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University of Diyala
College of Veterinary Medicine

Isolation and Identification of Some Zoonotic *Enterobacteriaceae spp*. from Poultry Meat in Diyala Province

A Thesis
Submitted to the Council of the College of Veterinary Medicine, University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master of Science in
Internal and preventive Veterinary Medicine -zoonosis

By

Ahmed Hussein Khamees Kache AL-Tamimi

B.V.M.S. - College of Veterinary Medicine - University of Diyala
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Supervised by

Prof. (Dr.) Nazar Jabbar AL-Khafaji

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق

الله العظيم

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We, the examining committee , certify that after reading this thesis entitled (**Isolation and Identification of Some Zoonotic Enterobacteriaceae spp. from Poultry Meat in Diyala Province**). was prepared by Ahmed Hussein Khamees kache AL-Tamimi in its contents , we think it is adequate for the award Degree of Master of Science in Internal and Preventive Medicine- Zoonosis.

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Prof .Dr .Nazar Jabbar AL-Khafaji
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Approved by Council of the College of Veterinary Medicine
University of Diyala

Assist. Prof. Dr.
Mohammed Youssef Mahmoud
head of department of
Internal and Preventive Medicine
College of Veterinary Medicine
University of Diyala

Prof .Dr.
Talib Jawed Kadhim
Dean of college of
Veterinary Medicine
University of Diyala
2021

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Supervisor

Prof. Dr.Nazar Jabbar AL-Khafaji

College of Veterinary Medicine

University of Diyala

2021

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Assist. Prof. Dr.

Khalid Ibrahim Abd

Vice dean Postgraduate Studies and Scientific Affairs

College of Veterinary Medicine

University of Diyala

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Dedication

In the name of Allah, the Creator Who illuminated the universe with His glorious light alone, Whom we worship alone, prostrate in reverence and thankful for His grace and favour upon me in completing this effort.

To the beacon and the enlightening of the nation and its prognostic intercessor of mankind the prophet Muhammad (peace be upon him).

To those who light the way for Science.

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To whom I see optimism and happiness in their laughter to those who looked forward to my success my ***(****brothers and sisters and my wife).*

To my, acquaintances and friends whom I respect and honour.

I *dedicate my research to you*

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Summary

The study was conducted on 250 samples, represented by 70 meat samples from each type of imported frozen and local fresh meat (Neck, Wing, Breast and Thigh), in addition to 110 swabs from worker's hands and equipment used in slaughterhouse and retail shops for meat in Diyala, from August 2020 – to April 2021. Samples were collected aseptically in clean polyethylene bag and transported to the laboratory in icebox and submitted to routine procedures of isolation, identification, specification, in depends on cultural, biochemical characteristics of colonies, level of contamination in addition to the sensitivities of isolates to 12 commonly used antibiotics. The results revealed that out of 250 samples, 54 (21.6%) were free from Enterobacteriaceae bacterial contamination; while 196 (78.4%) were contaminated, , single isolates 123/278(44.2%); others 155 (55.8%) in mixed forms, either in two isolates 65 (46.8%), or three 7 (7.6%) or four isolates 1 (1.4%) in a sample.

In current study E. *coli* was the highest 102/ 278 (36.7%); followed by *Klebseilla* spp. 84(30.2%), *Proteus* spp. 34(12.2%), *Enterobacter* 28(10.1%), *Salmonella* spp. 22 (7.9%), *Shigella* spp. 6(2.2%) and *Serratia* spp. 4(1.4%). From frozen meat, the highest isolates was *E. coli* 27/79 (34.2%); followed by *Klebsiella* spp. 21 (26.7%); *Proteus* spp. 11 (13.9%); *Enterobacter* spp. 10 (12.7%); *Salmonella* spp. 6 (7.6%); *Shigella* spp. 3(3.8%); and *Serratia* spp. 1 (1.3%). While in Fresh meat, the highest one was *Klebsiella* spp. 30/78(38.5%), followed by *E. coli* 26 (33.3%); *Enterobacter* spp. 10 (12.8%), *Proteus* spp. 8 (10.3%), and *Salmonella* spp. 4 (5.1%). Meanwhile from equipment the highest isolate was *E. coli* 49/ 121 (40.5%), followed by *Klebsiella* spp. 33(27.3%), *Proteus* spp. 15(12.4%) , *Salmonella* spp. 12(9.9%) , *Enterobacter* spp. 8(6.6%), *Shigella* spp.and *Serratia* spp. each 2 (1.7%).

The highest viable bacterial counts from frozen meat, were from neck log $_{10}$(5.50 ±0.01); wing (5.40±0.03); thigh (5.36± 0.03) and breast (5.18± 0.09). While from fresh meat, from breast, log $_{10}$ (4.85± 0.01); followed by thigh (4.70 ±0.04); neck (4.60 ±0.03); and wing (4.50± 0.07). Coliform count from frozen meat, neck $\log_{10}$ (5.46± 0.03); wing (5.00 ±0.04); thigh (4.99 ±0.10) and breast (4.79± 0.08). While from fresh meat, the highest was from thigh $\log_{10}$ (4.42± 0.01); breast (4.41 ±0.07); wing (4.36± 0.02); and neck 3 (4.32± 0.08). The best antibiotics to which all isolates were sensitive were Amikacin (AK30µg); Chloramphenicol (C30 µg); Ceftriaxone (CRO 30 µg) Cefepime (FEP 30 µg). [At the same time all isolates were resistant to Amoxicillin (AML25 µg) and Ampicillin (AMP 10 µg). The sensitivities to other antibiotics were in between].

