International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(4): 447-450

www.veterinarypaper.com
Received: 02-06-2023
Accepted: 08-07-2023

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Cultural studies of clinical mastitis in goats

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Abstract
During the investigation "Cultural studies of clinical mastitis in goats'' was under taken to study the diagnostic approach of clinical mastitis in goats based on cultural examination. Out of 260 quarters of 130 goats screened for clinical mastitis (CM), 54 quarters of 33 goats were found positive for clinical mastitis based on bacterial culture examination. The following bacteria were isolated from clinical mastitis affected milk samples as single infection which included coagulase negative *Staphylococcus spp., Escherichiacoli spp., Klebsiellaspp., Streptococcus spp.* and coagulase positive *Staphylococcus spp.,* were identified in 29.63,20.37,11.11, 9.26 and7.41percent. In mixed infection, the bacteria identified were coagulase negative *Staphylococcus spp.* in along with *Escherichia coli*, Coagulase negative *Staphylococcus* spp with *Klebsiella* spp., *E. coli* with *Streptococcus spp*., Coagulase negative *Staphylococcus spp*. with *Streptococcus spp.* and coagulase positive *Staphylococcus spp., with Escherichia coli* 7.41, 5.56, 3.70, 3.70 and 1.85 percent respectively. The drug sensitivity patterns of the above bacterial isolates revealed that, organisms were highly susceptible to Cefoperazone and Ceftriaxone followed by Amoxyclavacid (85%), Enrofloxacin (70%), Gentamicin (60%), Doxycycline (45%) and Ampicillin (35%). While, all the bacterial isolates (100%) whole milk cultures were resistant to penicillin G.

Keywords: Goats, clinical mastitis, cultural examination, antibiogram

1. Introduction
The use of unconventional feedstuffs especially in monogastric animal feeding continue to Mastitis is a complex disease with varied etiology and which is defined as inflammation of mammary gland parenchyma causing chemical, physical and bacteriological changes in milk and in the glandular udder tissues with pathological changes [1]. Mastitis is caused by bacteria, fungus, mycoplasma, and yeast, as well as stress-reduced resistance, udder and teat form, animal heredity, and the environment, including milking and feeding systems, chemical, mechanical, or thermal damage [2]. In general, Mastitis is presented is of two types Sub-clinical form and clinical (overt).Clinical mastitis is again subdivided into purulent-catarrhal , gangrenous, serous-catarrhal, and subclinical (hidden). Sudden onset of swelling, reduced and altered milk secretion, pain, and redness of the udder are charactersistic of Clinical mastitis (CM). The organisms involved with mastitis have been studied widely. However, still research should be focused on , as the etiological agents of isolated species changes over time. Almost hundreds of microorganisms are responsible for udder inflammation [3]. To hasten diagnostic methods, isolation of Bacteria and PCR identification has to be done. The bacterial flora of clinical mastitis has to be studied to develop a relevant line of treatment based on antibiotic sensitivity.
Generally, antibiotics were selected depending on their *in vitro* sensitivity testing, availability, and cost. However, increased illness in an animal herd leads to increased antimicrobial usage, which raises the risk of antimicrobial resistance and antibiotic residues in milk. Therefore, culture and antibiotic sensitivity testing (ABST) must be performed before administering antimicrobial drugs to prevent antimicrobial resistance [4].

Materials and Methods
The investigation conducted to study cultural Examination , antibiotic sensitivity testing (ABST) of clinical mastitis in goats that are presented to the Veterinary Ambulatory Clinic of VCC and Veterinary clinical complex, College of Veterinary Science, Rajendranagar, Hyderabad period of twelve months *i.e.*, from August, 2021 to July, 2022.

Suspected milk samples were collected in sterile vials from the affected goats with aseptic precautions and then submitted to bacteriological investigation for etiological agents isolation. The Edwards medium and blood agar was used in lab as per method decribed by [5] and *Staphylococcus spp.* and *Micrococcus spp.* differentiation media was prepared as per procedure given by [6]. The Bergey's Manual of Determinative Bacteriology was used to identify bacteria based on type of hemolysis, shape of the colony and gram's staining. Cultures in pure form are recognised at genus level [7]. For bacterial identification, 24-48 hour old pure culture was used, where in motility test, Coagulase test, Catalase activity, Haemolysin test, Sugar fermentation test, Indole test, Methyl red test, Voges - Proskauer test, Citrate Utilisation tests and Gram's staining were performed.

The antibiotic sensitivity of whole milk sample cultures was tested In-vitro on brain heart infusion (BHI) agar plates using the disc diffusion technique. To assess sensitivity, BHI agar plates were infected with a 6-8 hour pure culture which was cultured in nutrient broth. Using a sterile swab, the culture was transferred on to BHI agar. The BHI agar plates kept covered in room temperature 15 minutes to allow the inoculum to dry. The sensitivity pattern of isolates to various antibiotic discs was read by calculating width of the zone of inhibition in millimeters as per the manufacturer's chart after 18-24 hours of incubation at 37 °C of inoculated BHI plates.

