Isolation and Identification of *Staphylococcus Aureus* from Bovine Mastitis Milk and Their Drug Resistance Patterns in Silchar Town Dairy Farms, N.E India

Indu Sharma[a], A. Brinty[a]
[a]Microbiology department, Assam University, Silchar-788011, India
Corresponding author:
Indu Sharma

Abstract

A cross-sectional experimental study was conducted from December 2012 - May 2013 to estimate the prevalence of bovine mastitis caused by *Staphylococcus aureus* to assess the associated risk factors and to determine the antimicrobial resistance pattern in Silchar town dairy farms, North-East India. A total of 120 milk samples were collected aseptically and subjected towards California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT) to identify the subclinical mastitis from dairy cows. All the CMT, WST and SRMT high scored and clinically positive samples were investigated microbiologically. Rate of isolation of *Staphylococcus aureus* was determined and susceptibility of 8 antibiotics was evaluated using Kirby-Bauer disc diffusion method. Out of 36 pure isolates of *S. aureus*, resistance was detected for Norfloxacin (64.28%), Penicillin (76.78%), Ciprofloxacin (73.21%), Vancomycin (94.64%), Nalidixic acid (91.07%) and Ampicillin (50%). The results of antimicrobial susceptibility testing revealed that *S. aureus* was highly susceptible towards Choramphenicol and Gentamicin exhibiting 71.5% and 78.58% susceptibility. In conclusion, this study confirms the importance of *S. aureus* as a mastitis causing bacterium and identifies the risk factors associated with the disease in the Silchar dairy herd environment.

KEYWORDS: Bovine mastitis, drug resistance, *S. aureus*, NE India.

Introduction

Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms, especially pathogenic bacteria. However, health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues (Bradely *et al.*, 2002). The quality of milk may be lowered by a numbers of factors such as adulteration, contamination during and after milking and the presence of udder infections (Esron *et al.*, 2005). Pathogenic organisms in milk can be derived from the cow itself, the human hand or the environment (Bradely *et al.*, 2002).

Mastitis, despite of many years of research, even today is regarded and often considered as one of the most common dairy diseases (Rajala-Schultz *et al.*, 1999) because of its high incidence and heavy economic lossess which includes the loss of milk production, decrease in milk sale, increased culling rates and aims at high cost for veterinary treatments (Owens *et al.*, 1990). The intra mammary inflammatory response associated with mastitis not only results in a decrease in milk production, it also produces a decrease in quality of milk and manufactured products. Mastitis caused by contagious pathogens is transmitted directly from cow to cow (Erskine, 2001) and consumption of such milk from affected cows may lead to food poisoning or in rare cases provide mechanisms for spread of disease to humans (Radostits *et al.*,

2000). Incidence of mastitis caused by contagious pathogens depends on the dose, type of microbes to which a cow is exposed, physical barriers and the innate and acquired type of immunity. *Staphylococcal* mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. Therefore, the objectives of this study were to estimate the prevalence and identify the associated risk factors of bovine mastitis in Silchar town, N.E India, and to isolate, identify *S. aureus* from mastitic milk and to conduct *in vitro* antimicrobial susceptibility test on the isolates.

Materials and Methods

The study was conducted in Silchar town, Assam, North-East India where a total number of 120 milk samples were collected from lactating cows at early morning hours. Most of the farms were small holder dairy farms having an average of two to three lactating cows each; therefore, all the lactating cows from the dairy farms were taken into consideration for this study.

Study methodology

Data regarding the different potential risk factors (age, lactation stage, housing conditions and previous history of mastitis) were collected for 120 lactating cows from farm records when available. Questionnaires were distributed among the educated farm owners along with their personal interview. Clinical examination of the udder and screening of the cows using the California mastitis test (CMT) (Quinn *et al*., 1994), White Side Test (WST) (Murphy and Hanson, 1941), Surf Field Mastitis Test (SFMT) (Muhammad *et al*., 1995) and bacteriological examinations were also carried out.

Clinical inspection of the udder

Udders of the cows were examined by visual inspection and palpation for the presence of any lesion, pain, heat and swelling. In addition, milk from each quarter was withdrawn and checked for any change in colour and consistency.

Milk sample collection

Milk samples were collected according to the National Mastitis Council NMC (1990). After a quarter had been washed with tap water and dried (in cases when there was a considerable amount of dirt to be removed) the teat end was swabbed with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was then collected aseptically from clinical and subclinical (CMT positive) mastitic cows into sterile MacCartney bottles after discarding the first three milking streams. Samples from each quarter were transported on ice to microbiology laboratory where they were immediately cultured or stored at 4°C for a maximum of 24 h until cultured on standard bacteriological media.

