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College of Veterinary Medicine

Isolation and Identification of the most common zoonotic bacteria from workers, meat and meat products with study the pathogenicity of *Klebsiella pneumoniae* in Rabbits

A Thesis

Submitted to the Council of the College of Veterinary Medicine, University of Diyala in Partial Fulfillment of the Requirements of the Degree of M.Sc. in Internal and Preventive Medicine- Zoonosis

By

Jasim Hadi Mousa Al-Anbagi

Supervised by

Prof. Dr. Al-Khafaji Nazar Jabbar

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بسم الله الرحمن الرحيم

نَرْفَعُ دَرَجَاتٍ مَّنْ نَّشَاءُ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ

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I certify that this thesis which is entitled **(Isolation and Identification of the most common zoonotic bacteria from workers, meat and meat products with study the pathogenicity of *Klebsiella pneumoniae* in Rabbits)** has been prepared under my supervision at the Department of Medicine, College of Veterinary Medicine, University of Diyala, in a partial fulfillment of the requirements for the Degree of M.Sc. in Veterinary Medicine- Zoonosis.

Supervisor

Prof. Dr. Nazar Jabbar Al-Khafaji

College of Veterinary Medicine

University of Diyala

2020

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

Assist. Prof. Dr. Khalid Ibrahim Abd

Vice Dean of post graduate studies and science affairs

College of Veterinary Medicine

University of Diyala

2020

Examination Committee Certification

We, the examining committee, certify that after reading this thesis entitled **(Isolation and Identification of the most common zoonotic bacteria from workers, meat and meat products with study the pathogenicity of *Klebsiella pneumoniae* in Rabbits)** was prepared by Jasim Hadi Mousa Al-Anbagi 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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Member

Prof. Dr. Nazar Jabbar AL-Khafaji

Member and Supervisor

Approved by the council of the College Veterinary Medicine - University of Diyala

Assist. Prof. Dr. Mohammad Yousif Mahmood

Head of Department of

Internal and Preventive Medicine

College of Veterinary Medicine University of Diyala

2020

Prof. Dr. Talib Jawad Kadhim

The Dean College of

Veterinary Medicine

University of Diyala

2020

Dedication

I would like to dedicate this work to

My beloved father and mother, their encouraging, directing words and moral support to reach success and progress

My wife and children who supported me during study.

My brothers and sisters who were supporting me throughout my life.

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Summary

To investigate the common zoonotic bacteria that contaminated meat and meat products during handling, processing and transportation, till it reached the consumers, in Diyala province, Iraq. A total 251 samples were collected, 35 sheep's and cow's meat and meat products, 41 samples poultry's meat, and 175 swabs from workers and their equipment that are used in butchery shops, in the period from August, 2019 to April, 2020.

The samples submitted to laboratory investigation to isolate and identified , the contaminated bacteria, according to their cultural morphology and biochemical properties. Count the total viable bacteria. In addition to study the sensitivity of these bacteria to sixteen commonly used antibiotics. Moreover, the pathogenicity of *Klebsiella pneumoniae,* which was one of the common isolates in current study, was studied in rabbits.

The results revealed that *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *E. coli, Salmonella* spp., *Listeria*, *Enterobacter*, *Citrobacter*, *Proteus* spp., *Yersinia* and *Shigella* were common isolates. In 175 isolates, from workers and equipment, *Klebsiella* spp. was the predominant bacteria 44 (25.1%); followed by *Staphylococcus* spp. 42 (24.0%); *E. coli* 34 (19.4%); *Pseudomonas* spp. 26 (14.9%); *Proteus* spp. 14 (8.0%); *Citrobacter* and *Yersinia* each of 8 (4.6%); *Enterobacter* 7 (4.0%); *Streptococcus* 6 (3.4%); *Salmonella* spp. 4 (2.3%); *Listeria* 3 (1.7%) and *Shigella* 1 (0.6%).

From sheep's and cow's meat, 22 isolates were isolate, from which , *Staphylococcus* spp. was the highest, 12 (54.5%); followed by *Klebsiella* 72 (31.8%); *Pseudomonas* 6 (27.3%); *Listeria* 4 (18.2%); *E. coli* and *Salmonella* each of 3 (13.6%). From sheep's and cow's meat products 13 isolates were isolated, from *Staphylococcus* 7 (53.8%); was the highest

isolate; followed by *Klebsiella* 6 (46.2%); *Pseudomonas* 3 (23.1%); *Listeria* 2 (15.4%); *E. coli*, *Citrobacter* and Enterobacter each of 1 (7.7%). Total isolates from sheep's and cow's meat and meat products was 35; from which *Staphylococcus* 19 (54.3%); *Klebsiella* 13 (37.1%); *Pseudomonas* 9 (25.7%); *Listeria* 6 (17.1%); *E. coli* 4 (11.4%); *Salmonella* 3 (8.6%); *Citrobacter* and *Enterobacter* each of 1 (2.9%).

