



Cairo University
Faculty of Veterinary Medicine
Department of Microbiology



**Sources of bacterial contamination in slaughterhouses with
special reference to the methods of their control**

Thesis presented by

Ahmed Hany Mohamed Fathy

(B. V. Sc., Faculty of Veterinary Medicine, Cairo University, 2003)

(M. V. Sc., Faculty of Veterinary Medicine, Cairo University, 2012)

**For Ph. D. Degree in
Veterinary Medical Science
(Microbiology)**

Under Supervision of

Prof. Dr. Saad Ahmed Attia
Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Mahmoud Essam Hatem
Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Osama Kamel Zahran
Professor of Animal, Poultry and Environmental Hygiene
Faculty of Veterinary Medicine
Cairo University

Dr. Ahmed Samir Shehata
Assistant Professor of Microbiology
Faculty of Veterinary Medicine
Cairo University

2018



Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Name: Ahmed Hany Mohamed Fathy

Date of birth: 12/10/1981

Degree: Ph.D.

Supervisors:

Prof. Dr. Saad Ahmed Attia – Prof. of Microbiology, Fac. of Vet. Medicine - Cairo Univ.

Prof. Dr. Mahmoud Essam Hatem – Prof. of Microbiology, Fac. of Vet. Medicine - Cairo Univ.

Prof. Dr. Osama Kamel Zahran – Prof. of Animal Hygiene, Fac. of Vet. Medicine - Cairo Univ.

Dr. Ahmed Samir Shehata – Assistant Prof. of Microbiology, Fac. of Vet. Medicine - Cairo Univ.

Nationality: Egyptian

Place of birth: Giza

Specialization: Microbiology

Title of thesis: Sources of bacterial contamination in slaughterhouses with special reference to the methods of their control.

Abstract:

A swab samples were collected from different suspected sources of bacterial contamination in El-Monieb slaughterhouse in Giza Governorate. The results showed that out of the 345 samples, *S. aureus* was detected in 40.6%. The mean count between the different sources of contamination was $4.9 \pm 1.5 \log^{10}$ CFU/ cm². The antimicrobial susceptibility pattern showed that all isolates of *S. aureus* have antimicrobial resistance. By PCR amplification, *mecA* was detected confirming the isolation of methicillin resistant *S. aureus* in percentage 13%. *E. faecalis* were isolated by 35.4% with an average mean count $5.73 \pm 1.7 \log^{10}$ CFU/ cm². The antimicrobial susceptibility pattern showed that all isolates were also resistant. The *vanA* gene was detected confirming the isolation of vancomycin resistant *E. faecalis* in 21.3%. *Salmonella* was isolated in 1.4%. There were different sources of contamination in the slaughterhouse before, during and after the slaughtering process which could threaten public health and play a potential role in food safety. Different disinfectants were tested by spraying method to decrease the bacterial load on the surface of the utensils used in slaughtering, floor and walls inside the slaughterhouse. H₂O₂ plus peracetic acid was found to be the most effective disinfectant.

Key words: bacterial contamination - slaughterhouses – control of bacterial contamination in slaughterhouses – MRSA – VRE – *Salmonella* – *mecA* – *vanA* – *invA* – relative risk - disinfectants - H₂O₂ plus peracetic acid – CO₂ dry ice under pressure.

Dedication

*I would like to express my sincere gratitude and thanks to **My Family***

My Thanks and highest consideration for my Mother, my Father, my wife and my cute children Hana and Mostafa.

Special Thanks for my sister and my brother

I appreciate their encouragement and support. I pray with supplication for all of them to achieve pleasure and success in their life

Acknowledgement

*I would like to express my sincere gratitude and thanks asking for mercy to soul of my **Professor Dr. Mahmoud Essam Hatem Ahmed** for his valuable supervision and his great support throughout this work.*

*I would like to express the assurance of my highest consideration and sincere gratitude and thanks to my **Professor Dr. Saad Ahmed Atteya** for his valuable supervision and his great support throughout this work.*

*I would like to express the assurance of my highest consideration, my sincere gratitude and thanks to **Professor Dr. Osama Kamel Zahran** for his valuable technical backstopping.*

*I would like to express my consideration, sincere gratitude and thanks to **Professor Dr. Ahmed Samir Shehata** for his active and helpful guidance during work.*

I acknowledge the efforts of all who play a recognized role to help me.

