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Authors :	Marwa Mahmoud Radwan|Mohamed Mohamed Mousa|Nashwa mohamed helmy|

Assessment of proteomic and molecular techniques in isolation of *Listeria monocytogenes* in minced meat.

Marwa M. Radwan [1], , Nashwa M. Helmy[2] , Mohamed M. Mousa[3]

[1] Alex food inspection lab, Alexandria Provincial Laboratory, Animal Health Research Institute , Giza, Egypt.

[2] Department of biotechnology, Animal health research institute ,Giza, Egypt.

[3] Department of food Hygiene, Faculty of Veterinary Medicine, Alexandria University

*** Corresponding author**

Marwa M.Radwan

Phone: 01112611447

Fax: none

E-mail: marwa2011vet@gmail.com

Address: Alex food inspection lab, Alexandria Provincial Laboratory, Animal Health Research Institute

ABSTRACT

Listeria monocytogenes is one of the emerging pathogens worldwide. The aim of this study was to evaluate and Assess different techniques; Conventional cultural methods; Biochemical identification; Automated Biochemical identification (Vitek 2 compact); Proteomic identification MALDI-TOF MS and Molecular identification (RT- PCR) in Isolation and Identification of *Listeria monocytogenes* in minced meat retailed in local markets in Alexandria City, Egypt. Prevalence of suspected listeria spp. isolated from examined samples of minced meat by conventional culture method were 65% (65/100), while prevalence of *L. monocytogenes* isolated

from examined samples of minced meat 30.8% (20/65), 23.07% (15/65) and 53.8% (35/65) by ViTeK2 compact; MALDI-TOF MS and RT-PCR, respectively, with overall prevalence 53.8 % (35/65) considering molecular detection results which depends on gene detection which offer the highest Sensitivity in identification of *L. monocytogenes* . Identification of *L. monocytogenes* within (Vitek 2 Compact) were as followed, *L. monocytogenes* 30.8% (20/65), while *L. innoca* 23,07%(15/65).,while within MALDI -TOF MS were as followed *L. monocytogenes* 23,08% (15/65), while *L. innoca* 20%(13/65).The prevalence of *L. innoca* lower than *L. monocytogenes* , *L. monocytogenes* was most commonly detected in minced meat. The high isolation rate of *L. monocytogenes* among the examined minced meat highlight the risk of transmission of infection to human consumer and enforcing quality assurance programs as Hazard Analysis Critical Control Points (HACCP) program during processing, handling and storage of minced meat.

Key words: Minced meat, *L .monocytogenes*, VITEC, MALD-TOF MS, RT-PCR.

1. INTRODUCTION

Foodborne listeriosis is one of the most serious and severe foodborne diseases (WHO, 2015). *Listeria monocytogenes* possesses a significant public health significance due to frequent contamination of food products (Ahmed et al., 2017). In Egypt, *L. monocytogenes* enters into human food chain primarily through contaminated meat and chicken products (Abd El-Malek et al., 2010). In particular, the ability of *L. monocytogenes* to grow in the refrigerator or cooled condition in varieties of food products makes the pathogen difficult to control (Vinothkumar et al., 2013). *Listeria* is psychrophilic, gram positive, non- spore forming bacteria. it includes many species as *L.grayi, L. innocua, L. welshimeri, L. ivanovii, L. seeligeri, L. fleischmannii and L. weihenstephanensis. L. monocytogenes and L. ivanovii.* They are well known to be pathogenic to humans and animals causing clinical manifestations such as gastroenteritis, encephalitis,

meningitis and abortion (Lakićević et al., 2014; Al-Nabulsi et al., 2015; Ruppitsch et al., 2015). In addition to, *listeria* can produce a biofilm at low temperatures to facilitate persistent dissemination of this pathogen during food production (Allerberger et al., 2015). In contrast to other enteric pathogenic bacteria, *Listeria* can multiply at refrigeration temperature zones, which indicates that refrigeration of meat to 4ºC could not hinder the growth of *listeria* (Pal et al., 2008). Also *Listeria* can grow in broad zones of temperature ranging from 1.5ºC to 45ºC, pH started from 4.3 till 9.40 and a salt level till 10%. The ubiquitous feature of *listeria* led to contamination of various processed meat and fermented meat products at the different stage of processing and storage (Guillier et al., 2005; Zanette et al., 2015).

Various studies have reported the prevalence of *Listeria* spp. in a broad range of meat and its products (Abd El-Malek et al., 2010; Ismaiel et al., 2014; Al-Nabulsi et al., 2015; Mazza et al., 2015; Hesham et al., 2017). The bacteria is also considered as a potential contaminant source for chilled and marine food products (Vinothkumar et al., 2013) and unpasteurized dairy products and has been detected in recent outbreaks and sporadic cases of listeriosis (Kim et al., 2018). Determination *of listeria monocytogenes* in minced meat has become remarkably important as a food-borne pathogen.

