VII. BIBLIOGRAPHY


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ABSTRACT
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The present study was carried out with objectives to screen milk samples for subclinical mastitis (SCM) by employing somatic cell count and electrical conductivity tests, to isolate and identify major bacterial pathogens *Staphylococcus aureus*, *Escherichia coli* and predominant Streptococcal species from SCM cases and to standardize simplex and multiplex PCR for rapid detection of these pathogens in milk samples. Out of 246 milk samples screened for SCM, 186 milk samples were subjected for isolation and 85 Streptococci, 95 *S. aureus*, 95 CoNS and 48 *E. coli* isolates were obtained. Polymerase chain reaction was standardized targeting *tuf* gene to identify Streptococci and Staphylococci at genus level, 16S rRNA gene to identify *S. agalactiae*, *S. dysgalactiae*, *S. uberis* and further, *sip* and *pauA* gene to identify *S. agalactiae* and *S. uberis* respectively. The screening of 85 Streptococcal isolates revealed seven isolates as *S. agalactiae*. *Staphylococcus aureus* was identified by *nuc* and *sodA* gene based PCR. Screening of 95 *S. aureus* isolates revealed the presence of *nuc* gene in all isolates and *sodA* gene in 87 isolates. Forty eight isolates of *E. coli* were screened and confirmed by *alr* gene based simplex and by a multiplex PCR (m-PCR). A two tube m-PCR was standardized to simultaneously detect the five major mastitis pathogens from milk samples. Screening of 147 bulk milk samples detected major pathogens in 81 bulk milk samples. *Staphylococcus aureus* was the predominant pathogen detected, followed by *E. coli*, *S. dysgalactiae* and *S. uberis*. The m-PCR assay developed in the present study was an easy and rapid method to simultaneously detect the five major mastitis pathogens in bulk milk. The regular analysis of bulk milk by m-PCR developed in the study may become an useful tool for determining the herd status in the detection of contagious and environmental mastitis pathogens.