From poultry's meat and meat products 41 isolates was isolated from which , the highest isolate was *Staphylococcus* 16 (39.0%); followed by *E. coli* and *Salmonella* each of 12 (29.3%); *Klebsiella* and *Pseudomonas* each of 9 (22.0%); *Listeria* 7 (17.1%); *Citrobacter* and *Enterobacter* each of 5 (12.2%).

The order of the bacterial species isolated from the 251 samples were as the follows: *Staphylococcus* 77 (30.7%); *Klebsiella* 66 (26.3%); *E. coli* 50(19.9%); *Pseudomonas* 44 (17.5%); *Salmonella* 19 (7.6%); *Listeria* 16 (6.4%); *Citrobacter* and *Proteus* 14 (5.6%); *Enterobacter* 13 (5.2%); *Yersinia* 8 (3.2%); *Streptococcus* 6 (2.4%) and *Shigella* 1 (0.4%).

The highest number was of isolates obtained from workers 197/251 (78.5%); then those from poultry's meat and meat products 75/251 (29.9%); and from sheep's and cow's meat and meat products 56/251 (22.3%).

The total number of bacterial isolates was 328 from which there were 134 (40.9%) isolates in a single form, while the remaining were in more than one isolate; two isolates 110 (33.5%) and three isolates 84 (25.6%).

The highest viable bacterial counts was from chicken's raw meat (8.34±0.02 $\log_{10}$ cfu /g); followed by Roast meat (8.32 ±0.06); sheep's raw meat (8.31±0.03); cow's raw meat (8.30 ± 0.07); sausages from chicken (8.29±0.02); hamburger (8.22 ±0.03); liver of chicken (8.20 ±0.03); burger from chicken (8.16±0.06); shawerma from chicken (7.89±0.04); kebab from

chicken (7.85±0.05); kebab from meat (7.77±0.05); worker's ear (4.81±0.04) and the lowest count was from worker's hand(4.78 ±0.07). While coliform count were as follows: chicken 's raw meat (8.27±0.01); roast meat (8.27±0.05); sheep's raw meat (8.24±0.01); cow's raw meat (8.15±0.01); sausage – chicken (8.11±0.08); hamburger (8.08±0.02); liver chicken (8.10±0.05); burger – chicken (8.04±0.07) shawerma – chicken (7.79±0.03) ; kebab chicken (7.80±0.01); kebab meat (6.57±0.02); worker's ear (4.79±0.02) and worker's hand (4.72±0.01).

All tested isolates were sensitive to Norfloxacin (Nor 10) and Gentamycin (Cn10) except *Listeria.* But to Clindamycin (Da10); Cloxacillin (Cx10); Metromidazole (Met 30); Rifampin(RA 54); Amoxicillin (Ax10) ; Piperacillin (Prl 100) all isolates were resist. The tested isolated exhibit a resistant to 2/3 of tested antibiotics (9-12 antibiotics) and *Listeria* was resist to all tested antibiotics.

Clinically the animals exposed to *Klebsiella pneumoniae* exhibits signs of anorexia, depression, dyspnea, engorge blood vessels, three of them died. Heart rates and respiratory rates increased, body temperature and body weight non - significantly changed.

Hematologically; Packed cell volume (PCV%) decreased, Hb showed no significant changes. Total leucocyte count significantly increased. Lymphocytes% increased significantly. Heterophils increased in Monocytes significantly increased. Basophils% and Eosinophils% no significant changes.

Postmortem findings: The main gross lesion were: Lung sever, congestion, with presence of fluid and un-clotted blood. Heart, flabby, enlarged, severely congested. Liver enlarged and congested. Patchy hemorrhages on gastric mucosa, sever congestion of gastrointestinal mucous membranes. Kidneys enlarged and congested, with retention of urine.

Histopathologically: Lung showed expansion of alveoli and destruction of the alveolar walls, this referred to pulmonary emphysema. Amyloid present in some area of the lung, Necrosis, destruction of alveoli, hemorrhage in alveolar wall and thickness of alveolar wall in some area lung showed infiltration of the inflammatory cells Heterophils with a few mononuclear cells.

Heart showed infiltration of fibrocytes in the myocardium Liver showed necrotic area, Kupffer cells a rounded glomeruli with the fatty vacuoles, hepatocyte hyperplasia, and presence of fatty change. Kidneys showed infiltration of inflammatory cells (Heterophils), with a few of mononuclear cell (lymphocytes and mesengial cells.

