Subclinical mastitis in machine milked dairy farms in Punjab: prevalence, distribution of bacteria and current antibiogram

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Abstract

Aim: Mastitis, a complex disease, even at subclinical stage has a major economic impact on the dairy industry. The disease pattern at machine milking which has recently been introduced in Indian system is to be studied. Therefore the present study was conducted to see the prevalence, distribution and sensitivity pattern of bacteria at subclinical level in machine milked dairy farms in Punjab state.

Materials and Methods: The study involved 10 machine milked dairy cow herds in Ludhiana, Patiala, Moga, Bathinda and Ferozpur districts of Punjab. A total 218 HF × Sahiwal cross-bred dairy cow in milk were studied. About 872 quarter foremilk samples were collected to observe the prevalence of disease, distribution of udder pathogens and antibiotic sensitivity pattern.

Results: Prevalence of specific subclinical mastitis was 57.80 % and 30.73% on animal and quarter basis respectively. In specific subclinical mastitis *Staphylococci* (41.04%) were the main organisms while in case of latent infections *Corynebacteria* (36.81%) were found to be chief isolates. Erythromycin, Enrofloxacin and Gentamicin were found to be most sensitive, and Streptomycin was found to be the least sensitive *in vitro* antibiotic.

Conclusion: *Staphylococci* were the main organisms in specific subclinical mastitis while *Corynebacteria* were found to be chief isolates in case of latent infections. Erythromycin, Enrofloxacin and Gentamicin were found to be most sensitive, and Streptomycin was found to be the least sensitive *in vitro* antibiotic.

Keywords: antibiogram, bacterial distribution, machine milking, prevalence, subclinical mastitis.

Introduction

Mastitis is the inflammation of mammary gland; it is a complex disease which continues to have a major economic impact on the dairy industry throughout the world [1, 2]. The losses due to mastitis in USA alone are 1.7 billion dollars [3] while in India the losses are estimated to be about Rs. 7,165 crores [4]. The term mastitis comprises two relatively independent health problems: clinical and subclinical mastitis [5]. Subclinical mastitis is bereft of any obvious manifestation of inflammation and is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases and there is change in milk composition [6]. Subclinical mastitis is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds [2].

Mastitis is produced by interplay of the variety of pathogenic agents and bacteria are one of the major etiological agents of the mastitis. The infectious causes of mastitis are vast and at least, 137 infectious causes of bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* spp. and Coliforms [7]. Mastitis is considered as one of the major cause of antibiotic use in dairy animals, conventional antibiotic treatment is fast losing relevance due to development of resistance to the antibiotics among the mastitis pathogens, cost consideration and undesirable residues in milk [8]. Identification of mastitis causing pathogen and the results of antibiotic resistance pattern of the isolated bacteria are important prerequisites for implementation of effective control of mastitis.

Therefore the present study was conducted to see the prevalence, distribution and sensitivity pattern of bacteria in machine milked dairy farms.

Materials and Methods

Ethical approval: Not necessary.

Samples: The present study involved 10 machine milked dairy cow herds in Ludhiana, Patiala, Moga, Bathinda and Ferozpur districts of Punjab. A total 218 HF × Sahiwal cross-bred dairy cow in milk were studied. About 872 quarter foremilk samples were collected and subjected to bacteriological examination and California Mastitis Test (CMT) to observe the prevalence of subclinical mastitis on the guidelines of International Dairy Federation (IDF). Quarters were classified into different health groups' *viz.*, healthy, latent, non-specific and specific mastitis. An animal was considered positive irrespective of number of quarter(s) affected.

Analytical tests: The various analytical procedures

Table-1: Defining udder health status

CMT score	Microbial pathogen	
	Not detected	Detected
< 1	Healthy	Latent infection
≥ 1	Non-specific mastitis	Specific mastitis

Specific mastitis means the quarter is bacteriologically positive and having high somatic cell count indicated by positive CMT reaction. Latent infection means only bacteria is present but no cell reaction is present, while non-specific means only cell reaction is there but no bacteria present.

