Incidence and Duplex PCR for detection of *S. aureus* and *L. monocytogenes* in Meat Products

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**ARTICLE INFO**

**Keywords**  
*S. aureus*  
*L. monocytogenes*  
nuc gene  
hlyA gene  
duplex PCR

**ABSTRACT**

One hundred random samples of raw meat products (minced meat and sausage, 25 of each) and treated meat products (luncheon and basterma, 25 of each) were gathered from various markets in the provinces of Cairo and Giza for a period of 6 months. Accurately, 25 samples from each product were gathered from various markets in the provinces of Cairo and Giza. Traditional bacteriological methods and duplex polymerase chain reaction were used for detection of *S. aureus* and *L. monocytogenes* in such examined samples. Actually, *S. aureus* was isolated from 24%, 28%, 16% and 12% in minced meat, sausage, luncheon and basterma, respectively. Only, *L. monocytogenes* was detected in 4% of minced meat. Characterization of *S. aureus* by (nuc) gene and *L. monocytogenes* by (hlyA) gene using duplex Polymerase Chain Reaction. Concerning duplex PCR results, the incidence of *S. aureus* in minced meat, sausage, luncheon and basterma was (24%, 24%, 16%, 8%), respectively. While the incidence of *L. monocytogenes* in minced meat, sausage, luncheon and basterma was (4%, 0%, 0%, 0%), respectively. Duplex PCR showed a great agreement with the results of bacteriological method which proves the utility of the molecular technique in detection of food pathogens.

**1. INTRODUCTION**

Staphylococcus aureus can contaminate foods and cause illness humans; it's often linked to food poisoning. Furthermore, *S. aureus* can develop and convey virulence in a wide range of foods, including meat and meat products. The ability of certain *S. aureus* strains to generate heat stable enterotoxins that cause staphylococcal food poisoning, one of the most common causes of gastroenteritis worldwide (Saad et al. 2019).

Serious symptoms of listeriosis, such as sepsis, encephalitis, and meningitis, are common. The most vulnerable to severe infection are the elderly or immunocompromised, as well as pregnant women and their unborn children. Because of the severity of the symptoms and the high case fatality rate, *L. monocytogenes* research and control are critical for food safety worldwide (Thomas et al. 2015). The meat is exposed to many sources of contamination during the slaughtering process, including the atmosphere, machinery, and the hands of the staff. The sanitary condition of animals prior to, during, and after slaughter may have a significant impact on the quality of the finished product (Darweesh, 2008).

Microbiological testing is essential in determining food safety and quality. These methods are labor intensive, take a long time to process, and expensive. The fact that results can take up to three days to appear is a major drawback of this process (Jasson et al. 2010). The polymerase chain reaction (PCR) is an effective technique that has revolutionized molecular biology research. It can be used to diagnose pathogens in food samples. In comparison to culturing methods, duplex PCR has the advantage of being able to use more than one pathogen DNA in a single PCR reaction where it is time consuming, fast and reliable technique (Kim et al. 2010). Therefore, this study was carried out to optimizing a rapid duplex PCR method for detection of *S. aureus* and *L. monocytogenes* directly from the examined meat products not from bacteriological culture.

**2. MATERIAL AND METHODS**

2.1. Sampling

In reality, 100 raw and cooked meat samples (minced meat, sausage, luncheon, and basterma) were collected from various markets in Cairo and Giza governorates (25 of each) for a period of 6 moths. The collected samples were transferred to the Animal Health Research Institute's laboratory in sterile containers in a Stomacher bag for analysis.

2.2. Sample preparation

To achieve a $10^4$ dilution, 25 g of each sample was put in a sterile blender jar with (225 ml) peptone water (0.1 percent), the blender was turned on at 3000 rpm for no more than 2.5 min, and then (1 ml) of the original solution was transferred into a separate tube containing (9 ml) peptone water, from...
which a (10) fold serial dilution was performed (ICMSF, 1978).

2.3. Bacteriological isolation

According to Quinn et al. (2002) for *S. aureus* isolation, the samples were cultured on peptone water for 24 hours at 37°C, then a loopful was taken and cultured on Nutrient agar, 5% sheep blood agar, mannitol salt agar, and then Baird Parker medium (Oxoid). After incubating all inoculated plates at 37°C for 24-48 hours, colonies with an oval, smooth, convex, wet, 2-3 mm diameter, gray to jet-black, light-colored margin, surrounded by opaque zone, and sometimes with an outer transparent zone were suspected to be *S. aureus*. All suspected colonies are biochemically confirmed and checked.

