DEVELOPMENT OF A THERMOSTABILIZED MULTIPLEX PCR FOR THE SIMULTANEOUS DETECTION OF SALMONELLA ENTERITIDIS (SE) AND VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) IN POULTRY STOOL AND MEAT SAMPLES

ABSTRACT

Salmonella Enteritidis (SE) and vancomycin resistant *Enterococcus* (VRE) are food borne pathogen of primary concern worldwide. The incidences of SE and VRE outbreaks have been reported in meat and poultry products and its transmission to human often occurs during handling or consumption of poultry products. Conventional method for detection of SE (5-14 days) and VRE (3-4 days) was found to be more time consuming and labor intensive. Hence, the objective of this study was to develop a thermostabilized multiplex PCR assay for the rapid and sensitive identification of SE and VRE simultaneously to facilitate surveillance in poultry farms. A multiplex PCR consisting of four sets of specific primers were designed and BLAST analyzed to check the specificity. This assay simultaneously detects 4 genes namely 16S rRNA of *Enterococcus* genus (~1178bp), vancomycin-resistant genotype *van*A (~931bp), *Salmonella* genus (~429bp) and *S.* Enteritidis (~299bp) with an incorporated internal amplification control (IAC). The multiplex PCR assay optimized and also thermostabilized. An accelerated stability test was carried out to evaluate the stability of the thermostabilized PCR mix at room temperature and 4°C. Diagnostic evaluation was also performed using poultry stool samples

(n=226) and also with meat spiked samples. The multiplex PCR assay was standardized with known reference strains of SE and VRE. The assay was tested with 46 strains and showed an analytical sensitivity and specificity of 100%. The Limit of Detection (LoD) of the multiplex PCR assay was 10 ng/µl at DNA level for the wet PCR, while the thermostabilized PCR showed a LoD of 1ng/ µl. At the bacterial level the LoD was 10^3cfu/ml with respect to SE while 10^6cfu/ml with respect to VRE. The sensitivity of the assay with poultry stool sample was 62 % and 67 % for SE and VRE respectively. The spiked meat samples were positive by the multiplex PCR assay though the detection of VRE was 10^6CFU/ml. The thermostabilized PCR mix was estimated to be stable currently upto 24 days at 4°C. In conclusion, a thermostabilized multiplex PCR mix for the detection of *Salmonella* Enteritidis and vancomycin resistant *Enterococcus* was developed. The thermostabilized multiplex PCR mix provided a rapid and sensitive detection method for the same day diagnosis compared to conventional method with even better reliability. Thus, it helps the poultry farmers to easily identify and contain poultry farms that are infected with these bacteria in the nick of time and at the same time helps to boost the poultry industry of the country.