

## **DEDICATION**

To

The soul of my dearest father and mother

Allah bless and mercy for them

To

My loving brothers and sisters

## **ACKNOWLEDGEMENTS**

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## List of Abbreviations

<u>Abbreviation</u>	<u>Symbol Full Name</u>
ATCC	American Type Culture Collection
RTE	Ready-to-eat
PCR	Polymerase Chain Reaction
Taq	<i>Thermus aquaticus</i>
U.V	Ultraviolet
%	Percent
°C	Celsius
µm	Micrometer
bp	Base Pair
cfu	Colony Forming Unit
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleoside Triphosphates
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
ml	Milliliter
HACCP	Hazard Analysis and Critical Control Points
JFDA	Jordanian Food and Drug Administration
ca	Approximately
spp.	species
ALOA	Agar Listeria according to Ottaviani and Agosti
rpm	Round per Minute
β	Beta
<u>Abbreviation</u>	<u>Symbol Full Name</u>

<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>R. equi</i>	<i>Rhadococcus equi</i>
mm	Millimeter
s	Second
µg	Microgram
TSYEA	Tryptone Soya Yeast Extract Agar
BaSO <sub>4</sub>	Barium Sulphate
H <sub>2</sub> SO <sub>4</sub>	Hydrogen Sulphate
g	Gram
G+ve	Gram Positive
No.	Number
UVM Agar	University of Vermont Agar
α	Alpha
min	Minute

## ABSTRACT

### **Detection of *Listeria Monocytogenes* in Dressed Broiler Chicken and Ready-to-Eat Meat Products in Sudan**

By

**Ayman Daif Allah Ismail Alsheikh Eid**

The aim of this study was to detect and identify *Listeria monocytogenes* from raw dressed broiler chicken, frozen, shock frozen dressed broiler chicken, ready-to-eat (RET) meat products, frozen and shock frozen raw dressed broiler chicken and from breeding and slaughter houses for broiler chicken in Khartoum State - Sudan, using conventional methods (ISO 11290-1, 2004) and biochemical methods, and further confirmation by using Microbact™ Listeria 12L Kit System (Oxoid, MB1128A) and polymerase chain reaction technique (PCR).

The samples subjected to the isolation and identification of *Listeria monocytogenes* included the following: a total of five hundred (500) samples, of fresh raw dressed broiler chicken, and five hundred (500) samples, of frozen raw dressed broiler chicken, two hundred fifty (250) samples, ready-to-eat (RET) meat (frozen chicken-burger, chicken-sausage, frozen chicken meat balls (kofta), chicken-shawerma, and mortadella), three hundred (300) samples (100 fresh raw dressed broiler chicken, 100 frozen raw dressed broiler chicken and 100 shocked frozen raw dressed broiler chicken) and one hundred (100) swabs from breeding and slaughter houses for broiler chicken in Khartoum state – Sudan.

Also two hundred forty (240) samples raw dressed broiler chicken artificially infected with *listeria monocytogenes* in the laboratory were divided into two groups, in one group 120 samples were exposed to shock - freezing at (– 40° C) for six hour then kept at (– 18° C), in the other group 120 samples were kept at (– 18° C). Each month ten samples from each part were used to detect the presence of *listeria monocytogenes*. in frozen and shock - frozen artificially infected chicken meat. The isolate (97) of *listeria monocytogenes* were subjected to antibiotics sensitivity by using Microbroth Dilution Method.

From five hundreds (500) samples of fresh raw dressed broiler chicken 204 samples (40.8%) yielded *listeria* spp. these were distributed as follows: *L. monocytogenes* 68 samples (13.6%), *Listeria ivanovii* 99 samples (19.8%), *Listeria grayi* 23 samples (4.6%), *Listeria seeligeri* 5 samples (1%) and *Listeria welshimeri* 10 samples (2%).

