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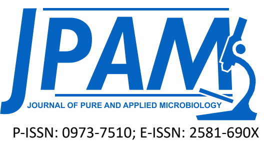

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RESEARCH ARTICLE OPEN ACCESS

Rapid Detection of *Listeria monocytogenes* In Chicken Meat By Real-time PCR without Culture Enrichment

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Abstract

Foodborne pathogens can easily contaminate chicken meat due to its high nutritional content, and these pathogens can infect humans. One of the most important pathogens contaminating chicken meat and causing severe public health problems is *Listeria monocytogenes*, which would be responsible for Listeriosis. Therefore, rapid and sensitive detection of *L. monocytogenes* in chicken meat samples is of great significance. In the current study, the presence of *L. monocytogenes* in chicken meat samples collected from several markets in Erzurum was detected by comparing two different DNA isolation methods with the Real-time PCR. As a result of the analyses, it was determined that 34% of the chicken meat samples collected were positive for *L. monocytogenes* in both two methods. According to the comparison analyses of the Bland-Altman method, no significant difference was found between the thermal lysis method and the DNA isolation method by commercial kit. As a result of this study, it has been shown that the thermal lysis method can be successfully applied for the detection of foodborne pathogens in chicken meat when evaluated in terms of workload and cost. The current study is the first report on the comparison of thermal lysis method and DNA isolation by commercial kit in the detection of *L. monocytogenes* from chicken meat by Real-time PCR.

Keywords: Chicken Meat, *Listeria monocytogenes,* Public Health, Real-time PCR

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INTRODUCTION

Chicken meat is consumed worldwide due to its high nutritional value, low-cost production, and short cooking process in a widespread manner. It is an excellent source of animal protein with a low lipid content, containing all essential amino acids and unsaturated fatty acids essential for human beings.[1,2] This feature of chicken meat also allows the development of different bacteria. Various pathogenic microorganisms such as *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Listeria* spp. contaminate chicken meat.[3-5] It is a known fact that these bacteria also become a serious threat to public health. Foodborne diseases kill almost two million people annually worldwide.[6] Chicken meat is a possible reservoir of foodborne pathogens. This causes infectious diseases in humans.[7] In order to reduce the risk of foodborne infection, biosecurity and pathogen control throughout the food chain becomes more of an issue. Complete elimination or low levels of pathogenic bacteria in foods is crucial for human health.[8] Therefore, advanced laboratory diagnostic techniques aimed at the identification of specific pathogens in the correct way as soon as possible are extremely valuable. Generally, bacterial isolation in different culture media, as well as phenotypic and serological characterization, are used to detect foodborne bacteria. Despite the fact that these traditional microbiological methods are considered the gold standard, they are laborious and require more than one step and reagents.[9] These time-consuming analytical processes can take days to reach a final result. In addition, late-growing bacteria are difficult to isolate and require extra analytical methods.[10] These traditional methods are also unquantified; hence, it is difficult to estimate contamination risk effectively.

As a consequence, today, DNA-based methods such as Real-time PCR (qPCR) and LAMP are increasingly being developed and used to detect foodborne pathogens. DNA-based methods provide rapid and reliable results for the qualitative and quantitative analysis of food pathogens in biological samples, simply by pre-enrichment.[11]

In this study, it is aimed to develop a procedure based on Real-time PCR for the detection and quantification of *Listeria monocytogenes* from chicken meat samples collected from the Erzurum market without pre-enrichment.

MATERIALS AND METHODS

Collection of Chicken samples

Fifty chicken meat samples collected at different times from butchers and markets in Erzurum were brought to the laboratory under aseptic circumstances.

Homogenization of Chicken samples

The collected chicken meat samples were cut into small pieces with a sterile scalpel blade. Subsequently, 25 grams of each sample were taken into filtered stomacher bags. 225 mL of sterile physiological water containing 0.85% NaCl was added and homogenized in a stomacher mixer for 5 minutes.[12] Thus, the microbial load in the chicken meat samples was provided to pass into the solution.

Preparation of DNA template

Thermal Lysis method

1 mL of the homogenized samples was centrifuged at 12.000 rpm for 10 minutes. Then, the pellet was resuspended in 0.1 mL of sterile Milli-Q water. Lysozyme enzyme solution (10 mg/mL) was added to each tube for lysis of the cell walls of the bacteria. Subsequently, the samples were left for incubation at 37°C for 1 hour. The suspension was boiled at 100°C for 10 minutes and immediately cooled on ice for 5 minutes. Then, after it was centrifuged at 12.000 rpm for 5 minutes, the resulting supernatant was used as template DNA.[9,13]

DNA isolation by commercial kit

One mL of the homogenized samples was centrifuged at 12.000 rpm for 2 minutes. Afterward, DNA isolation from the bacteria remaining in the pellet was performed by means of the WizardR Genomic DNA Purification Kit (Promega, UK, A2360).[14] The purified DNA samples were then stored at a temperature of -20°C, in anticipation of being employed as a template DNA.

