A REAL-TIME MULTIPLEX PCR ASSAY FOR THE DETECTION OF SALMONELLA ENTERITIDIS

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Introduction: Salmonellosis is an infectious disease caused by various serotypes of Salmonella. Salmonella enterica serotype Enteritidis and Salmonella enterica serotype Typhimurium are implicated in majority of Salmonella infections in Kazakhstan. For epidemiological studies, strain identification is necessary and such investigations have traditionally relied on biochemical and serological methods. Alternative method, such as real-time PCR can be used as an effective and reliable tool for detection of Salmonella. The aim of the study is to develop a real-time multiplex PCR assay for the detection of the S. Enteritidis serotype.

Methods: A total of 75 Salmonella enterica serotype Enteritidis and serotype Typhimurium strains were isolated at the Infectious diseases hospital (Astan, Kazakhstan) during 2016-2017. The isolation of DNA from inactivated bacterial cultures was carried out by phenol-chloroform extraction. The quantitative DNA content was carried out on a spectrophotometer (Nanodrop 1000) and evaluated by electrophoresis in a 1% agarose gel. Sequence-specific TaqMan probes and primers for the invA and sefA genes were designed using FastPCR 6.5 and Beacon Designer 8.0 software. All primer sequences were tested for complementarity using the database http://blast.ncbi.nlm.nih.gov/Blast.cgi. Real-time PCR was performed on a CFX96 (Bio-Rad) instrument. The fluorescence detection was set up after each annealing step and the results were analyzed using CFX Manager 2.1 software (Bio-Rad).

Results: The proposed assay is based on a duplex real-time PCR using competing TaqMan probes that are complementary to the nucleotide sequence of S. Enteritidis. The invA gene target is used for Salmonella detection, while sefA gene target sequence is specific for S. enteritidis. InvA gene is known to be in amplification control, while sefA gene encodes the fimbrial antigen SEF14 that is specific for S. enteritidis. All 75 Salmonella strains were positive for the invA target sequence. Fifty-one strains were positive for the sefA target sequence of S. Enteritidis (100% inclusivity, 95% exclusivity).

Conclusion: A duplex real-time PCR assay was developed for the detection of S. Enteritidis. The inclusivity and exclusivity were between 100 and 95% analyzing 75 bacterial strains.