Prevalence and antibiotic susceptibility of *Staphylococcus aureus* from bovine mastitis

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Abstract

Malabari Goat populations of Tanur, Thalassery and Badagara were studied for haemoglobin polymorphism. Two variants were observed for haemoglobin, Hb A and Hb B with a frequency of 0.987 and 0.012, respectively, suggestive of three phenotypes, viz. Hb AA, Hb AB and Hb BB, and indicating the predominance of Hb A in the pooled population. Hb B variant was observed only in the Thalassery population (gene frequency 0.038).
Key words: polymorphism, haemoglobin, phenotype

Introduction

Worldwide, mastitis is one of the most important diseases in the dairy sector. The bovine mastitis caused by Staphylococcus aureus has increased in many herds of urban and rural areas of the country. Staphylococcus aureus constitute the majority of disease causing bacteria. Staphylococcus aureus originates from the cow's environment and infects the udder via the teat canal. Many authors have reported Staphylococcus aureus as the most common etiological agent causing mastitis in cows following *E.coli* (1). For many years, Methicillin resistant *Staphylococcus aureus* (MRSA) was considered only a human pathogen, until a report of a MRSA infection in a dairy cow surfaced in 1972 (2). There is now increasing evidence that MRSA can be transmitted in both directions, from human to animal and from animal to human. Once exposed to MRSA, animals can become colonized, and may serve as reservoirs to transmit the infection to other animals and also to their human handlers (3, 4, 5, 6, and 7). This has been documented in both the general community and in animal nosocomial environments. (3,5, 8). Data have indicated that owners and veterinary personnel that come into contact with MRSA-infected animals may become colonized by MRSA.

There is a concern that antimicrobial treatment of MRSA in companion animals may increase antimicrobial resistance, and have a subsequent effect on the zoonotic transmission or re-transmission to humans, especially if the humans involved are already in an immunocompromised state. (4). The possibility that there may be a transmission route of MRSA from animals to humans via animal food products requires further investigation to determine its public health significance. (9 and 10)

Antimicrobial therapy plays a role in mastitis control by reducing the levels of herd infection and by preventing new infections. However, bacteriological cure rate against Staphylococcus aureus for antimicrobial therapy is relatively low due to pathogen characteristics such as the ability to survive inside the host cell and pathological changes induced in chronic infections (11). Some studies showed that increased frequency of penicillin resistance. Myllys et al., in 1998 reported that the proportion of S. aureus isolates resistant to at least one antibacterial agent increased from 36.9 to 63.6% in the period of 1988 to 1995, and most of the increase in antibacterial resistance was due to ß-lactamase producing strains (12). The penicillin resistance for Staphylococcus aureus was reported as 52.1% in 2004 (13). Therefore the present investigation was undertaken to study the prevalence and antimicrobial susceptibility of Staphylococcus aureus from bovine mastitis.

Materials and Methods

Animals: The lactating cows of the dairy farms of the Hubli-Dharwad region has been examined from dairy herds in different smallholder farms as well as large scale farms. Random number sampling has been used in selecting the cows on the farms visited. Information on age, parity, lactation stage and previous history of mastitis has been gathered. Cows have been kept in

semi-confinement open housing and milked twice daily

Sampling

Quarter foremilk samples were collected aseptically for bacteriological assay as described by Honkanen-Buzalski (14) . Before sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. The milk samples were transported on ice to the laboratory of the P. G. Department of studies in Microbiology and Biotechnology, Karnatak University, Dharwad for analysis.

Analysis of Milk Samples : From each sample, 0.01 mL of milk was cultured on blood-esculin agar and incubated for 48 h at 37°C; the plates were examined after 24 and 48 h of incubation. Bacterial species were identified using accredited methodology based on National Mastitis Council standards (15) and procedures described by Honkanen-Buzalski (14). A quarter was considered bacteriologically positive when growth of 500 cfu/mL was detected from a sample. Samples yielding >2 bacterial species were considered to be contaminated (16, 15).

Phenotypic characterization: The isolated organisms has been studied for identification by carrying out Gram's staining, microscopic observations and biochemical tests for catalase, Oxidation-Fermentation, Phosphatase; coagulase tests etc, according to the standard methods the isolates have been identified.

Antibacterial Susceptibility Testing: Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted for antibiogram. The antibiotic discs and Mueller- Hinton Agar were purchased from Hi-Media, Mumbai. The plates were prepared as per the manufacturer's instructions and checked for sterility by incubating the plates overnight at 37°C. The antibiotics discs were kept at room temperature for 1 hour. The agar plates were overlaid with inoculums of Staphylococcus aureus showing the turbidity equivalent to that of a 0.5 McFarland standard.

Results and Discussion

In this study for the isolation of S.aureus a total of 105 samples were collected from the dairy farms. Total 80 isolated colonies of Staphylococcus were subjected to biochemical analysis for confirmation of Staphylococcus aureus. A total of 68 colonies of Staphylococcus have shown positive for catalase, nitrate utilization, mannitol fermentation, Gelatin hydrolysis, MRVP and coagulase tests and were confirmed as Staphylococcus aureus based on conventional methods by studying the biochemical characters. The high prevalence of Staphylococcus aureus in the present study is in accordance with work of several other authors (17, 18, 19, and 20).

In the present study, the confirmed strains of Staphylococcus aureus were subjected to heamolytic activity test. Different patterns of heamolytic activities a, ß and non-heamolytic were observed among the isolates of Staphylococcus aureus, 20.58%, 75% and 4.41% respectively. The penicillins and synthetic penicillins those are resistant to ß-lactamase account for most of the treatments in the study farms. This is in line with the report from Tenhagen et. al.,(21). The antibiotic susceptibility testing of Staphylococcus aureus to various antibiotics revealed that the highest 86.76% isolates were resistant to penicillin followed by ampicillin 70.50%, amoxicillin 63.23%, gentamycin 47.05%, amikacin 30.80%, erythromycin 27.94%, Ciprofloxacin 26.47%, methicillin 23.52%, cefotaxime 20.58% and the lowest resistant was shown in ceftriaxone 19.11%. The present study demonstrated the existence of alarming level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents in the study farms. Therefore, it is very important to implement a systematic application of an in vitro antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

Among the isolates the susceptibility to various antibiotics have showed that the highest numbers of Staphylococcus aureus were susceptible to ceftriaxone 80.88% followed by cefotaxime 79.41%, methicillin 76.47%, ciprofloxacin 73.52%, erythromycin 70.05%, amikacin 69.11%, gentamycin 52.94%, amoxicillin 36.76%, ampicillin 29.41%, and the lowest susceptibility was shown in penicillin 13.23% . Overall, the proportion of isolates that were resistant to the antimicrobial agents tested was within the range of other reports from Germany using the same breakpoints (22, 23, 24, and 25). Reports based on the agar gel-diffusion method are difficult to compare with those performed with dilution methods because there is only limited agreement between the results of the 2 methods (26, 27).

The prevalence of Staphylococcus aureus is increasing and the antimicrobial resistance determined in our study was in line with other reports. The high number of ß-lactamase–producing isolates found in present study suggests that the administration of ß-lactams, especially penicillin and related drugs, should be carefully considered for mastitis control. This finding indicates the need for further investigation of the epidemiology of resistance against penicillin in Staphylococcus aureus isolated from bovine mammary glands.

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