

Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Diyala

College of Veterinary Medicine



**Isolation and Identification of Certain Zoonotic Bacteria
from Human, Milk, and Milk Products with study the
Pathogenicity of *Salmonella typhimurium* .**

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

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2020 A. D.

1442 A.H.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً ۖ نُسْقِيكُمْ مِمَّا فِي بُطُونِهِ مِنْ
بَيْنِ فَرْثٍ وَدَمٍ لَبَنًا خَالِصًا سَائِغًا لِلشَّارِبِينَ (66)

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We certify that the thesis entitled (**Isolation and Identification of Certain Zoonotic Bacteria from Human, Milk and Milk Products with study the Pathogenicity of *Salmonella typhimurium***) has been prepared under my supervision at the Department of Medicine, College of Veterinary Medicine, University of Diyala, as partial fulfillment of the requirements for the Degree of Master of Science in Internal and Preventive Medicine- Zoonosis.

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Dedication

*To the spirit of my father who planted in us the love of science from
childhood*

To my angel in life...

*To the meaning of love and the meaning of compassion and
dedication ... To the smile of life and the secret of existence my*

Mother...

To whom I see optimism and happiness in their laughter...

*To those who looked forward to my success, my brothers and
sister...*

I dedicate this humble Work.

Rofeida

Acknowledgment

Firstly, thanks to ﷻALLAHﷻ, the most gracious, the most merciful, for helping me to finish my study.

I am heartily thankful to my supervisor **Prof. Dr. Nazar Jabbar AL-Khafaji** for all of his time, advice, and guidance throughout my study.

I would like to thank the Deanery of the College of the Veterinary Medicine ﷻ University of Diyala, represented by **Prof. Dr. Talib Jawad Kadhim** for their outstanding help and support.

I would like to express my appreciation to **Msc walaa Mahmood** for her help in many areas of the project.

My thank should go to **Msc Mohammad Abed** for their help and guidance in the anatomical and Histopathological procedure.

Great thanks also extend to the staff members in Laboratories of College of Veterinary Medicine, the University of Diyala, especially **Mas Salman**, for their support.

I would like to express my appreciation for the **Assist. Prof. Dr. Raad Mahmood Al-Zubaedi** for his kindness help in the practical parts of the project.

Special thanks go to my best friend, **Jassim Hadi** and **Mohammed Jassim**, for their encouragement and help.

Finally, I would like to thank everyone who helped me and apologies to those I forgot to mention.

Summary

To investigate, the bacteria of zoonotic importance, contaminated milk, and milk products, a total of 1000 samples, represent 100 raw milk from cows, buffaloes, sheep, and goats; 100 milk products, cheese, yogurt, and 100 swabs from workers and their equipment in retail shops of milk products, were collected in the period from August 2019 to April 2020. The samples were submitted to laboratory investigations, for isolations and identifications of contaminated bacteria, according to their cultural morphology, biochemical properties. Counting of total viable bacteria. Sensitivity to sixteen antibiotics. In addition to investigating the pathogenicity of *Salmonella typhimurium* which was one of the common isolates in the current study. The results revealed, that from 200 isolates that were isolated in the current study; the highest isolate was *Staphylococcus* spp. 100 (20.0%) followed by *Salmonella* spp. 80 (40.0%) *Lactobacillus* 22 (2.2%) *Pseudomonas* spp. and *E. coli* each 20 (10.0%) *Klebsiella* spp. 2 (1.0%) *Enterobacter* 2 (1.0%) *Proteus* spp. and *Citrobacter* each 1 (0.5%) *Streptococcus* 1 (0.5%) and *Listeria* 1 (0.5%). From a total of 100 raw milk samples collected from the udder and bulk tank (cows, buffaloes, sheep, and goats), 102 isolates were isolated; from which the highest isolate was *Salmonella* spp. 20 (19.6%) followed by *Staphylococcus* spp. 20 (19.6%) *Lactobacillus* 22 (21.6%) *Enterobacter* 10 (9.8%) *E. coli* 10 (9.8%) *Citrobacter* and *Proteus* each 2 (1.96%) *Klebsiella* 1 (0.98%) and *Pseudomonas* 1 (0.98%). While from a total of 100 samples of milk products (Cheese and Yogurt), 102 isolates were isolated, the highest isolate was *Lactobacillus* 22 (21.6%) *Salmonella* 2 (1.96%) *E. coli* 10 (9.8%) *Staphylococcus* 1 (0.98%) *Pseudomonas* 1 (0.98%) *Proteus* 1 (0.98%) *Klebsiella* 1 (0.98%) *Enterobacter* 1 (0.98%) and *Citrobacter* 1 (0.98%). Meanwhile, from a total of 100 swabs obtained from

workers and their equipment, 11 isolates were isolated. The highest isolate was *Staphylococcus* 22.0 followed by *Pseudomonas* 20.0, *Klebsiella* 20.0, *E. coli*, and *Streptococcus* each 10.0, *Listeria*, *Enterobacter*, and *Citrobacter* each 5.0.

From a total of 100 samples, 20 samples were free from isolates, while from others, 80 samples 80% 20 isolates were isolated, from which 20% were in a single form, and others in more than one isolate in a sample—two isolates 20%, three isolates 20% and four isolates 20%. The highest isolates were from raw milk 20%, then, workers 20%, and milk products 20%.

