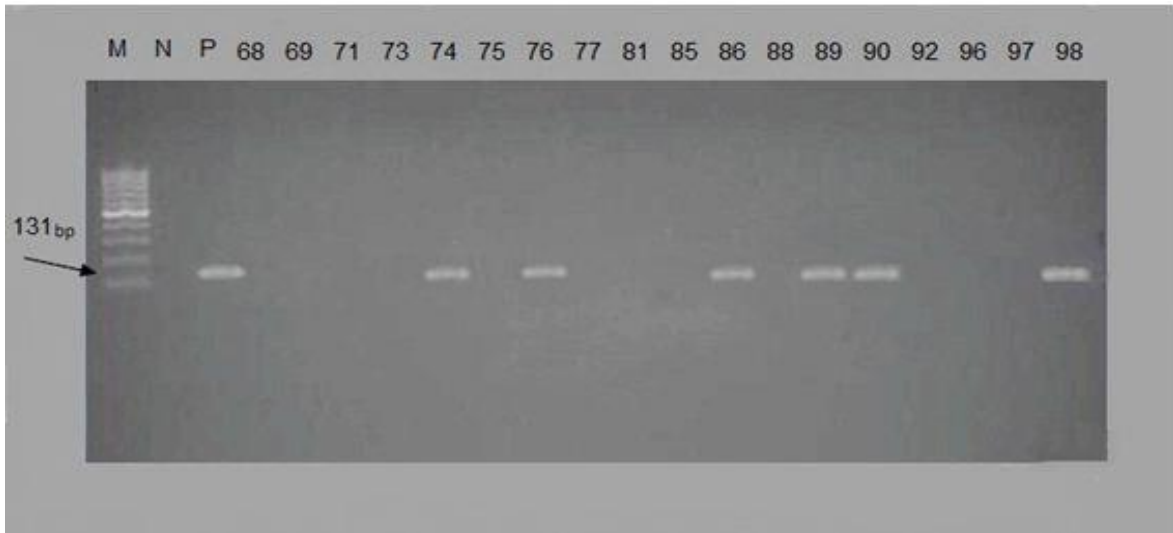


Figure 29: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 74, 76 and 86: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station one, Lane 89 and 90: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station two, Lane 98: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three.

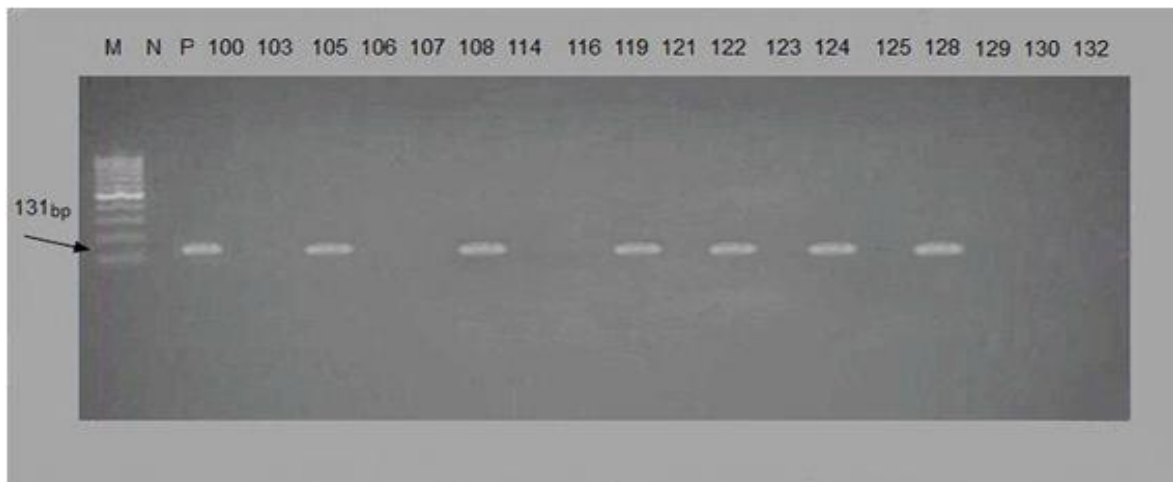


Figure 30: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 105: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station three, Lane 108 and 119: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station four, Lane 122, 124 and 128: viewing confirmed *L. monocytogenes* isolated from second experiment, frozen raw dressed broiler chickens, station five.



