

ORIGINAL ARTICLE

Prevalence and Antibiotic Susceptibility of Bacterial Isolates associated with Cow and Buffalo Mastitis in Udaipur of Rajasthan

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ABSTRACT

Mastitis disease has been known as one of the most costly diseases of dairy cattle and observed as an inflammatory disease of cow and buffalo udder. Mastitis inadequately affected animal health, quality of milk and economics of milk production along with cause's great economic loss. Bacteria have been representing the most common etiological agents of mastitis. The antibiotic sensitivity test was important to get accurate treatment of mastitis. The aim of present research work was to explore prevalence and antibiotic susceptibility pattern of bacterial isolates recovered from cow and buffalo clinical mastitis milk sample. During the period of April 2010 to March 2014, total 1487 clinical mastitis milk samples of cow and buffalo were tested to check the prevalence of mastitis causing by bacterial isolates. Milk samples were collected aseptically from the udder at the time of morning milking. The result showed most prevalent bacterial isolates were *Staphylococcus aureus* (24.34%) followed by Coliform bacteria (15.87%), Coagulase negative *Staphylococcus aureus* (13.85%), Non coliform bacteria (13.05%), Mixed infection (12.51%), *Streptococcus spp.* (10.96%). Out of 1487, 140 (9.42%) mastitis milk samples showed no growth on culture media. Identification of bacteria made on the basis of standard microbial features and procedures. Antibiotic susceptibility of bacterial isolates was investigated by Kirby-Bauer disk diffusion method. In vitro Antibiotic susceptibility test of bacterial isolates revealed higher sensitivity to Gentamicin (74.6%), Ciprofloxacin (62.1%) and Amikacin (59.4%). The lower susceptibility was shown to Amoxicillin (21.6%), Erythromycin (26.4%) and Ceftizoxime (29.9%). Antibiotic sensitivity pattern revealed Gentamicin is the possible effective antibiotic against the major prevalent mastitis pathogens. Present research work would be helpful in increase production, quality and quantity of milk, increase annual income of dairy owners and improve health of cow and buffalo.

Keywords: Antibiotic, Buffalo, Cow, Mastitis, Prevalence.

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INTRODUCTION

Mastitis is the single most costly disease of dairy livestock and a major monetary drain on the dairy Industry. It is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissue of the udder. It cause decrease production and quality of milk and increased use of medicaments and veterinary fees. Clinical mastitis indicates to there are visible changes in the udder such as swelling, heat, redness, pain and disturbed function, and visible changes in the milk such as clots or watery secretions and systemic reactions in varying degrees [1, 2, 3, 4, 5, 6 and 7].

Cow and buffalo mastitis, distinct as inflammation of the mammary gland, can have an infectious or non-infectious etiology. Inflammatory reaction caused by various types of bacteria that expand entry into the

teat canal and mammary gland and it is the most significant economic use up on the world wide dairy industry. Many infective agents have been implicated as cause of mastitis in dairy cattle. It is customary to divide them for enhanced relativity to control them. It is classified as contagious mastitis and environmental mastitis. Contagious mastitis is caused by *Streptococcus agalactiae* and *Staphylococcus aureus* who living on skin of teat and inside the udder, but environmental mastitis caused by environmental pathogens *Escherichia coli*, *Streptococcus uberis*, *Klebsiella* spp., who found in soil and feed [8, 9, 10 and 11].

Mastitis in cow and buffalo has become exceedingly complex and expensive disease so antibacterial therapy treating mastitis is the most common cause for antibacterial use in dairy cattle. *In vitro* antimicrobial sensitivity test of cow and buffalo mastitis pathogens are often used by bovine practitioners to guide animal or herd level treatment decisions [12, 13, 14 and 15].

The purpose of this study was to check prevalence of clinical mastitis of Udaipur and investigates the susceptibility pattern for bovine mastitis pathogens isolated from mastitis milk of cow and buffalo. Present research work would be helpful in increase production, quality and quantity of milk, increase annual income of dairy owners and improve health of cow and buffalo.

MATERIALS AND METHODS

In the present study, total 1487 clinical milk sample were tested for mastitis from Udaipur. Physical characters like clots, flakes, wateriness and colour change of milk were noted which is useful for detection of clinical mastitis. Milk sample were inoculated into brain heart infusion (BHI) broth and cultured on the Blood agar and Macconkey's agar plates. Plates and BHI broth were incubated micro aerobically at 37 °C for 24 h. The bacterial isolates were identified on the basis of Gram's staining, their cultural, morphological characteristics, catalase & oxidase test and biochemical reactions. Secondary test performed by IMVIC test for characterization pathogenic bacteria. Coagulase test for the differentiation of *Staphylococcus aureus* and Coagulase negative *Staphylococcus aureus*.

