# Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction

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**A B S T R A C T**

A total of 100 random samples of meat products including raw minced meat, raw sausage, luncheon and basterma (25 samples of each) were collected from different markets in Cairo and Giza governorates to be examined bacteriologically for detection of *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli*. These samples were examined for isolation of such pathogens by conventional bacteriological methods and by polymerase chain reaction (PCR). Concerning *S.aureus* bacteriological results revealed the prevalence in minced meat, Sausage, luncheon and basterma was (24%, 24%, 20%, 4%) respectively. While *L. monocytogenes* revealed the prevalence in minced meat, sausage, luncheon and basterma was (4%, 0%, 0%, 0%) respectively and *E. coli* revealed the prevalence in minced meat, sausage, luncheon and basterma was (20%, 20%, 24%, 20%) respectively. The results cleared that PCR is an ideal method for identification of foodborne pathogens, as it was effective, less labor, more sensitive, reduces effort and time after using gradient PCR in validation of each microbe.

**Keywords**: foodborne pathogens, optimization, polymerase chain reaction (PCR), bacteriological isolation, Meat products.

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## 1. INTRODUCTION

Food poisoning is an illness caused by eating food that has been contaminated. Foodborne pathogens which have been recognized as the most common sources causing food poisoning worldwide and in severe cases cause death. Meat and meat products are highly perishable and can spoil easily and soon become unfit to eat and possibly dangerous to health through microbial growth, chemical changes and breakdown by endogenous enzymes (Judge et al., 1990).

Microbiological assessment is important to determine the safety and quality of food. In the past detection and identification of microorganisms have relied mainly on cultural techniques. These methods are the most reliable and accurate in the detection of foodborne pathogens. However, they are labour intensive, have long processing times and are costly. Major disadvantage to this method is that it can take 2- 3 days for any results to show up and up to 7- 10 days for confirmation (Jasson et al., 2010). Polymerase chain reaction (PCR) is a powerful technique that has revolutionized molecular biology research and has application in the diagnosis of microbial infections and genetic diseases, as well as in detection of pathogens in food samples.

PCR is an advantage against the culturing methods as you can use numerous amounts of selective DNA in one PCR reaction. Recent reports have shown that PCR greatly improves specificity and sensitivity for the detection of pathogens (Huang et al., 2009).

## 2. MATERIAL AND METHODS

*2.1.Collection of samples*

Actually, 100 samples of raw meat and cooked meat (Minced meat, Sausage, Luncheon and Basterma) were collected from different markets in Cairo and Giza governorates. Samples were submitted to the lab in Animal Health Research Institute for bacteriological examination in sterile containers in a Stomacher bag.

*2.2.Sample preparation*

Actually, 25 grams of each samples were taken under aseptic condition into sterile blender jar to which (225ml) peptone water (0.1%) was added, then the blender was operated to give 3000 rpm for not more than 2.5 m to get a dilution of $10^{-1}$, after that (1ml) from the original solution was transferred into separate tube containing (9 ml) peptone water from which (10) fold serial dilution (ICMSF, 1978).

*2.3.Bacteriological isolation*

According to Koneman et al (1996) and Quinnet al (2002), the samples were cultured for isolation of *S. aureus* onto peptone water for 24 hours at 37ºC and then a loopful was taken and cultured onto Nutrient agar, 5% sheep blood agar, mannitol salt agar and then onto Baird parker medium. All inoculated plates were incubated at 37ºC for 24-48 hours then colonies were identified. The colonies characterized by circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone. All suspected colonies are tested and confirmed biochemically. According toISO 11290, the samples were cultured for isolation of *L. monocytogenes* onto peptone water for 24 hours at 37ºC and the initial suspension prepared and incubated at 30 °C for 24 hours ±2hours. A black coloration was developed during the incubation. After incubation of the initial suspension (primary enrichment) for 24 hours ±2 hours, 0.1 ml of the culture obtained was transferred to a tube containing 10 ml of secondary enrichment medium (Full Fraser broth), then incubated at 35 °C or 37 °C for 48 hours ± 2 hours. From the primary enrichment culture incubated for 24 hours ± 3 hours at 30 °C , a portion of the culture was inoculated on the surface of the selective plating medium OXFORD. The seeded plates were incubated to obtain well separated colonies. Colonies showing morphological characters as dew drop-like, black with brown hallow, or dark brown colonies 1-2 mm in diameter. All suspected colonies are tested and confirmed biochemically.

