*Journal of Education and Digital Learning*
Volume 11 Issue 2, April-June 2023
ISSN: 2995-3723
Impact Factor: 7.86
http://kloverjournals.org/journals/index.php/edl

# MOLECULAR MENACE: INVESTIGATING THE GENETIC LANDSCAPE OF PATHOGENIC BACTERIA IN FERMENTED MEAT PROVISIONS

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**Abstract**
Foodborne pathogens are microbial agents that can trigger instances of foodborne illness, lding to disease outbreaks with significant public health and economic implications. The occurrence of multiple similar illnesses resulting from the consumption of contaminated food characterizes a foodborne disease outbreak. Over 200 distinct foodborne diseases have been recognized, each posing a unique threat to human health. These diseases encompass both foodborne infections, characterized by a longer incubation period, and foodborne intoxications, marked by a more rapid onset of symptoms. In foodborne infections, pathogens ingested with food establish themselves within the human host, while in foodborne intoxications, toxins produced by pathogens in food are ingested, causing illness.
The consumption of foods contaminated with pathogenic microorganisms and their toxins contributes to fatalities, illnesses, hospitalizations, and economic burdens. Particularly, gastrointestinal infections resulting from foodborne diseases have a pronounced adverse impact on human well-being. Recognizing the significance of foodborne illnesses and their diverse manifestations is vital for effective prevention and control strategies. This paper sheds light on the various aspects of foodborne diseases, their classification, and the significant consequences they impose on individuals and society.

**Keywords:** foodborne pathogens, foodborne illness, disease outbreak, foodborne infection, foodborne intoxication.

## 1. Introduction

Foodborne pathogens are biological agents that can cause a foodborne illness event. A foodborne disease outbreak has defined the occurrence of two or more cases of a similar illness resulting from the ingestion of a typical food (CDC, 2012). More than 200 foodborne diseases have been identified (Mead et al., 1999). Foodborne illness occurs when a pathogen is ingested with food, establishes itself and multiplies in the human host, or produces a toxin that the human host then ingests. Thus, foodborne illness is classified into foodborne infection and foodborne intoxication. In foodborne infections, the time from ingestion of the human host until symptoms occur is much longer than foodborne intoxications (Bintsis, 2017). The consumption of foods contaminated with foodborne pathogenic microorganisms and microbial toxins is responsible for deaths, illnesses, hospitalization, and economic

losses. Due to their widespread nature, foodborne diseases (FBD), especially gastrointestinal infections, negatively affect human health (Abd El-Aziz, 2015).

Bacteria and fungi usually contaminate meat and meat products. Foodborne pathogenic bacteria as *Salmonella, Shigella, Escherichia, Listeria, Clostridium,* and *Vibrio* and their toxins have been health problems (Abd El-Aziz and Yousef, 2017, Abd El Aziz and Yousef, 2018). *Salmonella* spp., *S. aureus*, *E. coli* O157: H7 and *L. monocytogenes* are the predominant bacteria species that cause public health problems worldwide, and they are the primary pathogens involved in food poisoning (Lei et al., 2008). The presence of pathogenic bacteria such as *Salmonella, Listeria monocytogenes, E. coli*, and toxigenic fungi in foods poses a poisoning threat (Darwish et al., 2008). As well, the growth of bacteria can lead to organoleptic changes in food, including off-colors and off-odors, rendering it unacceptable to the human consumer (Duffy et al., 2006).

Several PCR tests for detecting pathogens in foods have been validated, harmonized, and commercialized to make PCR, a standard tool used by food microbiology laboratories (Maurer, 2011; Postollec et al., 2011). PCR based on oligonucleotide primers has been developed quickly and more quickly than the bacterial culture (Abd El-Aziz, 2013). So, the main objective of this study was to detect the prevalence of foodborne pathogenic bacteria in fermented meat products. To confirm the identification of the bacterial isolates, the DNA lysate of pure colonies was amplified by PCR- based method using specific primers for each genus.

## 2. Materials and Methods

### 2.1. Collection of meat product samples

Twenty fermented meat products: Hotdog, pepperoni, salami, sausage, and luncheon (four from each) were included in the study. These samples were purchased from different Assiut cities, Egypt, and collected from September to December 2018. The samples were transferred in an icebox to the laboratory and kept frozen until microbiological analyses for pathogenic bacteria. The following primers, which were used to PCR-amplify specific genes, were synthesized by Invitrogen, Germany (Table 1).