Bacteriological examination of milk samples

Bacteriological examination was done according to the standard protocols of Quinn *et al*. (1994), NMC (1990) and National Committee for Clinical Laboratory Standards (NCCLS) (1997). A loopful of milk sample was streaked on Sheep blood agar (Oxoid, UK), nutrient agar, MacConkey agar and mannitol agar, using the quadrant streaking method for each quarter. All the plates were incubated aerobically at 37°C for 24 - 48 h. The plates were examined for gross colony morphology, pigmentation and haemolytic characteristics at 24 - 48 h. The Morphological, cultural and biochemical characteristics of the (Cruickshank et al., 1975) bacteria were identified according to their Gram reaction, the catalase test, tube coagulase test (4 h), haemolysis, mannitol and maltose fermentation. Samples were considered positive for *S. aureus* when at least one colony was identified as *S. aureus.*

Antimicrobial resistance pattern test

Antimicrobial susceptibility test was conducted on randomly selected *S. aureus* isolates and tested against antimicrobials using the Kirby-Bauer disk diffusion method (Quinn *et al*., 1994; NCCLS, 1997). The following antimicrobial disks (HI-MEDIA, India) with their corresponding concentrations were used: Chloramphenicol (50µg), Gentamicin (10 µg), Norflaxacin (10 µg), Penicillin (10 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Nalidixic acid (30 µg) and Ampicillin (10 µg). The inhibition zone diameters were measured to the near millimetre and the strains were characterized as susceptible (S), intermediate (I) or resistant (R) to the above antibiotics based on the guidelines of NCCLS (1997).

Results

Bacterial isolation

A total of 120 samples were collected and 36 isolates were isolated from positive mastitic cows. Large, smooth, shiny and opaque colonies were observed on nutrient agar plates; golden colored colonies on mannitol agar and pink colored colonies on MacConkey's agar plates followed by clear hemolysis on sheep blood agar plates respectively.

Related risk factors

Related potential risk factors revealed that bovine mastitis most likely occurred in those cows that were above 6 years of age and were kept in muddy house in comparison to those cows that were kept in houses with concrete floors and were at the early lactation stage.

Antimicrobial susceptibility

Antimicrobial susceptibility tests were performed on 36 S. aureus isolates isolated in the present study. It was observed that *S. aureus* isolates were found to be highly susceptible towards Choramphenicol and Gentamicin exhibiting 71.5% and 78.58% susceptibility and resistance was detected for Norfloxacin (64.28%), Penicillin (76.78%), Ciprofloxacin (73.21%), Vancomycin (94.64%), Nalidixic acid (91.07%) and Ampicillin (50%).

Discussion

In this present study out of 120 milk samples, 36 *S. aureus* were isolated from 43 positive milk samples and the rest 77 samples were tested as negative. The failure of negative finding of cultures in high percentage of samples may be due to premedication of the animals with antibiotics, non-bacterial causes and the type of media that did not support the growth of whole range of bacteria associated with mastitis (Quinn *et al*., 1994). The main source of the infection being the udder of infected cows where the *S. aureus* organisms can easily colonize in teat orifices, infection thus, may also be transferred via milker's hands while milking and washing or cleaning the udder with only plain water without using any antiseptic solutions, through utensils, towels and the environment (floor) in which the cows are kept (Radostitis *et al.,* 1994). It has been found and observed by earlier workers that *Staphylococci* organisms are mostly responsible for causing prevalent mastitis globally (Workineh *et al.*, 2002) which primarily resides in the mammary gland of cows is also supported by the questionnaire survey of the present studies (Iqbal et. al., 2003). It was observed that in those herds, where poor hygienic and milking practices was practised, the incidence of *Staphylococcus* sp. (30%) was higher that other organisms since these organisms exist in the mammary gland of the cow (Esron et al., 2005). It was also observed that contamination of teat ends was another major predisposing risk factor in the development of environmental mastitis, since the environmental pathogens can survive and multiply in organic bedding materials and

housing conditions that influences teat contamination rates (Kerro and Tareke 2003). In the present study, 36 isolates of *S. aureus* were obtained from 43 mastitic milk samples and the frequency of S. *aureus* alpha and beta hemolysins were also evaluated (Busato *et al.*, 2000). Of the 36 isolates, 16 isolates exhibited beta hemolysins and none of the isolates were alpha hemolysin positive. It has been observed that since beta hemolysin is the main toxin detected in the bovine mastitis isolates, but *S. aureus* isolates isolated from per-acute and acute mastitis have reported to produce larger amounts of beta toxins than *S. aureus* isolated from chronic infections (Busato *et al.*, 2000).

The antimicrobial susceptibility tests carried out in this study against different antimicrobial agents represented the prevalence of susceptibility and resistance of *S. aureus*. The results indicated that the resistance for Norfloxacin was 64.28% followed by Penicillin (76.78%), Ciprofloxacin (73.21%), Vancomycin (94.64%), Nalidixic acid (91.07%) and Ampicillin (50%). Further, antimicrobial susceptibility testing revealed that *S. aureus* was highly susceptible towards Choramphenicol and Gentamicin exhibiting 71.5% and 78.58% susceptibility. The pattern of susceptibility and resistance exhibited variations in the present study which may be due to different geographical and environmental conditions. Also prolonged and indiscriminate usage and prescriptions of particular drugs often leads to possible resistance development in the animals (Edward *et al.*, 2002; Gentilini, 2000), thus urging the veterinarians, farmers and the field assistants to implement a systemic application of an *in vitro* antibiotic susceptibility test prior to the use of antibiotics for successful treatment and prevention of intra-mammary infections against mastitis of the cows (Green and Bradely, 2004).

