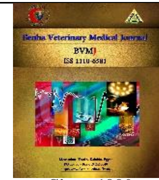
Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/



Since 1990

Original Paper

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

George, Armany[1], Hemmat, Ibrahim[2], Reham, Amin[2], Naglaa, Hagag[1]

[1]*Genome Research Unit., Animal Health Research Institute, Dokki, Egypt*

[2]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*

ARTICLE INFO

Keywords

S. aureus

L. monocytogenes

nuc gene

hlyA gene

duplex PCR

Received 01/06/2021

Accepted 20/06/2021

Available On-Line 01/10/2021

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^{-1} 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

* Corresponding author: georgearmany006@gmail.com

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 dew drop-like colonies, black with brown hallow 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 Oligoneucleotide 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.

Target pathogen	Target gene	Primer	Sequence (5` - 3`)	Amplicon size bp	Reference
S. aureus	*nuc*	Forward Reverse	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	270	*Pinto et al. (2005)*
L. monocytogenes	*hylA*	Forward Re+verse	GCGATTGATGGTGATACGGTT GCAGTTGCAAGCGCTTGGAGTGAA	456	*Paziak-Domanska et al. (1999)*

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).

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

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

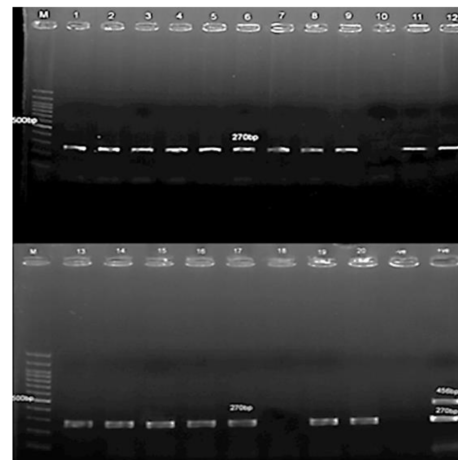


Figure 1 Duplex PCR for S. aureus positive meat product samples. M: marker (100bp). Lane 1-6: 6 positive minced meat samples for *S. aureus.*Lane 7-13: 7 positive sausage samples for *S. aureus.* Lane 14-17: 4 positive luncheon samples for *S. aureus.*Lane 18-20: 3 positive basterma samples for *S. aureus.*-ve: Negative Control.+ve: Positive Control Control (for *S. aureus* → (270bp) & *L. monocytogens* → (456bp).

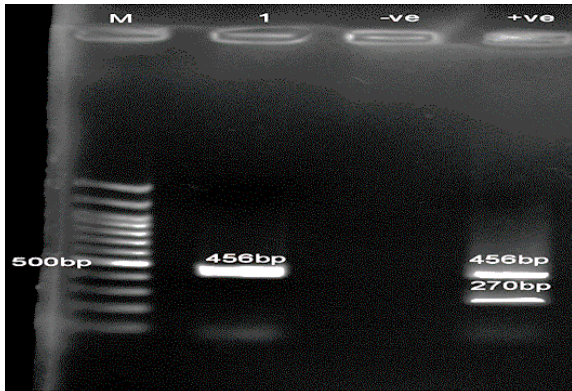


Figure 2 Duplex PCR for L.monocytogenes positive meat product sample. M: marker (100bp). Lane 1: minced meat sample positive for *L. monocytogenes*. -ve: Negative Control.+ve: Positive Control (for *S. aureus* → (270bp) & *L. monocytogens* → (456bp).

4. DISCUSSION

As shown in Table (2), the percentages of *S. aureus* isolated from the examined minced meat, sausage, luncheon, and basterma samples were 24%, 24%, 16%, 12%, 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 Hassanien-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 Hassanien-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 Sanlibaba 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.
However, the lower incidence of *L. monocytogenes* may be due to addition of spices, curing agents and the temperature used during processing, and good sanitation.
In general, inadequate cooking of meat products can lead to a possible risk of *L. monocytogenes* especially if present in large numbers (>10^5 cfu/g). 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 listerisois (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* which 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. monocytogenes*, where the gene is selective to that species (Paziak-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. monocytgenes*).
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 (Jeníkova 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.

