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Short Communication

Studies on detection of mastitis causing organisms and their antibiotic resistance

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Abstract

Mastitis is one of the major concerns of the dairy industry, especially for milch animals, as it leads to significant economic losses. Many organisms, including bacteria, viruses, fungus and even managemental problems, lead to mastitis. Among the different bacterial agents implicated, *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*), *Corynebacterium bovis* (*C. bovis*), *Mycoplasma* spp., *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Serratia marcescens* (*S. marcescens*), *Citrobacter freundii* (*C. freundii*), *Enterobacter aerogenes* (*E. aerogenes*), *Streptococcus uberis* (*S. uberis*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are the common ones. In this study, a total of 60 milk samples were screened from clinical/subclinical mastitis cases to study the prevalence of bacteria in mastitis milk. From the study, a total of thirty *S. aureus*, twelve *S. uberis*, nine *E. coli*, six *K. pneumoniae*, four *P. aeruginosa* and one *Acinetobacter baumannii* (*A. baumannii*) were isolated. Further, the milk samples subjected to antibiotic sensitivity test revealed resistance against methicillin, oxacillin, cefpodoxime, penicillin-G, ampicillin, amoxycillin, vancomycin, nalidixic acid, erythromycin, sparfloxacin, ciprofloxacin and co-trimoxazole. However, they were sensitive for tetracycline, ampicillin-sulbactum, doxycycline, amoxyclav, amikacin, gentamicin, enrofloxacin, co-trimoxazole, streptomycin, cefoperazone, ciprofloxacin, sparfloxacin and vancomycin.

Key words: Antibiotic resistance, *Escherichia coli*, Mastitis, *Staphylococcus aureus*, *Streptococcus uberis*

Highlights

- Out of 60 mastitis milk samples screened for bacteria, a total of 39 samples revealed bacterial growth.
- Out of the 39 samples yielding bacterial growth, thirty *S. aureus*, twelve *S. uberis*, nine *E. coli*, six *K. pneumoniae*, four *P. aeruginosa* and one *A. baumannii* were isolated.
- Antibiotic sensitivity test indicated maximum resistance against methicillin, oxacillin, cefpodoxime, penicillin-G, ampicillin, amoxycillin, vancomycin, nalidixic acid, erythromycin, sparfloxacin, ciprofloxacin and co-trimoxazole.

Mastitis is an economically important disease of milch animals resulting in huge downpours, both in terms of animal health and production benefits, losses including therapeutics, service costs etc. The milk production loss due to mastitis is estimated to be between 100 and 500 kg/cow each lactation (Radostits *et al*., 2000), whereas it accounts for approximately 38.0% of the total direct expenses of common industrial illnesses globally (Kossaibati and Esslemont, 1997). The cost of clinical mastitis alone is estimated to be Rs.7165.51 crores per year (NAAS, 2013). Though caused by various organisms, the important udder pathogens associated with mastitis are divided into three groups, viz., (1) Contagious (*S. aureus*, *S. agalactiae*, *C. Bovis* and *Mycoplasma* spp.), (2) Environmental (*E.*

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coli, *K. pneumoniae*, *S. marcescens*, *C. freudii*, *E. aerogenes*, *S. uberis*, *S. bovis* and *S. dysgalactiae*) and (3) Coagulase-Negative Staphylococcal species (CoNS), *P. aeruginosa*, *Nocardia asteroides*, *Prototheca* spp., *Candida* spp., *Arcanobacterium pyogenes* (Sharma *et al*., 2012). In fact, 30% of overall losses in milk production are contributed by clinical mastitis, which is mostly caused by coliforms such as *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. aerogenes* and *Streptococcus* spp. manifesting acute, subacute or chronic infection. As there is no effective vaccine currently available against bovine mastitis for prevention, treatment is the only option available for the infected animals. The problem is more due to emergence of antibiotic resistance, which act as a speed-breaker in the therapeutic process. Therefore, the present study was carried out to know the prevalence of different mastitis causing bacteria and their antibiotic resistance pattern.