According to ISO 11290-1, (1996) for *L. monocytogenes* isolation the samples were cultured on peptone water for 24 hours at 37°C, and the initial suspension was prepared and incubated at 30°C for 24 hours±2 hours. During the incubation, a black coloration developed. After incubating the initial suspension (primary enrichment) for 24 hours ±2 hours, 0.1 ml of the culture was transferred to a tube containing 10 ml of secondary enrichment medium (Full Fraser broth), which was then incubated for 48 hours ±2 hours at 35°C or 37°C. A part of the primary enrichment culture was inoculated on the surface of the selective plating medium Oxford (Oxoid) after being incubated for 24 hours ±3 hours at 30°C. The seeded plates were incubated to obtain colonies that were well differentiated. Colonies with morphological characteristics such as daw drop-like colonies, black with brown hollow colonies, or dark brown colonies with a diameter of 1-2 mm. All suspected colonies are biochemically confirmed and checked.

2.4. DNA extraction

To obtain purified DNA, Thermo Scientific Genomic DNA extraction kit was used to extract DNA directly from meat products samples.

2.5. PCR Primers

The Oligonucleotide primers that were designated using Integrated DNA technologies and used for amplification of the nuclease (*nuc*) gene of *S. aureus* and the hemolysin (*hlyA*) gene of *L. monocytogenes*. The primers were re-suspended in sterilized water to a final concentration of 100 pmol/l after being lyophilized. These primers are thought to amplify a particular 270 and 456 bp, respectively (Table 1).

Table 1 Detailed descriptions of the oligonucleotide primers used for PCR system.

| Types of Samples | Number of examined samples | Bacteriological finding | No. of positive samples | L. monocytogenes% |
|------------------|--------------------------|------------------------|------------------------|------------------|
| Minced meat      | 25                       | 6/24%                  | 1/4%                   |
| Sausage          | 25                       | 7/28%                  | 0/0%                   |
| Luncheon         | 25                       | 4/16%                  | 0/0%                   |
| Basterma         | 25                       | 3/12%                  | 0/0%                   |
| Total            | 100                      | 20/29%                 | 1/4%                   |

Table 2 Number and percentages of S.aureus and L.monocytogenes detected in the examined samples of meat products.

2.6. PCR amplification

The reaction mix is 6.25 µl Verso master mix (2x), 1.75 µl PCR grade water, 0.5 µl forward primer from each gene, 0.5 µl reverse primer from each gene, 2.5 µl template DNA to achieve a final amount of 12.5 µl in the reaction, according to Verso master mix (Thermo Scientific).

2.7. Duplex PCR

Thermocycling conditions included primary denaturation at 95°C for 5 minutes, proceeded by 40 cycles of heat denaturation at 95°C for 15 seconds, primer annealing at (59°C) for 45 seconds, and DNA extension at 72°C for 1 minute, followed by final extension at 72°C for 10 minutes.

2.8. UV Visualization

On a 1.5 % agarose gel, 5 ul of the PCR substance was resolved. The gels were stained with Ethidium bromide (0.2g/ml) and photographed in a gel documentation device with UV transillumination (BioRad). (Sambrook et al. 1989).

3. RESULTS

3.1. Results of culture isolation

Table (2) showed the incidence in minced meat, sausage, luncheon, and basterma for *S. aureus* was (24%, 28%, 16%, 12%), respectively. While for *L. monocytogenes* was (4%, 0%, 0%, 0%), respectively.

3.2. Results of duplex Polymerase Chain Reaction (PCR)

For *S. aureus*, 20 random positive meat product samples detected by conventional method were re-examined by duplex PCR, there were great agreement between results of conventional method and duplex PCR technique in 18 random samples. While two samples were positive by conventional method for *S. aureus*, showed negative for (*nuc*) gene one from sausage and one from basterma negative for (*nuc*) gene as shown in fig. (1).

For *L. monocytogenes*, there was only one positive sample of minced meat had been detected by both conventional method and duplex PCR technique as shown in fig. (2).

![PCR result](image-url)
Ahmed, (2001). On the contrary, higher findings were nearly similar to those obtained by L. monocytogenes Sanlibaba et al. (2000) (12.2%); Van Coillie et al. (2004) (42.1%) and et al. (2013) (2.8%), and lower than those reported by Inoue (5%).