L.monocytogenes causes listeriosis in humans and animals and it is associated with high hospitalization and outbreaks of food-borne illness worldwide (Osman et al., 2019), as *L.monocytogenes* is ranked as one of the major causative agents for high mortality rates among all foodborne bacterial infections. The instance of listeriosis has been sporadic in African countries. Recently, there has been an expansion in the prevalence of human listeriosis, comprising food contamination in Egypt which iteratively detected in several dairy products, ready-to-eat foods, fish and fish products, meat and meat products (Ismaiel et al., 2014; Kamar et al., 2016; Mohamed

et al., 2016). Traditionally, Listeria diagnosis is primarily based on isolation and biochemical characterization, which is time consuming. Molecular approaches such as PCR provide rapid and specific techniques for the diagnosis of *L. monocytogenes* (Abd El-Malek et al., 2010).

Therefore, this study was conducted to evaluate and Assess different techniques: Conventional cultural methods; Biochemical identification; Automated Biochemical identification (Vitek 2 compact); Proteomic identification MALDI-TOF MS and Molecular identification (RT- PCR) in isolation and identification of *Listeria monocytogenes* in examined retailed minced meat.

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 100 minced meat samples size of each sample: 250 g. were collected randomly from supermarkets, groceries, and street-vendors at Alexandria City, Egypt. The samples were obtained as sold to the public and transferred as soon as possible in an insulated icebox at $4 \pm 1^{o}C$ to the laboratory. The samples were collected from October 2018 to October 2019.

2.2. Isolation and identification of Listeria monocytogenes (ISO, 2017)

Twenty–five grams of each examined sample were transferred into 225 ml of the primary enrichment medium (half-Fraser broth) pre-warmed to room temperature before use, to obtain tenth fold dilution, and homogenized. The incubation carried out at 30 °C for 25 ± 1 h Aseptically, 0.1 ml of incubated half-Fraser broth added to 10 ml Fraser broth followed by incubation at 37 °C for 24 ± 2 h. Loopful of Fraser broth (after incubation 37 °C for 24 ± 2 h) was streaked onto the first selective medium, Agar Listeria according to Ottaviani and Agosti (ALOA), and the second selective medium (Oxford agar). All the plates were incubated at 37 °C for 48 h. Suspected colonies (Blue-green colonies surrounded by an opaque halo zone)were transferred to tryptone soya yeast extract agar (TSYEA), for further biochemical identification (Beta-haemolysis , L-Rhamnose and D-Xylose).

2.3. Automatic biochemical identification by VITEK 2 Compact (BioMérieux, USA)

The VITEK2 compact is an automated microbiology system utilizing growth-based technology. The system accommodates colorimetric reagent cards that are incubated and interpreted automatically. This format focuses on the industrial microbiology-testing environment while also having application for low to middle volume clinical laboratories. Features specifically developed for industrial microbiology include 21 CFR Part 11compliance (for electronic records and signatures) and colorimetric reagent cards used to identify GP (*L. monocytogenes*).

2.4. MALDI-TOF MS (BioMérieux, France)

MALDI-TOF MS was performed at time-points 3, 6, 8, 10 and 12 h until a pathogen was successfully identified. The spectra were acquired using the MALDI-TOF Vitek

MS (bioMérieux) and analysed on Vitek MS IVD system (bioMérieux; Marcy l'Etoile, France). Samples were performed in duplicate, with tests performed simultaneously on the same

target slide. Part of single colony was transferred to an individual spot on the 48-well Vitek MS-DS disposable target slide. Each spot was covered with 1 µl ready-to-use Vitek MS alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix (bioMérieux, France). The target plate was then read and analyzed by the Vitek MS IVD system.

The protein profile of each specimen with an m/z of 3,000 to 15,000 was produced and profiles were further matched with the Vitek MS reference CE-IVD certified database (>20,000 spectra). Matching results with confidence percentages 90 to 98% confidence were considered for genus level, while results of>98% confidence, were considered for the species level, while <90% confidence were unacceptable identification. For specimens that showed different identification under the same genus, we identified to the genus level only, with more than one genus or family identifications, they were considered unacceptable.

2.5. Molecular identification Quantative Polymerase Chain Reaction (qPCR) (Real time PCR)

2.5.1. DNA extraction: DNA were extracted by using G-spin ^{TM} Total DNA Extraction kit

The extraction procedures were carried out according to producer instruction from Cultivated animal cells, tissues, Gram negative bacteria and Blood using silica membrane

2.5.2. Real time PCR

Real time DNA amplification was done according to the manufacturer's protocol of mericon® Pathogen Detection Handbook instructions manual

The reaction was conducted in 20 µl total volume as shown in table (1). A positive and negative control must be analyzed for every run.

1- Keep all samples and reaction tubes on ice during setup.