From five hundreds (500) samples of frozen raw dressed broiler chicken 195 samples (39%) yielded *listeria* spp. these were distributed as follows: *L. monocytogenes* 64 samples (12.8%), *Listeria ivanovii* 97 samples (19.4%), *Listeria grayi* 20 samples (4%), *Listeria seeligeri* 5 samples (1%) and *Listeria welshimeri* 9 samples (1.8%).

From two hundred fifty (250) samples of ready-to-eat (RET) meat. (frozen chicken-burger, chicken-sausage , frozen chicken meat balls (kofta), chicken-shawerma, and mortadella) 95 samples (38%) yielded *listeria* spp. these were distributed as follows: *L. monocytogenes* 34 samples (13.6%), *Listeria ivanovii* 52 samples (20.8%), *Listeria grayi* 4 samples (1.6%), *Listeria seeligeri* 2 samples (0.8%) and *Listeria welshimeri* 3 samples (1.2%).

From three hundred (300) samples, of ( 100 fresh raw dressed broiler chicken, 100 frozen raw dressed broiler chicken and 100 shocked frozen raw dressed broiler chicken) 111 samples (37%) yielded *listeria* spp. these were distributed as follows: *L. monocytogenes* 39 samples (13 %), *Listeria ivanovii* 54 samples (18%), *Listeria grayi* 11 samples (3.6%), *Listeria seeligeri* 5 samples (1%) and *Listeria welshimeri* 4 samples (1.3%).

From one hundred (100) swabbing samples from Breeding and Slaughter house broiler chicken 41 samples (41%) yielded *listeria* spp. these were distributed as follows: *L. monocytogenes* 12 samples (12%), *Listeria ivanovii* 23 samples (23%), *Listeria seeligeri* 5 samples (5%) and *Listeria welshimeri* 11 samples (11%). *listeria monocytogenes* was isolated mainly from hand workers and water in both breeding house and slaughter house. *Listeria monocytogens* survived freezing (- 18° C) and shock - freezing (- 40° C) for twelve month in all samples examined (240).

As a conclusion the conventional method showed that out of total 1650 samples, 646 (39.15%) were found to be contaminated with *Listeria* spp. 217 (13.15%) were found to be contaminated with *Listeria monocytogenes*, 68 (13.6%) samples fresh raw dressed broiler chicken, 64 (12.8%) frozen raw dressed broiler chicken, 34 (13.6%) samples ready-to-eat (RET) meat, 39 (13%) samples frozen and shocked frozen raw dressed broiler chicken and 12 (12%) samples from breeding – slaughter house broiler chicken.

On the bases of the act A gene (827bp), hyl A gene (417bp) and iap gene (131bp), PCR method identified only 97 out of 217 as *Listeria monocytogenes*.

*Listeria monocytogenes* strains isolated from raw dressed broiler chicken, Frozen, shock frozen dressed broiler chicken and read to eat (RTE) meat products and breeding – slaughter house broiler chicken were susceptible to ampicillin, Chloramphenicol, Doxycycline, Imipenem, Teicoplanin, Rifampicin, Trimethoprim and Vancomycin and resistant to Fosfomycin.

The results presented in this study indicate the potential risk of contamination of raw dressed broiler chicken, frozen dressed broiler chicken, ready-to-eat (RTE) meat chicken products by *Listeria monocytogenes*.

# اكتشاف جراثيم الليستيريا مونوسايتوجينز المغزولة من الدجاج اللاحم المذبوح ومنتجات اللحوم الجاهزة للأكل في ولاية الخرطوم - السودان

إعداد: أيمن ضيف الله إسماعيل الشيخ

## الملخص

تهدف هذه الدراسة إلى تحديد وتشخيص جراثيم الليستيريا مونوسايتوجينز من الدجاج اللاحم المذبوح الطازجه والمجمدة ومن حضائر تربية الدواجن ومذبح الدواجن ومنتجات اللحوم الجاهزة للأكل في ولاية الخرطوم - السودان وأخذت العينات من المطاعم والاسواق وحللت باستخدام الطرق التقليدية والبيوكيميائية وتأكيد تشخيصها بالطرق السريعة (Kit) وباستخدام التقنية الجزيئية (PCR).