As shown in Table (2), the percentages of S. aureus obtained from the examined minced meat, sausage, luncheon, and basterma samples were 4%, 0%, 0%, 0%, respectively. The obtained results of S. aureus in the examined samples of those of minced meat were nearly similar to those of Saad et al. (2019) (25%) of minced beef, higher than those obtained by Heredia et al. (2001) (2.3%); Omar et al. (2009) (14.6%), while lower than those of Gundogan et al. (2005) (53.3%), and Yi Li, (2010) (34%).

In the examined sausage samples, the obtained results were nearly similar to those of Zakaria, (2007) (25%), and higher than those obtained by Soultos et al. (2003) (19.4%), while lower than those of Saad et al. (2019) (36%).

On the other hand, the obtained results of the examined luncheon samples were nearly similar to those reported by Hassani-Fatin, (2004) (16%), while lower than those of Ismail-Seham et al. (2013) (32%). They speculated that contamination may occur during supermarket luncheon meat slicing and packing.

The obtained results of the examined basterma samples were lower than those of Hassani-Fatin, (2004) and Zakaria, (2007) (36% and 25%), respectively.

In general, S. aureus is widely known to be one of the main causes of foodborne diseases in meat products. Contamination of foodstuffs may occur directly from contaminated animals processing foodstuffs or may result from inadequate hygiene during manufacturing processes, or from food retailing and storage (Normanno et al. 2007).

As shown in Table (2), the percentages of L. monocytogenes obtained from the examined minced meat, sausage, luncheon, and basterma samples were 4%, 0%, 0%, 0%, respectively. The obtained results of the examined minced meat samples were nearly similar to those of El-lawandy-Hanan, (2001) (5%). While they were higher than those obtained by Zarei et al. (2013) (2.8%), and lower than those reported by Inoue et al. (2000) (12.2%); Van Coillie et al. (2004) (42.1%) and Sanibaba et al. (2020) (8.75%).

On either, the ready-to-eat beef samples that were assessed, L. monocytogenes was not found (luncheon and basterma). Such results were nearly similar to those obtained by Ahmed, (2001). On the contrary, higher findings were obtained by Saad et al. (2001) (7.5%) in the luncheon samples analyzed and Ahmed-Hanaa et al. (2012) (26.6%) in the analyzed basterma samples.

In general, inadequate cooking of meat products can lead to a possible risk of L. monocytogenes especially if present in large numbers (>10⁵ cfug). Therefore, proper time-temperature monitoring for meat cooking should be observed. Food should be cooked to an internal temperature of 70°C for more than 20 minutes to guarantee that L. monocytogenes is eradicated in order to decrease human listeriosis (Luth et al. 2020).

The current study revealed that the duplex PCR technique was very convenient to take DNA directly from the samples of meat products, and there is no need to take them from the bacterial culture as it is time-consuming, labor-intensive and very costly, as stated by Chen et al. (2012) and Kim et al. (2014) Who tested without the use of bacterial cultures directly from the food samples.

The selection of specific target genes for each of the target pathogens is essential for developing the m-PCR assay as shown in Table (1). The nuclease (nuc) gene of S. aureus was used in several laboratories for the detection of S. aureus isolates (Pinto et al. 2005). The hemolysin (hlyA) gene is most widely used for detecting L. monocytogens, where the gene is selective to that species (Pazia-Domanska et al. 1999).

The current results are very similar to those of Latha et al. (2014) who developed duplex PCR on detection of S. aureus and L. monocytogenes, where (nuc) gene and (hlyA) gene were used. Thus, this study was initiated to support a rapid and efficient duplex PCR assay for simultaneous detection of two pathogens for gram +ve (S.aureus & L. monocytogens).

The inclusion of spices, curing methods that suppress the influence of PCR, and the existence of PCR inhibitors in food. In addition, inefficient DNA extraction and incomplete extraction of bacterial cells contribute to false negative PCR findings (Jenikova et al. 2000).

5. CONCLUSION

There was a lot of consensuses between the results of duplex PCR and traditional culture methods, indicating that duplex PCR was a relatively reliable and effective tool for rapid screening of these pathogens. To summarize, the duplex PCR assay has the potential to be used in standard diagnostic laboratories and may also be used as a rapid screening tool in food testing laboratories to quickly identify food samples, particularly in the case of outbreaks.

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