2. Close the PCR tubes or strips and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.

3. Program the thermal cycler. Table(2).

4. Start the PCR run.

3. RESULTS AND DISCUSSION

Detection of the bacteria in food products at retail outlets indicates a major defect in the quality control measures (El-Demerdash & Raslan, 2019). Contamination of the meat with *L. monocytogenes* generally occur after the slaughter and may come from the skin of the animals, the hands of the workers, the equipment and the tools used (Marinsek & Grebenc, 2002). It is also important to comment that the presence of any Listeria spp. may be indicative of poor hygiene and cross-contamination scenarios which cold favor the persistence of *L. monocytogenes* (Azevedo et al., 2005).

The current study revealed that table (3) prevalence of suspected listeria spp. isolated from examined samples of minced meat by conventional culture method were 65% (65/100).

The results obtained from table (4) showed that prevalence of *L. monocytogenes* isolated from examined samples of minced meat 30.8% (20/65), 23.07% (15/65) and 53.8% (35/65) by ViTeK2 compact; MALDI-TOF MS and RT-PCR, respectively with overall prevalence 53.8 % (35/65) considering molecular detection results which depends on gene detection which offer most sensitive and specific technique, that agree with Somer and Kashi (2003) , Jantzen et al. (2006), Abd El-Malek et al. (2010), Chen et al. (2010), El-Demerdash and Raslan (2019), and Abdeen et

al. (2021) who demonstrated the accuracy and sensitivity of PCR techniques in isolation of *L. monocytogenes* from various food products.

Figure (1) show the amplification plot of *L. monocytogenes* through RT- PCR .

The high prevalence of *L. monocytogenes* in examined minced meat may attribute to exposure of minced meat to various processing steps and manipulation throughout its processing (Tompkin et al., 1992; Uyttendaele et al., 1997).

The presence of *L. monocytogenes* in minced meat may occur due to contamination of meat through insignificant hygienic conditions, throughout slaughtering, evisceration, processing, and vending (Mahmoud et al., 2019). Also, it may come from the skin of the animals, the hands of the workers, the equipment and the tools used (Marinsek & Grebenc, 2002 and Mahmoud et al., 2019). It is also important to comment that the presence of any Listeria spp. may be indicative of poor hygiene and cross-contamination scenarios which cold favor the persistence of *L. monocytogenes* (Azevedo et al., 2005; Yücel et al., 2005).

The results of our study higher than studies reported by Marinsek and Grebenc (2002) who isolated *L. monocytogenes* from 6.81% of minced meat samples collected from Slovenia. Akpolat et al. (2004),Yücel et al. (2005) who obtained lower incidence of *L. monocytogenes* 5% and 4.7% ,respectively, from minced meat in turkey . Molla et al. (2004) who isolated *L. monocytogenes* from 1.6% minced meat in In addis Ababa, Ethiopia. Abd El-Malek et al. (2010) who studied 4% *L. monocytogenes* from minced meat that collected from retail supermarkets in Assiut (Egypt). Mohamed et al. (2016) , Reda et al. (2016) who found that *L. monocytogenes* in minced meat (4%) that collected from Giza Governorate, Egypt . and Hassan et al. (2019) who reported that the minced meat samples that collected from three different Egyptian companies collected from local supermarkets at Alexandria Governorate were negative for *L. monocytogenes*.

the results in our study in concurrence with El-Demerdash and Raslan (2019) who reported high prevalence of L. monocytogenes in minced meat (56%) in urban and rural areas of Egypt. , Mahmoud et al. (2019) who isolated *L.monocytogenes* (15%) from minced meat that collected from Qena Governorate, Egypt and Abdeen et al. (2021) who reported that *L. monocytogenes* was most commonly detected in minced meat (14%) in Menoufiya governorate in Egypt. The variation in the detection rate of *L. monocytogenes* among different studies could be explained by food sample size, geographic area and the degree of sanitary measures applied during food processing and manufacture.

The results obtained from table (5) showed that identification of *L. monocytogenes* (n =65), within biochemical identification (Vitek 2 Compact) were as followed, *L. monocytogenes 30,8% (20/65),* while *L. innoca* 23,07%(15/65).

On the other hand, the results obtained from table (6) showed that identification of *L. monocytogenes* (n =65), within MALDI -TOF MS were as followed L. monocytogenes 23,08% (15/65), while *L. innoca* 20%(13/65).

The overall prevalence of *L. innoca* 23,07 %(15/65) considering (Vitek 2 Compact) results which offer more sensitivity than MALDI -TOF MS as it showed in table (7). The prevalence of *L. innoca* lower than L. monocytogenes , *L. monocytogenes* was most commonly detected in minced meat. The results of our study are agree with Garedew et al. (2015) who isolated 12% L. monocytogenes and 8% of L. innoca from minced meat & Abdeen et al. (2021) who isolated 6.8% L. monocytogenes and 3.2% of L. innoca from minced meat. while the results are not agree with Molla et al., (2004) who isolated 1.6% L. monocytogenes and 34.4% of L. innoca from minced meat, Abd El-Malek et al. (2010) who isolated 4% L. monocytogenes and 28% of L. innoca from

minced meat &Mahmoud et al. (2019) who isolated 15% L. monocytogenes and 20% of L. innoca from minced meat.

