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Prevalence of major pathogenic bacteria from mastitis buffaloes

Deeksha and Sonia Sindhu

Abstract
The study was carried out at the animal farms of the ICAR- Central Institute of Research on Buffaloes (ICAR-CIRB), Hisar across two breeds during winter season to compare the efficacy of infrared thermography with different diagnostic techniques for early detection of mastitis. The two organized herds included the Murrah breed animals (n=104) at the main campus located at Hisar, Haryana and the Nilli-Ravi herd (n=100) at ICAR-CIRB sub campus located at Nabha, Punjab. CMT was used as initial classifier for animal as normal/non-mastitis, subclinical/clinical mastitis. Milk samples from thirty-eight mastitis cases were examined for the isolation and identification of bacterial agents. Among bacterial agents isolated from thirty-eight mastitis cases, prevalence of Coagulase-negative Staphylococci was 65.79%, mixed infection was 21.05%, S. aureus and Escherichia coli was 5.26% each and Streptococcus spp. was 2.63%. Prevalence of subclinical mastitis in herd of CIRB was 10.29% and Clinical mastitis was 8.33%.

Keywords: Infrared thermography, CMT, mastitis, coagulase-negative staphylococci, S. aureus

Introduction
Animal husbandry is an integral component of agriculture sector and plays a multifaceted role in uplifting Indian economy. It provides employment and empowerment to rural population and acts as moving bank during financial crisis. Livestock provides sustained income to small and landless farmers throughout the year, thus contributing in their socio-economic development. Livestock sector contributes 4.4 percent to GDP and 25.6 percent of total agricultural GDP (*National Accounts Statistics-2016; Central Statistical Organisation; GoI*) [15]. India possesses world's largest livestock population and largest producer of milk but still not self-sufficient in milk. Milk production during 2017-18 and 2018-19 is 176.3 million tonnes and 187.7 million tonnes respectively showing an annual growth of 6.47%. (Annual report 2019-20, DAHD & F, GOI) [1] This growth is mostly the result of an increase in numbers of producing animals rather than a rise in productivity per head. Low productivity is due to poor animal health, inferior management practices and ineffective diagnostic techniques. Contagious disease affects animal health and productivity.
Worldwide most frequently occurring calamitous disease of dairy herd is mastitis (Ojo *et al.* 2009) [16] causing major economic losses to the farmers. Disease causes inflammation of affected mammary gland. The major pathogens of both contagious and environmental mastitis are; *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, and *Klebsiella*, causes fever, inflammation, swelling, milk composition and color changes, and presence of somatic cells etc. Intensity of the inflammation can be classified into sub-clinical, clinical and chronic forms. Inflammation might differ in severity due to many factors such as pathogen type, animal health status, age and lactation cycle of the animal. In addition, mastitis being a potent zoonotic disease, poses serious hazard to human health (Blum *et al.* 2008) [2].

Impacts of mastitis
Reduction or complete loss in milk production affects farmer's income. Milk has to be discarded for 3 days during treatment and 3 days post treatment for withholding period (Huijps, Lam and Hogeveen, 2008) [10]. The quality of milk is deteriorated due to elevated somatic cell count and decreased milk fat. Antibiotic treatment and veterinary care impose additional financial burden on livestock holders. The minimised organoleptic properties affects the selling price of milk and milk products. The meat of mastitis affected animals fetch lower prices due to reduced quality and carcass yield. Mastitis affected animals are culled and replaced with healthy stock.

The premature replacement of stock largely contributes to economic losses (Halasa *et al.*, 2007; de Graves and Featrow, 1993; Hortet and Seegers, 1998) [8, 5, 9].

Experimental setup

The study was carried out at the animal farms of the ICAR-Central Institute of Research on Buffaloes (ICAR-CIRB), Hisar across two breeds during winter season. The two organized herds included the Murrah breed animals (n=104) at the main campus located at Hisar, Haryana and the Nilli-Ravi herd (n=100) at ICAR-CIRB sub campus located at Nabha, Punjab. All animals studied were maintained as per standard practices of ICAR minimum feeding standards with ad-libtum drinking water availability round the clock. All buffaloes studied were milked twice daily.

