RESEARCH ARTICLE

Prevalence of Subclinical Mastitis and Associated Bacterial Pathogens in Milkshed Districts of Odisha, India

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Abstract

The present study deals with subclinical mastitis (SCM) with respect to prevalence, etiology and antibiogram pattern. A total of 1273 cross-bred Jersey lactating cows in four milkshed districts of Odisha *viz*. Jagatsinghpur, Puri, Khordha and Cuttack were screened through modified california mastitis test during the period from September 2019 to January 2020. Overall 57.58% prevalence of SCM was recorded. Microbiological examination of 72 milk samples positive for SCM revealed association of *Staphylococcus aureus* in maximum (65.27%) cases followed by *Streptococcus uberis* (9.7%), *Streptococcus agalactiae* (8.3%), *Pseudomonas aeruginosa* (5.55%) and *Escherichia coli* (2.76%). *In vitro* antibiotic sensitivity test results indicated > 95 % *S. aureus* isolates were quite sensitive to the antibiotics like gentamicin, ceftizoxime, cefuroxime and ceftiofur.

Keywords: Antibiotic Sensitivity Test, Cross-bred Jersey cows, Prevalence, Subclinical Mastitis.

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Introduction

Mastitis, an inflammatory condition of udder, is prevalent among the dairy cows throughout the world. The direct and indirect economic losses associated with the disease are enormous. It includes losses due to discarded milk, reduced milk yield, poor quality milk, costs of treatment and premature culling or replacement of the stock. Milk from infected animals may contain microorganisms that may be pathogenic to humans. In India, due to mastitis the gross annual losses estimated were to the tune of about Rupee 7165 crore (Bansal and Gupta, 2009). Prevalence of the subclinical mastitis (SCM) is 30–40 times more as compared to clinical mastitis (Bhanderi and Garg, 2012) and infected cows serve as a major source of infection for healthy cows (Ruegg, 2017). Around 75% of the economic losses from SCM is attributed to drop in milk production. The incidence of subclincal mastitis in cows increases with increasing number of lactations, increased milk production and poor hygiene (Dasohari *et al*., 2017). More than 200 microbial species, sub-species and serotypes have been isolated from bovine mammary gland (Balakrishnan *et al*., 2004) and identified as causative agents of mastitis. The present study was designed to detect the current prevalence status of subclinical mastitis and identification of bacterial pathogens in milk samples collected from subclinical mastitis affected cows of four major milkshed districts of Odisha.

Materials and Methods

Four sample villages each were selected randomly from four major milkshed districts of Odisha *viz*. Jagatsinghpur, Puri, Khordha and Cuttack and included in the present study. Cross-bred Jersey cows between 2-4 calvers, 1-4 months of their lactation and having a minimum yielding record of 6.5 L/day were included in the study. Dairy cows (n=1273) fulfilling above criteria and having the history of gradual loss of milk yield and normal appetite were screened through cowside test for detection of SCM. Preliminary diagnosis of subclinical mastitis was done by general clinical and mammary gland examination followed by using modified california mastitis test (MCMT) (Schalm and Noorlander, 1957) and modified Newman's stain (HiMedia).

Out of 733 milk samples positive for sub clinical mastitis, 82 milk samples received in proper pack and condition were selected for microbiological examination. Individual

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milk sample was inoculated into nutrient broth, incubated at 37°C and cultured on media such as nutrient agar, Blood agar, Macconkey's agar, Eosin Methylene Blue agar and *Staphylococcus* medium no. 110. The inoculated plates were incubated aerobically at 37°C for 24–48 hours. Then the bacterial isolates to the species level were identified based on their colony and hemolytic characteristics, Gram's staining, morphological characteristics, growth on media and various biochemical reactions (Quinn *et al*., 2002; Barrow and Feltham, 2004).

Milk samples containing *Staphylococcus aureus* isolates were processed for *in vitro* antibiotic sensitivity test. Based on the availability of antibiotics for use in veterinary practice, the antibiotic discs *viz.* gentamicin, streptomycin, tetracycline, cloxacillin, ceftizoxime, cefoperazone, penicillin G, ceftriaxone, ceftriaxone + sulbactam, ciprofloxacin, cefuroxime, amoxycillin, ampicillin and ceftiofur were selected.

