Design a Multiplex PCR for Detection of Multi-Drug Resistant Gram-Negative Bacteria

Reza Heidari[1*], Mohammad Foad Heidari[2], Satar Gorgani Firuzjae[2], Mojtaba Yousefi Zoshk[3], Fatemeh Khodabandehloo [1]

1. Research Center for Cancer Screening and Epidemiology, AJA University of Medical Sciences, Tehran, Iran.
2. Department of Medical Laboratory Sciences, School of Allied Health Medicine, AJA University of Medical Sciences, Tehran, Iran
3. School of Medicine, AJA University of Medical Sciences, Tehran, Iran

Abstract:

Introduction: The increasing prevalence of multidrug-resistant Gram-negative bacteria (MDR-GNB) infections is a clinical problem in presents many challenges. An effective treatment must be performed as soon as possible, which requires rapid and efficient diagnosis. This has highlighted the need for molecular methods with improved sensitivity. To this end, we have developed a multiplex PCR kits that target a **β**-lactamase genes of Gram-negative bacterial.

Material and Method: Our kit has two main lines, in the first line we detect 6 gene of **β**-Lactamase (CTX-M, IMP, KPC, NDM, OXA-48 and VIM) of the and in the second line we have identifies 6 gene of ampC (MOX, ACC, FOX, DHA, EBC, and CMY-2) families associated with plasmid-mediated mechanisms of antibiotic resistance. DNA extraction from **β**-lactamase-positive and negative bacterial cultures (n=15) that had been previously characterized by single PCR. Then in order to optimization multiplex PCR primer, the mix of DNA sample provided and characterized by multiplex PCR. Sensitivity and specificity of the assays were calculated based on the ability to correctly identify the positive and negative clinical isolates, respectively. The amplification program consisted of an initial denaturation of 95°C for 15 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 15sec, and 72°C for 15sec. All isolates included in the study were positive for internal control (IC) included in all the PCR mixes.

Result: The sensitivity and specificity of **β**-Lactamase is reported to be about 90% and 93%. Also, the result show that sensitivity and specificity for detection of ampC gene in the clinical sample was 91.2 % and 93%, respectively.

Conclusion: Multiplex PCR method was developed for detection of 12 of multi-drug resistant gram-negative bacteria in Iran. This method allows for detection and differentiation of clinical isolates of multi-drug resistant gram-negative bacteria and does not require prior phenotypical characterization, constituting a rapid avaluable tool in the management of infections in hospitals.

Keywords: Multidrug-resistant Gram-negative bacteria (MDR-GNB), Multiplex PCR, β-Lactamase, antibiotic resistance

***Corresponding Author:** Reza Heidari, PhD, Assistant Professor, 1. Research Center for Cancer Screening and Epidemiology, AJA University of Medical Sciences, Tehran, Iran.

Tel: +98-9358644606, E-mail: r-heidary@ajaums.ac.ir