Brief Report

Comparison of PCR and culture methods for detecting of Listeria monocytogenes in vaginal samples

Mohtaram Nasrollahi1, Zeynab Marzhoseyni2, Tahoora Mousavi3, Mohammad Ahanjan4*

1. Professor in Microbiology, Department of Microbiology, Mazandaran University of Medical Science, Mazandaran, Sari, Iran.

2. MSc in Microbiology, Department of Microbiology, Mazandaran University of Medical Science, Mazandaran, Sari, Iran.

3. Ph.D. Student in Molecular and Cell Biology, Student Research Committee, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

4. Associate Professor, Department of Microbiology, Mazandaran University of Medical Science, Mazandaran, Sari, Iran.

*correspondence: **Mohammad Ahanjan**, Associate Professor, Department of Microbiology, Mazandaran University of Medical Science, Mazandaran, Sari, Iran. E-Mail: ahanjan2007@gmail.com

Abstract:

Listeria monocytogenes is an opportunistic intracellular pathogen that has become an important cause of human food borne infections worldwide. The aim of this study is the determinate sensitivity of diagnostic methods of this organism and achieving a test that can detect Listeria monocytogenes in all circumstances in clinical samples. Materials and Methods: In this study 88 vaginal swabs were collected from women with age of (15-45) referred to gynecologic clinics in the hospital (Sari, Iran). Vaginal swabs were examined by culture on specific PALKAM agar medium and PCR technique by specific primers. Results: Out of 88 studied vaginal swabs, 8 cases with culture method and 27 cases were positive with PCR technique. The sensitivity of PCR and culture is reported %100, %29 respectively. Conclusion: The results show that PCR is a more sensitive, easier, and faster method in comparison to in clinical samples.

Keywords: Listeria monocytogenes, PCR, culture methods

Introduction:

Listeria monocytogenes is a Gram-positive bacterium that causes meningitis, encephalitis, septicemia, abortion, and prenatal infection. Serological Methods for Identification of Listeria are not very sensitive because sometimes antigenic cross-reaction between L.monocytogenes and other Gram-positive bacteria can be seen (1). PCR detection has been proposed and it is replaced with a time-consuming culture based on classical techniques and serological tests (2). The aim of this study is to introduce a new method (PCR) for identification of L. monocytogenes in vaginal samples and compare this method with culture.

Methods:

During 2014-2015 a total of 88 vaginal swabs were collected from women. Briefly, vaginal swabs were streaked on to PALKAM Agar medium and inoculated at 37ºC for 24-48h, then all of the samples were incubated at 4 ºC. Morphologically typical colonies confirmed by diagnostic tests. The standard strains of L. monocytogenes (ATCC 7835) and Staphylococcus aureus (ATCC 25923) were obtained from Pasture Institute of Iran.

Chromosomal DNA was extracted following the protocol of DynaBio blood/tissue DNA Extraction Mini Kit (Takapouzist, Iran). The concentration and purity of DNA were determined by spectrophotometer and the ratio of the absorbance calculated at 260 and 280 nm.

Bioneer, Korea synthesized the primers for detection of hemolysin gene (hlyA) of L. monocytogenes. (F: 5'- GCA GTT GCA AGC GCT TGG AGT GAA -3', R: 5'- GCA ACG TAT CCT CCA GAG TGA TCG -3') with the size of 456 bp (3). PCR was performed in a reaction volume of 14µl containing 7x PCR buffer (100 mM Tris (pH 9.0), 500 mM KCl, 15 mM MgCl2, 0.1% gelatin, 2 mM MgCl2, 0.2 mM dNTPs),0.75µl forward and reverse primers, ,2µl of DNA template and sterilized milliQ water to make up the reaction volume(3.5µl). under the following cyclic conditions: initial denaturation at 94ºC for 4 min, 35 cycles in sequence 94 ºC for 30 s, 54ºC for 30 s and 72 ºC for 30 s, and a final extension at 72 ºC for 7 min. The reaction mixture with no DNA template was incorporated as a negative control in each run. The DNA amplification reaction was performed in Master Cycler Gradient Thermocycler (Applied Biosystems, USA) with a preheated lid. The PCR products were analyzed by 2% agarose gel electrophoresis, stained with the green viewer, visualized by UV transilluminator.

Findings:

In our study, out of 88 samples, 8 (9%) vaginal swabs had positive results and 27(61%) samples were positive In PCR. On the other hand, the sensitivity of PCR and culture is reported %100, %29 respectively. In this study, the target genes specific for L. monocytogenes produced PCR products with the size of 456 bp is showed in Fig. 1.

Discussion:

In this study, our result shows that PCR method is more sensitive than culture test. According to Malik (2007) findings, the PCR procedure is a rapid and sensitive method and suitable for identifying bacterial strains and clinical laboratories (4).

The PCR technique employed an enzyme and oligonucleotide primers were found to be simple, rapid, less laborious, and more

reliable in comparison to culture method (5). Conventional methods for routine detection of L. monocytogenes involve enrichment culture in liquid medium (usually Fraser or PALCAM broth), plating on selective agar medium and biotyping that takes some days (6). The high specificity and sensitivity of multiplex PCR are demonstrated in some epidemiological studies of diseases.7 Studies show that PCR is an advantage and a helpful diagnostic method other than culture test in clinical microbiology. PCR method is fairly quick and organism does not require being available. The PCR assay provides a useful tool for detection of sampling transported to the laboratory within the 2h period, while anaerobic cultures need 7-8 day (7).

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

References:

1. Doijad S, Barbuddhe SB, Garg S, Kalekar S, Rodrigues J, D'Costa D, Bhosle S, Chakraborty T. (2011) Incidence and genetic variability of Listeria species from three milk processing plants. Food Control. 31;22(12):1900-4.

2. Gottschalk M. (2015) The challenge of detecting herds sub-clinically infected with Actinobacillus pleuropneumoniae. The Veterinary Journal. 31;206(1):30-8.

3. Soni DK, Singh M, Singh DV, Dubey SK.(2014) Virulence and genotypic characterization of Listeria monocytogenes isolated from vegetable and soil samples. BMC microbiology. 8;14(1):241.

4. Malik SV, Barbuddhe SB, Chaudhari SP. (2002) Listeric infections in humans and animals in the Indian subcontinent: a review. Tropical animal health and production. 1;34(5):359-81.

5. Mengaud J, Vicente MF, Chenevert J, Pereira JM, Geoffroy C, Gicquel-Sanzey B, Baquero F, Perez-Diaz JC, Cossart P.(1988) Expression in Escherichia coli and sequence analysis of the listeriolysin O determinant of Listeria monocytogenes. Infection and Immunity. 1;56(4):766-72.

6. Bohnert M, Dilasser F, Dalet C, Mengaud J, Cossart P. (1992) Use of specific oligonucleotides for direct enumeration of Listeria monocytogenes in food samples by colony hybridization and rapid detection by PCR. Research in microbiology. 1;143(3):271-80.

7. Jamali H, Chai LC, Thong KL.(2013) Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media. Food Control. 31;32(1):19-24.

Figures:

Figure 1: left to right: Ladder, positive control, negative control, 1, 2, 3, 4, 5 are samples.