According to Quinn et al (2002) samples were cultured for isolation of *E. coli* and inoculated separately into buffer peptone water were incubated at 37˚C for 18 hours ±2 hours under aerobic condition. A loopful from the broth of each sample was streaked onto MacConkey's agar and Eosin Methylene Blue agar. The inoculated plates were incubated at 37℃ for 24 hours. Suspected *E. coli* colonies were purified and kept for further identification. Where the colonies appear red on MacConkey's agar and appear as green metallic sheen EMB agar. All suspected colonies are tested and confirmed biochemically.

*2.4.Optimization of the Polymerase Chain Reaction (PCR)*

DNA extraction and purification direct from the meat products samples by Thermo Scientific Genomic DNA extraction kit used to obtain purified DNA.

Table (4) show the Oligoneucleotide primers which were designated according to Integrated DNA technology and were used for amplification, the nuclease (nuc) gene of *S. aureus*, hemolysin (hlyA) gene is essential for virulence of L.*monocytogenes* and universal stress protein (uspA) gene which encodes for a highly conserved universal stress protein present in all *E. coli*. The primers were received in lyophilized form and resuspended in sterilized water to reach a final concentration of 100 pmol/µl. These

primers suspected to amplify specific segment of 270, 456 and 884 bp. The mix used in validation of PCR on detection of foodborne pathogens according to AmpliTaq Gold 360 mastermix (Applied Biosystems) where the reaction is 6.25µlAmpliTaq Gold 360 mastermix (2x), 0.75µl PCR grade water,1.5µlforward primer, 1.5µl reverse primer,2.5µl template DNAto reach a final volume of 12.5µl in the reaction. According to the methods of Henegariu et al (1997), amplifications conditions for thermocycling were 95˚C for 5 min for primary denaturation and followed by 40 cycles of heat denaturation at 95˚C for 15 sec, primer annealing at (56˚C - 63 ˚C) for 45 s and DNA extension at 72˚C for 1 min, this was followed by final extension at 72˚C for 10 min. After PCR reaction, 5µl of the PCR product was resolved on 1.5% agarose gel. The gels were stained with Ethidium bromide (0.2µg/ml) and photographed under UV transillumination in gel documentation system (BioRad).

## 3. RESULTS

### *3.1. Results of Bacteriological isolation*

Table (1) shows the results of isolation of *S.aureus* revealed the prevalence in minced meat, sausage, luncheon and basterma was (24%, 24%, 20%, 4%) respectively.

Table (1): Number and percentage of S. aureus of positive samples from meat products.

| Types of samples | No. of examined samples | Bacteriological finding No. of positive samples | % |
|---|---|---|---|
| Minced meat | 25 | 6 | 24% |
| Sausage | 25 | 6 | 24% |
| Luncheon | 25 | 5 | 20% |
| Basterma | 25 | 1 | 4% |
| Total | 100 | 18 | 18% |

Table (2) shows the results of isolation of *L. monocytogenes* revealed the % of prevalence in minced meat, sausage, luncheon and basterma was (4%, 0%, 0%, 0%) respectively.

Table (2): Number and percentage of L. monocytogenes of positive sample from meat products.

| Types of samples | No. of examined samples | Bacteriological finding No. of positive samples | % |
|---|---|---|---|
| Minced meat | 25 | 1 | 4% |
| Sausage | 25 | 0 | 0% |
| Luncheon | 25 | 0 | 0% |
| Basterma | 25 | 0 | 0% |
| Total | 100 | 1 | 1% |

Table (3) shows the results of isolation of *E.coli* revealed the prevalence in minced meat, sausage, luncheon and basterma was (20%, 20%, 24%, 20%) respectively.

Table (3): Number and percentage of E. coliof positive sample from meat products

| Types of samples | No. of examined samples | Bacteriological finding No. of positive samples | % |
|---|---|---|---|
| Minced meat | 25 | 5 | 20% |
| Sausage | 25 | 5 | 20% |
| Luncheon | 25 | 6 | 24% |
| Basterma | 25 | 5 | 20% |
| Total | 100 | 21 | 21% |

### *3.2. Results of Polymerase Chain Reaction (PCR)*

Figure (1) shows the optimization for validation of PCR on different gradient annealing temperatures from 56˚C to 63 ˚C

Table (4): A detailed descriptions of the designed oligonucleotide primers used.