تم جمع خمسمائة (500) عينة من الدجاج اللاحم المذبوح الطازجه وخمسمائة (500) عينة من الدجاج اللاحم المجمد ومنتان وخمسون (250) عينة مختلفة من منتجات اللحوم الجاهزة للأكل (برغر الدجاج، صوصج الدجاج، كفتة الدجاج، شاورما الدجاج ومرنديلا الدجاج) و ثلاثمائة (300) عينة تشمل الأتي ١٠٠ من الدجاج اللاحم المذبوحة الطازجه و ١٠٠ من لحوم الدجاج المجمدة و ١٠٠ من لحوم الدجاج المجمدة بطريقة التجميد السريع. و كذلك جمعت مائه (١٠٠) عينة (مسحه) منها ٤٠ من محطة لتربية دجاج اللاحم و ٦٠ مذبج الدجاج.

العينات جمعت من محطة لتربية دجاج اللاحم و خمس مسالخ للدواجن والمطاعم والأسواق المحلية في ولاية الخرطوم - السودان.

كما تمت اختبار أثر التجميد - ١٨ ° م والتجميد السريع - ٤٠ ° م على 240 عينة تم ثلويتها بجراثيم الليستيريا مونوسايتوجينز وفحصت كل عشرة منها شهريا ولمدة اثنا عشر شهراً لمعرفة مدي مقاومة البكتريا . جراثيم الليستيريا مونوسايتوجينز المغزولة من طريقة الغزل التقليدية تم تأكيدها باستخدام التقنية الجزيئية . استخدمت الجينات *act A* (827 bp), *hyl A* (417 bp) و *api* (131 bp) وتم عمل اختبار الحساسية للمضادات الحيوية لمعرفة المضادات الحيوية ذات الكفاءة لقتل جراثيم الليستيريا مونوسايتوجينز المغزولة.

أظهرت نتائج طريقة الغزل التقليدية تلوث العينات بجراثيم الليستيريا كما يلي:

تلوث ٢٠٤ عينات (٤٠,٨%) بجراثيم الليستيريا من أصل ٥٠٠ عينة من لحوم دجاج اللاحم الطازجه وكان توزيع عثرات الليستيريا كما يلي: جراثيم الليستيريا مونوسايتوجينز ٦٨ عينة (١٣,٦%) ، جراثيم الليستيريا الايفانوفي ٩٩ عينة (١٩,٨%) ، جراثيم الليستيريا جري ٢٣ عينة (٤,٦%) ، وجراثيم الليستيريا سليجري ٥ عينات (١%) و جراثيم الليستيريا ولشمري ١٠ عينات (٢%).

تلوث ١٩٥ عينات (٣٩%) بجراثيم الليستيريا من أصل ٥٠٠ عينة من لحوم دجاج اللاحم المجمدة وكان توزيع عثرات الليستيريا كما يلي: جراثيم الليستيريا مونوسايتوجينز ٦٤ عينة (١٢,٨%) ، جراثيم الليستيريا الايفانوفي ٩٧ عينة (١٩,٤%) ، جراثيم الليستيريا جري ٢٠ عينة (٤%) ، وجراثيم الليستيريا سليجري ٥ عينات (١%) و جراثيم الليستيريا ولشمري ٩ عينات (١,٨%).

تلوث ٩٥ عينات (٣٨%) بجراثيم الليستيريا من أصل ٢٥٠ عينة من منتجات لحوم الدجاج اللاحم الجاهزة للأكل وكان توزيع عثرات الليستيريا كما يلي: جراثيم الليستيريا مونوسايتوجينز ٣٤ عينة (١٣,٦%) ، جراثيم الليستيريا الايفانوفي

٥٢ عينة (٢٠,٨ %) ، جراثيم الليستيريا جري ٤ عينة (١,٦ %) ، وجراثيم الليستيريا سليجيري ٢ عينة (٠,٨ %) و جراثيم الليستيريا ولشمري ٣ عينات (١,٢ %).