6. REFERENCES

1. Ahmed-Hanaa, A., Mohamed-Amal, A. and Omran-Gihan, M. 2012. Molecular detection of pathogenic genes of Listeria monocytogenes as food poisoning micro-organism isolated from ready to eat meals in Port-Said city markets. Assuit Veterinary Medicine Journal 132(5), 51-67.

2 . Ahmed, S. 2001. Prevalence of Listeria monocytogenes in meat and some meat products. Ph.D Thesis, Veterinary Sciences, Meat Hygiene, Faculty of Veterinary Medicine, Assiut University.

3. Chen, J., Tang, J., Liu, J., Cai, Z. and Bai, X. (2012): Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. Applied Microbiology Journal 112, 823–830.

4. Darweesh, F. 2008. Assessment of Microbial quality of meat and its products designed for retailed sale. PhD Thesis Meat Hygiene, Faculty of Veterinary Medicine, Alexandria University.

5. El-Lawandy-Hanan, T. 2001. Occurrence of listeria species and survival of Listeria monocytogenes in meat and meat products. Ph.D Thesis, Veterinary Sciences, Meat Hygiene Faculty of Veterinary Medicine, Zagazig University (Benha Branch).

6. Gundogan, N., Citak, S., Yucel, N. and Devren, A. 2005. A note on the incidence and antibiotic resistance of Staphylococcus aureus isolated from meat and chicken samples. Meat Science 69 (1), 807 –810.

7. Hassanien-Fatin, S. 2004. Bacterial Hazards Associated with Consumption of Some Meat Products. Benha Veterinary Medicine Journal 15(2), 129-141.

8. Heredia, N., García, S., Rojas, G. and Salazar, L. 2001. Microbiological condition of ground meat retailed in Monterrey, Mexico Journal of Food Protection 64(8), 49-51.

9. ICMSF International Commission on the Microbiological Specification for food. 1978. Microorganism in Foods, Their Significance and Method of Enumeration .2nd Ed University of Toronto Press. Toronto Canada.

10. Inoue, S., Nakama, A., Kokubo, Y., Maruyama, T., Saito, A., Yoshida, T., Terao, M., Yamamoto, S. and Kumagai, S. 2000. Prevalence and contamination levels of Listeria monocytogenes in retail foods in Japan. International Food Microbiology 59 (1), 73 - 77.

11. Ismail-Seham, A.; Shehata-Amal, A. and El-diasty, M. 2013. Microbiological Quality of Some Meat Products in Local Markets with Special Reference to Mycotoxins. Global Veterinaria 10(5), 577-584.

12. ISO 11290-1, 1996. International Organization for Standardization. Microbiology of food and animal feeding stuffs–horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1: detection method .

13. Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A. and Uyttendaele, M. 2010. Alternative microbial methods: An overview and selection criteria. Food Microbiology 27, 710–730.

14. Jeníkova, G.; Pazlarova, J. and Demnerova K. 2000. Detection of Salmonella in food samples by the combination of immunomagnetic separation and PCR assay. International Journal of Microbiology 3(1), 225–229.

15. Kim, J., Rhim, S., Kim, K., Paik, H. and Lee, J. 2014. Simultaneous Detection of Listeria monocytogenes, Escherichia coli O157: H7, Bacillus cereus, Salmonella spp. and Staphylococcus aureus by Multiplex PCR. Korean Journal of Food Science Animal Resources 34(5), 715–716.

16. Kim, W., Kim, H., Rhim, R., Lee, A., Kim, J. and Paik, D. 2010. A Multiplex PCR Assay for the Detection of Foodborne Pathogens in Meat Products. Korean Journal for Food Science of Animal Resources 30(4), 590-596.

17. Latha, A., Sunil, A. and Jolly, D. 2014. Multiplex PCR assay for the simultaneous detection of four common food pathogens in meat. Journal of Foodborne and Zoonotic Diseases 2(3), 45–49.