A total of 60 milk samples were collected (August-October, 2021) from milch animals with clinical or subclinical mastitis from Ludhiana, Punjab. The milk samples were collected aseptically from all the quarters of infected cows in sterile containers and were transferred to the laboratory under cold chain and processed immediately. To examine the bacterial growth characteristics, the samples were first individually inoculated on a basal media, i.e., Brain Heart Infusion (BHI) agar and incubated at 37°C for 12 h. The colonies on BHI agar were subjected to Gram's staining. Thereafter, the organisms were inoculated on differential or selective media, viz., MacConkey's Lactose agar (MLA) for *E. coli*, *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter* spp., Mannitol Salt Agar (MSA) for *S. aureus* and incubated at 37ºC for 12 h for observing cultural characteristics. Further, biochemical tests, viz., indole, methyl red, Voges-Proskauer, citrate, triple sugar iron test, oxidase and catalase tests, were carried out for confirmation of all the isolates. All the isolates were also tested to identify genus as well as species using MALDI-TOF bacterial identification system version 4.1.100 (Bruker Daltonics GmbH & Co., Germany). The bacterial isolates were subjected to antibiotic sensitivity test as per disc diffusion method described by Bauer *et al*. (1966). Twenty three antibiotic discs were used in this study viz., amikacin (30 mcg), amoxyclav (30 mcg), ampicillin (10 mcg), ampicillin-sulbactum (10/10 mcg), tetracycline (30 mcg), erythromycin (15 mcg), nalidixic acid (30 mcg), streptomycin (10 mcg), gentamicin (10 mcg), amoxycillin (30 mcg), methicillin (5 mcg), cefpodoxime (10 mcg), oxacillin (1 mcg), penicillin-G (10 mcg), doxycycline (30 mcg), enrofloxacin (10 mcg), ceftriaxone (30 mcg), trimethoprim (5 mcg), co-trimoxazole (25 mcg), cefoperazone (30 mcg), ciprofloxacin (5 mcg), sparfloxacin (5 mcg) and vancomycin (30 mcg). Individual bacterial isolates were cultured overnight in BHI broth and dispersed uniformly on Mueller-Hinton agar. Under aseptic conditions, all the antibiotic discs were evenly placed on the plates and incubated for 12 h. The diameter of zone was measured in millimeters (mm), and isolates were categorized as sensitive, intermediate, or resistant based on their zone of inhibition as per Clinical and Laboratory Standards Institute guidelines (CLSI, 2018).

Out of total 60 milk samples streaked, 39 milk samples (65.0%) revealed bacterial growth and were confirmed on the basis of morphological characters, biochemical characters as well as with MALDI-TOF. Among the 39 samples, thirty *S. aureus*, twelve *S. uberis*, nine *E. coli*, six *K. pneumoniae*, four *P. aeruginosa* and one *A. baumannii* were detected. Further, out of these 39 samples, 22 (56.41%) samples had growth of single bacterium, viz., *S. aureus* (38.46%), *E. coli* (5.13%), *S. uberis* (5.13%), *P. aeruginosa* (2.56%), *K. pneumoniae* (2.56%) and *A. baumannii* (2.56%) whereas, 17 samples had mixed growth of bacteria. In mixed growth, *S. aureus* and *S. uberis* were present in four samples (10.26%), *E. coli* and *S. aureus* in three (7.69%), *S. aureus*, *S. uberis* and *E. coli* in two (5.13%), *P. aeruginosa*, *S. aureus* and *S.*

Table 1. Prevalence of organisms from mastitis milk

Sl. No.	Organism	Number	%
	Single bacteria (22)		
1	*S. aureus*	15	38.46
2	*E. coli*	2	5.13
3	*S. uberis*	2	5.13
4	*P. aeruginosa*	1	2.56
5	*K. pneumoniae*	1	2.56
6	*A. baumannii*	1	2.56
	Mixed bacteria (17)		
7	*S. aureus*+ *S. uberis*	4	10.26
8	*E. coli* + *S. aureus*	3	7.69
9	*S. aureus*+ *S. uberis* + *E. coli*	2	5.13
10	*P. aeruginosa* + *S. aureus* + *S. uberis*	2	5.13
11	*K. pneumoniae* + *S. aureus*	2	5.13
12	*K. pneumoniae* + *S. aureus* + *S. uberis*	2	5.13
13	*E. coli* + *P. aeruginosa*	1	2.56
14	*E. coli* + *K. pneumoniae*	1	2.56
	Total	**39**	**100.00**

uberis in two (5.13%), *K. pneumoniae* and *S. aureus* in two (5.13%), *K. pneumoniae*, *S. aureus* and *S. uberis* in two (5.13%), *E. coli* and *P. aeruginosa* in one sample (2.56%) and *E. coli* and *K. pneumonia* in one sample (2.56%) (Table 1).