Coagulase Test: The Coagulase test was performed with rabbit plasma 0.1 gm per vial (HiMedia Cat. No.FD248). Rehydrated the contents of one vial aseptically with 3 ml sterile distilled water. Added 0.5 ml of rehydrated rabbit plasma in a tube and added approximately 0.05 ml of overnight broth culture of test organisms. Mixed gently & incubates at 37°C in the incubator for up to 4 hours. Observe for clot formation in the tube at regular intervals. Any degree of clotting within 4 hours considered as positive results.

All the bacterial isolates were tested *In vitro* for their sensitivity to different antibiotics commonly used in veterinary practices by the Kirby bour disk diffusion assay. Disk diffusion assay performed on Muller Hinton agar.

RESULTS

Out of 1487 clinical mastitis milk sampled 362 (24.34%) were examined as *Staphylococcus aureus* bacterial isolates which showed most prominent mastitis causing bacteria in milk of cow and buffalo. Different type of mastitis microorganisms isolated shown in Table 1. After most frequent *Staphylococcus aureus* other microorganism shown accordingly coliform 236 (15.87%), Coagulase negative *S. aureus* 206 (13.85%), Non coliform 194 (13.05%), Mix infection 186 (12.51%), *Streptococcus* spp. (10.96%). Within all 1487 clinical mastitis milk sample 140 (9.42%) sample showed no growth. Coliform bacteria include *Escherichia coli*, *Klebsiella* spp. & *Enterobacter* spp. and non coliform bacteria comprise *Pseudomonas* spp., *Pasteurella* spp. & *Proteus* spp.

Table 1: Isolation of microorganism from clinical mastitic milk samples of cow and buffalo (April 2010 - March 2014)

Isolates		Number of isolates	Percentage of results (%)
Gram Positive	<i>Staphylococcus aureus</i>	362	24.34
	Coagulase Negative <i>S. aureus</i>	206	13.85
	<i>Streptococcus</i> spp.	163	10.96
Gram Negative	Coliform	236	15.87
	Non coliform	194	13.05
Mix Infection		186	12.51
No Growth		140	9.42
Total		1487	100

All isolates were tested *In vitro* for their antimicrobial susceptibility as illustrated in Table 2. The highest rate of susceptibility was found to gentamicin 74.6% among all the pathogens, followed by ciprofloxacin 62.1%, Amikacin 59.4%, Enrofloxacin 58.3% and Tetracyclin 57.8%. Whereas, the highest rate of resistance among the isolates was against Amoxicillin 76.5%, Ceftizoxime 67.3%, A. cloxacillin 67.2% and Ceftriaxone 55.7%, respectively.

In vitro susceptibility pattern of each bacterial strains are shown in Table 3. Isolate of *S. aureus*, Coagulase negative *S. aureus*, Streptococcus spp., Coliform and Non coliform showed higher susceptibility to Gentamycin accordingly 69.70 %, 70.28 %, 77.78 %, 81.82% and 77.27 %. In mix growth of microorganism showed 69.51 % sensitivity against gentamicin.

Table 2: *In vitro* antimicrobial susceptibility test against microorganism present in cow and buffalo mastitic milk (April 2010 - March 2014)

Antibiotic	Susceptible	Intermediate	Resistance
Gentamycin (G)	74.6	12.9	12.5
Ciprofloxacin (CIP)	62.1	12.1	25.8
Amikacin (Ak)	59.4	9.0	31.6
Enrofloxacin (Ex)	58.3	9.8	31.9
Tetracyclin (Te)	57.8	12.5	29.7
Azythromycin (AZM)	54	12	34
Oxytetracyclin (O)	50	6.3	43.7
C. salbactum (CFS)	47.7	10.0	42.3
Streptomycin (S)	47.5	21.2	31.3
Ceptriaxome S. (CIS)	43.0	12.3	44.7
Ceftriaxone (CTR)	35	9.3	55.7
A. cloxacillin (Ax)	31.4	1.4	67.2
Ceftizoxime (CZX)	29.9	2.8	67.3
Erythromycin (E)	26.4	23.9	49.7
Amoxicillin (AMX)	21.6	1.9	76.5

Table 3: Most antibiotic susceptibility pattern among clinical isolated microorganisms associated with cow and buffalo mastitis (April 2010 - March 2014)

Isolates	Antibiotic	Percentage (%)
<i>Staphylococcus aureus</i>	Gentamycin	69.70
Coagulase negative <i>S. aureus</i>	Gentamycin	70.28
Streptococcus spp.	Gentamycin	77.78
Coliform	Gentamycin	81.82
Non coliform	Gentamycin	77.27
Mix	Gentamycin	69.51

In vitro resistance patterns of each bacterial strain shown in Table 4. *S. aureus* and Coagulase negative *S. aureus* recovered from mastitis milk sample showed highly resistance against Ampicillin cloxacillin antibiotic in that order 68.42% and 71.36%. Streptococcus spp. showed high resistance against Ceftriaxone, Coliform bacteria against Erythromycin and Non coliform bacteria most resistance against Amoxacillin antibiotic. Streptococcus spp. showed 66.67 % resistance against Ceftriaxone, Coliform 81.82 % resistance to Erythromycin and Non Coliform 80% resistance against Amoxacillin antibiotic. Mix microbial growth showed 81.40 % resistance pattern against Ceftriaxone antibiotic.