Table-2: Udder health status of dairy cows at various machine milked dairy farms

Farm ID	No. of animals involved	No. of quarters involved	Percent distribution with respect to udder health (quarter wise)				
			Blind/ Others	Healthy	Latent infections	Non-Specific mastitis	Specific mastitis
Herd I	17	68	0.00	47.06	16.18	19.12	17.65
Herd II	14	56	0.00	48.21	14.29	23.21	14.29
Herd III	45	220	7.73	25.45	12.27	17.73	36.82
Herd IV	20	80	0.00	58.75	6.25	11.25	23.75
Herd V	19	76	2.63	53.95	3.95	9.21	30.26
Herd VI	12	48	6.25	22.92	20.83	10.42	39.58
Herd VII	21	84	0.00	39.29	34.52	1.19	25.00
Herd VIII	23	92	0.00	30.43	20.65	11.96	36.96
Herd IX	18	76	1.32	22.37	28.95	19.74	27.63
Herd X	18	72	1.39	30.56	13.89	12.50	41.67
Overall	218	872	2.75	36.01	16.51	13.99	30.73

Cows positive for specific mastitis = 126/218 × 100 = 57.80%.

used were as follows:

Isolation and identification of pathogenic bacteria: The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council [9].

Cultural sensitivity testing: The organisms isolated from quarter foremilk samples were tested for sensitivity to various antimicrobial agents as per method of Ward and Bates [10].

Defining udder health status: The quarter health status was assessed and defined on the basis of bacteriology and SCC estimate (CMT score) of quarter foremilk samples as given in Table-1.

Results and Discussion

Prevalence of subclinical mastitis: The average prevalence of specific subclinical mastitis at the machine milked dairy farms under study was found to be 57.80% on animal basis and 30.73% on quarter basis (Table-2).It is in accordance with the studies [11-13] found the prevalence of subclinical mastitis in cows at machine milked dairy farms as 53.78%, 49.29% and 41.77%, respectively. Similar results were also observed by other studies [14, 15] who also found the animal's level prevalence of subclinical mastitis 48.6 to 55.1% while quarter level prevalence was 34.8 to 42.7% respectively. However, the other studies [16-18], reported a lower prevalence of 29.62%, 36.75% and 14.60% respectively.

The difference in prevalence of subclinical mastitis in present and earlier studies may be attributed to various factors like climate, season and farm managemental practices such as general hygiene, care of teat injuries, care and working of milking machine, and adoption of various mastitis control measures including teat dipping and dry cow therapy etc. Also the basis of declaring a quarter or animal positive for mastitis, and criteria of classifying organisms into pathogenic or non-pathogenic effects the results to great extent. For example, present study unlike the studies reported above has considered quarters with specific mastitis i.e. positive for CMT as well as bacteriology. Also, in present study quarters infected with *Streptococci*, *Staphylococci* (both coagulase positive and negative), *Corynebacteria* and *Bacillus* spp. were taken as pathogenic where as many times coagulase negative *Staphylococci* and *Bacillus* spp. are taken as non-pathogens [19].

Distribution of organisms: The organisms isolated from specific subclinical mastitis constituted *Staphylococci* (41.04%), *Corynebacteria* (30.60%), *Streptococci* (21.27%), and *E. coli* and others (7.09%). However, in case of latent infections *Corynebacteria* (36.81%) were found to be chief isolates followed by *Staphylococci* (27.78%), *Bacillus* (20.83%), *Streptococci* (9.72%) and *E. coli* and others (4.86%). The higher prevalence of staphylococcal mastitis has been reported by many workers [17, 14] over the years. The higher incidence of *Staphylococci* indicates unhygienic milking practices as this pathogen is mainly spread during milking via milkers' hands [20].It may also be due to indiscriminate use of antibiotics which have resulted in emergence of more resistant strains (L-form). It is asserted [21] that *S. aureus* is well adapted to survive in the udder and usually establishes a mild subclinical infection of long duration from which it shed in milk facilitating transmission to healthy animals mainly during milking. Also, the organism can survive for longer periods in environment, are widely distributed over the body surface of lactating

Table-3: Cultural Sensitivity pattern of the bacterial species isolated from cases of specific mastitis.