Antibiotic sensitivity test helps to understand the resistance and susceptibility of bacteria towards a particular drug and thus helps in the choice of drug for treatment of infection. In this study, the samples were directly subjected to antibiotic sensitivity testing against twenty three different antibiotics as mentioned earlier. Tetracycline was found to be most sensitive, followed by ampicillin-sulbactum, doxycycline, amoxyclav, amikacin, gentamicin, enrofloxacin, co-trimoxazole, streptomycin, cefoperazone, ciprofloxacin, sparfloxacin and vancomycin. Antibiotic resistance was observed against methicillin, oxacillin, cefpodoxime, penicillin-G, ampicillin, amoxycillin, vancomycin, nalidixic acid, erythromycin, sparfloxacin, ciprofloxacin and co-trimoxazole (Table 2). It was observed, invariably from the study that *S. aureus* was the major causative agent in mastitis on the basis of cultural isolation and identification, which is in tandem with the results of earlier studies of Kour *et al*. (2017) and Singh *et al*. (2018) where both reported higher prevalence of staphylococcal organisms. The high rate of isolation of *S. aureus* may be attributed to the fact that the principal reservoirs of *Staphylococcus* spp. are udder and milk. In addition, *Staphylococcus* spp. has the capacity to penetrate into the tissue, producing deep-seated foci protected by a tissue barrier (Ranjan *et al*., 2010). Also, the high frequency of staphylococcal mastitis is due to the existence of inadequate hygiene in the dairy industry, poor animal health services, and lack of proper attention to the health of the mammary gland in general.

Previously many researches had shown observations similar to the present study. Gonzalez *et al*. (1980) documented that bacteriological examination of milk samples revealed 43% *S. aureus*, 21% *S. epidermidis*, 19% *S. uberis*, 13% *S. agalactiae*, 9%

Table 2. Antibiotic sensitivity results from mastitis milk

Sl. No.	Antibiotics	%Sensitive	%Intermediate	%Resistant
1	Methicillin	0.00	0.00	100.00
2	Oxacillin	0.00	0.00	100.00
3	Cefpodoxime	01.33	01.33	97.34
4	Penicillin-G	09.33	0.00	90.67
5	Ampicillin	14.34	02.22	83.44
6	Amoxycillin	15.33	08.89	75.78
7	Vancomycin	27.33	0.00	72.66
8	Nalidixic acid	20.44	11.78	67.78
9	Sparfloxacin	31.17	05.00	63.83
10	Trimethoprim	20.78	16.56	62.67
11	Ceftriaxone	26.22	13.78	60.00
12	Erythromycin	07.33	36.00	56.67
13	Ciprofloxacin	45.56	0.00	54.44
14	Co-trimoxazole	45.22	05.00	49.78
15	Cefoperazone	36.56	14.44	49.00
16	Streptomycin	43.00	10.00	47.00
17	Enrofloxacin	53.78	13.89	32.33
18	Doxycycline	76.56	06.00	17.44
19	Ampicillin-sulbactum	77.67	04.89	17.44
20	Amikacin	59.11	24.22	16.67
21	Gentamicin	68.89	14.67	16.44
22	Amoxyclav	78.22	09.34	12.44
23	Tetracycline	85.00	05.00	10.00

S. dysgalactiae and 1.7% coliforms. Dego and Tareke (2003) in their study found a prevalence of 39.2% for *Staphylococcus* spp., 23.6% for *Streptococcus* spp. and 14.1% for coliforms. Wadhwa *et al*. (1996) found that *Staphylococcus* spp. (68.83%), *Streptococcus* spp. (16.88%), *E. coli* (7.8%), *Klebsiella* spp. (2.59%), *Proteus* spp. and *Corynebacterium* spp. (1.3%) were the main organisms implicated in mastitis. Sharma and Prasad (2003) reported *Staphylococcus* spp. (54.05%) followed by *Streptococcus* spp. (14.41%) and *E. coli* (11.71%) as the predominant isolates. Mohanty *et al*. (2013) reported the presence of *Staphylococcus* spp. in 47% samples followed by *Streptococcus* spp. and *E. coli* in 32% and 21% of the samples respectively, which is also similar to the findings of this study. In a study by Sayed *et al*. (2014), 26 samples harbored more than one microorganism indicating the presence of mixed infection, which is in accordance with the findings of the present study. The presence of mixed infection/more than one organism in the samples has been reported earlier by Kour *et al*. (2017) and Singh *et al*. (2018) which also correlates with the results of this study.

From the study, it could be concluded that *S. aureus* followed by *S. uberis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were found to be the most prevalent microorganisms in mastitis milk. The milk samples tested for antibiotic sensitivity test revealed resistance against methicillin, oxacillin, cefpodoxime, penicillin-G, ampicillin, amoxycillin, vancomycin nalidixic acid,

erythromycin, sparfloxacin, ciprofloxacin and co-trimoxazole.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: AG: Performed the experiment and prepared rough draft; MC: Planned the study and analyzed the data; DN: Helped in planning the study; AKA: Helped in planning the study; DKG: Helped in collection of samples.

Ethical approval: The current study was ethically approved by Institutional Animal Ethics Committee, vide GADVASU/2019/1941 dated 13/09/2019.

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