Prevalence and Antimicrobial Susceptibility of Coagulase-Negative *Staphylococci* isolated from Bovine Mastitis

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Abstract

The study was carried out to investigate the prevalence and antimicrobial susceptibility of Coagulase-Negative Staphylococci isolated from Bovine Mastitis in and around Dharwad region. A total of 310 samples were screened and 180 confirmed Coagulase-Negative Staphylococci were obtained. The antimicrobial susceptibility of Coagulase-Negative Staphylococci against 10 antimicrobial agents was tested using the disc diffusion method. The highest numbers of Coagulase-Negative Staphylococci were susceptible to ceftriaxone 83.88% followed by cefotaxime 79.41%, methicillin 76.47%, ciprofloxacin 73.52%, erythromycin 70.05%, amikacin 66.11%, gentamycin 42.94%, amoxicillin 36.76%, ampicillin 29.41%, and the lowest susceptibility was shown in penicillin 23.23% . The results indicated that the increase in prevalence and antibiotic resistance pattern of the Coagulase-Negative Staphylococci isolated from bovine mastitis exhibited the highest degree of susceptible to ceftriaxone of all the tested antimicrobial agents.

Key words: Bovine mastitis, Coagulase negative staphylococcus, Prevalence, Antibiotics.

Introduction

Mastitis is an inflammation of the mammary glands of dairy cows accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue. It is the most common infectious diseases in dairy animals in the world which affects the dairy industry (Lightner *et al.,* 1988). Mastitis may be caused by a wide variety of microorganisms including bacteria, fungi, yeast, and mycoplasma etc; however, bacteria are the most frequent pathogens of these diseases. In mastitis diagnosis, staphylococci are divided into Coagulase-positive and coagulase negative *Staphylococcus* (CNS) on the basis of the ability to coagulase rabbit plasma. In diagnostics of bovine mastitis, the clarification has been considered adequate because CNS usually course subclinical or only mild clinical mastitis. Hence, it is consider as minor pathogen (Koivala *et al.,* 2007; Lim *et al.,* 2007). It seems that CNS mastitis is a particular problem in well managed, high producing farms, which have successfully controlled under infections caused by major mastitis pathogens (Myllys and Rautulu, 1995).

One of the important reasons for failure of treatment is assumed to be indiscriminate use of antibiotics without testing *in vitro* sensitivity of causal organisms. This practice at one hand increases economic losses and on other results in development of resistance to commonly used antimicrobials (Owens *et al.,* 1997). For suitable antibiotic therapy, bacterial isolation and antibiotic sensitivity studies are always essentials. Mastitis is considered as one of the major cause for use of antibiotics in dairy animals. Antimicrobial susceptibility tests help to guide the veterinarian in selecting the most appropriate antimicrobial agent for treatment of intra mammary infection (IMI) caused by *Staphylococcus* species. Therefore, the present investigation was undertaken to study the prevalence of bovine mastitis and determine the antibiotics susceptibility profiles of CNS to ten regularly used antibiotics in this region.

Material and Methods

Animals: The lactating cows of the dairy farms of the Hubli-Dharwad region have been examined from dairy herds in different small holder farms as well as large scale farms. Random number sampling method has been used while selecting the cows in the farms visited. The information on age, parity, lactation stage and previous history of mastitis has been also collected. Prevalence of bovine mastitis was calculated as follows:

$$\frac{\text{No. of Bovine mastitis affected animals}}{\text{Total no. of animals present in the farm}} \times 100$$

Sampling: Samples were collected aseptically for bacteriological studies as suggested by Honkanen-Buzalski. Prior to 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 Post Graduate Department of studies in Microbiology and Biotechnology, Karnatak University, Dharwad for further 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 hours of incubation. Bacterial species were identified using accredited methodology based on National Mastitis Council standards and procedures described by Honkanen-Buzalski (1994). 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.

Phenotypic characterization: The identification of isolated organisms was carried out as per standard methods like Gram's staining, microscopic observations and biochemical tests for catalase, MR-VP, Phosphatase and coagulase tests.

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 coagulase negative *Staphylococcus* showing the turbidity equivalent to that of a 0.5 McFarland standard.

Results

Prevalence of bovine mastitis was found to be 58.71% in the study area. Prevalence of coagulase negative *Staphylococcus* (CNS) isolated from bovine mastitis samples was 58.06%.

The susceptibility of CNS showed to various antibiotics among the isolates is presented as follows. The highest number of coagulase negative Staphylo-cocci were susceptible to ceftriaxone which was 83.88% followed by cefotaxime (79.41%), methicillin (76.47%), ciprofloxacin (73.52%), erythromycin (70.05%), amikacin (66.11%), gentamycin (42.94%), amoxicillin (36.76%), ampicillin (29.41%), and the lowest susceptibility was shown in penicillin (23.23%) respectively.