Organism	No.	G	A	Amoxi	N	T	P	C	E	S	Enro
Staphylococci	59	93.22	67.80	74.58	87.93	81.36	76.27	72.41	94.83	51.79	87.50
Streptococci	27	77.78	66.67	62.96	74.07	77.78	62.96	33.33	85.19	52.00	94.74
Corynebacteria	31	61.29	74.19	74.19	61.29	75.61	58.06	36.00	93.55	41.38	77.27
Others*	06	100	33.33	50	100	83.3	33.3	60	75	75	100
Overall	123	82.11	67.48	70.73	78.69	78.95	66.67	54.78	91.80	50.86	86.96

G: Gentamicin (10µg), A: Ampicillin (10µg), Amoxi: Amoxicillin (10µg), N: Neomycin (30µg), T: Tetracycline (10µg), P: Pencillin (10Units), C: Cloxacillin (10µg), E: Erythromycin (10µg), S: Streptomycin (25µg), Enro: Enrofloxacin (10µg); *E.coli.: 01; Bacillus spp.: 02; Proteus spp. : 01; Pseudomonas spp.

animals, teat cups of the milking machine and so can easily gain entry into the teat canal.

The one unusual and different finding from that of previous experience at hand milked dairy farms under local conditions is the high share of *Corynebacteria*, *Streptococci* and Gram positive aerobic bacilli isolations in machine milked dairy cows. The reason for this difficult to explain, but it is probably related to changes in herd management and consequent bacteriological ecology in the herd environment e.g., it has been observed that suboptimal postmilking teat disinfection may result in a high herd prevalence of these minor pathogens [22]. *C. bovis* persists with routine application of teat disinfection but is eliminated with dry cow antibiotic therapy [23]. The higher incidence of *Streptococci* may be due to environmental *Streptococci* that are considered to become in-habitant in milking machine. A clear linkwas observed between milking machine faults and higher incidence of *Streptococcus uberis*, an organism regarded as environmental pathogen [24]. She concluded that the increase in *S. uberis* might have been due to teat end damage resulting from the failure of pulsation applied in that environment. Also *C. bovis* infected quarter's represent a significant risk factor for environmental *Streptococcal* infections. It has been reported that the presence of *C. bovis* will reduce the likelihood of subsequent infection with *S. aureus* but may increase the risk of infection with *S. agalactiae* and environmental *Streptococci* [21].

Culture sensitivity pattern: A total of 123 isolates from specific subclinical cases of mastitis were tested for sensitivity to different chemotherapeutic agents (Table-3). Overall, Erythromycin, Enrofloxacin and Gentamicin were found to be highly sensitive, and streptomycin was found to be the least sensitive *in vitro* antibiotic. *Staphylococci* were found highly sensitive to Erythromycin, Gentamicin, Neomycin, Enrofloxacin and Tetracycline. Enrofloxacin was the most effective drug against *Streptococci* followed by erythromycin and tetracycline. Sensitivity pattern of *Corynebacteria* showed Erythromycin, Enrofloxacin, Tetracycline and Ampicillin as the most effective antibiotics. *E. coli* and other gram negative bacteria were least sensitive to ampicillin and cloxacillin but were highly sensitive to Enrofloxacin, Gentamicin and Neomycin. The present findings of sensitivity pattern can be compared favourably with the findings of [25, 26, 27] who also

revealed similar sensitivity pattern for different organisms to one or more of these antimicrobial agents. However in another study [12] observed Gentamicin (93.08%) as the most effective while Pencillin (52.31%) as the least sensitive antibiotic. *Staphylococci* were seen to be highly sensitive to Gentamicin while *Streptococci* and *Corynebacteria* were highly sensitive to Cephalexin.

The different antibiotic sensitivity pattern observed in the present study may be due to the emergence of newer strains of organisms over the years. Also, it may be due to the differences in the distribution pattern of the organisms. In present work an increased prevalence of environmental organisms like *Corynebacteria*, *Streptococci* and gram positive aerobic bacilli was noted; *Staphylococci* was the only major pathogen isolated in earlier studies. The higher percentage of sensitivity to Erythromycin and Enrofloxacin could be due to their comparatively less use in veterinary practice. On the other hand, lower degree of sensitivity to Penicillin, Cloxacillin, Streptomycin and Ampicillin may be due to their prolonged and injudicious use in veterinary practice under field conditions.

Conclusion

Staphylococci were the main organisms in specific subclinical mastitis while *Corynebacteria* were found to be chief isolates in case of latent infections. Erythromycin, Enrofloxacin and Gentamicin were found to be most sensitive, and streptomycin was found to be the least sensitive *in vitro* antibiotic.

Authors' contributions

BKB supervised the research. AQM and DKG were involved with sample collection and processing of samples in the laboratory. AQM and BKB participated in draft and revision of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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