تلوث ١١١ عينات (٣٧%) بجراثيم الليستيريا من أصل ٣٠٠ عينة من لحوم الدجاج اللاحم الطازجة والمجمدة والمجمدة تجميد سريع وكان توزيع عثرات الليستيريا كما يلي: جراثيم الليستيريا مونوسايتوجينز ٣٩ عينة (١٣ %) ، جراثيم الليستيريا الايفانوفي ٥٤ عينة (١٨ %) ، جراثيم الليستيريا جري ١١ عينة (٣,٦ %) ، وجراثيم الليستيريا سليجيري ٥ عينات (١,٦ %) و جراثيم الليستيريا ولشمري ٤ عينات (١,٣ %).

تلوث ٤١ عينة (٤١%) بجراثيم الليستيريا من أصل ١٠٠ عينة (مسحة جرثومية) من حضيرة تربية دجاج اللاحم ومن مسلخ الدجاج وكان توزيع عثرات الليستيريا كما يلي: جراثيم الليستيريا مونوسايتوجينز ١٢ عينة (١٢ %) ، جراثيم الليستيريا الايفانوفي ٢٣ عينة (٢٣ %) ، جراثيم الليستيريا جري ٥ عينة (٥ %) و جراثيم الليستيريا ولشمري ١١ عينات (١١ %). جراثيم الليستيريا مونوسايتوجينز غزلت من أيدي العمال ومن الماء في حضيرة تربية دجاج اللاحم ومسلخ الدجاج.

و خلاصة نتائج طريقة العزل التقليدية كانت كالآتي : تلوث 646 عينة (39.15%) من أصل ١٦٥٠ عينة بجراثيم الليستيريا، وتلوث ٢١٧ عينة (١٣,١٥%) من أصل ٦٤٦ عينة بجراثيم الليستيريا مونوسايتوجينز ، ٦٨ عينة (١٣,٦%) من الدجاج اللاحم الطازجة ، ٦٤ عينة (١٢,٨%) من الدجاج اللاحم المجمدة ، ٣٤ عينة (١٣,٦%) من الدجاج الجاهزة للأكل و ٣٩ عينة (١٣%) من الدجاج (المذبوحة الطازجه ، التجميد ، التجميد السريع ) و (١٢%) من حضيرة التربية و مسلخ الدواجن.

وجراثيم الليستيريا مونوسايتوجينز أظهرت تحملاً لدرجة حرارة التجميد - ١٨ ° م والتجميد السريع - ٤٠ ° م لمدة ١٢ شهر لكل العينات (٢٤٠ عينة).

أكدت نتائج التقنية الجزيئية تشخيص ٩٧ عينة (٦,٢٥%) من جراثيم الليستيريا مونوسايتوجينز من ال ٢١٧ عينة. هذه ال ٩٧ عينة الايجابية مفصلة كالآتي : ٣٧ عينة (٧,٤%) من الدجاج اللاحم الطازجة ، ٣٥ عينة (٧%) من الدجاج اللاحم المجمدة ، ١٤ عينة (٥,٦%) من الدجاج الجاهزة للأكل و ١١ عينة (٣,٦%) من (الدجاج المذبوحة الطازجه و التجميد والتجميد السريع).

أبدت عزلات جراثيم الليستيريا مونوسايتوجينز المعزولة من اللحوم الطازجة والمجمدة و منتجات اللحوم الجاهزة للأكل حساسية للمضادات الحيوية التالية: الأمبسلين ، الكلوروفينيكول ، الدوكسي سايكلين ، الإيمبيبينيم ، التيكوبلانين ، الريفامبيسين ، الستربتومايسين سلفات والفانكوميسين. كما ابدت مقاومتها للفوسفوميسين.

ان النتائج الممثلة في هذه الدراسة تبين الخطورة المحتملة من تلوث اللحوم الطازجة والمجمدة والجاهزة للأكل بجراثيم الليستيريا مونوسايتوجينز.