18. Luth, S., Halbedel, S., Rosner, B., Wilking, H., Holzer, A., Roedel, A. and Kleta, S. 2020. Backtracking and forward checking of human listeriosis clusters identified a multiclonal outbreak linked to Listeria monocytogenes in meat products of a single producer. Emerging Microbes and Infections 9(1), 1600–1608.

19. Normanno, G., Corrente, G., Dambrosio, A., Quaglia, C., Parisi, A., Greco, G., Bellacicco, L., Virgilio, S. and Celano, V. 2007. Methicillin-resistant Staphylococcus aureus (MRSA) in foods of animal origin product in Italy. International Journal of Food Microbiology 117(2), 219-22.

20. Omar, S., Ikbal, A. and Entesar. A. 2009 Extraction of Staphylococcus aureus toxin from minced meat in Mosul City. The Iraq Journal for Veterinary Medicine 23(1), 27-32.

21. Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M. and Kur, J. (1999): Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of Listeria monocytogenes in meat foods. FEMS Microbiology Letter 171(1), 209–214.

22. Pinto, B., Chenoll, E. and Aznar, R. 2005. Identification and typing of foodborne Staphylococcus aureus by PCR-based techniques Systematic and Applied Microbiology 28(1), 340–352.

23. Quinn, J., Markey, K., Carter, E., Donnelly, C., Leonard, C. and Maguire, D. 2002. Veterinary Microbiology and Microbial diseases. Published Blackwell science (1st Ed.) London.

24. Saad, S., Fahim, S., Abo El-Roos, N. and Saber, E. 2019. Incidence of Staphylococci and E. coli in Meat and Some Meat Products. EC NUTRITION Research Article. Ec Nutrition 4(1), 171-179.

25. Saad, S., Ibrahim, H., Niazi, Z. and El-Lawandy, H. 2001. Prevelance of Listeria spp. in meat and meat products. Veterinary Medicine Journal 49(4), 543-552.

26. Sambrook, J., Fritscgh, E. and Meniates, E. 1989. Molecular cloning. A laboratory manual. (1). Cold Spring Harbor Laboratory press, New York.

27. Şanlibaba, B., Uymaz Tezel, A., Çakmak, K. and Akcelik. M. 2020. Occurence of Listeria spp. and Antibiotic Resistance Profiles of Listeria monocytogenes from Raw Meat at Retail in Turkey. Italian. Journal of Food Science 32(2), 234–250.

28. Soultos, N., Abrahim, A. and Abrosiadis, I. 2003. Incidence of some food-borne bacterial pathogens in

traditional sausages produced in northen Greece. Archives of Food Hygiene 54(3), 55-57.

29. Thomas, K., Vriezen, R., Farber, M., Currie, A., Schlech, W. and Fazil, A. 2015. Economic cost of a Listeria monocytogenes outbreak in Canada, 2008. Foodborne Pathogens and Disease 12(12), 966-971.

30. Van Coillie, E., Werbrouch, H., Heyndrickx, M., Herman, L. and Rijpens, N. 2004. Prevalence and typing of Listeria monocytogences in ready to- eat food products on Belgian market. Journal of Food Protection 67 (11), 2480- 2488.

31. Yi Li, 2010. Isolation and Characterization of antimicrobial resistant Staphylococcus aureus in retail ground meats The Degree of M. V. Sc. University of Maryland, College Park.

32. Zakaria, H. 2007. Studies on food poisoning microorganisms in some meat products. The Degree of M. V. Sc. (Meat Hygiene & control). Faculty of Veterinary Medicine, Menoufia University (Sadat branch).

33. Zarei, M., Basiri, N., Jamnejad, A. and Eskandari, H. 2013. Prevalence of Escherichia coli O157:H7, Listeria monocytogenes and Salmonella spp. in Beef, Buffalo and Lamb Using Multiplex PCR. Jundishapur Journal of Microbiology 6(8), 1–5.