| Target pathogen | Target gene | Primer | Sequence (5` - 3`) | Amplicon sizebp | Reference |
|---|---|---|---|---|---|
| S. aureus | nuc | Forward<br>Reverse | GCGATTGATGGTGATACGGTT<br>AGCCAAGCCTTGACGAACTAAAGC | 270 | Pinto et al., (2005) |
| L. monocytogenes | hlyA | Forward<br>Reverse | GCAGTTGCAAGCGCTTGGAGTGAA<br>GCAACGTATCCTCCAGAGTGATCG | 456 | Paziak-Domanska *et al.,* (1999) |
| E. coli | uspA | Forward<br>Reverse | CCGATACGCTGCCAATCAGT<br>ACGCAGACCGTAGGCCAGAT | 884 | Chen and Griffiths (1998) |

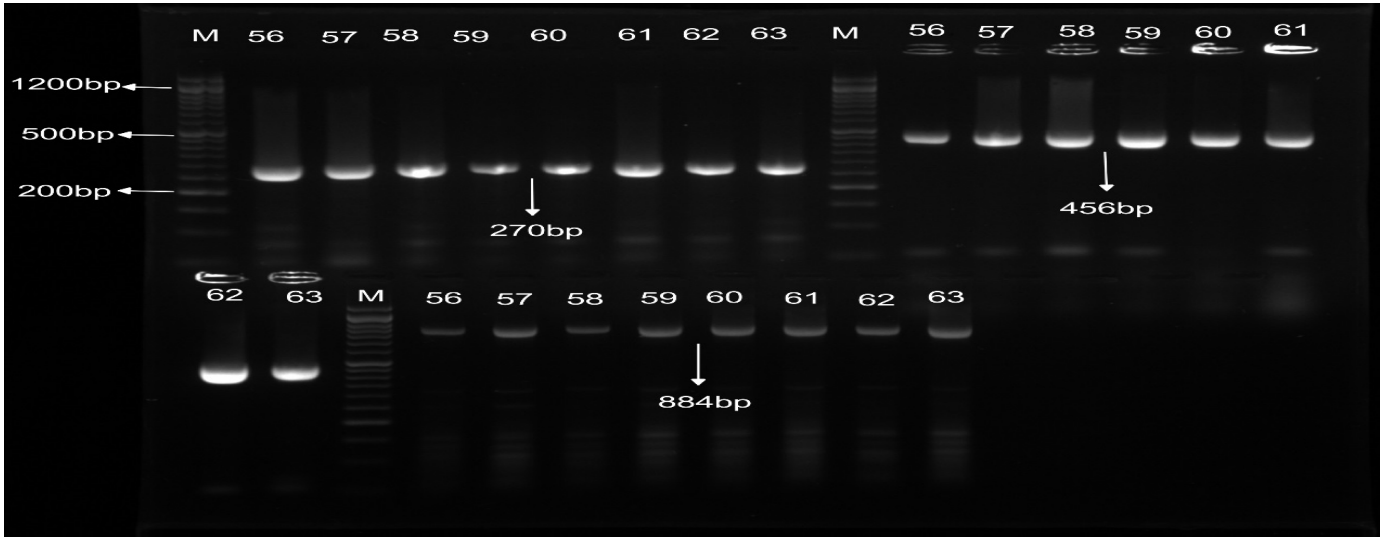


**Figure (1):** Uniplex gradient PCR using different annealing temperatures from (56 º to 63º).
M:marker (50bp).
1st group: annealing temperatures for staph. aureus from 56 º to 63º → (270bp).
2nd group: annealing temperatures for L. monocytogenes from 56 º to 63º → (456bp).
3rd group: annealing temperatures for E.coli from 56 º to 63º → (884bp).

on foodborne pathogens concerned in this study. The nuclease (nuc) gene of *S. aureus*amplify at specific band of 270bp, hemolysin (hlyA) gene of *L. monocytogenes* amplify at specific band of 456bp, and universal stress protein (uspA) gene of *E. coli* amplify at specific band of 884bp.

## 4. DISCUSSION

The foodborne pathogens are likely to cause serious public health problems, especially in developing countries where they lead to high level of morbidity and mortality rates. Quick, sensitive, specific and easy techniques for detection of the foodborne pathogens are needed for the effective implementation of food safety. Since its advent in the 1980s, polymerase chain reaction (PCR) has become indispensable tool in molecular diagnostics and can be very efficiently used in rapid detection of food-borne pathogens (Pinto et al., 2005).

