



*Benha University
Faculty of Vet. Med. Moshtohor
Dept. of Bacteriology, Immunology and Mycology*

Molecular Diagnosis of Some Bacterial Foodborne Pathogens in Meat by Multiplex Qualitative PCR.

A thesis presented by

Abdelhalim Abdelaty Elshawadfy Abdelrazek

B. V. Sc., Fac. Vet. Med., Minofiya Univ., Sadat City branch, (2003)

M.V. Sc., Fac. Vet. Med., Benha Univ., (2014)

A Thesis

Submitted to

Faculty of Veterinary Medicine

Benha University

For the Degree of PhD

(Bacteriology, Immunology and Mycology).

Under supervision of

Prof. Dr. Ashraf Awwad Abd El -Tawab

*Prof. and Head of Bacteriology, Immunology
and Mycology Department.*

Fac. Vet. Med., Benha Univ.

Prof. Dr. Hanan Ali Fahmy

Prof. of Biotechnology.

Deputy Director for Research and Environmental Health.

Animal Health Research Institute, Dokki – Giza.

Fac. Vet. Med., Benha University

(2020)

Index

Content

No.	Title	Page
1.	Introduction	1
2.	Review of literature	4
2.1.	Incidence of Foodborne Pathogens in Meat & Meat Products	5
2.2.	Sources of Contamination of Meat & Meat Products with Food-Borne Pathogens	7
2.3.	Bacteriological Characters of Food-Borne Pathogens	11
2.4.	Public Health Hazards of Isolated Microorganisms	23
2.5	Molecular Detection of Foodborne Pathogens	30
2.6.	DNA Sequence and Phylogenetic Analysis	39
3.	Material and Methods	46
3.1.	Materials	46
3.1.1	Samples	46
3.2.	Methods	56
4.	Results	76
4.1	Isolation and Identification of Foodborne Pathogens	76
4.2	Results of Multiplex PCR Assay of <i>E. coli</i> and <i>S. aureus</i>	86
4.3	Results of DNA Sequence and Phylogenetic Analysis	91
5.	Discussion	99
6.	Conclusion	106
7.	Summary	107
8.	References	110
9.	Arabic Summary	\

List of Tables

No.	Title	Page
1	Antisera Used in Serological Identification of <i>E. coli</i>	52
2	Oligonucleotide primers sequences source Metabion (Germany)	54
3	The biochemical characters of the isolated members of <i>Staphylococcus</i> spp.	64
4	Component of Multiplex PCR Reaction Mix	69
5	Cycling Conditions of The Different Primers During Multiplex PCR	69
6	Instruction of the manufacture Big dye Terminator V3.1 cycle sequencing kit	73
7	Thermal Profile Used As in Sequence Reaction	73
8	Total Number and Percentage of Positive Samples for Pathogens Isolation from Studied Samples	76
9	Percentage of Foodborne Pathogens in Examined Samples	78
10	Percentage of <i>E. coli</i> Strains Isolated from Examined Samples	80
11	Percentage of <i>S. aureus</i> strains isolated from examined samples	81
12	Biochemical reaction of <i>E. coli</i> isolates	83
13	Serological typing of <i>E. coli</i> strains isolated from different examined samples	84
14	Results of Biochemical Identification of <i>S. aureus</i>	86
15	Multiplex PCR Results of previously examined meat products by conventional bacteriological method	89
16	Results of Multiplex PCR for detecting <i>E. coli phoA</i> Genes & <i>S. aureus clfA</i> .	90

List of Figures

No.	Title	Page
1	Total Number and Percentage of Positive Samples for Pathogens Isolation	77
2	Total Number of Single and Mixed Isolates of Positive Samples for Pathogens Isolation	77
3	Total percentage of foodborne pathogens isolated from examined samples	79
4	Percentage of Foodborne Pathogens Isolated from Frozen Meat, Minced Meat and Burger Examined Samples	79
5	Total number and percentage of <i>E. coli</i> isolated from examined samples	80
6	Total number and percentage of <i>S. aureus</i> isolated from examined samples	81
7	Percentage of different <i>E. coli</i> serotypes	84
8	Validation of multiplex PCR.	87
9	Agarose Gel Electrophoresis of Multiplex PCR for <i>S. aureus clfA</i> & <i>E. coli phoA</i> Genes Amplified at 638 bp and 720 bp	88
10	<i>E. coli</i> Nucleotide alignment report representing of the partial sequence <i>phoA</i> gene for 1 isolate with other related <i>E. coli</i> reference strains.	92
11	Phylogenetic tree representing the partial nucleotide sequences of the <i>phoA</i> gene for 1 isolate with other related <i>E. coli</i> reference strains using Neighbor-joining method of Mega5 software	93
12	Amino acids Identity percent representing of the <i>phoA</i> gene for 1 isolate with other related <i>E. coli</i> reference strains.	93
13	<i>E. coli</i> amino acids alignment report representing of the partial sequence <i>phoA</i> gene for 1 isolate with other related <i>E. coli</i> reference strains	94
14	<i>Staphylococcus aureus</i> Nucleotide alignment report representing of the partial sequence <i>clfA</i> gene for 1 isolate with other related <i>S. aureus</i> reference strains	96
15	Phylogenetic tree representing the partial nucleotide sequences of the <i>clfA</i> gene for 1 isolate with other related <i>Staphylococcus aureus</i> reference strains using Neighbor-joining method of Mega5 software	97