Bacterial strains

The *Listeria monocytogenes* ATCC 7644

reference strain used as a control in this study was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The reference strain was grown in Mueller-Hinton medium and incubated at 37°C for 48 hours. Then, a dilution series of this bacterium at different concentrations (10^0-10^7 CFU/mL) was prepared. A standard chart was created to determine the ct values of each dilution tube.

PCR primers

The *L. monocytogenes*-specific primer sequences used in the study are given in Table 1, and the primers synthesized by Methabion (Martinsried, Germany) were adjusted to 5 µM concentration for PCR analyses by dissolving in nuclease-free water.

Statistical analysis

The linear regression model, which considered the basic approach in calculating bacterial counts and modeling bacterial count data, was used. All measurements were taken in triplicate for each group (n = 3), and the results were compared statistically using unpaired t-test analysis. The comparison of "Thermal lysis method" and "DNA isolation with kit" groups was done using the Bland-Altman test using the GraphPad Prism version program.

RESULTS

Linear-regression analysis of standard pathogenic bacteria

A linear regression graph was drawn by means of Real-Time PCR to identify the number of *Listeria monocytogenes* in the collected chicken meat samples (Figure 1).

The Ct values determined as a result of Real-Time PCR analysis of 50 chicken meat samples were estimated using linear equations of standard graphics, and the amount of pathogenic bacteria in chicken meat samples was determined.

Table 1. Primer sets were used in the study to detect *Listeria monocytogenes*

Strains	Primer sequence	Annealing temp. (°C)	Gene	GenBank ID
Listeria monocytogenes	5'-TCGCAAACAGATCTAGACCAAGTT-3' 5'-GTTCAAGTATTCCAATCCATCGATAG-3'	60	inlA	OP686910.1

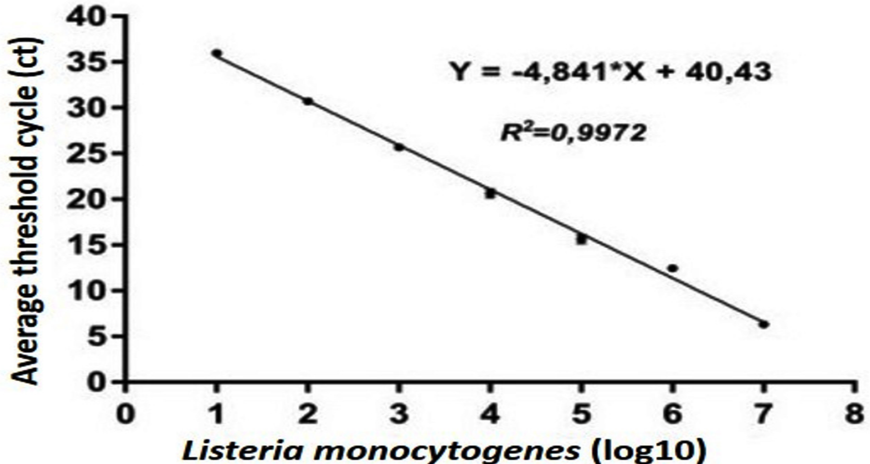

Figure 1. Graphs of the linear regression of *Listeria monocytogenes*. GraphPad software (San Diego, CA, USA) was used to generate a standard curve of serial decimal dilutions (10^1-10^7) of *L. monocytogenes*. The R2 and the linear equation were calculated automatically by the software

Quantitative analysis of pathogenic bacteria in ground mince samples

Listeria monocytogenes were discovered in 17 of 50 chicken samples in all two groups (Using the thermal lysis method and DNA isolation by the kit). Both methods were compared with one-way ANOVA analysis. Table 2 shows the average, median, lower, and higher 95% CI values.

Moreover, the 'Bland-Altman' method comparison analysis method was used to compare the 'Thermal lysis method' and the 'DNA-isolated' groups. With regard to comparison analysis, the Bland-Altman method revealed that there was no significant difference between the DNA extraction methods. Further results are shown in Figure 2.

Table 2. The number of pathogenic bacteria present in chicken meat samples

	Thermal lysis method	With DNA isolation
L. monocytogenes (n=50)		
Positive samples	17/50	17/50
Mean (CFU/mL)	4853	4886
Median (CFU/mL)	192	220
Lower 95% CI	-1503	-1498
Upper 95% CI	11210	11269

