

Zagazig University Faculty of Vet. Medicine Food Control Department

## STUDIES ON SOME FOOD BORNE PATHOGENS IN MEAT AND MEAT PRODUCTS

By

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# List of abbreviations

A. flavus	Aspergillus flavus
A. niger	Aspergillus niger
A. terreus	Aspergillus terreus
A.fumigatus	Aspergillus fumigatus
A.ochraceus	Aspergillus ochraceus
A.parasiticus	Aspergillus parasiticus
AGE	Aqueous garlic extract
ALOA	Agar Listeria according to Ottaviani and Agosti
AMEs	Aminoglycoside-Modifying Enzymes
ANOVA	Analysis of variance
APC	Aerobic Plate Count
АРНА	American Public Health Association
ATCC	American Type Culture Collection
BHI	Brain Heart Infusion Broth
BLEB	Buffered Listeria Enrichment Broth
bp	Base pair
CAMP	Christie-Atkins-Munch-Peterson test
CDC	Center of Disease Control and prevention
cfu	Colony Forming Unit
DNA	Deoxy Ribo- Nucleic Acid
dNTP	Deoxy Nucleotide TriPhosphate
E. coli	Escherichia coli
EC	European Commission
Ed.	Edition
EDTA	Ethylene Diamien Tetracetic Acid
EFSA	European Food Safety Agency
Ео	Essential oil
Eos	Essential oils
ES	Egyptian Organization For Standardization
FAO	Food and Agriculture organization

FDA	Food and Drug Administration
g	Gram
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
h	Hour
НАССР	Hazard Analysis and Critical Control Points
HIV	Human Immunodeficiency Virus
hylA	haemolysin
iap	invasive associated protein
ICMSF	International Committee on Microbiological
	Specifications for Foods
ISO	International Organization for Standardization
Kg	Kilogram
L. grayi	Listeria grayi
L. innocua	Listeria innocua
L. ivanovii	Listeria ivanovii
L. monocytogenes	Listeria monocytogenes
L. seeligeri	Listeria seeligeri
L. welshimeri	Listeria welshimeri
Log	Logarithm
Max.	Maximum
MDR	Multidrug resistant
MEA	malt extract agar media
Min.	Minimum
ml	millilitre
MLA	Meat and Livestock Australia
MRSA	Methicillin Resistant Staphylococcus aureus
n	Number of examined samples
NARMS	National Antimicrobial Resistance Monitoring
	System
NCCLS	National Committee for Clinical Laboratory
	Standards

NICD	National Institute for Communicable Diseases
OCLA	Oxoid Chromogenic Listeria agar
OXA	Oxford agar
P.L.	Permissible Limits
PBS	phosphate buffered saline
PCR	Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
pН	Potency of Hydrogen
PI	Propidium iodide
R. equi	Rhodococcus equi
r.p.m.	Round Per minute
RNA	Ribo-Nucleic Acid
RTE	Ready to eat
RT-PCR	Real time polymerase chain reaction
S. aureus	Staphylococcus aureus
SAC	S-allylcysteine
SMAC	S-allyl mercaptocysteine
SPP	Species
ТВА	Thiobarbituric Acid
TSA-YE	Trypticase Soya Agar-Yeast Extract
TSB	tryptone soya broth
TSB-YE	Trypticase Soya broth-Yeast Extract
TVN	Total Volatile Nitrogen
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
°C	Degree Centigrade
μg	Microgram

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## SUMMARY

The objectives of this study were:

#### Part I (survey):

- 1- Evaluate the organoleptic properties of examined raw meat, fresh minced meat, luncheon, beef burger and sausage samples.
- 2- Evaluate the chemical quality (pH, TVN, TBA) of examined raw meat, fresh minced meat, luncheon, beef burger and sausage samples.
- 3- Bacteriological evaluation of examined samples:

a- Detection of aerobic plate count (APC).

b- Isolation and identification of *L. monocytogenes* with molecular characterization of the existed *L. monocytogenes* isolates using PCR for detection of their virulent genes.

4- Mycological evaluation of examined samples: Through isolation and identification of some mould genera. Their ability to produce lipase and protease enzymes was also detected.

### Part II (Experimental study):

Improving the sanitary status of meat and meat products via evaluation the antimicrobial effect of clove, thyme, garlic natural Eos and potassium sorbate in the contaminated minced meat and enhancement of its shelf life.