Milk sample collection

CMT was used as initial classifier for animal as normal/non-mastitis, subclinical/clinical mastitis. Composite and quarter-wise (10ml each) milk samples in sterile polypropylene tubes of all animals were collected and preserved for bacterial isolation and identification. During collection, udder of each animal was properly disinfected with 70% alcohol and dried before sample collection. First few streaks of milk were discarded and milk sample from each teat was collected in sterile test tubes and properly labelled with buffalo's identification number and teat position (i.e. fore-right, fore-left, hind-right and hind-left).

Bacteriological Culture Examination

Milk sample from each quarter (approx. 0.1 ml of milk) was inoculated using sterile cotton swab in a zigzag pattern onto the surface of blood agar, MacConkey Lactose agar, Mannitol salt agar and incubated at 37^0C for 24 hours under aerobic conditions. The isolates were identified by their cultural characteristics, microscopic examination in their Gram stained slides, catalase test, and oxidase test. Identification and characterization of bacteria was performed as per the method described by Cowan and Steel, 1970.

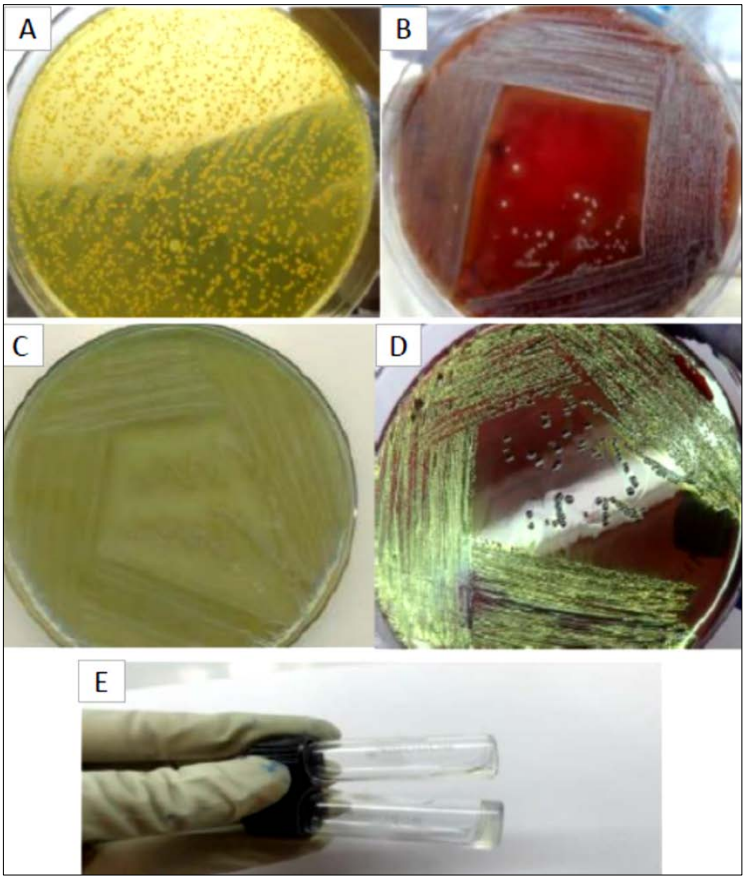

Fig 1: A. Primary isolation of *Staphylococcus* on Mannitol salt agar. Presumptive *S. aureus* ferments mannitol and produces yellow colored colonies B. Presumptive isolation of *Streptococcus spp.* on 5% bovine blood agar C. Green pigmentation of presumptive *Pseudomonas* colonies on nutrient agar D. Metallic sheen of *E. Coli* on Eosin Methylene Blue agar E. Tube coagulase test using rabbit plasma to confirm coagulase-positive *S. aureus*.