Contents

Chapter (1): Introduction	1
Aim of the work	4
Chapter (2): Review of literature	5
Determination of sources of bacterial contamination in slaughterhouse	5
Microbiological characteristics and public health significance	27
PCR detection of antimicrobial resistance genes and common genes	32
Microbiological risk assessment	38
Methods of contamination control in slaughterhouses using different disinfectants	40
Chapter (3): Material and methods	48
Chapter (4): Results	83
Chapter (5): Discussion	122
Chapter (6): Conclusion and recommendations	132
Chapter (7): Summary	135
Chapter (8): References	137

List of tables

No.	Tables	Page
1.	Number of swabs / samples, sampling site and time of sampling in cattle hall	54
2.	Interpretation of species results of KB004 HiStaph Identification Kit	59
3.	Results interpretation chart of the color results “KB004 HiStaph Identification Kit”	60
4.	Interpretation of species results of KB005A HiStrep Identification Kit	63
5.	Results interpretation chart of the color results “KB005A HiStrep Identification Kit”	63
6.	Interpretation of <i>Salmonella</i> species using KB003 Hi25 <i>Enterobacteriaceae</i> Kit (HiMedia)	68
7.	Results interpretation chart of the color results “KB003 Hi25 <i>Enterobacteriaceae</i> Kit”	69
8.	Reference primer for <i>mecA</i> gene	74
9.	Reference primer for <i>vanA</i> gene	75
10.	Reference primer for <i>invA</i> gene	76
11.	Name, commercial name and composition of the used disinfectants	79
12.	Number & percentage of <i>S. aureus</i> isolates from different sampling sites in the slaughterhouse	88
13.	Total Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and % prevalence of <i>S. aureus</i> in the different sources of sampling in the	89

	slaughterhouse	
14.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of <i>S. aureus</i> in the different sampling sites from the animals before and after slaughtering process	91
15.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of <i>S. aureus</i> in the different sources of sampling from butchers and utensils before and after slaughtering process	92
16.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of <i>S. aureus</i> in the different sources of sampling from the slaughterhouse environment	93
17.	Number & percentage of positive <i>E. faecalis</i> isolates from different sampling sites in the slaughterhouse	98
18.	Total Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ Standard deviation), 95% confidence interval of mean and % prevalence of positive <i>E. faecalis</i> in the different sources of sampling in the slaughterhouse	99
19.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of positive <i>E. faecalis</i> in the different sampling sites from the animals before and after slaughtering process	101
20.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of positive <i>E. faecalis</i> in the different sources of sampling from butchers and utensils before and after slaughtering process	102

21.	Mean counts (\log^{10} CFU/ $\text{cm}^2 \pm$ standard deviation), 95% confidence interval of mean and prevalence of positive <i>E. faecalis</i> in the different sources of sampling from the slaughterhouse environment	103
22.	No. (%) of <i>S. aureus</i> isolates showing antimicrobial susceptibility pattern	107
23.	No. (%) of <i>E. faecalis</i> isolates showing antimicrobial susceptibility pattern	107
24.	Number of methicillin resistant <i>S. aureus</i> (MRSA) isolates recovered from different sources in the slaughterhouse	110
25.	Number of vancomycin resistant <i>E. faecalis</i> (VRE) isolates recovered from different sources in the slaughterhouse	111
26.	Log reduction in MRSA count after spraying method of different disinfectants on floor and utensils	116
27.	Log reduction in VRE count after using of spraying method of different disinfectants on floor and utensils	118
28.	Log reduction in <i>Salmonella</i> Typhimurium count after using of spraying method of different disinfectants on floor and utensils	119
29.	Log reduction in recovered isolates count after using of spraying method of different disinfectants on floor surface	120
30.	Log reduction in recovered isolates count after using of spraying method of different disinfectants on utensils surface	121

List of figures

No.	Figure	Page
1.	Dry CO2 ice: Extinguisher machine 35 bar	81
2.	Stainless steel coupons: with dimensions 1cm × 2 cm	81
3.	Cement coupons: 2 × 2 × 1cm ³	81
4.	Isolation and counting of <i>S. aureus</i> on Baird-Parker agar with egg yolk plus potassium tellurite 3.5%	85
5.	Microscopical examination of <i>S. aureus</i>	85
6.	Culturing of <i>S. aureus</i> on sheep blood agar	85
7.	Culturing of <i>S. aureus</i> on Mannitol salt agar	86
8.	Coagulase test for <i>S. aureus</i>	86
9.	Culturing of <i>S. aureus</i> on DNase agar	87
10.	HiStaph Identification strip – Biochemical tests for <i>S. aureus</i>	87
11.	Isolation and counting of <i>E. faecalis</i> on KF <i>Streptococcus</i> agar	96
12.	Microscopical examination of <i>E. faecalis</i>	96
13.	Culturing of <i>E. faecalis</i> on sheep blood agar	96
14.	Culture of <i>E. faecalis</i> on bile esculin agar	97
15.	HiStrep Identification strip – Biochemical tests for <i>E. faecalis</i>	97
16.	Isolation <i>Salmonella</i> species on XLD	105
17.	Biochemical identification for <i>Salmonella</i> species	105
18.	Hi25 <i>Enterobacteriaceae</i> Identification Kit – Biochemical tests for <i>Salmonella</i> species	105

19.	Determination of antimicrobial susceptibility for <i>S. aureus</i>	108
20.	Determination of antimicrobial susceptibility for <i>E. faecalis</i>	108
21.	Agarose gel electrophoresis of PCR product for <i>mecA</i> gene (310 bp)	112
22.	PCR for <i>mecA</i> gene	112
23.	Agarose gel electrophoresis of PCR product for <i>vanA</i> gene (732 bp)	113
24.	PCR for <i>vanA</i> gene	113
25.	Agarose gel electrophoresis of PCR product for <i>invA</i> gene (284 bp)	114