Results and Discussion

Prevalence of Bovine Subclinical Mastitis

Out of 1273 milk samples from lactating cows screened, 733 (57.58%) milk samples showed positive reaction to MCMT as well as Newman's staining (Table 1 and Fig. 1). There is no significant difference among the districts, however numerically highest prevalence (62.42%) was recorded in Jagatsinghpur and lowest (51.06%) in Khordha. Results on the prevalence of SCM in the present study are in close approximation to the earlier studies where about half of the lactating cattle population had SCM during the period under study (Tanwar *et al*., 2001; Shrestha and Bindari, 2012; Tripathi 2015; Suleiman *et al*.,2018).High prevalence of SCM in the present study could be attributed to a number of factors such as milking methods, calf suckling practices, production level, daily milk production, stage of lactation, calving interval, teat lesion and other influential factors could be housing system, climatic conditions and maintenance of hygiene due to lack of awareness of owner towards the disease (Mulshet *et al*., 2017; Sahoo *et al*., 2020)

Table 1: District-wise prevalence of subclinical mastitis among cross-bred Jersey lactating cows

Sl. No.	*District*	*No. of cows examined*	*No. of cows affected with subclinical mastitis*
1.	Jagatsinghpur	346	216 (62.42%)
2.	Puri	225	138 (61.33%)
3.	Khordha	376	192 (51.06%)
4.	Cuttack	326	187 (57.36%)
	Total	1273	733 (57.58%)

Isolation and Identification of Bacterial Species

Out of 733 positive milk samples to MCMT, 82 samples were further processed for identification of bacterial pathogen through standard microbiological procedure, in which 87.8% (72/82) milk samples found positive for one or more pathogens i.e., as highest bacterial isolate (65.27%) *Staphylococcus aureus* was present followed by *Streptococcus uberis* (9.7%), *Streptococcus agalactiae* (8.3%), *Pseudomonas aeruginosa* (5.55%) and *Escherichia coli* (2.76%). Mixed infection was recorded in 6 (8.33%) samples (Table 2). The remaining 10 (12.2%) samples did not exhibit any evidence of bacterial growth after 48 hours and these milk samples were classified as sterile or contaminated with nonbacterial pathogens. The findings of present study almost corroborated with findings of Tripathi (2015) who found maximum involvement of *Staphylococcus* spp. (66.90%), followed by *Streptococcus* spp. (23.23%), *E. coli* (2.81%) and mixed infection (7.04%). On the contrary, Patel *et al*. (2012) reported variable percentage of pathogens from milk samples i.e., *S. aureus* (30.64%), followed by *S. agalactiae* (20.00%), *E. coli* (10.21%), *Pseudomonas*

Table 2: Distribution of different bacterial isolates recovered from 82 subclinical mastitis samples

Sl. No.	*Bacteria isolated from the milk samples positive for SCM*	*No. of positive isolates (%)*
1.	Staphylococcus aureus	47/72 (65.27%)
2.	Streptococcus uberis	7/72 (9.72%)
3.	Streptococcus agalactiae	6/72 (8.33%)
4.	Pseudomonas aeruginosa	4/72 (5.55%)
5.	Escherichia coli	2/72 (2.76%)
6.	Mixed bacterial infection with two bacteria of different genera	6/12 (8.33%)
	Total	72/82 (87.80%)

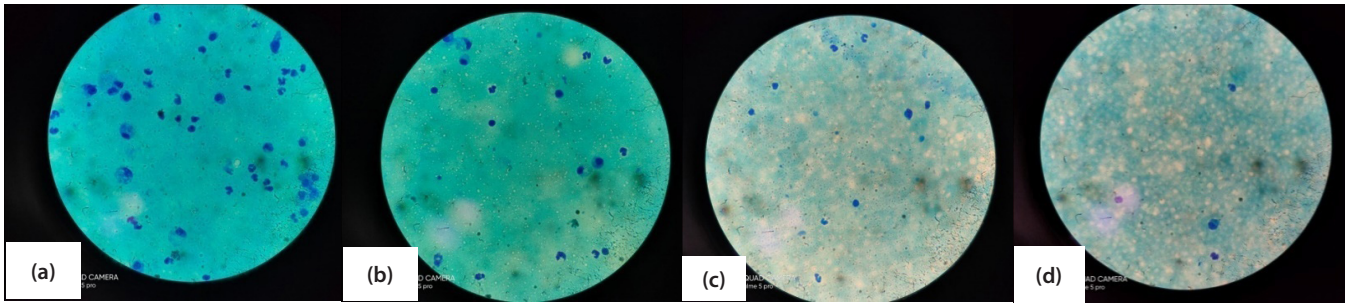

Fig 1: Air dried milk films under oil immersion objective (100x) stained with Newman's stain depicting somatic cells corresponding to MCMT score ; (a) +++ (b) ++ (c) + (d) –