Stomach showed amyloid deposition in the lamina propria with hyperplasia in mucosal gland.. Small intestine showed hemorrhage nearly the microvilli of intestine, and presence of vacuolation in submucosal layer, this result in hemorrhagic enteritis.

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List of Abbreviations

Aberrations	Key or full name
μm	Micromole
ANOVA	Analysis of variance
B cells	B lymphocyte
BPW	Buffer peptone water
CFU	Colony forming unit
EFSA	European Food Safety Authority
GIT	Gastrointestinal tract
Gm	Gram
H_2S	Hydrogen sulphate
H_2O	Water
ID	Infective dose
IND	Indol test
mg	Milligram
ml	Mole
MLNS	Mesenteric lymph node
T cells	T lymphocyte
TSI	Tribble sugar iron
URE	Urease test
VP	Voges–Proskauer
USA	United States America
UK	United kingdom
ICMSF	International Commission and Microbiological Specification for Foods
G	Grams
S	Second
CDC	Centers for disease Control and Prevention
PW	peptone water
M ± S.E	mean ± standard error

Chapter one
Introduction

1.1. Introduction

Consumers are looking for healthy food with high nutritional value, red meat is one of the most important food for humans, which containing high levels of proteins, fats, and vitamins (Prescott., 2002 ; Yaseen, 2006) .Contaminated raw meat and meat products causes (90%) of food-borne illnesses in the human and animals (Barbuddhe *et al*.,2003; Bhandare *et al*.,2007; Podpečan *et al*.,2007; Arul and Saravanan, 2014).

local markets in Iraq usually contains various kinds of food including meat and meat products from several countries regardless of whether or not this food is valid for consumption by human (Jarallah, *et al*.,2014 ; Jarullah, 2015).The inappropriate storage, transport and retail marketing of these food products can increase the opportunity of contamination with various biological, chemical and physical contaminants, this threat of food contamination may lead to serious health complications, particularly by the imported food (Al-jasser,2012;Haleem,*et al.*,2013).

The refrigerated chicken meat can be contaminated during the long period of storage due both of actions of microorganism and the biochemical changes inside such meat (Octavian, *et al.*,2010; Al-jasser,2012 ; Noori, and Alwan, 2016).

There are several factors contribute to the increased zoonotic infections including Temperature abuse, eating habits, storage and transport for long distances (Hedberg, *et al*.,1992). World Heath Organization has defined the food-borne diseases as these disease that are occurred due to consumption of contaminated food by infectious bacteria or their toxin (le Loir *et al*., 2003).

The International Meat Secretariat Newsletter (2005) reported that meat consumption has been increased dramatically in the last 2-3 decades due to the improvement in the living standards, the increase in the meat demands is attributed to the modern urbanization, high personal income , Therefore, the

meat hygiene has become one of the major concerns of the society in this era (Sofos, 2008).

Meat from healthy animals is free of microorganisms cross contamination of meat and meat products can occurs during different stages of processing , handling, and storage , particularly chicken meat due to its high fluid contents compared to cooked ones (Javadi and Saeid, 2011; Koffi-Nevry *et al*., 2011; Darshana, *et al.*,2014).

Klebsiella pneumonia causes infections to nasal mucosa and pharynx that lead to primary pneumonia which is called nosocomial infection, this type of pneumonia contributes to (30-33%) of cases in the surgical and medical intensive care units (Richards *et al*., 2000; Ko *et al*.,2002). This pathogen can produce beta lactamase which is enable this bacteria to resist various antibacterial drugs (Abdulhasan *et al*., 2015). Hyper-virulent strain of *K. pneumonia* is highly infectious to healthy human which can lead to death or community-acquired bacterial infections such as meningitis, pyogenic liver abscess, necrotizing fascilitis, pneumonia and endophthalmitis (Shon and Russo . 2012; Shon *et al*., 2013).

There is little studies of the contaminated bacterial species in the raw meat of beef, lamb and chicken meat and their processed products in Diyala province. Thus, the current study was designed to isolation and identification the most of common zoonotic bacterial species and determination the level of contamination in the meat and meat products with sensitivity of these bacterial species to the most commonly used antibiotics ,with special emphasis to studying the pathogenicity of *Klebsiella pneumoniae* in the rabbit.

1.2 The aims of current study were:

1. Isolation and identication the most common zoonotic bacteria from sheep, cows, poultry meat , meat products , workers and their equipment.
2. Counting the viable bacteria in the meat and meat products
3. Determtion the sensitivity of isolates to most commonly used antibiotics.
4. Studying the pathogenicity of *Klebsiella pneumoniae* in rabbits