L. innoca is well known to be non pathogenic to human and animals but its presence indicate to poor hygiene (Abd El-Malek et al. 2010, Ismaiel et al. 2014; Mazza et al. 2015 and Hesham et al. 2017**)**

The results of our study obtained from table (7) demonstrated the assessment of the different technique used in isolation of *L. monocytogenes* from examined minced meat. Molecular identification through Real-time PCR was the highest specificity, Sensitivity and agreement in identification of *L. monocytogenes* while the conventional cultural methods and biochemical identification were the lowest specificity, Sensitivity and agreement.

MALDI TOF-MS have the same specificity as Real-time PCR in isolation of *L. monocytogenes* but lower in sensitivity than RT-PCR and this may attribute to MALDI TOF-MS enables the identification of isolated microorganisms based on their protein profile. However, the method has the following limitations: difficulty in identifying microorganisms having high phylogenetic relatedness, lack of protein profile of some species in the database, and the possibility of technical errors, for example when the sample is applied to the plate for analysis (Cieślik & Wróblewska 2018). In addition to, MALDI TOF-MS is affected by type of bacteria if the Gram-positive strains or the Gram-negative strains and the type of food in identification of micro-organisms which may cause changes in the intensity and area of the peaks in the mass spectrum and even led to the disappearance of certain peaks (Nicolaou et al., 2012).

This result agree with Nucera et al. (2006), Bagge et al. (2010), Wieser et al. (2012), Body et al. (2018), Rychert (2019),Abd El-Aziz et al. (2021), Ramatla et al. (2021).

while, our results were not agree with Angelakis et al. (2011), Böhme et al. (2011), Nicolaou et al. (2012), Quintela-Baluja et al. (2013), Król et al. (2018), Pomastowski et al. (2019), Bucka-Kolendo et al. (2020), Khater et al. (2021) who detected foodborne pathogens in food samples by MALDI-TOF MS and reported that MALDI-TOF in comparison with other isolation techniques is the most efficient tool for identifying foodborne pathogens.

Vitek 2 compact system have the same specificity as Real-time PCR in isolation of *L. monocytogenes* but lower in sensitivity than RT-PCR, as the PCR technique is very convenient to target specific genes and take the nucleic-acid templates whether directly from the tissue samples after nucleic-acid Extraction or from the culture. lower sensitivity of vitek2 compact system may due to the low number of bacterial load which can't be detected by microbiological assay, high population of competitive bacteria, and the inhibitory effect of some food additives.

This result agree with Chen et al. (2012) , Kim et al. (2014), Moustafa et al. (2016), Hernández-Durán et al. (2017) , Shaltout (2020), , who detected a false negative results by Vitek2 compact system and positive by PCR.

4. CONCLUSIONS

Finally, the current study allows concluding that the high prevalence of *L. monocytogenes* in examined minced meat that collected from Alexandria city and *L. innoca* indicate to poor hygiene and cross-contamination that may occur to minced meat during various processing steps and manipulation throughout its processing. Molecular identification through Real-time PCR was the highest Sensitivity in identification of *L. monocytogenes* . Detection of *L. monocytogenes* by MALDI_TOF MS could be retarded by the type of the food. Choosing the technique used in

diagnosis of Foodborne-pathogen could be affected by type of examined food, type of pathogen, cost and time consuming.

5. **ABBREVIATIONS: MALDI_TOF MS :** Matrix-assisted laser desorption ionization-time of flight mass spectrometry ; **L**: Listeria ; **RT-PCR** : Real Time Polymerase chain Reaction

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7. REFERENCES

Abd El-Aziz, N. K., Gharib, A. A., Mohamed, E. A. A., & Hussein, A. H. 2021. Real-time PCR versus MALDI-TOF MS and culture-based techniques for diagnosis of bloodstream and pyogenic infections in humans and animals. J. Appl. Microbiol. 130(5): 1630-1644.

Abd El-Malek, A. M., Ali, S. F. H., Hassanein, R., Mohamed, M. A., & Elsayh, K. I. 2010. Occurrence of Listeria species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of Listeria monocytogenes. Vet. World. 3(8): 353-359.

Abdeen, E. E., Mousa, W. S., Harb, O., Fath-Elbab, G. A., Nooruzzaman, M., Gaber, A. . . . & Abdeen, A. 2021. Prevalence, Antibiogram and Genetic Characterization of Listeria monocytogenes from Food Products in Egypt. Foods. 10(6): 1381.