**Table 1:** Primer nucleotide sequences for amplification of target genes from isolated foodborne pathogenic bacteria.

| Primer Name | Bacterial strain | Amplified product [bp] | Characteristics Annealing Ta | Primer Sequence 5`- 3` |
|---|---|---|---|---|
| F .FLICH7<br>R<br>FLICH7 | *E. coli* 0157:H7 | 247 | 48 °C | TACCATCGCAAAAGCAACTCC-<br>GTCGGCAACGTTAGTGATACC- |

| | | | | |
|---|---|---|---|---|
| 907R 27F | *Listeria* | 900 | 48 °C | CCGTCAATTCCTTTGAGTTTAGAGTTTGATCCTG GCTCAG- |
| *hIy*A-F *hIy*A-R | *Listeria monocytoge nes* | 200 | 48 °C | CCGTGCGCCCTTTCTAACTT- TTTGTTCAGTTTTGAGAGGT- |
| ST11-F ST15-R | *Salmonella* | 429 | 48 °C | GCCAACCATTGCTAAATTGGCGCA- GGTAGAAATTCCCAGCGGGTACTG G- |
| F-16S R-16S | *S. aureus* | 228 | 52 °C | GTAGGTGGCAAGCGTTATCC- CGCACA TCA GCG TCA G- |
| *mec*A1 *mec*A2 | *S. aureus mecA* | 532 | 50 °C | AAAATCGATGGTAAAGGTTGC- AGTTCT GCA GTA CCG GAT TTGC- |

**2.2. Isolation procedures of foodborne pathogenic bacteria**

**2.2.1. Isolation of Enterohemorrhagic *E. coli* O157:H7**

Each meat sample was enriched at a 1:10 ratio in Vancomycin Trypticase Soy Broth (VTSB) and shaked for 2 min in stomacher then incubated for 24 h at 37 °C (Samadpour et al., 2002; Ethelberg et al., 2009). One loopful from each enrichment VTSB culture was streaked on Sorbitol MacConkey agar plates then incubated at 37 °C for 24 h. *E. coli* O157:H7 colonies are sorbitol negative (appear pale yellow as compared with pink sorbitol positive).

**2.3. Biochemical reactions of *E. coli* O157:H7**

**2.3.1. Sugar fermentation test**

The pure culture of *E. coli* O157:H7 inoculated in peptone water contains 1% sorbitol using phenol red as an indicator, and then incubated at 37 °C for 24 h (Cowan and Steel., 1974). The appearance of no yellow color could not ferment sorbitol and be suspected to be *E. coli* O157:H7.

**2.3.2. Isolation of *Listeria* spp.**

Each meat sample was enriched at a 1:10 ratio in *Listeria* Enrichment Broth (LEB) and shaked for 2 min, then incubated at 35 °C for 48 h. A Loopful from LEB culture was streaked on oxford agar plates and incubated at 35 °C for 48 h. Gray colonies with black centers were suspected to be *Listeria* spp. were picked up onto nutrient agar slants for further confirmation.

**2.3.3. Isolation of *Salmonella* spp.**

Each meat sample was pre-enriched at a 1:10 ratio in lactose broth and blended for 2 min in stomacher, then incubated at 35 °C for 24 h. For enrichment, after incubation, the mixture was shaken well, and 1 mL was transferred to a sterile test tube containing 10 mL Rappaport Vassiliadis broth (R.V broth), then incubated at 35 °C for 24 h. A loopful from enrichment R.V broth culture was streaked on the surface of Salmonella-Shigella agar plates, and then the plates were incubated at 37 °C for 24 h. The

small colorless colony with a black center was picked up onto nutrient agar slants to confirm *Salmonella* (APHA, 1992).

### 2.3.4. Isolation of *Staphylococcus aureus*

Sodium chloride 10% broth tubes (Finegold and Martin, 1982) were inoculated with an appropriate amount from each prepared sample. Inoculated tubes were incubated at 37 °C for 24 h. Loops from incubated tubes were streaked on mannitol salt agar (MSA) plates (Finegold and Martin, 1982). Inoculated plates were incubated at 37 °C for 24 h. Mannitol fermented colonies (surrounded by yellow halo) were picked up onto nutrient agar slants for further confirmation.

