PCR BASED DETECTION OF COAGULASE GENE ENCODING STAPHYLOCOCCUS AUREUS ISOLATED FROM SUBCLINICAL MASTITIS CASES IN BUFFALOES

Malmarugan ,S. Arthanarieswaran, M. and Suresh, P.

Department of Veterinary Microbiology
Veterinary College and Research Institute, Namakkal, (T.N.)

ABSTRACT :

The present study was carried out to characterize *Staphylococcus aureus* (S. aureus) isolated from mastitic cases in buffalo based on coagulase gene. A total of 18 strains of S. aureus were isolated during processing of 81 milk samples from 21 buffaloes. The S. aureus strains were characterized phenotypically, and were further characterized genotypically by polymerase chain reaction using oligonucleotide primers that amplified genes encoding coagulase (Coa).

Amplification of the Coa gene yielded a product size of 730bp in all 15 coagulase positive isolates. The phenotypic and genotypic findings of the present strategies might provide an understanding of the distribution of the cogulase positive S. aureus clones among buffaloes mastitis isolates, and might aid in the development of steps to control S. aureus infections in buffaloes.

KEY WORDS: Buffaloes, *Staphylococcus aureus*, sub-clinical mastitis, coagulase gene

INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the common causes of subclinical mastitis worldwide, which is of economic importance to the dairy industry (Miles et al., 1992). Raw milk is a potential source of S. aureus in milk and milk products, especially in the case of defective pasteurization. The main reservoir of S. aureus seems to be the infected quarter. Molecular epidemiological analysis of the bovine S. aureus population suggested that a small number of clonal types were responsible for most infections, and that strains had a broad geographic distribution (Kapur et al., 1995; Fitzgerald et al., 1997 and Salasia et al., 2004).

S. aureus has a capacity to produce a large number of putative virulence factors (Foster, 1995 Foster et al., 1997 and Fitzgerald et al., 2000). Some of these factors may be more important than others in different diseases or at different stages of the pathogenesis of particular infections, as not all factors are produced by each strain. Among these the most important factor is coagulase.

At present, there is no reported literature on the prevalence of coagulase positive S. aureus isolates from buffaloes in India. The present study was conducted to characterize S. aureus isolates in milk samples from buffaloes with subclinical mastitis based on coagulase gene.

MATERIALS AND METHODS

Samples

A total of 81 quarter milk samples were collected from 21 buffaloes selected randomly from 9 farms in Namakkal, Tamilnadu over a three week period. The samples were tested by the California mastitis test (CMT) for subclinical mastitis, and were graded as negative, trace, weak, distinct, or strongly positive (Radostits, et.al., 2003). Isolation of *Staphylococcus* was attempted from the CMT positive milk samples.

Phenotypic characterization

The isolates were phenotypically characterized using various cultural, morphological, and biochemical tests such as tube coagulase, catalase, urease and sugar fermentation (Cowan and Steel, 1970 & Cruickshank, et.al., 1975). The strains of S. aureus were further examined for DNase production (Lachica et al., 1971).

Genotypic characterization

Chromosomal DNA of the isolates was extracted and purified as described by Kalorey et al., (2007). The purified DNA was precipitated with isopropanol and sodium acetate (3M) solutions, washed in 70% ethanol, and suspended in 50 µl of TE buffer. To identify and characterize coagulase positive S. aureus, the specific primers for Coa gene (Coa F: 5'- ATA GAG ATG CTG GTA CAG G -3' CoaR: 5'- GCT TCC GAT TGT TCG ATG C-3') were used.

PCR master mix (Genei, Bangalore) was used in PCR assay . The amplified products were separated by agarose gel electrophoresis (1.5% agarose containing 0.5 mg ethidium bromide in 0.5 x Tris-EDTA electrophoresis buffer) at 5 V/cm for 2 h and photographed under UV illumination.

RESULTS

Isolation of Staphylococcus was attempted from CMT positive milk samples. Out of 81 milk samples collected from 21 buffaloes of 9 farms, 70 (86.41%) samples from 19 buffaloes were found to be CMT positive. Of these 68 milk samples, 18 buffaloes harbored *Staphylococcus* sp. On the basis of cultural and biochemical properties, 15 isolates were identified as S. aureus. All 15 isolates were positive for the tube coagulase test. Other strains were identified as coagulase negative *staphylococc*i. All the 15 isolates yielded an amplicon of approximately 730bp of the Coa gene.

DISCUSSION

S. aureus has been recognized as a pathogen in human and animal infections. In the present stur dy, 15 S. aureus strains isolated from subclinical mastitis cases in buffaloes were identified and further characterized by PCR amplification of virulence gene encoding coagulase activity. Comparable PCR-based detection studies of the coagulase genes have been described by other investigators in bovine mastitis. (Kalorey et.al. 2007 and Aslantas et.al., 2007). In the present study, S. aureus isolates from buffaloes with mastitis were found to be similar in their amplification of Coa gene patterns. The results are in agreement with the findings of Aslantas et al., 2007 but contrast to this Kalorey et al., (2007) and Coelho et al., (2009) obtained three to four different polymorphism with Coa gene in bovine strains.

The present report of investigation of Coa gene from mastitic buffalo samples suggest that mastitis in buffaloes from Namakkal region may be caused by S. aureus strains with the same Coa genotype. This Phenotypic and genotypic identification based on Coa gene might provide a better understanding of the distribution of the prevalent S. aureus pathogenic clones among buffalo mastitis isolates which may aid in the development of control stratagies for S. aureus infections in buffalo herds.

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