Conclusion

The elimination of *S. aureus* infections in conventional herds has proven to be difficult, even with the use of current antibiotics available, but treatment for mastitis cannot be outlined here, as there are large differences in antibiotics, thus, effective steps, preventive and control measures are required to avoid the spread of contagious pathogens in all herds. Also continuing education programs both in visual and audible forms to the farmers are required from time to time for better mastitis control programs.

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References:

1. Bradley A, 2002. Bovine mastitis: an evolving disease. Vet. J., 164: 116- 128.
2. Busato A, Trachsel P, Schallibaum M, Blum JW (2000). Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. Prev. Vet. Med. 28:205-220.
3. Cruickshank, R.; Duguid, J.P.; Marmion, B.P. Swain, R.H.A. Medical Microbiology. 12th Edn. Edinburg, London and New York. Churchill Livingstone 1975.
4. Erskine R. J., (2001). Mastitis control in dairy herds. In: Radstits O.M. (Ed.) Herd Health. Food Animal Production Medicine. W.B. Saunders Company, Pennsylvania, USA.
5. Esron D, Karimuebo E, Lughano T, Kusiluka RH, MelegelaAglowisye M, Kapaa M, Kalvin S (2005). Study on Mastitis, milk quality and health risk associated

with consumption of milk from pastoral herds in Dodoma and Morgora region, Tanzania. Journal Veternary science. 6: 213-221.

6. Edward M, Anna K, Michal K, Henryka L, Krystyna K (2002). Antimicrobial susceptibility of staphylococci isolated from mastitic cows, Bull. Vet. Inst. Pulawy pp. 289-294.
7. Green M, Bradely A (2004). Clinical Forum- *Staphylococcus aureus* mastitis in cattle UK. VET. 9: 4.
8. Gentilini E, Denamiel G, Llarente P, Godaly S, Rebuelto M, Degregorio O (2000). Antimicrobial susceptibility of *staphylococcus aureus* isolated from bovine mastitis in Argentina. J. dairy Sci. 83: 1224-1227.
9. Iqbal, M., M. Amjed and M.A. Khan (2003). Comparative study of various laboratory diagnostic tests of sub-clinical mastitis in dairy cows and buffaloes. MSc Thesis, NWFP Agri. Univ., Peshawar, Pakistan.
10. Kerro OD, Tareke F (2003). Bovine Mstitis in selected areas of Southern Ethiopia. Trop. Anim. Health Prod. 35: 197-205.
11. Muhammad, G., M. Athar, A. Shakoor, M.Z. Khan, Fazal-ur-Rehman and M. T. Ahmad, 1995. Surf field mastitis test: An inexpensive new tool for evaluation of wholesomeness of fresh milk. Pakistan J. Food Sci., 3(3-4): 91-93.
12. Murphy, J. M. and J. J. A. Hanson, 1941. Modified White Side Test for the detection of chronic bovin mastitis. Cornell Vet. , 32: 439.
13. National Committee for Clinical Laboratory Standards (NCCLS) (1997). Performance standard for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals and humans. Approved Standard, NCCLS document M 31-A, NCCLS, Villanova, PA.
14. National Mastitis, Council Inc. 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infections, National Mastitis Council Inc. 1840 Wilson Boulevard Arlington V. A. 2201, USA.
15. Owens WE, J.L. Watts, B.B. Greene. (1990). Minimum inhibitory concentrations and disk diffusion zone diameter for selected antibiotics against streptococci isolated from bovine intramammary infections. Journal of Dairy Science. 73:1225-1231.
16. Quinn PJ, Carter ME, Markey B, Carter GR (1994). Clinical Veterinary Microbiology, Wilfe Publishing, London pp. 95-101.
17. Radostitis OM, Blood DC, Gay CC (1994). Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th ed. BaillereTindall: London 8: 563-613.
18. Radostits, O. M., et al.(2000): Bovine mastitis, In: A Textbook of Cattle, Sheep, Pigs, Goat and Horse. Veterinary Medicine. 9th edn. pp. 563-618. ELBS and BaillereTindau.
19. Rajala-Schultz P J, Grohn Y T, McCulloch CE, GuardCL (1999). Effects of clinical mastitis on milk yield in dairy cows. Journal of Dairy Science 82: 1213–1220.
20. Workineh S, Bayleyegn M, Mekonnen H, Potgieter LND (2002). Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. Tropical Animal Health and Production, 34: 19-25.