### 2.3.5. Identification of foodborne pathogenic bacteria

The bacterial isolates were identified based on morphological examination include colony characteristics, shape, spore, motility, Gram's reaction and growth on differential medium (MacConkey agar, Endo agar medium). The bacterial identification was carried out following the standard methods described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

## 2.4. Molecular identification

### 2.4.1. Bacterial lysis and DNA extraction

A single colony of each isolate was picked and suspended in 200 µL of nuclease-free distilled water. After vortexing, the suspension was boiled for 5 min followed by snap chilling on ice for 10 min. 50 µL of the supernatant was collected after centrifuging for 10 min at 10956 × g. It was used as a template for PCR reactions. The concentration of DNA was measured using spectrophotometer Gene Quant1300 (Ali and Yousef, 2014).

### 2.4.2. Primers and PCR amplification

Specific primers were used for the amplification of each isolated foodborne pathogenic bacteria. The primer *fli*CH7was specific for *E. coli* O157:H7 (Samadpour et al., 2002; Wang et al., 2002). The primer*16SrRNA* gene was used for the detection of *the Listeria* genus. In addition, specific primers of the hemolysin (*hly*A) gene were used to detect *L. monocytogenes* isolates (Sanlibaba et al., 2018). The primer ST11-ST15 was specific for the genus *Salmonella* (Soumet et al., 1999). The primer *mec*A gene was used for the detection of methicillin resistance (MRSA) of *S. aureus (*Khan et al., 2012) (Table 1). The polymerase chain reaction was done by using a total volume of 25 µL. The optimal amplification reaction mixture contained 12.5 µL of master mix, 1 µL of forwarding primer (10 pmoL),1 µL of reverse primer (10 pmoL), 5 µL of DNAase and RNAse free water by using Deionizer water (Millipore-Direct-Q UV) and 5 µL of DNA (bacterial lysate). Go Taq ®Green Master mix is a premixed ready to use solution (Promega, USA): 608-274-4330 was used. PCR was carried out in a thermal cycler (Biometra, German).

The PCR products were separated on 1% agarose gel containing ethidium bromide at 100 volts for 1 hour. The results were analyzed by UV illuminator (viberloumat) and photographed by Gel documentation system, including Bio-Doc Analyze (BDA) software (Biometra) for measuring and analyzing the PCR products.

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## 3. Results and Discussion

### 3.1. Morphological identification of *E. coli* O157:H7

Examination of fermented meat samples revealed that one sample of 20 samples was positive for *E. coli* O157:H7 (Figure 1A). It was isolated from luncheon samples. It appears as Gram-negative short rods and facultative anaerobe. It can grow on sorbitol MacConkey agar medium. *E. coli* O157:H7 do not ferment sorbitol (colorless colony) (Cowan and Steel, 1974) (Figure 1A). Sheikh et al. (2013) isolated *E. coli* O157:H7 from ground beef hamburger. Also, *E. coli* O157:H7 was isolated by Chinen et al. (2001) from hamburger and ground beef.

### 3.2. Molecular identification of *E. coli* O157:H7

For confirming the identity of isolated *E. coli* O157:H7, the amplification PCR results of the target gene for Shiga toxinproducing *Escherichia coli* (STEC), *E. coli* O157:H7 was used to confirm the isolated colony. Specific PCR was used to determine the identities of *E. coli*O157:H7 through amplification of *fli*CH7 band gene fragments for *E. coli* O157:H7. Figure (1B) showed agarose (1%, w/v) gel indicating *fli*CH7 band gene fragments generated by PCR using genomic DNA extracted from *E. coli* O157:H7 isolated from luncheon. Gel electrophoresis of PCR products revealed the desired 247 bp fragment for the *fli*CH7 band. Abd El-Aziz (2015) found that 12 out of 90 meat-based sandwiches contain portions of *rfb* (O-antigen-encoding) regions of *E. coli* STEC serogroups O157 and O111. Also, Pavithra and Ghosh (2013) found 36 out of 215 samples from meat shops, fast foods, and fish stalls samples (16.7%) were identified with *E. coli*; 12 out of 100 samples were from fast foods. Out of 36 *E. coli* positive samples, nine (25%) possessed the gene encoding Shiga toxin *(stx*1) gene, including two samples from fast food (Baschera et al., 2019) as well as Hessain et al. (2015) isolated *E. coli* O157:H7 from beef burgers and chicken burgers. While Rasheed et al. (2014) and Ozbey et al. (2017) could not isolate STEC pathogen from the examined street vended samples, they stated that efficient cooking and other suitable hygienic prophylactic measures are needed decrease the incidence of STEC in food items.

