CONTENTS

Title	Page
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. Sources of contamination of some meat products with foodborne pathogens	4
2.2. Incidence of foodborne pathogens in some meat products	8
2.3. Public health hazards of foodborne pathogens	19
2.4. Multiplex PCR in the diagnosis of foodborne pathogens	28
3. MATERIAL AND METHODS	35
3.1. Collection of samples	35
3.2. Preparation of samples	35
3.3. Isolation and identification of foodborne pathogens by conventional culture method	35
3.4. Material used for Polymerase Chain Reaction	46
3.5. Methods used for Polymerase Chain Reaction	48
4. RESULTS	55

5. DISCUSSION	68
6. CONCLUSION AND RECOMMENDATIONS	78
7. SUMMARY	83
8. REFERENCES	85
9. ARABIC SUMMARY	-

LIST OF TABLES

Table	Title	Page
No.		
Α	Detailed description of the oligonucleotide primers used	48
В	PCR Mastermix for Uniplex PCR	51
С	PCR Mastermix for Multiplex PCR	52
D	Temperature and time conditions of different primers during PCR	53
1	Incidence of <i>Staph. aureus</i> in the examined meat product samples	55
2	Incidence of <i>L. monocytogenes</i> in the examined meat product samples	56
3	Incidence of <i>E. coli</i> in the examined meat product samples	57
4	Summary of bacteriological results and m- PCR results	67

LIST OF FIGURES

Figure No.	Title	Page
Α	Diagram of standard procedure used for detection of <i>Staph. aureus</i>	37
В	Diagram of standard procedure used for detection of L. monocytogenes	42
С	Diagram of standard procedure used for detection of <i>E. coli</i>	46
D	DNA Ladder Ready to Load	49
1	Incidence of <i>Staph. aureus</i> in the examined samples of meat product	55
2	Incidence of <i>L. monocytogenes</i> in the examined samples of meat product	56

3		57
5	Incidence of <i>E. coli</i> in the examined samples of meat	57
	product	
4	Uniplex gradient PCR using different annealing	58
	temperatures from 56 ° to 63°	
5	Uniplex PCR validation for Meat and Culture	58
		50
6	Validation of multiplex PCR by using one template	59
7	Validation for specificity of primers	60
	vandadon for specificity of primers	00
8	Validation for specificity of primers with other microbes	61
9	Sensitivity of multiplex PCR for <i>Staph. aureus</i> obtained by	62
	serial dilutions	
10	Sensitivity of multiplex PCR for <i>L. monocytogenes</i> obtained	62
10		02
	by serial dilutions	
11	Sensitivity of multiplex PCR for <i>E. coli</i> obtained by serial	62
	dilutions	
12	Validation for multiplex PCR	63
10		
13	Multiplex PCR for <i>Staph. aureus</i> positive meat product	63
	samples	
14	Multiplex PCR for <i>E. coli</i> positive meat product samples	64
17	Francipies I Civitor 2. con positive meat product samples	04
15	Multiplex PCR on positive meat product samples (mixed	65
	infection) and isolated L. monocytogenes positive meat	
	product sample	
16	Multiplex PCR on 20 random negative meat product	65
	samples	
17		
17	Uniplex PCR for confirmation of Fig. (16)	66

List of Abbreviations

Abbreviation	Word
BHI	Brain Heart Infusion
BLAST	Basic Local Alignment Search Tool
Вр	Base pair
Cfu	Colony forming unit
DNase	Deoxyribonuclease
EHEC	Enterohaemorrhagic E.coli
EIEC	Enteroinvasive E.coli
ЕМВ	Eosin Methylene Blue
EPEC	Enteropathogenic E.coli
ETEC	Enterotoxigenic E.coli
FDA	Food and Drug Administration
GMPs	Good Manufacturing Practices
НАССР	Hazard Analysis and Critical Control Point
нс	Hemorrhagic Colitis
HL	Heat labile
HS	Heat stable
HUS	Hemolytic Uremic Syndrome
hylA	Hemolysin gene
ICMSF	International Commission On Microbiological Specification For Foods
ISO	International Organization for Standardization
KFDA	Korea Food and Drug Administration
LEB	Listeria Enrichment Broth
LT	Thermolabile Toxin
MFB	Modified Fraser Broth
m-PCR	Multiplex PCR
MR	Methyl Red
ND	Not detected

nuc	Nuclease gene
P.mol	Picomol
PCR	Polymerase Chain Reaction
Pg	Pictogram
ppm	Part per million
rpm	Revolution per minute
RTE	Ready-To-Eat
SEs	Staphylococcal enterotoxins
SFP	Staphylococcal Food Poisoning
SLTI	Shiga Like Toxin I
SLTII	Shiga Like Toxin II
STEC	Shiga Toxin Producing <i>E.coli</i>
STx	Shiga Toxin
ТСТ	Tube Coagulase Test
tlh	Thermolabile Hemolysin
TNase	Thermostable Nuclease
TSA-YE	Trypticase soya Agar-yeast Extract
TSI	Triple Sugar Iron agar
TSST-1	Toxic shock syndrome toxin 1
ТТР	Thrombotic Thrombocytopenic Purpura
USA	United States of America
USEPA	United States Environmental Protection Agency
uspA	Universal Stress Protein gene
VTEC	Verocytotoxic E.coli
who	World Health Organization
μΜ	Micrometer

6. Conclusion and Recommendations

The obtained results in the present study indicated that the examined samples of meat products were more contaminated with *Staph. aureus*, *L. monocytogenes* and *E.coli*. The presence of these microorganisms in high percentage not only renders meat products of inferior quality and unfit for human consumption, but also considered as an indication for the presence of unhygienic conditions during processing and feccal contamination, which can cause foodborne illness and outbreaks.