16	Amino acids Identity percent representing of the <i>clfA</i> gene for 1 isolate with other related <i>S. aureus</i> reference strains	97
17	<i>Staphylococcus aureus</i> amino acids alignment report representing of the partial sequence <i>clfA</i> gene for 1 isolate with other related <i>S. aureus</i> reference strains	98

7. Summary

E. coli, *S. aureus* and *Salmonellae* are of the most common cause of food-borne human illness throughout the world.

The objective of this study was to achieve accurate and rapid identification of foodborne pathogens that is crucial for public health. To achieve our goals the following points were investigated:

- A grand of total of 210 random samples of frozen meat and meat products (minced meat, burger) (70 samples of each) were collected from different markets in Dakahleyah Governorate to be examined conventional bacteriological methods and m-PCR Technique for detection of *E. coli*, *S. aureus* and *Salmonellae* microorganisms.
- The results of Food borne pathogens isolation, revealed that, 29 out of 210 samples were positive for isolation (13.8%); represented as 10 positive samples (14.3%) from frozen meat samples, where 7 were single isolates and 3 were mixed contaminants, meanwhile, 6 (8.6%) from minced meat samples where 2 were single isolates and 4 were mixed isolates and 13 positive samples (18.6%) from burger samples, where 10 were single isolates and 3 were mixed isolates.
- From the 29 isolates of foodborne pathogens *E. coli* were the most isolated one (18= 8.6%) followed by *S. aureus* (11=5.2%).

- *Salmonellae* failed to be isolated in all tested samples.
- Regarding the serological identification of 8 isolated *E. coli* strains, one (12.5%) strain was typed as O₂₆, from burger sample, 3 O_{86a} (37.5%), one from frozen meat, one from minced meat and one from burger samples; 4 O₁₂₅ (50%), one from frozen meat, two from minced meat and one from burger samples.
- For *E. coli*, 4 positive meat products samples (1 minced meat, 2 frozen meat, 1 burger) by conventional method were reexamined by m-PCR, there were total agreement between results of conventional method and m-PCR technique.
- For *S. aureus*, 4 positive meat product samples (2 minced meat, 1 frozen meat, 1 burger) by conventional method were reexamined by m-PCR, the percent of agreement between results of both methods reached to 100 %.
- 6 positive samples showed mixed infection with *S. aureus* and *E. coli* (2 minced meat, 2 frozen meat, 2 burger) by both conventional method and m-PCR technique.
- On the other hand, when m-PCR technique was applied on 4 negative samples (1 minced meat, 1 frozen meat, 2 burger) by conventional method, there were no difference in results obtained by both methods.

- One selected sample was sequenced for *E. coli phoA* Gene using specific set of primers as *Escherichia coli* strain STEC388 with 97% nucleotide identity % and *Escherichia coli* O8:H8 16F5M1D1 DNA with 97% identity%. The isolate generated in this study was submitted to the GenBank database with accession number MT051989.
- Another sample was sequenced for *Staphylococcus aureus clfA* Gene using specific set of primers as *Staphylococcus aureus* strain 628 chromosome with 99% nucleotide identity percent and *Staphylococcus aureus* strain UP_1442 chromosome with 99% identity percent. The isolate generated in this study was submitted to the GenBank database with accession number MT051990.
- The results showed that multiplex PCR, is an ideal method for identification of foodborne pathogens, as it was effective, less labor, more sensitive, reduces effort and time. To sum up, the multiplex PCR assay has the potential to be used in routine diagnostic laboratories and also might be as rapid screening tool in food testing laboratories to quickly identify food samples especially in case of out breaks and urgency.