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Prevalence and phylogenetic characterization of *Listeria monocytogenes* isolated from processed meat marketed in Egypt

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Abstract Because of its high case fatality rate, listeriosis locates among the most frequent causes of death due to food-borne illness. In this study, a total of 150 processed meat samples were collected from Giza Governorate, Egypt. Phenotypic and genotypic identification of *Listeria monocytogenes* was performed using PCR incorporating listeriolysin O virulence gene hlyA followed by DNA sequence analysis. *L. monocytogenes* was confirmed in 4% of each of beef burger, minced meat, and luncheon samples. Phylogenetic analysis showed that all the six Egyptian isolates have high homology with Colombian isolate (EF030606), except one Egyptian isolate which showed high homology with Indian isolate (EU840690). The public health significance of these pathogens as well as recommended sanitary measures were discussed.

1. Introduction

The genus *Listeria* includes only two pathogenic strains, *Listeria monocytogenes* is a well-known cause of abortion, encephalitis and septicemia in man and animals. *Listeria ivanovii* is of major veterinary importance as a cause of abortions, still births and neonatal septicemias in sheep and cattle, and a rare human infection [24].

L. monocytogenes has become remarkably important as a food-borne pathogen. The ability to persist in diverse conditions such as low temperature and pH, high salt concentrations and multiplication under refrigeration temperatures makes it a serious threat to public health [7,12]. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness with the highest hospitalization rates (91%) and mortality rate up to 30% [13].

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The detection of this pathogen in food by routine culture methods is difficult by the sporadic or low levels of contamination, by the presence of a high level of background microflora [17]. Moreover, these methods are laborious and time consuming [1], while immediate action should be taken in case of contamination especially in the case of foods having short shelf-lives, such as meat or dairy products. In general, DNA-based typing approaches are recognized as simple and cost-effective methods that have better discriminatory power than phenotypic approaches and being better for investigating *L. monocytogenes* outbreaks [5]. The classification based on the nucleic acid sequence has become more common. The gene hly encoding listeriolysin O is one such target gene for specific detection of *L. monocytogenes* [21].

Molecular fingerprinting is by far considered the most precise method for studying the epidemiology of foodborne diseases and allows prediction of the relationships between Listeria isolates, regardless of the origin or the geographical location [9].

Usually, *L. monocytogenes* is susceptible to a wide range of antibiotics, but resistance to multiple antibiotics was recorded [18]. The presence of multiple key virulence factors (Virulence markers) such as listeriolysin O (LLO encoded by hlyA) significantly regulates the virulence and pathogenicity of *L. monocytogenes* [21]. Moreover, food and host environments may present variable expression of virulence genes, resulting in varied infectivity [4]. Therefore, it is essential to study the epidemiological significance and distribution of virulence determinants within these isolates [23]. Thus the aim of this study was to determine the prevalence and phylogenetic characterization of *L monocytogenes* isolated from processed meat collected from markets in Egypt.

2. Materials and methods

2.1. Samples

A total of 150 meat samples were collected from Giza Governorate over the period of October 2013 to September 2014. These samples included 25 minced meats, 25 luncheons, 50 sausages, 50 beef burger.

2.2. Isolation and identification of L. monocytogenes

L. monocytogenes was isolated from the examined food samples according to the International Organization for Standardization procedure [11]. Suspected colonies were transferred to tryptic soya agar plates with 0.6% yeast extract (TSA-YE) for further biochemical identification using *Listeria* Microbact 12L (Oxoid, UK). For DNA extraction, colonies were suspended in 500 µl of PBS, pH 7.2, washed 3 times in PBS. The cell suspension was centrifuged for 10 min at 800*g*, then the supernatant was discarded carefully and the pellet was dried and stored at −20 °C till use.

2.3. Polymerase chain reaction (PCR) analysis and DNA sequencing

2.3.1. DNA extraction

For extraction of DNA, bacterial pellets were re-suspended with 200 µl PBS. DNA was extracted from *L. monocytogenes* isolates using the DNA extraction Kits (GF-1, Vivantis Co., Malaysia) according to manufacturer's instructions.

2.3.2. PCR

This PCR amplifies a 234 bp region of the hlyA gene (α-Hemolysin, listeriolysin O) encoding listeriolysin O. PCR was performed according to Furrer et al. [8]. 50 µl volume containing 2 µl of each primer (10 µM), 25 µl of 2X Taq Master Mix (Cat. No. PLMM01,Vivantis Co., Malaysia), Primers, LMA: CGGAGG TTCC GCAAAAGATG and LMB: CCTCCAGAGTGATCGATGTT. Polymerase chain reaction (PCR) amplification conditions were: 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 45 s at 55 °C, 45 s at 72 °C and a final extension of five min at 72 °C. The PCR products were analyzed using 1% agarose gel electrophoresis.

The positive PCR products were then sequenced in MACROGEN Company (Korea) on 3730 × L sequencers (Applied Biosystem, USA). The accuracy of data was confirmed by two-directional sequencing with the forward and reverse primers used in PCR.

The nucleotide sequences obtained in this study were analyzed using the BioEdit 7.0.4.1 and ClustalW2 (http://www.clustal.org/) programs. The resulting sequences were aligned with hlyA gene of reference sequences of *Liateria* spp. using a neighbor-joining analysis of the aligned sequences implemented in the program CLC Sequence Viewer 6.

3. Results and discussion

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 [16]. 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* [2].

In the present study, PCR results showed that *L. monocytogenes* were confirmed in 8 out of 25 of beef burgers examined (4%), one out of 25 of minced meat (4%), and one out of 25 of Luncheon (4%) from the total 100 examined food samples. The sausage samples were totally negative.

This suggests the presence of a significant public health hazard linked to the consumption of this meat sold in Giza Governorate contaminated with *L. monocytogenes*.

3.1. Nucleotide sequence accession numbers

Six sequences PCR samples (Giza 1–6) used in this study have been deposited in the Gene Bank database under accession no: KR812472, KR812473, KR81274, KR534875, KR812475 and KR812476 respectively. Phylogenetic analysis showed that all the six Egyptian isolates have high homology with Colombian isolate (EF030606), except one Egyptian isolate which showed high homology with Indian isolate (EU840690). This may be due to the importation of animals and raw meat from the Latin America and India. This suggests the presence of a significant public health hazard linked to the consumption of this meat sold in Egypt contaminated with *L. monocytogenes*.

From Fig. 1, the differences in the sequences of the six Egyptian isolates (Giza 1–6) which we examined in comparison with the other Gene Bank isolates from different isolation