Results and Discussion

Milk samples from thirty-eight mastitis mastitis cases were examined for the isolation and identification of bacterial agents. Out of Milk samples from 38 mastitis cases, 08 samples were found containing mixed infection (*Coagulase-negative Staphylococci* + *Escherichia coli* -2, *Coagulase-negative Staphylococci* + *pseudomonas spp.*-2, *Coagulase-negative Staphylococci* + *Streptococcus spp.*-2, *S. aureus* + *Escherichia coli* -1, *S. aureus*+ *Streptococcus spp.*-1) and the rest 30 samples were associated with single infection.

Mastitis can be epidemiologically categorized into contagious and environmental mastitis caused by a wide spectrum of pathogens. Increase in the level of humidity and pollution in the environment of the barn also increases the load of bacterial pathogens in the environment. In present study among bacterial agents isolated from 38 mastitis cases, prevalence of *Coagulase negative Staphylococcus spp.* was 65.79%, mixed infection was 21.05%, *S. aureus* and *E. coli* was 5.26% each and *Streptococcus spp.* was 2.63%. Prevalence of *Coagulase negative Staphylococcus spp.* was

found highest in present study. The results were alike with Zeryehun and Abera, (2017) [20] as their study revealed bacteriological examination of milk samples where *Coagulase negative Staphylococcus species* (*CNS*) (34.2%) was the predominant species while *Streptococcus faecalis* (2.1%) was identified as the least bacteria. Prevalence of mastitis particularly the subclinical mastitis was major problem likewise *Coagulase negative Staphylococcus species* was observed as a main causative agent in mastitis (Moroni *et al.*, 2006; Saber *et al.*, 2017; Dhakal and Nagahata 2018; Boireau *et al.*, 2018) [13, 17, 6, 3]. Prevalence of subclinical mastitis was found more in present study as compared to clinical mastitis which was in agreement with the results of Isaea and Kurtu (2018) [11]. Present study results were in close resemblance with Mostafa *et al.* (2018) [14] who observed comparatively higher prevalence of *Coagulase negative Staphylococci* (CNS) (19.5%) followed by *Escherichia coli* (8.3%) and *Streptococcus spp.* (5.6%) in mastitis milk samples similarly very less prevalence of *E. coli* (6.7%) in mastitis was reported by Ferreira *et al.* (2018). Very less prevalence of *Streptococcus spp*. (2.4%) was also detected by Kasa *et al.* (2020) [12] in bovine clinical mastitis. The results were differing with the findings of Waseem *et al.* (2020) [19] as they observed highest prevalence of *Staphylococcus spp*. in clinical cases of Bovine mastitis. Correspondingly *Staphylococcus aureus* was the most frequently isolated bacterial species as per the findings of Srinivasan *et al.* (2013) followed by *Streptococcus agalactiae* and *Coagulase negative Staphylococcus species.*

Table 1: Prevalence of bacterial agents isolated from thirty-eight mastitis cases

Pathogen	N=38	Prevalence Rate
Coagulase-negative Staphylococci	25	65.79%
Staphylococcus aureus	2	5.26%
Streptococcus spp.	1	2.63%
Escherichia coli	2	5.26%
Mixed infection	8	21.05%

Table 2: Details of bacterial agents causing mixed infections

Mixed Infections	
Coagulase-negative Staphylococci + Escherichia coli	2
Coagulase-negative Staphylococci + Pseudomonas spp.	2
Coagulase-negative Staphylococci + Streptococcus spp.	2
Staphylococcus aureus + Escherichia coli	1
Staphylococcus aureus + Streptococcus spp.	1

Table 3: Prevalence of bacterial agents isolated from mastitis cases of Nilli-Ravi buffaloes

Pathogen	N=16	Prevalence Rate
Coagulase-negative Staphylococci	10	62.50%
Staphylococcus aureus	01	6.25%
Streptococcus spp.	01	6.25%
Escherichia coli	01	6.25%
Mixed infection	03	18.75%

Table 4: Details of bacterial agents causing mixed infection in mastitis cases of Nilli-Ravi buffaloes

Mixed Infections	
Coagulase-negative Staphylococci + Escherichia coli	02
Coagulase-negative Staphylococci + Streptococcus spp.	01

Table 5: Prevalence of bacterial agents isolated from mastitis cases of Murrah buffaloes