#### Part I (survey):

A total of 200 raw meat and meat products (minced meat, sausage, beef burger and luncheon) (40 of each) were collected from different

markets at Zagazig city, Sharkia governorate, Egypt. The collected samples subjected organoleptic, chemical microbiological to and were examinations. The examined samples accepted organoleptically and chemically (pH, TVN, TBA) according to the Egyptian Organization for Standardization. Mean values of aerobic plate count (APC) ( $\log_{10}$  cfu/g)  $4.92 \pm 0.18$ ,  $4.98 \pm 0.17$ ,  $4.39 \pm 0.11$ ,  $4.18 \pm 0.14$  and  $3.93 \pm 0.09$  in raw meat, minced meat, sausage, beef burger and luncheon, respectively. The overall isolation rate of Listeria spp. from the examined samples was 25.5% (51 out of 200 samples), the isolation rates from the examined raw meat, minced meat, beef burger, sausage, and luncheon were 22.5%, 27.5%, 55%, 15% and 7.5%, respectively. Serological identification of the isolated Listeria spp. revealed identification of six Listeria spp. namely, L. ivanovii, L. welshimeri, L. innocua, L. seeligeri, L. gravi, and L. monocytogenes. L. ivanovii was isolated only from one sausage sample at 0.5%. L. welshimeri was isolated from 13 out of 200 examined samples (6.5%). The isolation rates of L. welshimeri were 5%, 10%, 7.5%, 5%, and 5% from the examined raw meat, minced meat, beef burger, sausage, and luncheon, respectively. L. innocua and L. seeligeri were isolated from 14 out of 200 examined samples (7%), and 10 out of 200 examined samples (5%), respectively. The isolation rates of L. innocua were 5%, 10%, 5%, 10%, and 5% from the examined raw meat, minced meat, beef burger, sausage, and luncheon, respectively. While, the isolation rates of L. seeligeri were 5%, 5%, 7.5%, 5%, and 2.5% from these samples respectively. L. gravi was isolated from six samples at 3%. The isolation rates of L. gravi were 2.5%, 5%, 5%, and 2.5% from the examined raw meat, minced meat, beef burger, and sausage, respectively. L. monocytogenes was isolated from 7 out of 200 examined samples (3.5%). L. monocytogenes was isolated at 2.5%, 5%, 5%, 2.5%, and 2.5% from the

examined raw meat, minced meat, beef burger, sausage, and luncheon, respectively. All obtained *Listeria* isolates harbored *iap* (100%), while 5 out of 7 isolates (71.43%) expressed *hylA*. In the present study, *L. monocytogenes* isolates showed a complete resistance (100%) to both of streptomycin and neomycin. The percentage of the resistance among the examined antimicrobials was as following: oxacillin (85.74%), kanamycin (57.16%), erythromycin (57.16%), oxytetracycline (57.16%), cephalothin (42.87%), sulphamethoxazol (28.58%), enrofloxacin (28.58%).

Aspergillus species was the most prevalent species in beef burger, luncheon, minced meat, raw meat and sausage samples in 49%, 47.8%, 46%, 42.9% and 41.7%, respectively. *Penicillium species* were recovered at 25.5%, 25%, 23.9%, 20.6% and 19% in beef burger, sausage, luncheon, minced meat and raw meat respectively. Out of 233 (88.9%) positive fungal isolates for lipase enzyme production, 90 (38.6%) exhibited high lipase production. Among 262 isolates tested, 216 (82.4%) could produce the enzymes. From the positive isolates 73 (33.7%) exhibited high proteolytic, whereas 60 (27.7%) showed moderate production and 83 (38.4%) were weak producers

### Part II (Experimental study):

Trials applied on minced meat to improve its quality by clove, thyme, garlic Eos 0.5, 1% and potassium sorbate 0.3%.

Additives delay chemical deterioration of treated minced meat samples (pH, TVN, TBA).

All Eos significantly reduced *L. monocytogenes* counts starting from the second day. The highest reduction was achieved on the  $4^{th}$  day post-inoculation with a count range of 4.02 to 5.18. From the  $6^{th}$  day, *L*.

*monocytogenes* counts start to rise up again. The Eos and pot. sorbate 0.3% significantly reduced *A. flavus* counts starting from the third day.

Collectively, clove 1% and thyme 1% achieved the highest improvement percentages with values of 44.07% and 43.61%, respectively. Clove 1% and thyme 1% showed decreases in mean values of total *A*. *flavus* through  $3^{rd}$  day and  $6^{th}$  day until  $9^{th}$  day not detected. Clove 0.5%, thyme 0.5%, garlic1%, garlic 0.5% and potassium sorbate 0.3% showed decreases in mean values of total *A*. *flavus* through the  $3^{rd}$  day in which the lowest count recorded, then started gradually but still lower than the count of control in 0 day.