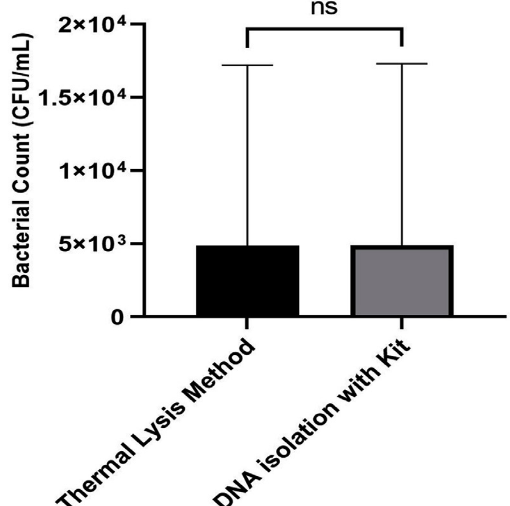

Figure 2. Comparison of two DNA extraction methods using Bland-Altman method comparison analysis. Data are expressed as mean ± SEM. All P values are derived from one-way ANOVA analysis using Prism software 7.0 (GraphPad Software, San Diego, CA)

DISCUSSION

L. monocytogenes is a foodborne pathogen commonly found in nature.[15] The groups most at risk of Listeriosis infection are pregnant women, newborns, individuals with compromised immune systems, and the elderly.[16,17] *L. monocytogenes* is found in a variety of foods, including cheese, meat, milk, vegetables and fish.[18] While the traditional methods are preferred for the detection of *L. monocytogenes* in food, these methods are time-consuming and labor-intensive since they require pre-enrichment, cultivation in selective media, serological and biochemical tests.[19] Due to some disadvantages of these methods, such as being labor-intensive and time-consuming, new methods are needed for sensitive, specific, and rapid detection of foodborne pathogens. Within this context, Real-Time PCR a nucleic acid-based method, is recognized to be a powerful molecular analysis that is highly sensitive, convenient, highly efficient, and requires less time.[20] The organization of credible procedure of DNA extraction is one of the main parameters influencing PCR sensitivity.[21] Herein, the thermal lysis method (without DNA isolation) using lysozyme and the extraction method using the WizardR Genomic DNA Purification Kit (Promega, UK, A2360) procedure were compared. In the present study, DNAs belonging to 50 chicken samples purchased from various butchers and markets were obtained by two different isolation methods without pre-enrichment, and then pre-enrichment, *L. monocytogenes* - a facultative intracellular pathogen that causes Listeriosis disease, was detected by Real-time PCR method. To that end, InIA, one of the two known forms (InlA and InlB) of internalin, which is a surface protein, was used to detect *L. monocytogenes*. This gene has been proven to be a virulence factor, bind to E-cadherin in the host epithelial cell, and help to cross the intestinal barrier. A large number of studies have used InIA as the target gene region for the determination of *L. monocytogenes* in food samples.[22,23]

As a consequence of real-time PCR analyses, in the study conducted with both DNA

extraction methods, *L. monocytogenes* were designated in 17 of 50 chicken meat samples (34%), and these data are similar to previous studies.[24-26] In addition, quantitative analyses of bacterial levels were performed using real-time PCR, and the bacterial counts obtained by both methods were compared. Although differences were observed between the bacterial count detected in the samples in which the thermal lysis method was applied and DNA isolated through the kit, it was revealed that there was no statistically significant difference by using the Bland-Altman comparison method.

Taking all this information into account, the presence and amount of *L. monocytogenes* in chicken meat samples was determined by real-time PCR, which would be performed without pre-enrichment.

As a result, it was determined that a reliable DNA isolation was carried out by thermal lysis, but the quantity and quality of the DNA obtained were lower than DNA extraction by the kit. The fact that this situation did not lead to a significant statistical change in the bacterial count revealed that the Thermal lysis method, which is cheaper and less labor-intensive, can be used instead of commercial DNA isolation by the kit in the determination of pathogenic microorganisms in foods by Real-time PCR.

CONCLUSION

The use of reliable and rapid detection methods is essential to control foodborne pathogens in nutrients and reduce public health concerns. Immunological and culture-based methods are among the most preferred methods for detection. However, genomic-based studies have attracted the attention of researchers because these methods cannot detect low numbers of pathogenic bacteria. It is stated that in studies in which the existence of pathogens in foods is investigated by Real-Time PCR, pre-enrichment and DNA extraction stages will be carried out in the procedure steps, which will cause extra workload, cost, and loss of time. In this study, detection of *Listeria monocytogenes* in chicken meat samples was carried out by Real-time PCR using the Thermal lysis method without pre-enrichment. It is stated that foodborne pathogens that would be capable of growing on foods can be designated precisely with lesser cost and workload as well as shorter amounts of time, thereby inhibiting the occurrence of microbial infections threatening human health while they are still in the production phase. Bacteria such as *L. monocytogenes, Staphylococcus aureus,* and *Campylobacter jejuni* are considered to be the most common pathogens in chicken and chicken products. Since they generally do not give rise to any significant change in chicken meat, people relievedly consume foods contaminated with these pathogens and therefore may get serious infectious diseases.

There are many researchers and many studies on this subject aiming to detect *L. monocytogenes*, one of the important pathogens found in chicken and chicken products that causes severe public health problems and product losses in the long term.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

None

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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