Ahmed, S., Tayeb, B., Ameen, A., Merza, S., & Sharif, Y. 2017. Isolation and molecular detection of Listeria monocytogenes in minced meat, frozen chicken and cheese in Duhok province, Kurdistan region of Iraq. J. Food. Microbiol. Saf. Hyg. 2(1): 118.

Akpolat, N., Elci, S., Atmaca, S., & Gül, K. 2004. Listeria monocytogenes in products of animal origin in Turkey. Vet. Res. Commun 28(7): 561-567.

Al-Nabulsi, A. A., Osaili, T. M., Awad, A. A., Olaimat, A. N., Shaker, R. R., & Holley, R. A. 2015. Occurrence and antibiotic susceptibility of Listeria monocytogenes isolated from raw and processed meat products in Amman, Jordan. CyTA-J. Food. 13(3): 346-352.

Allerberger, F., Bagó, Z., Huhulescu, S., & Pierzka, A. 2015. Listeriosis: the dark side of refrigeration and ensiling. In A. Sing (Ed.), Zoonoses - Infections Affecting Humans and Animals Focus on Public Health Aspects (p.p. 249–286). Heidelberg, Germany: Springer Verlag.

Angelakis, E., Million, M., Henry, M., & Raoult, D. 2011. Rapid and accurate bacterial identification in probiotics and yoghurts by MALDI-TOF mass spectrometry. J. Food. Sci. 76(8): M568-572.

Azevedo, I., Regalo, M., Mena, C., Almeida, G., Carneiro, L., Teixeira, P. . . . & Gibbs, P. A. 2005. Incidence of Listeria spp. in domestic refrigerators in Portugal. Food. Control. 16(2): 121-124.

Bagge, E., Persson, M., & Johansson, K. E. 2010. Diversity of spore-forming bacteria in cattle manure, slaughterhouse waste and samples from biogas plants. J. Appl. Microbiol. 109(5): 1549-1565.

Body, B. A., Beard, M. A., Slechta, E. S., Hanson, K. E., Barker, A. P., Babady, N. E. . . . & Rychert, J. 2018. Evaluation of the Vitek MS v3.0 Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry System for Identification of Mycobacterium and Nocardia Species. J. Clin. Microbiol. 56(6): e00237-00218.

Böhme, K., Fernández-No, I. C., Barros-Velázquez, J., Gallardo, J. M., Cañas, B., & Calo-Mata, P. 2011. Rapid species identification of seafood spoilage and pathogenic Gram-positive bacteria by MALDI-TOF mass fingerprinting. Electrophoresis. 32(21): 2951-2965.

Bucka-Kolendo, J., Sokołowska, B., & Winiarczyk, S. 2020. Influence of High Hydrostatic Pressure on the Identification of Lactobacillus by MALDI-TOF MS- Preliminary Study. Microorganisms. 8(6): 813.

Chen, B. Y., Pyla, R., Kim, T. J., Silva, J. L., & Jung, Y. S. 2010. Prevalence and contamination patterns of Listeria monocytogenes in catfish processing environment and fresh fillets. Food. Microbiol. 27(5): 645-652.

Chen, J., Tang, J., Liu, J., Cai, Z., & Bai, X. 2012. Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. J. Appl. Microbiol. 112(4): 823-830.

Cieślik, J., & Wróblewska , M. 2018. MALDI-TOF MS–new possibilities in routine microbiological diagnostics. Diagn. Lab 54: 99-104.

El-Demerdash, A. S., & Raslan, M. T. 2019. Molecular characterization of Listeria monocytogenes isolated from different animal-origin food items from urban and rural areas. Adv. Anim. Vet. Sci. 7(s2): 51-56.

Fayol, L., Beizig, S., Le Monnier, A., Lacroze, V., & Simeoni, U. 2009. Neonatal meningitis due to Listeria monocytogenes after 3 weeks of maternal treatment during pregnancy. Arch. Pediatr. 16(4): 353-356.

Guillier, L., Pardon, P., & Augustin, J.-C. 2005. Influence of stress on individual lag time distributions of Listeria monocytogenes. Appl. Environ. Microbiol. 71(6): 2940-2948.

Hassan, M. M., AbdAl-Aziz, S. A., Megahed, A. M., Abdel Rassoul, H. A., & Mabrouk, Y. M. 2019. Molecular Identification of Listeria Species from Some Processed Meat Products Using Specific Iap Gene and 16S rRNA Gene. Alex. Sci. Exchange. J. 40: 333-339.

Hernández-Durán, M., López-Jácome, L. E., Colín-Castro, C. A., Cerón-González, G., Ortega-Peña, S., Vanegas-Rodríguez, E. S. . . . & Franco-Cendejas, R. 2017. Comparison of the microscan walkaway and Vitek 2 compact systems for the identification and susceptibility of clinical gram-positive and gram-negative bacteria. Invest. Discapacid. 6(3): 105-114.