(9.36%). *Staphylococcus* spp. is the most common cause of SCM worldwide (Zuniga *et al*., 2015 and Hamid *et al*., 2017). High involvement of *Staphylococcus* spp. (65.27%) was observed during present investigation which corroborated with findings of Krishnamoorthy *et al*. (2017), Verma *et al*. (2018) and Zaatout *et al*. (2019). It may be due to the ability of the bacteria to colonize on the teat ends and teat canal and to their long-term survival on the skin before entering the upper parts of the mammary gland that causes new subclinical intramammary infection. Moreover, it had the ability to survive phagocytosis, form biofilm and invade into mammary epithelial cells that protect it from host immune response thus increasing infection rate (Janosi *et al.,* 2001; Barkema *et al.,* 2006). Many authors have isolated several *Streptococcus* spp. from mastitic milk samples (Jeykumar *et al*., 2013; Tripathi, 2015), whose entrance may be aided by cow-to-cow transfer, presumably via the milker's hands. In this study, *P. aeruginosa* was recorded in 5.55 % of cases, which is an opportunistic bacterium that causes mastitis in cows and has been classified as an environmental pathogen (Balakrishnan *et al*., 2004). *Escherichia coli* was found in 2.76 % of milk samples in the current investigation, which might have entered the udder via the teat canal after being transferred from the cow's environment (Burvenich *et al*., 2003).

In vitro Antimicrobial Sensitivity Test

S. aureus-associated subclinical mastitis was found to be 65.27% (47/72) in lactating cows. On further processing of MCMT positive samples for *in vitro* antimicrobial sensitivity test by disc diffusion method, more than 90% *S. aureus* isolates were found sensitive to the antibiotics; gentamicin, ceftizoxime, cefoperazone, cefuroxime, ceftriaxone and ceftiofur whereas antibiotics like streptomycin, tetracycline, cloxacillin, penicillin-G, amoxicillin, ampicillin, ceftriaxone + sulbactum and ciprofloxacin showed moderate degree of sensitivity. Ceftizoxime and cefuroxime were found to be most effective (97.8%) antibiotic against *S. aureus* in the present study. Similar observations have been made by Sahoo *et al*. (2020) who showed the antibiogram pattern to SCM sensitive to ceftiofur (98.32%) followed by cefuroxime (97.34%), gentamicin (95.52%) and ceftizoxime (94.09%). Sharma (2000) opined that sensitivity pattern could vary depending on the frequent and long-term therapeutic use of particular antibiotic in a region. Some pathogens exhibited poor sensitivity to certain antimicrobials and this can be attributed to prolonged use of antibacterial drugs in the absence of *in vitro* drug susceptibility testing (Sobiraj *et al*., 1999).

Conclusion

The prevalence rate of 57.58% subclinical mastitis in cows of four districts of Odisha i.e., Jagatsinghpur, Puri, Khordha and Cuttack districts during the study period was recorded. The isolate *S. aureus* was associated with subclinical mastitis maximum cases (65.27%)in lactating cows followed by *S. uberis* (9.7%), *S. agalactiae*(8.3%), *P. aeruginosa* (5.55%) and *E. coli* (2.76%). More than 95 % isolates of *S. aureus* were sensitive to gentamicin, ceftizoxime, cefuroxime and ceftiofur.

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ANNOUNCEMENT: SVSBT-NS-2022

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The ***IX Annual Convention*** and ***National Seminar*** of The Society for Veterinary Science & Biotechnology ***(SVSBT) on "Recent Biotechnological Advances in Health and Management to Augment Productivity of Livestock and Poultry"*** will be **organized at Ramayanpatti, Tirunelveli - 627 358, Tamil Nadu, during September 22-24, 2022** (Thursday, Friday & Saturday) by Veterinary College & Research Institute, Tirunelveli - 627 358, TANUVAS, (TN). The detailed Brochure cum Invitation showing Theme Areas/ Sessions, Registration Fee, Bank Details for online payment and deadlines, etc. has been floated on the Whats Apps and e-mails. Accordingly, the organizing committee of ***SVSBT NS-2022 invites abstracts*** of original and quality research work on theme areas of seminar limited to 250 words by e-mail on svsbttnns2022@gmail.com or mopandian69@gmail.com latest by 30th August, 2022 for inclusion in the Souvenir cum Compendium to be published on the occasion.

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