In addition, the conventional methods of isolation and identification of foodborne pathogens were time consuming. On the other hand, m-PCR was more sensitive, more accurate and rapid for foodborne pathogens isolation especially in case of outbreaks.

Special recommendations for m-PCR:

1-DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction product analysis, should be performed in separate areas.

2-A Laminar Flow Cabinet equipped with a UV lamp is recommended for preparing the reaction mixture.

3-Fresh gloves should be worn in DNA extraction and each reaction to avoid contamination.

4- Using high quality DNA templates greatly enhances the success of m-PCR.

5-It is advisable to incubate the meat products overnight to permit the enrichment of the microbes and obtain accurate results.

6-PCR primers are usually 15-30 nucleotides in length. Longer primers provide higher specificity.

7-Gradient annealing temperatures applied to ensure the best annealing temperature for the microbes.

8-Primers selected in multiplexing should be different in their molecular weight to avoid the false diagnosis.

9- Validation for primers specificity should be ensured to avoid the cross reaction between primers.

10- Using multiplex master mix is very effective to obtain accurate results.

11- Using control negative to confirm the absence of contamination.

Virtues of m-PCR:

1-High specificity and sensitivity.

2-High stability of DNA permits analysis of food samples with less contamination.

3-Numerous amounts of selective DNA for many foodborne pathogens can be used in one PCR reaction.

4-Constitute very valuable tools for routine applications especially in case of outbreaks.

5-Quantitative and qualitative test.

6-Cost reduction test.

In order to improve the meat products to be safe for human consumption, the following recommendations should be adopted:

Quality choice:

1-A good quality raw material should be used in manufacturing of meat products.

2-Raw materials should be inspected by trained staff.

3-Raw material should be stored under proper conditions.

4-High quality meat and additives should be used.

Personal hygiene:

The following advises should be followed:

1-Worker's hands come in contact with raw materials should be thoroughly washed and sanitized.

2-All jewellery and watches should be removed before starting the work.

4-Hands should be washed before work and after visiting the toilet.

3-Workers who have infected lesions specially boils and other pus containing lesions should not touch food.

4-Workers or meat handlers should have medical certificate to avoid cross contamination.

5-Food handlers should be turned away from food while coughing or sneezing.

Hygienic practices (rooms / equipment):

1-Periodical sanitation of kitchen and adjoining area by adequate sanitizers.

Frequent cleaning of working areas in kitchens.

2-Spreading dirt and other foreign matter to kitchen utensils and food should be avoided

3-Food area should be protected from insects.

4-Dangerous or poisonous substances, e.g. detergents, disinfectants, insecticides should be kept outside the kitchen area.

5-Rooms should have physical barriers for rodents.

Hygienic practices (food):

1-Perishable food should be always refrigerated.

2-In spite of cooling, perishable food may only be stored for a limited time.

3-The temperature of freezing should be checked periodically at -18°C.

4- Freezing of meat product must be considered for the shelf life of that product.

5-It is important to avoid put the power "off" overnight.

6-Thawing of frozen raw meat products should be recommended in refrigerator at temperature 5°C.

7-Meat products after cooling should not be held at room temperature.

8-Meat products must be thoroughly cooked to permit the internal temperature to be lethal to most vegetative bacteria (80°C for 3 minutes).

9-Keeping food warm below 60°C should be avoided.

10-All parts of cooked dishes should be reheated to at least 80°C before eating.

11-All working procedures in the kitchen have to be done speedily, especially after easily perishable food component have been taken out of the refrigerator.

12-Using of chemical preservatives should be utilized because these chemicals can interfere with serious pathogens combination of nitrite (50 ppm), potassium sorbate (0.2%) and nisin (60 ppm) is the most effective formula for destruction of these pathogens and the use of curing agents in case of basterma.

Education of meat handlers and consumers:

1-Education and training of the meat handlers are the key stones of effective quality control.

2-Mangers, veterinarians and supervisors in the processing group should train the workers for safe production of meat products.

3-Worker must be learned the basic principles of meat borne diseases and their control.

4-Consumers should understand the importance of adequate refrigeration of raw meat products, hand washing before preparation and proper cooking of such meat

products.

5-Industry can help by giving advice on the label wherever possible about handling and storage of the product.

6-Hazard Analysis Critical Control Point (HACCP) system as a hazards control system should be done in meat processing group to decide a Good Manufacturing Practice (GMP) is being done and to ensure a maximum safety to consumers.