The highest level of contamination was in cow's raw milk, as the count was $10^{10.0}$ log₁₀cfu/ml followed by buffalo 10^{9.0} yogurt 10^{8.0} cheese 10^{7.0} worker's ear 10^{6.0} and the lowest count was from worker's hand 10^{5.0}. While the coliform counts were in cow's raw milk 10^{10.0} buffalo's raw milk 10^{9.02} yogurt 10^{8.0} cheese 10^{7.0} worker's ear 10^{6.0} and worker's hand 10^{5.0}.

The sensitivity of isolates in the current study to sixteen commonly used antibiotics was of high variations. All the isolates were resistant to Clindamycin (DA), Cloxacillin (cx) and Piperacillin (pr) but all isolates were sensitive to norfloxacin or except *Staphylococcus* and *Listeria*. Also, about 20% of antibiotics were resisted by the tested isolates, and the *Listeria* was the isolate that resists nearly all tested antibiotic.

Clinically the animals exposed to *Salmonella typhimurium* exhibit signs of depression, anorexia, isolation from other animals, soft feces.

Heart rates significantly decreased in those of group II, but increased in rabbits of group III. Respiratory rates significantly increased in group II and group III. Body temperature non-significantly increased in group II and III body weight non-significantly decreased in group II and III.

Hematologically total leucocytes count was significantly increased in both group II and III. While Lymphocytes increases in those of group II then decreased, and in group III increased. Meanwhile, neutrophils decreased in group II then increased, but in group III increased then decreased. Monocytes significantly increased in both group II and III. Basophils and Eosinophils showed no significant changes.

The main gross lesions were in the liver, lung, heart, kidneys, and gastrointestinal tract. Severe congestions, with enlargement of the liver, heart flabby, enlarged, congested, gastrointestinal tracts congested, lungs emphysematous, congested. Kidneys are enlarged and congested.

The main histopathological changes were the stomach showed desquamation of mucosal surface with the bleeding, different areas in the mucosal and submucosal layers showed an area of fibrosis and infiltration of mononuclear cells neutrophils hyperplasia of the mucosal gland hemorrhagic gastritis Small intestine showed thread of mucus from the goblet cells, vacuolar degeneration of mucosal glands in the submucosa of the small intestine, and hemorrhage, mucosal erosion, and hyperplasia of peyer's patchy in the submucosa. Lungs showed edematous fluid with erythrocytes, emphysema which is characterized by highly alveolar wall destruction and thickening of the alveolar wall in some areas interstitial pneumonia due to the infiltration of inflammatory cells neutrophils with a few mononuclear cells. The kidney showed focal intertubular mononuclear cells MNCs infiltration and many vacuoles, infiltration of

multinuclear cells [mesengial cells] and severe vacuolar degeneration of occluded tubules, hemorrhagic area, the infiltration of mononuclear cells, and vacuolation, hemorrhagic nephritis. Heart showed infiltration of inflammatory cells in the interstitial and presence of vacuole.

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List of abbreviations

Aberrations	Key or full name
A□□VA	Analysis of variance
□ cells	□ lymphocyte
C□U	Colony forming unit
EM□	Eosin Methylene blue agar
E□SA	European □ood Safety Authority
E□SA	European Food Safety Authority “
□I□	□astrointestinal tract
gm	□ram
H2S	Hydrogen sulphate
I□D	Indol test
ID	Infective dose
Mac	MacConkey Agar
MSA	Mannitol Salt Agar
ML□S	Mesenteric lymph node
□m	Micromole
mg	Milligram
ml	Mole
□SPIs)	Salmonella pathogenicity islands
SS	Salmonella Shigella Agar
□SPC□	standard plate count
□ cells	□ lymphocyte

Chapter one

Introduction

1.1. Introduction

Milk and milk products are a source of numerous essential nutrients. Chemically, milk is a complex mixture, being rich in proteins, fats, carbohydrates, vitamins, minerals, essential amino acids, and other constituents (Haug *et al.*, 2007; Marjan *et al.*, 2014; van Hooijdonk and Hettinga, 2015). The high nutrient content of milk, all at a near-neutral pH and a high water activity, provides an ideal environment for the growth of many microorganisms. (Haridy, 1992; Frank, 1997; Sangoyomi *et al.*, 2010). Milk may serve as an ideal substrate for the growth and survival of an array of bacteria and fungi, thereby leading to the public health threat (Zucali *et al.*, 2011; Aebi *et al.*, 2015).

Milk secreted from healthy animal's udder is sterile, becomes contaminated throughout the milking, chilling, and loading (Younan *et al.*, 2005). Bacterial contamination of raw milk can originate from different sources, air, milking equipment, feed, soil, feces, and grass (Coorevits *et al.*, 2008). Microbes on the surface of milking and chilling device, milking workers, the environment of milking animals, and water used in different steps of processing are considered as a common source of contamination (Mbabazi, 2005).

The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed, and animal health (Rogelj and Mleko, 2003). Rinsing water for milking machines and milking equipment washing involves some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley and McKinnon, 1990).

Pathogenic bacteria are transmissible to humans through milk and milk products. attention is focused on milk, cheese, and ice cream contaminated

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with pathogenic bacteria, viz, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and enteropathogenic *E. coli* (Dasavasda, 1988).

1.2. Aims of the study:

1. Isolation and identification of the most common zoonotic bacteria from milk and milk products of, cows, buffaloes, sheep, and goats.
2. Total viable bacterial counts in milk and milk products
3. Determining the sensitivity of isolates to the most commonly used antibiotics.
4. Study the pathogenicity of one of the most common isolates in rabbits.