**B**

A

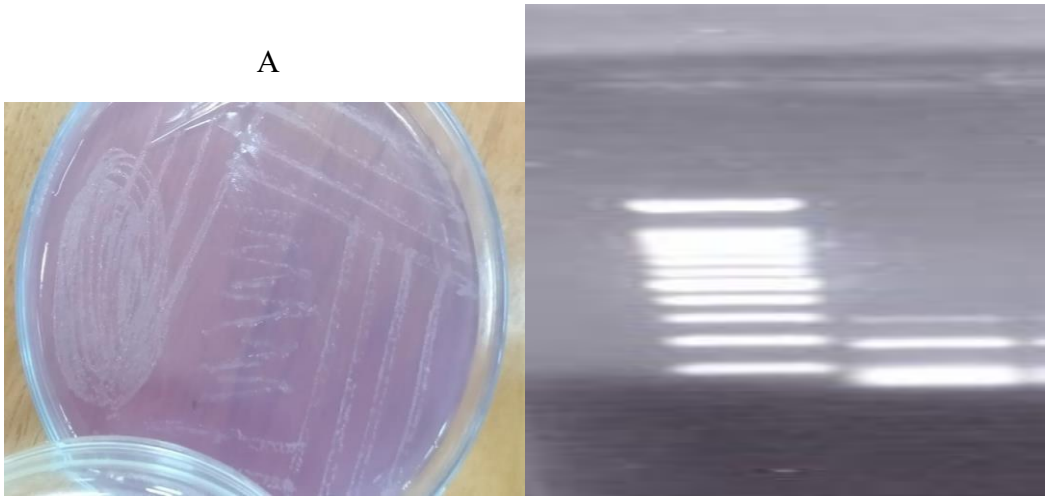

**Figure 1.** A; Streaking of *E. coli* O157:H7 on sorbitol MacConkey agar media. B; 1% agarose gel electrophoresis of PCR products to detect *E. coli* O157:H7 gene *fli*CH7: Lane 1: DNA Marker; Lane 2: PCR product of *E. coli* O157:H7 at 247 bp.

*Escherichia coli* are essential intestinal microbiota of humans and warm-blooded mammals. While *E. coli* typically harmlessly colonizes the intestinal tract, several *E. coli* strains can cause a variety of diseases within the intestinal tract and elsewhere in the host. The strains that because enteric infections are called diarrheagenic or pathogenic *E. coli* strains (Duffy et al. 2006; Abd El-Aziz, 2015).

A strain O157:H7 of *E. coli,* one that expressed O-antigen 157 and H-antigen 7, was shown to belong to a category of *E. coli* that produce toxins similar to Shiga toxin of *Shigella dysenteriae* and distinct from *E. coli* heat-stable and heat-labile toxins. Strain O157:H7 is estimated to cause 63,000 illnesses, 2,100 hospitalizations, and 20 deaths each year (Scallan, 2011). The principal reservoir for this zoonotic pathogen is the intestinal tract of cattle, and other animals may also serve as reservoirs. Transmission of *E. coli* O157:H7occurs when food or water contaminated with feces of infected animals or humans is consumed. Contamination of animal products often occurs during the slaughter and processing of animals or cattle manure as fertilizer for crops (Garcia et al., 2010). *E. coli* can survive for long periods in the environment and proliferate in vegetables and other foods (Garcia et al., 2010).

### 3.3. Isolation and molecular identification of *Salmonella* spp.

*Salmonella* spp. was isolated from two samples of chicken luncheon. It appears as a small colorless colony with a black center, Gram-negative short rods. The amplification PCR results of the target gene set for genus *Salmonella* confirmed the desired 429 bp fragment (Figure 2). Also, Tarabees et al. (2017) and Fahim et al. (2019) isolated *Salmonella* spp. from chicken meats. *Salmonella typhimurium was* isolated from chicken carcass meat and molecularly identified (Abd El-Aziz, 2013). Also, Ozbey et al. (2017) isolated *Salmonella* spp. from sausage.

