Bibliography
VII. BIBLIOGRAPHY


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Abstract
VIII. ABSTRACT

The present study was carried out with the objective of isolation, identification and study of antimicrobial resistance patterns of coagulase negative staphylococci (CoNS) associated with bovine mastitis. In all, 313 bovine milk samples collected from different organized/ unorganized sectors were screened for subclinical mastitis (SCM) by somatic cell count, electrical conductivity test, California mastitis test and bromo thymol blue strip tests. These tests revealed SCM status at 42.8% and 48.2%, 21.4% and 26.8%, respectively. On processing all the samples for isolation, a total of 152 CoNS isolates including 14 isolates from clinical mastitis samples (n=42) were obtained. Biochemical characterization of the isolates by HiStaph™ Identification Kit revealed *S. arlettae*, *S. fleurettii*, *S. equorum* and *S. epidermidis* as predominant CoNS species in SCM cases. Further, these isolates were subjected to two-tube multiplex PCR targeting *gap/rpoB/sodA* genes for species level identification, subsequent to the confirmation at genus level by the partial amplification of *tuf* gene. Of the 10 species targeted by m-PCR, *S. epidermidis*, *S. chromogenes* and *S. sciuri* were the major CoNS detected. Coagulase negative variants of *S. aureus* were also detected by *S. aureus* specific triplex PCR. The overall agreement between phenotype- and genotype-based speciation of CoNS was only 11%. Antibiogram studies of CoNS isolates revealed highest resistance to oxacillin/ methicillin, followed by ceftriaxone with sulbactum/ tazobactum, penicillin G and aminopenicillins. Highest sensitivity was recorded for chloramphenicol followed by enrofloxacin and gentamicin. Another multiplex PCR to detect antibiotic resistance genes and *coa* gene revealed *mecA*, *aacA-D* and *aph3' IIIa* genes in 10, 15 and 5 isolates, respectively. *Coa* gene was detected only in 15 of the 19 coagulase negative *S. aureus* isolates. The two-tube multiplex PCR assay developed in the present study is an easy and rapid method to simultaneously detect the ten major CoNS prevailing in bovine mastitis.