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

List of Tables

List of Figures

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 contaminant s, 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 **O_{26}**, from burger sample, 3 **O_{86a}** (37.5%), one from frozen meat, one from minced meat and one from burger samples; 4 **O_{125}** (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 pho*A 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 clf*A 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.