Hesham, T. N., Hanan, L. E., Fathi, A. T., Gehan, A. E., & Salem, F. A. 2017. Prevalence of Listeria spp. among dairy, meat and their products marketed in Tripoli, Libya. Int. J. Life Sci. Res. 5: 19-25.

International Organization for Standardization [ISO]. 2017. IISO 11290-1: Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes. Geneva, Switzerland: ISO. Available from: https://www.iso.org/standard/19268.html.

Ismaiel, A. A.-R., Ali, A. E.-S., & Enan, G. 2014. Incidence of Listeria in Egyptian meat and dairy samples. Food. Sci. Biotechnol. 23(1): 179-185.

Jantzen, M., Navas, J., Corujo, A., Moreno, R., López, V., & Martínez-Suárez, J. V. 2006. Specific detection of Listeria monocytogenes in foods using commercial methods: from chromogenic media to real-time PCR. Span. J. Agric. Res. 4(3): 235-247.

Jemmi, T., & Stephan, R. 2006. Listeria monocytogenes: food-borne pathogen and hygiene indicator. Rev. Sci. Tech. 25(2): 571-580.

Kamar, A. M., Torky, H. A., & El-Houshy, S. M. 2016. Phenotypic and Genotypic Characterization of Listeria Monocytogenes Isolated from Different Sources. Alex. J. Vet. Sci. 51(2): 1-19.

Khater, D. F., Lela, R. A., El-Diasty, M., Moustafa, S. A., & Wareth, G. 2021. Detection of harmful foodborne pathogens in food samples at the points of sale by MALDT-TOF MS in Egypt. BMC. Res. Notes. 14(1): 112.

Kim, J. H., Rhim, S. R., Kim, K. T., Paik, H. D., & Lee, J. Y. 2014. Simultaneous Detection of Listeria monocytogenes, Escherichia coli O157:H7, Bacillus cereus, Salmonella spp., and Staphylococcus aureus in Low-fatted Milk by Multiplex PCR. Korean. J. Food. Sci. Anim. Resour. 34(5): 717-723.

Kim, S. W., Haendiges, J., Keller, E. N., Myers, R., Kim, A., Lombard, J. E. . . . & Haley, B. J. 2018. Genetic diversity and virulence profiles of Listeria monocytogenes recovered from bulk tank milk, milk filters, and milking equipment from dairies in the United States (2002 to 2014). PLoS One. 13(5): e0197053.

Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., Walczak, J., & Buszewski, B. 2018. Microbiology neutralization of zearalenone using Lactococcus lactis and Bifidobacterium sp. Anal. Bioanal. Chem. 410(3): 943-952.

Lakićević, B., Bunčić, O., Katić, V., LEPŠANOVIĆ, Z., Petrović, L., Janković, V., & Ikonić, P. 2014. Detection of Listeria spp. during production and ripening of Petrovská klobása. Acta. Veterin. Beograd. 64(3): 367-377.

Mahmoud, H., Karmi, M., & Maky, M. 2019. Occurrence and Characterization of Listeria Species Isolated from Processed Meat in Qena, Egypt. Zagazig. Vet. J. 47(3): 267-276.

Marinsek, J., & Grebenc, S. 2002. Listeria monocytogenes in minced meat and thermally untreated meat products in Slovenia. Slov. Vet. Res. 39(2): 131-136.

Mazza, R., Piras, F., Ladu, D., Putzolu, M., Consolati, S. G., & Mazzette, R. 2015. Identification of Listeria spp. strains isolated from meat products and meat production plants by multiplex polymerase chain reaction. Ital. J. Food. Safety. 4(4): 5498.

Mohamed, Y., Reda, W. W., Abdel-Moein, K., Abd El-Razik, K. A., Barakat, A. M. A., El Fadaly, H. A. . . . & Hegazi, A. G. 2016. Prevalence and phylogenetic characterization of Listeria monocytogenes isolated from processed meat marketed in Egypt. J. Genet. Eng. Biotechnol. 14(1): 119-123.

Molla, B., Yilma, R., & Alemayehu, D. 2004. Listeria monocytogenes and other Listeria species in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop. J. Heal. Dev. 18(3): 208-212.

Moustafa, N. Y., Abd El-Hafiz, R. M., & ElBahy, E. F. 2016. Incidence of Staphylococcus aureus and Salmonella in Poultry Meat. Glob. Vet. 16(6): 570-578.

Nicolaou, N., Xu, Y., & Goodacre, R. 2012. Detection and quantification of bacterial spoilage in milk and pork meat using MALDI-TOF-MS and multivariate analysis. Anal. Chem. 84(14): 5951-5958.

Nucera, D. M., Maddox, C. W., Hoien-Dalen, P., & Weigel, R. M. 2006. Comparison of API 20E and invA PCR for identification of Salmonella enterica isolates from swine production units. J. Clin. Microbiol. 44(9): 3388-3390.