Table 4: Most antibiotic resistance pattern among clinical isolated microorganisms associated with cow and buffalo mastitis (April 2010 - March 2014)

Isolates	Antibiotic	Percentage (%)
<i>Staphylococcus aureus</i>	Ampicillin cloxacillin	68.42
Coagulase negative <i>S. aureus</i>	Ampicillin cloxacillin	71.36
Streptococcus spp.	Ceftriaxone	66.67
Coliform	Erythromycin	81.82
Non coliform	Amoxacillin	80
Mix	Ceftriaxone	81.40

DISCUSSION

Cow and buffalo mastitis is the most costly disease and a major monetary drain on the dairy industry [2, 6 and 8]. In our research result *Staphylococcus aureus* found most prominent bacteria causing cow and buffalo mastitis. The research finding indicates of high prevalence of *S. aureus* and concerning about spreading mastitis in cow and buffalo. Our result of high prevalence of *S. aureus* found similar with other studies [16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32]. The high prevalence of *S. aureus* could be due to its capability to evade and influence the host immune system by production of various enzymes and toxins that cause damage to mammary tissue and allow tissue invasion. Furthermore, *S. aureus* is able to survive in the keratin of the teat canal of healthy cow and buffalo. In addition, many *S. aureus* strains have the aptitude to resist antibiotic therapy by production of Beta lactamase an enzyme that inactivates penicillin and closely related antibiotics [30, 33 and 34].

In present research findings after most frequent *Staphylococcus aureus* other microorganism shown accordingly coliform 236 (15.87%), Coagulase negative *S. aureus* 206 (13.85%), Non coliform 194 (13.05%), Mix infection 186 (12.51%), Streptococcus spp. (10.96%). Present findings are similar to reports from earlier investigations in other countries [20, 28, 34, 35 and 36]. Within all 1487 clinical mastitis milk sample 140 (9.42%) sample showed no growth. Some mastitis milk samples showed no growth on cultured media agar plate can be further processed by anaerobically culture method.

Previous result showed that most of mastitis isolated pathogens were sensitive to Enrofloxacin, Ciprofloxacin, Gentamycin and Neomycin [1]. Beforehand researcher checked prevalence and antibacterial susceptibility in mastitis in buffalo and cow, reported that Gentamycin and Ciprofloxacin found highly sensitive in cow and buffalo [37]. Gentamicin reduce 90% the growth of biofilm forming *S. aureus* isolates recovered from clinical cases of cow and buffalo mastitis [30]. Present conclusions are similar to the results of previous study that Gentamycin, ciprofloxacin, Amikacin, Enrofloxacin and Tetracyclin showed higher sensitivity against all pathogens isolated from mastitis milk of bovine. Research judgment of susceptibility compare with previous work showed similarity. The most effective antibiotic against all the pathogens were Gentamycin. Among the entire pathogens Gentamycin antibiotic showed higher effectiveness against coliform bacteria. Our research data compare with other previous work done showed similar in case of *Staphylococcus aureus* [11, 38, 39 and 40]. Whereas, the highest rate of resistance among the isolates was against Amoxicillin, Ceptizoxime, A. cloxacillin and Ceftriaxone. In related to similar finding reported that Ceftriaxone, Ceptizoxime and Erythromycin showed low sensitivity against *S. aureus* isolated from cow and buffalo mastitic milk [30]. In case of *Staphylococcus aureus* and Coagulase Negative *S. aureus* most antibiotic resistance to Ampicillin cloxacillin. Coliform and non coliform isolates confirmed most resistance against Erythromycin and Amoxacillin, respectively. Ceftriaxone antibiotic showed most resistance against the Streptococcus spp. and Mix infection.

Present research conclusion is evaluated with international studies since no similar previous studies were conducted in Udaipur. However, variation in the environmental condition, herd size, management practices and other risk factors might have partake to the differences in prevalence rates of mastitis pathogens among the findings of preceded works.

CONCLUSION

Cow and buffalo mastitis is inflammation of the mammary gland that is almost always caused by microorganisms, frequently bacteria that invade the udder, multiply and produce inflammatory toxins. In present study result *Staphylococcus aureus* found most prevalent bacteria. Environmental pathogens also posses high prevalence rate due to lack of management include poor ventilation, inadequate manure removal and lack of farm cleanliness. In present research work Gentamycin confirmed most susceptibility to all the isolated recovered from the mastitis milk of cow and buffalo. Due to availability and low cost of antibiotic for the farmer present research work would be very useful to treat mastitis, improved management, increase milk production and quality.

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