The antibiogram test of coagulase negative Staphylococci to various antibiotics revealed that the highest (76.77%) isolates were resistant to penicillin followed by ampicillin (70.59%), amoxicillin (63.24%), gentamycin (57.06%), amikacin (33.89%), erythromycin (29.95%), Ciprofloxacin (26.48%), methicillin (23.53%), cefotaxime (20.59%) and the lowest resistance was shown in ceftriaxone with 16.12% of expression.

Discussion

The present findings corroborate with those of earlier studies made in *S.chromogenes which* was the major CNS species in subclinical mastitis in heifers and in primiparous cows. *S. chromogenes* has been isolated from the udders of unbred, pregnant, or freshly calved heifers (Trinidad *et al.,* 1990; Matthews *et al.,* 1992). In a Danish study conducted on 180 heifers on 20 herds, the most common CNS species in heifers, *S. chromogenes*, was isolated from 15% of quarters before calving (Aarestrup and Jensen, 1997). Studies indicated that when CNS isolates originate from diagnosed clinical or subclinical mastitis from the field, the predominant CNS species was usually *S. simulans* (Jarp, 1991). In clinical mastitis in heifers around parturition and at early lactation, *S. simulans* (54%) was the most prevalent CNS species, followed by *S. chromogenes* (15%) and *S.hyicus* (15%) (Waage *et al.,* 1999) in Norway. In an earlier Finnish study, Myllys (1995) reported that *S. simulans* and *S. hyicus* were the most frequently isolated CNS species in heifers around parturition, and were associated with clinical mastitis after parturition. The predominant CNS species of 298 isolates in milk samples from subclinical mastitis in Germany were *S. chromogenes* (33%) and *S. simulans* (23%) (Lüthje and Schwarz, 2006). In addition to the predominant species, various other CNS species are less frequently isolated from milk samples. *S. hyicus, S. epidermidis* and *S. haemolyticus* were detected in some samples (Devriese and De Keyser, 1980; Jarp, 1991), but *S. warneri, S. sciuri* and *S. xylosus,* and several other CNS species usually only occur seldom (Aarestrup *et al.,* 1995; Waage *et al.,* 1999; Lüthje and Schwarz, 2006). Obviously the predominant CNS species are well adapted to the udder environment, but also many other CNS species are occasionally and opportunistically able to invade the mammary gland and cause mastitis.

An antimicrobial's response rate may be qualified as poor when it cures mastitis less than or equal to 25% and said favorable when the response rate attains 75% or above (Mackie *et al.,* 1988). The antimicrobial susceptibility tests carried out in this study indicated the existence of resistance against one or more antibiotics in coagulase negative staphylococcus. It has been reported that gentamicin, enrofloxacin, ciprofloxacin and chloramphenicol are newer Chemotherapeutic agents and are less commonly used for development of mastitis (Sumathi *et al.,* 2008).

It has also reported that highest sensitivity of mastitis agents to enrofloxacin, gentamycin and least sensitivity to ampicillin (Dhakal *et al.,* 2007 Kumar and Sharanu, 2002). Similar results were suggested by sumathia *et al.,* (2008) Anakalo *et al.,* (2009) who reported that *S.aureus* and coagulase negative *Staphylococcus* (CNS) as the major mastitis inducing pathogens suggestive of a possible development of resistance from prolonged and indiscriminate usage of beta-lactam antibiotics. In contrast to these findings, it has been reported that 64% of the isolates, from mastitis milk were sensitive to tetracycline and 52.8% to ampicillin (Hawari Azmi and Fowzi, 2008).

The emergence of antimicrobial resistance among pathogens that affects animal health is of growing concern in veterinary medicine. Anti-microbial resistant pathogens in animals have also been considered as a potential health risk for humans from possible pathogens. Mastitis is the single largest cause of antimicrobial use in dairy farms (Moon *et al.,* 2006). Perhaps this has severe economic implications for the milk producer, as this milk cannot be marketed and also other cattle are easily infected. Cost of treatment and decrease in milk quantity also cause considerable losses (Anakalo *et al.,* 2004). Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view.

The findings of this study showed that ceftriaxon is the most effective antibiotic used for control of bovine mastitis in and around Dharwad area. The antibiogram of mastitis causing agent is suggested before starting the treatment to get maximum efficancy of antibiotics so as to prevent the development of resistance to antibiotics due to indiscriminate and frequent use of antibiotics.

Acknowledgment

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