Osman, K. M., Kappell, A. D., Fox, E. M., Orabi, A., & Samir, A. 2019. Prevalence, Pathogenicity, Virulence, Antibiotic Resistance, and Phylogenetic Analysis of Biofilm-Producing

Listeria monocytogenes Isolated from Different Ecological Niches in Egypt: Food, Humans, Animals, and Environment. Pathogens. 9(1): 5.

Pal, A., Labuza, T. P., & Diez-Gonzalez, F. 2008. Comparison of primary predictive models to study the growth of Listeria monocytogenes at low temperatures in liquid cultures and selection of fastest growing ribotypes in meat and turkey product slurries. Food. Microbiol. 25(3): 460-470.

Pomastowski, P., Złoch, M., Rodzik, A., Ligor, M., Kostrzewa, M., & Buszewski, B. 2019. Analysis of bacteria associated with honeys of different geographical and botanical origin using two different identification approaches: MALDI-TOF MS and 16S rDNA PCR technique. PLoS One. 14(5): e0217078.

Quintela-Baluja, M., Böhme, K., Fernández-No, I. C., Morandi, S., Alnakip, M. E., Caamaño-Antelo, S. . . . & Calo-Mata, P. 2013. Characterization of different food-isolated Enterococcus strains by MALDI-TOF mass fingerprinting. Electrophoresis. 34(15): 2240-2250.

Ramatla, T., Ngoma, L., & Mwanza, M. 2021. The Utility of MALDI-TOF-Mass Spectrometry, Analytical Profile Index (API) and Conventional-PCR for the Detection of Foodborne Pathogens from Meat. J. Food. Nutr. Res. 9(8): 442-448.

Reda, W. W., Abdel-Moein, K., Hegazi, A., Mohamed, Y., & Abdel-Razik, K. 2016. Listeria monocytogenes: An emerging food-borne pathogen and its public health implications. J. Infect. Dev Ctries. 10(2): 149-154.

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H. L., Allerberger, F. . . . & Mellmann, A. 2015. Defining and Evaluating a Core Genome Multilocus Sequence Typing Scheme

for Whole-Genome Sequence-Based Typing of Listeria monocytogenes. J. Clin. Microbiol. 53(9): 2869-2876.

Rychert, J. 2019. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. J. Infectiol. 2(4): 1-5.

Shaltout, F. 2020. Prevalence of some food poisoning bacteria in semi cooked chicken meat products at Kaliobyia governorate with using of recent Vitek 2 compact and PCR techniques. Benha. Vet. Med. J. 38(2): 88-92.

Somer, L., & Kashi, Y. 2003. A PCR method based on 16S rRNA sequence for simultaneous detection of the genus Listeria and the species Listeria monocytogenes in food products. J. Food. Prot. 66(9): 1658-1665.

Tompkin, R., Christiansen, L., Shaparis, A., Baker, R., & Schroeder, J. 1992. Control of Listeria monocytogenes in processed meats. Food. Australia. 44(8): 370-376.

Uyttendaele, M., Neyts, K., Lips, R., & Debevere, J. 1997. Incidence ofListeria monocytogenesin poultry and poultry products obtained from Belgian and French abbatoirs. Food. Microbiol. 14(4): 339-345.

Vinothkumar, R., Arunagiri, K., & Sivakumar, T. 2013. Studies on pathogenic Listeria monocytogenes from marine food resources. Int. J. Curr. Microbiol. App. Sci. 1(1): 86-93.

Walsh, D., Duffy, G., Sheridan, J., Blair, I., & McDowell, D. 1998. Comparison of selective and nonselective enrichment media for the isolation of Listeria species from retail foods. J. Food. Saf. 18(2): 85-99.

Wieser, A., Schneider, L., Jung, J., & Schubert, S. 2012. MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl. Microbiol. Biotechnol. 93(3): 965-974.

World Health Organization [WHO]. 2015. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. Geneva, Switzerland: WHO.

Yücel, N., Cıtak, S., & Önder, M. 2005. Prevalence and antibiotic resistance of Listeria species in meat products in Ankara, Turkey. Food. Microbiol. 22(2-3): 241-245.

Zanette, C., Dalla Santa, O., & Bersot, L. 2015. Effect of Lactobacillus plantarum starter cultures on the behavior of Listeria monocytogenes during sausage maturation. Int. Food. Res. J. 22(2): 844-848.