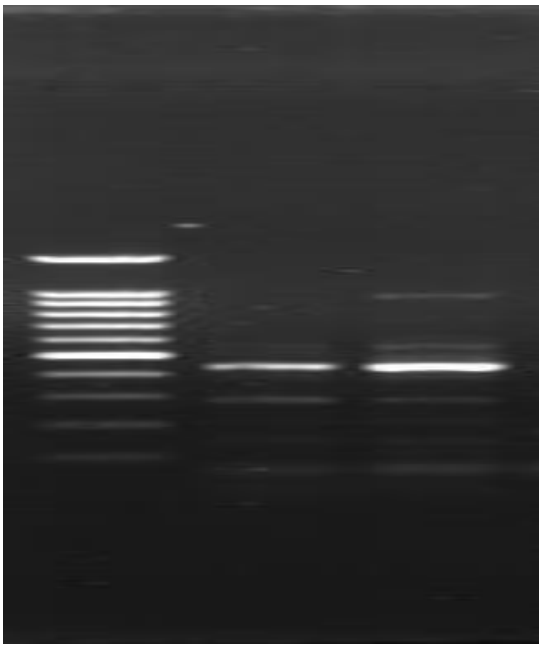

**Figure 2.** DNA gel electrophoresis on 1% agarose gel showing the PCR products: Lane 1: DNA size marker; lanes 2 & 3 PCR products of *Salmonella* spp. at 429 bp.

*Salmonella* spp, one group of Enterobactericiae, has pathogenic characteristics and is considered one of the most common causes of enteric infections (food poisoning) worldwide. *Salmonella* spp. lives in the intestines of most livestock and many wild animals. *Salmonella* spp. infection usually occurs when a person eats food contaminated with the feces of infected animals or humans. *Salmonella* outbreaks are commonly associated with meat, poultry, and eggs, but these bacteria can also contaminate other foods such as fruits and vegetables (Abd El-Aziz, 2013). Poultry products have consistently topped the incidence of salmonellosis in many developing countries, including India, Egypt, Brazil, and Zimbabwe (Yang et al., 2011). Contamination with *Salmonella* in poultry products can occur at multiple steps along the food chain, including processing, handling, preparation production, distribution, and retail marketing (Dookeran et al., 2012).

### 3.4. *Listeria monocytogenes*

*Listeria monocytogenes*was isolated from two samples of sausage. The genus of *Listeria* appears as Gram-positive short rods bacteria.

### 3.5. Molecular identification of *L. monocytogenes*

The amplification PCR results of the target genes for *Listeria* spp. were used to confirm the isolated colony. A total of 20 samples were examined for the presence of *Listeria* spp. Two samples of sausage were identified as *Listeria* spp. (Figure 3A). The amplification results of the target gene for the identification of *Listeria* spp. showed that 2 samples from 20 samples were defined as *Listeria monocytogenes* (Figure 3B). Hosseini et al. (2014) and Natratilova et al. (2004) isolated *Listeria monocytogenes*from sausages, salami, and burgers meat products. Ozbey et al. (2017) isolated *Listeria* sp. from sausage, salami, and sosis. Meloni (2015) reported that *L. monocytogenes*is among the most frequently detected pathogens in dry fermented sausages. Also, Bohaychuk et al. (2006) isolated *Listeria monocytogenes* from fermented sausages. B A