*Klebsiella pneumonia*e, *Proteus mirabilis* and *Enterobacter asburiae* 16S ribosomal RNA gene were registered after the correspondence of the National Center for Biotechnology Information and obtained accession number and became a reference to Iraq and the Middle East and the world.

The levels of contamination exceeded the limit level depended in this field. The highest contamination was with *E. coli* and *Klebsiella.* The isolates showed multiple antibiotics resistant Amoxicillin (AML25 µg) and Ampicillin (AMP 10 µg). Microorganisms that are transmitted through food from the family of Enterobacteriaceae, such as poultry meat. These organisms lead to diseases transmitted through contaminated food. example, shigellosis, Salmonellosis, the runs etc. Chloramphenicol showed the highest zones of inhibition, and all the *E.coli , Klebsiella ,proteus , Enterobacter salmonella Shigella Serratia* were susceptible to it, hence it should be preferred over novobiocin,. Which showed resistance in all . Sensitivity of Enterobacteriaceae group of microorganisms to known antibiotics is decreasing. Decreased sensitivity to carbapenem group of antibiotics is a matter of concern. The study that was conducted on fresh local chicken meat and frozen chicken meat of the Turkish type, the results show the pollution rate in both types is very high, and the authorities must. Regulatory and

health authorities move quickly to prevent this contamination in the markets. The spread of the Iraqi picture is very high .

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Abbreviation	Key or full name

BLAST	Basic local alignment search toolp
BP	Base Pair
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
ESBL	extended spectrum Beta lactamase
EDTA	Ethylene Diamine Tetra-acetic acid
EMB	Eosin Methylene blue Agar
EtBr	Ethidium Bromide
ID	Identifier number
Mac	MacConkey Agar
µm	Micromole
Mg	Milligram
MI	Mole
mM	Mill Molar
MDR	Multi Drug Resistant
NCBI	National Center for Biotechnology Information
nM	Nano molar
PBS	Peptone Buffer Solution
PCR	Polymerase Chain Reaction
RPM	Revolutions per minute
RNA	Ribonucleic Acid
SS	Salmonella Shigella Agar
TSI	Triple Sugar Iron
Taq	Themus aquaticus
TAE	Tries-acetate EDTA
TBC	Total bacterial count
TC	Total count
TE	Tries-EDTA
Tm	Melting temperature
TDB	Dry Block thermostat
XLD	Xylose-lysine Desoxycholate Agar
UV	Ultra Violet
WHO	World Health Organization

List of abbreviation

1.1. Introduction:

Food safety issues are becoming more important in international trade (Kenneth H. Mathews, Jr. *et al 2014*). Outbreaks of food – borne diseases have leading to

considerable illness and even death (Quintavalla and Vicini 2002). It has found that every year there are between 24 to 81 million cases of food- borne illness every year and out of which 50% are associated with meat and poultry (Gravani 1987; Mcbean, 1988; Albrecht 1986)

The increase in chicken meats' consumption is associated with the high nutritional value, lower cost, conveniences, and various aspects for the consumer (Islam *et al*., 2004).

It is believed that meat from healthy animals is free from microorganisms. Meat contamination occurs due to the handling of raw meat and transmitted by the liquid in raw meat more than cooked meat (Javadi and Saeid, 2011; Koffi-Nevry *et al*., 2011; Darshana *et al.*, 2014).

Slaughter shops in the markets usually has various sources of meat contamination with microbes as skin, attached soil, and the contents of the animal's digestive tract. Fecal materials are a significant source of contamination via direct deposition and indirect contact through contaminated tools used in slaughtering, cutting and transport, as well as people working in the slaughterhouses (Holzapfel, 1998; Borch and Arnder, 2002; Mead, 2004; Salihu *et al.,* 2010; Keshab, 2015).

The refrigerated poultry meat would be spoilage when stored for an extended period due to the microorganism actions and the biochemical transformations inside the product (Asghar *et al.,* 1988).

In Iraq, poultry slaughtered manually, therefor contaminated by different microorganisms bacteria, fungus even parasites from soil or contaminated earth with other poultry wastes (Fayad and Naji, 1989).

Multiplex PCR technique is used to identify the organisms (Edel *et al*., 2008; Lee *et al*. 2009). This technique detects the microorganism with high sensitivity

and specificity (Anbazhagan *et al*., 2010). The mechanism work of PCR depends on replicating the DNA segment for the identification of microorganisms (White *et al*., 2013).

1.2. Aims of study:

1. Isolate and Identified Enterobacteriaceae of zoonotic importance from chicken's meat.
2. Simultaneously detect some of the Enterobacteriaceae isolates from chicken's meat through Multiplex PCR based method.
3. Determine the level of meat contamination
4. Determine the sensitivity of isolates to most commonly used antibiotics.

2. Literature Review

2.1. Introduction

Poultry meat is considered a main source of meat in the world (Kearney, 2010). Chicken and turkey are the major types of poultry meat. Chicken meats comprise