List of charts

No.	Chart	Page
1.	Mean counts (\log^{10} CFU/ cm^2) and prevalence of <i>S. aureus</i> in different sources of sampling in the slaughterhouse	90
2.	Prevalence of <i>S. aureus</i> in different sampling sites in the slaughterhouse	93
3.	Mean counts (\log^{10} CFU/ cm^2) and prevalence of positive <i>E. faecalis</i> in different sources of sampling in the slaughterhouse	100
4.	Prevalence of <i>E. faecalis</i> in different sources of sampling in the slaughterhouse	103
5.	Log reduction in MRSA count after using of spraying method of different disinfectants on floor and utensils	117
6.	Log reduction in VRE count after using of spraying method of different disinfectants on floor and utensils	118
7.	Log reduction in <i>Salmonella</i> Typhimurium count after using of spraying method of different disinfectants on floor and utensils	120

List of abbreviations

AMR	Antimicrobial Resistance
AMHB	Aerobic Mesophilic Heterotrophic Bacteria
APC	Aerobic Plate Counts
CFU	Colony Forming Unit
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographic Information System
HACCP	Hazard Analysis Critical Control system
MRSA	Methicillin Resistant <i>S. aureus</i>
QRA	Quantitative Risk Assessment
OIE	World Organization for Animal Health
TCC	Total Coliform Counts
TFC	Total Faecal Counts
TVC	Total Viable Count
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

Chapter (1)
Introduction

Introduction

In Egypt, slaughtering of animals is challenged by severe hygienic problems with different sources of contamination which results in heavy bacterial loads in the slaughterhouse environment leading to contamination of the produced meat. The improper practices such as slaughtering on the ground, skinning and evisceration in the same place with the same equipment are considered a threat to food safety and consumers' health and have been implicated in many cases of foodborne illness. **(Zhao *et al.*, 2001).**

Many microorganisms are excreted in the feces and can be found on the hides and fleeces of the live animal. Bovine carcasses can be contaminated during the slaughter process through the contact with the animal's skin and hair, limbs, blood, stomach, gut contents, bile and other excretions, facilities, equipment and workers' hands and clothes **(Sofos, 2008)**. Effluents from slaughterhouses are known also to contribute in contamination of both surface and groundwater during processing in abattoir. Blood, fat, manure, urine and meat tissues are discharged to the wastewater streams meaning that the abattoir environment and slaughtering processes play a vital role in meat safety **(Bello and Oyedemi, 2009)**.

The common bacterial contaminants in large animal slaughterhouses are *S. aureus*, *E. coli*, *B. cereus*, *P. aeruginosa*, *Klebsiella* species, *Enterococcus* species, *Enterobacter* species and *Citrobacter* species. The higher rate of contamination of meat with these organisms is an indication of unacceptable state of poor hygienic and sanitary practices during slaughtering, transportation, butcher shops and processing **(Haileselassie *et al.*, 2012)**. It is also found that temperature abuses during distribution and meat contamination by infected handlers are important contributors to the post-slaughter contamination of bovine meat **(Eugene *et al.*, 2015)**.

By understanding outbreak data which was published internationally from 1996 to 2005, it is noted that 12.7 % of reported foodborne outbreaks were attributable to bovine meat. *Salmonella* and pathogenic *E. coli*, respectively, were identified as the causal agents in 32.9 and 34.6 % of foodborne outbreaks of bacterial origin attributable to beef (**Scallan *et al.*, 2011**).

Staphylococci are ubiquitous in the environment. Natural populations of staphylococci are associated with skin, skin glands and mucous membranes of warm-blooded animals. They have been isolated from animal products such as meat, milk and cheese, and other sources such as soil, sand, seawater, fresh water, dust and air. Some *Staphylococcus* species are known to be frequently encountered in severe infections. *S. aureus* was considered to be pathogenic by production of variety of enzymes and toxins which are the best known and frequently implicated in the etiology of a series of infections and intoxications in animals and humans (**Harris *et al.*, 2002**). For a long time, *S. aureus* has been a devastating pathogen in hospitals, where it is known for its resistance to several antibiotics and for its ability to infect immunocompromised patients. The ability of *S. aureus* to survive under nutrient limiting and stressful conditions increases its ability to survive and spread in the hospital environment. The spreading of methicillin resistant *S. aureus* (MRSA) outside hospitals has been encountered recently as a major problem. This has been observed, for example, in veterinary staff and farmers. An increasing number of reports showed that food producing animals can be carriers of methicillin resistant *S. aureus* where, more than 4% of antimicrobial resistance in human is thought to be associated with animal sources (**Bergenstrahle, 2015**).

Enterococci can grow and survive in harsh environments, and can persist almost anywhere including soil, plants, water, and food. Enterococci can survive 5 days to 4 months on dry inanimate surfaces and it could be used as indicators of fecal contamination and implicated in outbreaks of foodborne illness.