Figure 31: Agarose Gel Electrophoresis showing positive samples. M: DNA Marker, N: negative control, P: positive control, Lane 139: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken burger, Lane 141 and 143: viewing confirmed *L. monocytogenes* isolated from third experiment, RET frozen chicken sausages.

4.5.4 Presence of confirmed *L.monocytogenes* using PCR technique.

Table 67: No. Of *L.monocytogenes* isolates positive for each primer set specific for its virulence genes

Primer	Total detected isolates
<i>actA</i>	37
<i>hlyA</i>	32
<i>Iap</i>	28

4.5.5 Comparison of *L. Monocytogenes* Detection Using ISO Culturing Method, and PCR based Molecular Technique.

The detection efficiency of *L. monocytogenes* in samples was higher through using ISO method. The presence of *L. monocytogenes* was confirmed in 68 (13.6%) fresh broiler chicken, 64 (12.8%) frozen broiler chicken, 34 (13.6%) RTE chicken products and 39 (13%) RTE chicken products, through using PCR technique compared to the presence of *L. monocytogenes* that was confirmed in 37 (7.4 %) Fresh broiler chicken, 35 (7 %) Frozen broiler chicken, 14 (5.6 %) RTE chicken products and 11 (3.6 %) RTE chicken products through using ISO methods. Table (68)

Table 68: Summary of the comparison of *L. Monocytogenes* Detection Using conventional method (ISO Culturing Method), and PCR Molecular Technique

Experimental No	Sample name	No. of sample tested	Conventional	Total PCR Isolates (%)	PCR profile		
					<i>actA</i> (%)	<i>hylA</i> (%)	<i>Iap</i> (%)
First experiment	Fresh broiler chicken	500	68 (13.6)	37 (7.4)	12 (2.4)	12 (2.4)	13 (2.6)
Second experiment	Frozen broiler chicken	500	64 (12.8)	35 (7)	12 (2.4)	11 (2.2)	12 (2.4)
Third experiment	RTE chicken products	250	34 (13.6)	14 (5.6)	7 (2.8)	4 (1.6)	3 (1.2)
Fourth experiment	Effect of temperature on broiler	300	39 (13)	11 (3.6)	6 (2)	5 (1.6)	0
Sixth experiment	Breeding and Slaughter	100	12 (12)	0	0	0	0
Total		1650	217 (13.15)	97 (6.25)	37 (2.38)	32 (2.06)	28 (1.80)

4.5.6 Presence of confirmed *L. monocytogenes* in fresh, frozen, shock frozen raw dressed broiler chickens and RTE chicken meat products.

According to confirmed PCR technique gene analysis output, out of the total 217 samples examined, only 97 (44.7%) samples were found positive for *L. monocytogenes*.table (68)

4.5.7 Susceptibility to antimicrobial agents-microbroth dilution method.

The Seventy nine confirmed PCR *L. monocytogenes* isolates (ATCC 7644) were tested for susceptibility to eighteen antibiotics. The minimum inhibitory concentration (MIC) for *L. monocytogenes* (ATCC 7644) was as follows: Amoxicillin 0.25µg/ml, Benzylpenicillin 0.125µg/ml, Ciprofloxacin 1µg/ml, Clindamycin 0.25µg/ml, Chloramphenicol 2µg/ml, Doxycycline 0.0625µg/ml, Fosfomycin 128µg/ml, Fusidic Acid 8 µg/ml, Imipenem 2µg/ml, Teicoplanin 0.5µg/ml, Oxacillin 1 µg/ml, Rifampicin 0.5 µg/ml, Streptomycin 2µg/ml, Tetracycline 0.25 µg/ml, Trimethoprim 0.25 µg/ml, Vancomycin 0.5 µg/ml .Table (69)

The Seventy nine confirmed PCR *L. monocytogenes* isolates showed difference in antimicrobial susceptibility from that of the reference strain.

All *L. monocytogenes* isolates were sensitive to nine antibiotics; ampicillin, Chloramphenicol, Doxycycline, Imipenem, Teicoplanin, Rifampicin, Trimethoprim and vancomycin, and more than 88% of isolates were sensitive to three antibiotics: benzylpenicillin, ciprofloxacin and Streptomycin.

All the isolates were resistance to fosfomycin and. 91.3% of the isolates were resistance to oxacillin. Table (69)