



Prevalence of Arcobacter species in meat

ThesisPresented

By

Heba Allah Ahmed Mahmoud Ali

(M. V. Sc. 2016, Assiut University)

Submitted for

Ph.D. degree in Veterinary Medical Sciences

Department of Food Hygiene

(Hygiene of Meat, Fish and Their Products and Animal By-products)

Under supervision of

Prof. Dr. TalaatSayedAlyEl-khateibProf. Dr. Ashraf MohamedAbd El-Malek

Professor of Meat HygieneProfessor of Meat Hygiene Department ofFood Hygiene Department of Food Hygiene Faculty of Veterinary Medicine Faculty of Veterinary Medicine Assiut University Assiut University

> **Prof. Dr. Ramadan SayedRefaey** Head Researcher Animal Health Research Institute- Assiut-Lab.

Department of Food Hygiene Faculty of Veterinary Medicine Assiut University

2020

Contents

Subject	page
Introduction	1
Review of literature	5
I-Historical Background to Genus Arcobacter	5
II- General Characteristics	6
III- Isolation and identification of Arcobacter species	8
IV- Incidence of Arcobacterspecies in meat	11
V- Virulence factors of Arcobacters	15
VI-Antibiotic sensitivity and emerging drug resistance	18
VII-Sources of contamination	21
VIII- Ways of transmission in humans	23
VIIII- Public health significance of Arcobacters	24
Antibacterial properties of some plant essential oils against A.butzleri	28
A-Cinnamon	30
B-Rosemary	32
C-Thyme	33
Material and Methods	35
Results	58
Discussion	76
Conclusion and Recommendations	94
Summary	96
References	99
Arabic summary	

List of Tables

NO.	TITLE	PAGE NO.
1	Phenotypic characteristics of Arcobacterspp.	40
2	Antimicrobial discs, concentration and interpretation of their action on the isolated <i>Arcobacter</i> spp.	42
3	Score system for sensory evaluation	50
4	Incidence of <i>Arcobacter</i> spp. in fresh chicken meat samples.	58
5	Incidence of Arcobacter spp. in fresh meat samples	59
6	Incidence of Arcobacter spp. in minced beefsamples.	60
7	Incidence of virulence genes of <i>A. butzleri</i> isolated from the examined samples.	62
8	Antimicrobial susceptibility of Arcobacterspp.	66
9	Antimicrobial resistance profile of Arcobacter strains.	67
10	Minimum inhibitory (MIC) and minimum lethal (MLC) concentrations of different essential oils used against <i>A.butzleri</i> .	70
11	Sensory evaluation of minced meat treated with 0.5% and 1% essential oil of cinnamon.	71
12	Effect of 0.5% and 1% essential oil of cinnamon on <i>A. butzleri</i> in refrigerated mincedmeat.	72
13	Reduction % in <i>A. butzleri</i> count in minced meat treated with 0.5% and 1% essential oil of cinnamon.	74

List of Figures

NO.	Title	Page NO.
1	Comparison between incidences of <i>Arcobacter</i> spp. isolated from different samples.	61
2	Agarose gel electrophoresis of multiplex PCR for characterization of <i>A. cryaerophilus, A.butzleri and A.</i> <i>skirrowii</i> .	63
3	Agarose gel electrophoresis of PCR ofmultiplex tlyA(230 bp) gene as virulence factor for <i>A. butzleri</i> .	64
4	Agarose gel electrophoresis of multiplex PCR ofcadF (283 bp) and irgA gene as virulence genes for <i>A. butzleri</i> .	65
5	Inhibition zones of different essential oils used by agar well diffusion method.	68
6	Petridish plate representing inhibition zone diameter of different essential oils.	69
7	Effect of essential oil of cinnamon on viable cell count of <i>A. butzleri</i> in minced beef during storage at 4 c.	73
8	Reduction % in <i>A. butzleri</i> count in minced meat treated with 0.5% and 1% essential oil of cinnamon.	75

Summary

The present studyreavealed the prevalence of *Arcobacterspp*. in 200samples of meat, including100 poultry meat (50 thighs and 50 breasts), 50 fresh meat and 50 minced meat that were collected from retail shops throughout Assiut City.

Arcobacterwas recovered from 8 % of fresh chicken breast and could be isolated from chicken thigh in a percentage of 14%. The identified species were *A.butzleri*which could be isolated only from 2 % of chicken breast samples and 4 % of chicken thigh samples; *A. cryaerophilus* with a percent of 2 % chicken breast samples and 6 % of chicken thigh samples and *A. skirrowii* which could be isolated from 4 % of each chicken breast and chicken thigh samples.

In case of fresh meat samples *Arcobacter* spp. isolates represented 4 %. The recovered isolates were *A. cryaerophilus* with a percentage of 2% and *A. skirrowii* in a ratio of 2% also, but *A.butzleri* failed to be isolated from fresh meat samples.

Regarding to minced meat samples, *Arcobacter* spp. were recorded in in 6% of the examined samples. In addition, *A.butzleri* and *A. cryaerophilus*were isolated from the positive samples in incidences of 2 % for each. While, *A.skirrowii*was not detected in the examined minced meat samples.

The biochemically identified *A. butzleri*strains were subjected to confirmation by using PCR and the results were compatible with the biochemical identification.

In the present study, PCR was carried out for screening of some putative virulence genes in the isolated *A. butzleri* strains. The obtained data revealed that the detected genes were tlyA and cadF andirgA.

The antibiotic sensitivity test for Arcobacterspp.showed that the resistance of Arcobacterspp. isolates against Penicillin G and Cephalothin was found to 100% followed byOxacillin Sulphamethoxazol(93.7% be and each), Erythromycin(81.3%) and Ampicillin (75%). Besides, resistance to Nalidixic acid (68.8%),Ciprofloxacin (62.5%), Cefotaxime (50%), Amikacin (37.5%) and

Enrofloxacin (31.3%) was evident whereas low percentage of *Arcobacterspp.* isolates demonstrated resistance to Doxycycline (18.8%), Gentamicin and Tetracycline (6.3% each).

An experimental study was conducted to investigate the antimicrobial activity of Cinnamon, Rosemary and Thyme EOin growth media and in minced meat (food model).

The investigation revealed that within the tested antimicrobials; the cinnamon essential oil (EO) was the most efficient against tested *A.butzleri* strain in growth media. The cells were killed by a concentration of 0.5 %. The minimum inhibitory concentration (MIC) of cinnamon EO was 0.25%. Regarding rosemary and thyme EO, MIC was 2 % and 0.5%, respectively. While, the MLC was 4% and 1% for rosemary and thyme EO, respectively.

The essential oil which had the best inhibitory effects against *A.butzleri* in the laboratory medium and in-vitro study, should be chosen and tested for the food model study (in vivo) by using minced meat which inoculated with adjusted initial inoculum of the pathogen ($1 \ge 10^7 \text{cfu/g}$) and stored at 4°C for 24 hr. The obtained results showed that cinnamon EO (0.5 and 1%) has highest inhibitory effect against *A.butzleri* after the 24hr of storage with reductionrate of (99 and 99.98%), respectively. Thesensory evaluation of minced meat treated with these levels showed that the sensory attributes of treated minced beef samples during cold storage (4°C) were improved by using different concentrations of cinnamonEO.compared to the control samples during the storage period.

The public health significance of the isolated *A.butzleri* was discussed and the suggestive measures to protect the consumers were outlined.