Pathogen	N=22	Prevalence Rate
Coagulase-negative Staphylococci	15	68.18%
Staphylococcus aureus	01	4.54%
Escherichia coli	01	4.54%
Mixed infection	05	22.72%

Table 6: Details of bacterial agents causing mixed infections in mastitis cases of Murrah buffaloes

Mixed Infections	
Coagulase-negative Staphylococci + Pseudomonas spp.	02
Coagulase-negative Staphylococci + Streptococcus spp.	01
Staphylococcus aureus + Escherichia coli	01
Staphylococcus aureus + Streptococcus spp.	01

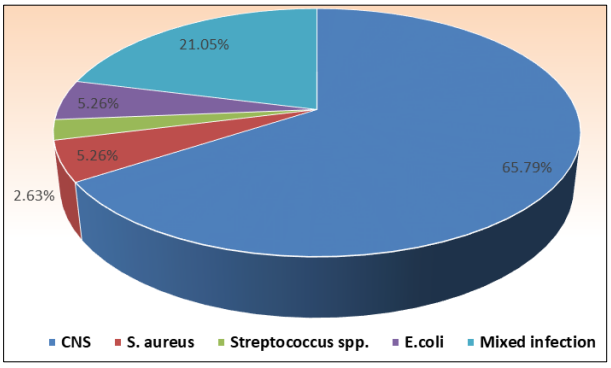

Fig 2: Prevalence rate of bacterial agents isolated from 38 mastitis cases

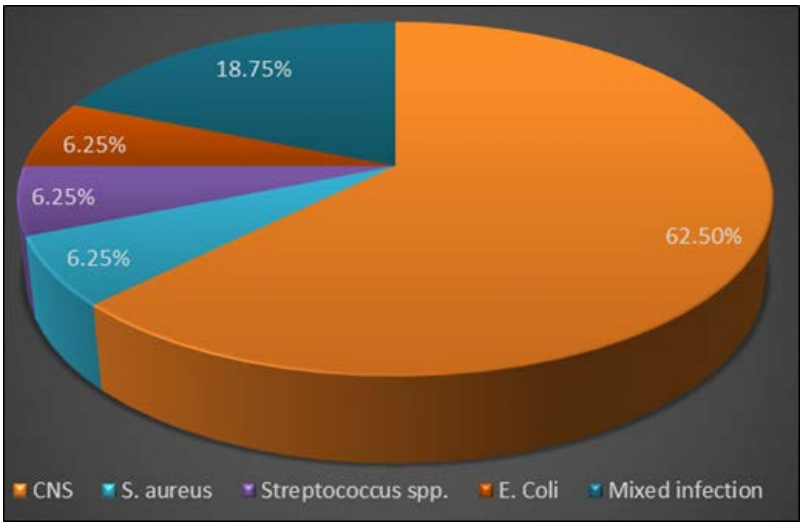

Fig 3: Prevalence rate of bacterial agents isolated from mastitis cases of Nilli-Ravi buffaloes

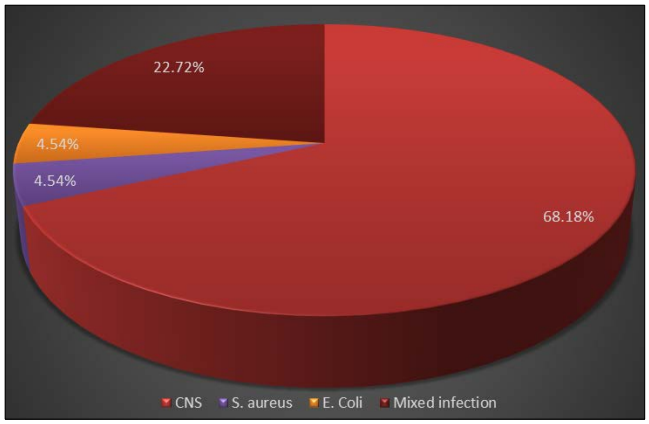

Fig 4: Prevalence rate of bacterial agents isolated from mastitis cases of Murrah buffaloes

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