Figure:

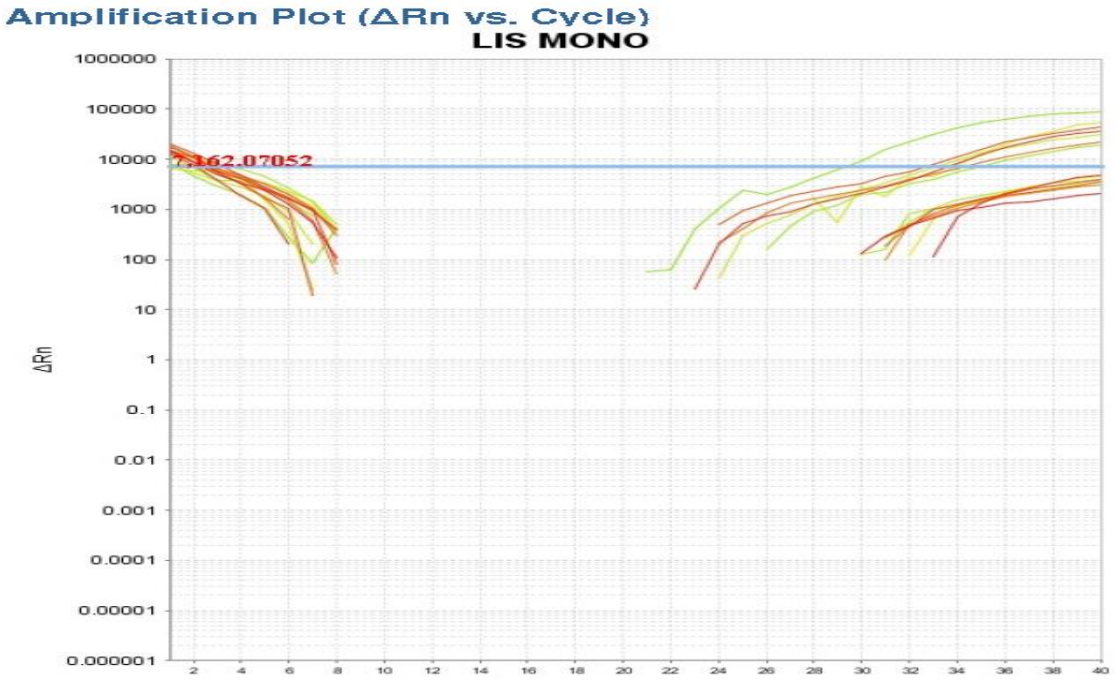

Figure (1): Amplification plot of *Listeria monocytogenes* through RT-PCR

Tables:

Table (1): Setup of sample and control reactions

Component	Sample	Positive PCR Control	Negative PCR Control
Reconstituted mericon Assay	10 µl	10 µl	l0 µl
Sample DNA	10 µl	-	-
Dissolved Positive Control DNA	-	10 µl	-
QuantiTect Nucleic Acid Dilution Buffer or RNase-free Water	-	-	10 µl
Total Volume	20 µl	20 µl	20 µl

Table (2). Cycling protocol for real-time cyclers other than Rotor-Gene Q

Step	**Time**	**Temperature**	**Comments**
Initial PCR activation Step	5 min	95°C	Activation of HotstarTaq plus DNA polymerase
3-Step Cycling:			
Denaturation	15s	95°C	Data Collection at 60°C
Annealing	23s*	60°C	
Extension	10s	72°C	
Number of Cycles 40			
Detection	**Reporter**	**Excitation / emission**	**Channel**
Target	FAM	495/520nm	FAM
Internal control	MAX	524/ 557nm	VIC

Table (3): Prevalence of suspected *Listeria spp.* isolated from examined samples of minced meat by conventional culture method .(n=100)

Isolate	Incidence by using conventional culture methods (ALOA) -Blue green colonies surrounded by an opaque halo zone .		Biochemical identification	
	Positive sample		Positive sample	
L. monocytogenes	NO.	%	NO.	%
	65	65	65	65

Table (4) Prevalence of *Listeria monocytogenes* isolated from examined samples of minced meat by different isolation techniques. (n=65)

Isolate	Techniques of Identification					
	Automated Biochemical identification (ViteK 2) compact		Proteomic identification MALDI-TOF MS Bruker Daltonik MALDI Biotyper		Molecular Identification RT-PCR	
	Positive Sample		Positive Sample		Positive Sample	
L. monocytogenes	NO.	%	NO.	%	NO.	%
	20	30.8	15	23.07	35	53.8

Table (5): Identification of suspected *listeria spp.* by Automated Biochemical identification (Vitek 2 compact) (n=65)

Type	No. of Sample	%
Listeria monocytogenes	20	30.8
Listeria innocua	15	23.07

Table (6): Identification of suspected *listeria spp.*by MALDI-TOF MS (n=65)

Type	No. of Sample	%
Listeria monocytogenes	15	23.08
Listeria innocua	13	20

Table (7): Sensitivity, specificity, and agreement of isolation and identification of *Listeria monocytogenes* with confirmatory RR-PCR technique in examined minced meat samples

Identification techniques	Methods	Sensitivity %	Specificity %	Agreement %
Conventional cultural methods	**ALOA agar**	100	53.8	70
Biochemical Identification	**Biochemical tests**	100	53.8	70
Automated Biochemical identification	**Vitek 2 compact**	57.1	100	85
Proteomic identification	**MALDI-TOF MS**	42.9	100	80
Molecular identification	**RT- PCR**	100	100	100