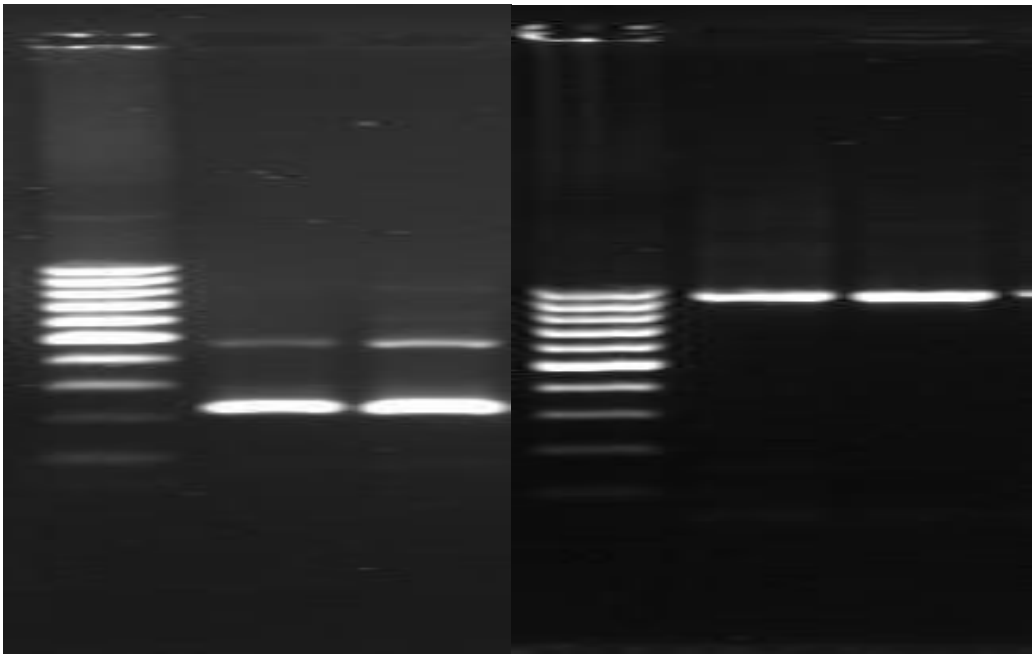

**Figure. 3.** A-PCR results of 16S rRNA gene for the detection of *Listeria spp*. Lane 1: DNA Marker; lanes 2, 3 amplification of *Listeria* spp. at 900 bp. B; 1% agarose gel electrophoresis showing the PCR results: Lane 1: DNA Marker; Lanes 2, 3 PCR products *hly* gene of *Listeria monocytogenes* at 200 bp.

*Listeria monocytogenes*is one of the leading causes of death from foodborne pathogens, especially in newborns, pregnant women, the elderly, and immuno-compromised individuals. The bacteria *Listeria spp* have been found in various raw foods, such as vegetables, uncooked meats, and contaminated foods after cooking or processing. It causes meningitis, septicemia and gastroenteritis (Ferreira et al., 2014; Buchanan, 2017). Listeriosis is a severe infection usually caused by eating food contaminated with *L. monocytogenes*. Although it is a relatively rare disease with a high mortality rate (20-30%), it is one of the deadliest food-borne threats (Jemmi and Stephen, 2006). Unlike many other pathogenic bacteria, *Listeria* multiplies in cold environments such as refrigerators and has tolerance to freezing temperature, high salt, and low pH (Ghandhi and Chikindas, 2007; Raheem, 2016). Ready-To-Eat (RTE) foods pose a higher risk for listeriosis as they are ingested without any further processing, such as cooking, that could kill *L. monocytogenes* (Raheem, 2016).

### 3.6. *Staphylococcus aureus*

Out of 20 samples, S. aureus was isolated from 6 samples of chicken luncheon and sausage (three from each). *S. aureus* is nonmotile, Gram-positive cocci that appear singly or in pairs, tetrads, short chains, or characteristic "grapelike" clusters and facultative anaerobes. All *S. aureus* samples were tested for the presence of the 16S rRNA gene in order to ensure the correct interpretation of toxin-negative isolates. Detection of the mecA gene by the polymerase chain reaction is considered the "Gold standard" in MRSA diagnosis. The primer *mec*A gene was used for the detection of methicillin resistance of *S. aureus*.