In this study a total of 100 samples of meat products; minced meat and sausage (which represent raw meats), luncheon and basterma (which represent cooked meats) were examined bacteriologically to reveal the prevalence of foodborne pathogens as *S. aureus*, *L. monocytogenes and E. coli* and the percentage of prevalence was (18%, 1%, 21%) respectively.

The obtained results of minced meat were nearly similar to (Vorster et al., 1994)they found *S.aureus*in (23.4%) of minced beef in south Africa, and more than **(**Omar et al., 2009) who isolated *S.aureus*in a percentage of (14.6%), and less than (Depourcq and Poucke, 1991)who determined the bacteriological quality of 52 samples of minced meat, *S.aureus*were found in all samples which represents (100%). In sausage results were nearly similar to (Soultos et al., 2003) who reported incidence of *S.aureus* in sausage (19.4%) and less than (El-Khateib, 1997)who reported higher incidence of *S.aureus* in sausage (29%). While On the other hand, our results of isolation from luncheon were nearly similar to those reported by(Fatin, 2004)who isolated *S.aureus*in (16%), less than (Seham et al., 2013) who isolated *S.aureus*in 32%. They mentioned that contamination may occur during the slicing and packaging of luncheon meat in supermarkets**.** The results of basterma examination were less than (Zakaria, 2007) who isolated *S.aureus*in (25%).

The obtained results of minced meat were nearly similar to(El-gaml et al., 1998)who detected *L. monocytogenes* by 2 (4%) of examined raw minced meat samples, and more than (Hua Wang et al., 1992) who failed to isolate L. monocytogenes in China, and less than (Inoue et al., 2000)in percentage of 12.2 of examined minced meat samples. On the other hand, *listeria. monocytogenes* failed to be detected in the examined ready to eat meat products samples (luncheon and basterma), such result was similar to that obtained by (Gomez et al., 1999**).** On the contrary, (Furrer et al., 1991)who revealed the presence of L. monocytogenes in and (6%) of examined luncheon samples and (Hanaa Kader et al., 2012)who revealed the presence of L. monocytogenes in (26.6%) of examined basterma samples. However the low percentage may be due to addition spices, curing techniques and the temperature used during manufacture as well as good hygiene.

The obtained results of minced meat were nearly similar to(Ahmed, 1992) who examined 30 samples of minced meat where *E. coli* was isolated at (17.5%), and more than (Blanco and Blanco, 1996)whorevealed that 3 (5%) of 58 minced beef samples were positive, and less than (Shawki, 1990)who examined 25 minced meat samples where the percentage was (44%)examined minced beef for detection of *E. coli*. In Sausage the obtained results were nearly similar to (Fathi et al., 1992) who examined sausage for *E.coli* where the incidence was (20%), and more than (Ahmed, 1992) who examined 30 samples

of sausage *E. coli* was isolated at (6.6%), and less than (Mousa et al., 1993) isolated *E.coli* (45%) of the examined sausage samples. While On the other hand, our results of isolation from luncheon were more than (Fawzy, 2004) who isolated *E.coli* from (8%) of luncheon examined, and less than (Fathi et al., 1992)isolated *E.coli* (41.67%) from luncheon. The results of basterma examination were nearly similar to (Fathi et al., 1992) who examined basterma for *E.coli* and the incidence was (19.37%), and more than (Afaf, 2009) who examined 30 samples of basterma and found that *E.coli* incidence was (13.3%).

On the other hand, PCR methods offer a sensitive and specific detection of pathogens. In the last 10 years, many authors have proposed the use of PCR for the detection of foodborne pathogens to replace the time consuming culture based classical techniques. They are rapid, easy to handle, sensitive and specific and therefore constitute very valuable tools for routine applications**.**The result of optimization for validation of PCR on different gradient annealing temperatures from 56˚C to 63 ˚C on foodborne pathogens concerned in this study show great accordance with the results of (Chen et al., 2012);(Thapa et al., 2013)and(Kim et al., 2014) where they used gradient PCR on validation and detection of *S. aureus*, *L. monocytogenes* and *E.coli* but with different primers used in this study while(Guan et al., 2013) and (latha et al., 2014)used gradient PCR on validation and detection of *S. aureus* and *L. monocytogenes* with same primers used in this study.On the other hand, (Kupradit et al., 2013)used gradient PCR on validation and detection of*L. monocytogenes* and *E.* coli with only the (uspA) gene of *E. coli* with the prfA gene of *L. monocytogenes*.

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