*Journal of Education and Digital Learning*
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http://kloverjournals.org/journals/index.php/edl

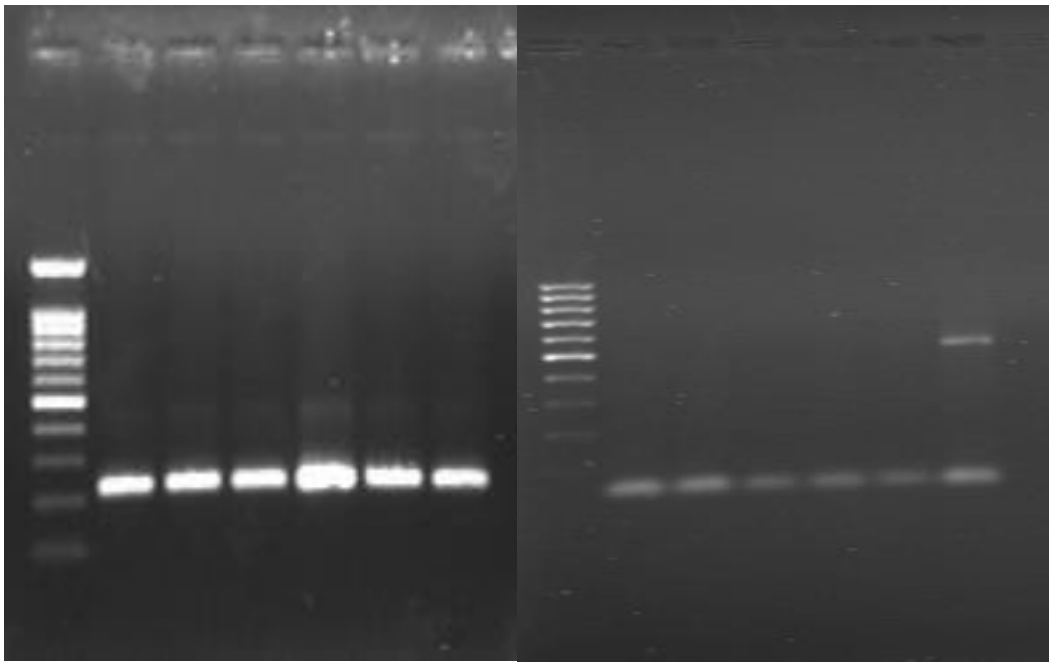


**Figure 4.** A: PCR results of 16S rRNA gene for the detection of *S. aureus* Lane 1: DNA Marker; lanes 2 to 7 amplification of *S. aureus*. B: Electrophoresis on 1% agarose gel showing the PCR results: Lane 1: DNA Marker; Lane7: MecA of *S. aureus*

### 3.7. Molecular identification of *S. aureus*

The amplification PCR results of the target genes for *S. aureus* was used to confirm the isolated colony. A total of 20 samples were examined for the presence of *S. aureus*. Six samples were identified as *S. aureus* (Figure 4A). The amplification results of the target gene for the identification of *S. aureus* showed that one sample from 20 samples defined as *S. aureus* contains *mec*A gene, which was isolated from sausage (Figure 4B). *S. aureus* was isolated from the beef burger and sausage (Mohammad et al., 2018). Bacon and Sofos (2003) reported that *S. aureus* was recorded in pork, ground beef, sausage, ground turkey. Most Staphylococcal food poisoning cases being traced to food contamination during preparation because of inadequate refrigeration, inadequate cooking or heating, or poor personal hygiene (Bacon and Sofos, 2003). After ingestion of the enterotoxin and an incubation period of less than 6 and up to 10 h, symptoms may include headache, vomiting, nausea, abdominal cramps, dizziness, chills, perspiration, general weakness, muscular cramping and prostration, and diarrhea that may or may not contain blood (Bacon and Sofos, 2003). *S. aureus* in food is considered a public health hazard because of its ability to produce enterotoxin and the subsequent risk of food poisoning. They are challenging to inactivate with heat because temperatures required to inactivate them are higher than

those needed to kill the organism (Bacon and Sofos, 2003). *S. aureus* is considered one of the most resistant non-spore-forming pathogens (FDA, 2012).

## 4. Conclusion

The results showed the moderate incidence of foodborne pathogenic bacteria in the examined meat samples, and greater emphasis should be applied in prevention and control of contamination during processing for reducing foodborne risk for consumers. Also, the results cleared that PCR is an ideal method for identifying foodborne pathogenic bacteria, as it was effective, more sensitive, reduces effort and time. PCR can be used as a diagnostic tool to correct foodborne pathogenic bacteria from meat samples.

## Acknowledgment

Authors would thanks to Research Finance Unit, Faculty of Science, Assiut University, Egypt, for their funding of this research.

## Conflict of interest

We declare that we have no conflict of interest.

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