Results and Discussion

In the present study, 54 quarters from 33 goats were diagnosed with clinical mastitis based on a examination of bacterial culture. The examination of bacterial was done to identify the etiology and 54 milk samples from affected quarters were positive for bacteria which is pathogenic in nature. Bacteria isolated from clinical mastitis-affected quarter milk samples as single infections were coagulase-negative *Staphylococcus spp.*, *Escherichia coli spp.*, *Klebsiella spp.*, *Streptococcus spp.* and coagulase-positive *Staphylococcus spp.*, identified in 29.63, 20.37, 11.11, 9.26 and 7.41 percent. The bacteria identified among mixed infection were coagulase-negative *Staphylococcus spp.* Along with *Escherichia coli*, Coagulase-negative *Staphylococcus spp.* with *Klebsiella* spp., *E. coli* with *Streptococcus spp*., Coagulase-negative *Staphylococcus spp.* with *Streptococcus spp.* and coagulase-positive *Staphylococcus spp., with Escherichia coli* 7.41, 5.56, 3.70, 3.70 and 1.85 percent respectively. (Table 1 and Fig 1, 2 &3).

Table 1: Bacteria identified among clinical mastitis in goats

S. No.	Bacteria isolates	Total no. of samples (n= 54)	Percentage (%)
1	Coagulase-negative *Staphylococcus spp.*	16	29.63
2	*Escherichia coli*	11	20.37
3	*Klebsiella spp.*	6	11.11
4	*Streptococcus spp.*	5	9.26
5	Coagulase-positive *Staphylococcus spp.*	4	7.41
6	Coagulase-negative *Staphylococcus* spp. and *E. coli*	4	7.41
7	Coagulase-negative *Staphylococcus spp.* and *Klebsiella spp.*	3	5.56
8	*E.coli and Streptococcus spp.*	2	3.70
9	Coagulase-negative *Staphylococcus spp.* and *Streptococcus spp.*	2	3.70
10	Coagulase-positive *Staphylococcus* spp. and *E. coli spp.*	1	1.85

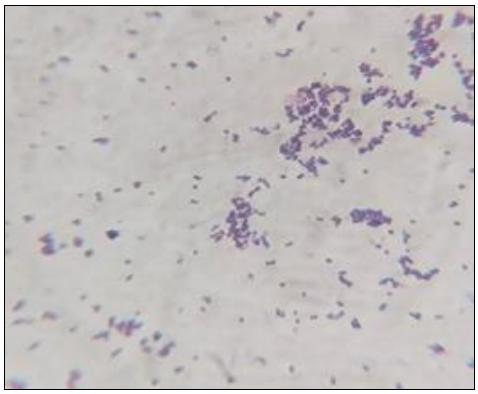

Fig 1: Coagulase positive *Staphylococcus spp.* (Gram's stain)

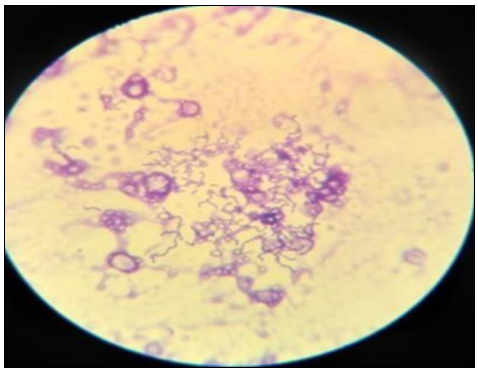

Fig 2: *Streptococcus spp.* in chains (Gram's stain)

Fig 3: *E. coli* (Gram's stain)

These results were in almost agreement with [8, 9, 10, 11, 12, 13] who reported highest prevalence of *Staphylococcus spp.* and concluded that , *Staphylococcus spp* is the more predominant organism in the milk samples collected from CM affected goats. Bacteriological analyses of milk samples revealed Gram-positive *Staphylococci spp.* among clinical mastitis [3] [10]. Coagulase-negative *Staphylococci*, upon isolation was the more predominant bacteria among all identified bacterial organisms [12]. *Streptococcus spp*. was more predominant bacteria along with *Staphylococcus spp*. may be because of poor managemental dairy practices and infected other healthy

animals of the herd due to its contagious nature [2]. Common pathogens isolated in goats with acute and per-acute mastitis were either *E. coli* alone or a combined infection of *Staphylococcus aureus* and *E. coli.* [14]. Highest occurence of *Staphylococcus spp*. in the study may be due its ubiquitous nature and adaption to live in the udder to cause a mild form of Sub-Clinical Mastitis with long period [15].

Whereas, reduced occurence of *Streptococcus spp*. as compared with *Staphylococcus spp*. might be because of the poorest survival rates of bacteria outside the environment. *E. coli* isolation from the bacterial culture was attributed to contaminated feed bad farm managemental practices, milking machine [10]. In the investigation, mixed infections were also present along with single infection concluded that both the sub-clinical mastitis a and clinical mastitis affected udder-halves had single infection *viz*., Sub-clinical 71.43% and Clinical 76.27%) mastitis and mixed (sub-clinical 24.49% and clinical 16.95%) infection [16]. *E. coli* , *Staphylococcus spp* and *Klebsiella spp* constitutes mixed infection in the udder and are the most common cause of intramammary infections and hence bacteriological culture is routinely used in the confirmation of mastitis [17].

Therapeutic management of a clinical case of mastitis using selected antibiotics with broad-spectrum was recommended. Injudicious and indiscriminate usage of antibiotics along with improper management of mastitis with various antibiotics caused multiple drug resistance. As of now, various types of antibiotics have been used on mastitis causing pathogens along with or without isolation, identification and *In-vitro* sensitivity [18]. Generally, therapy success relies on the best selection of effective antibiotics based on In-vitro sensitivity along with good supportive management to decrease indiscriminate usage of antibiotics along with antimicrobial resistance [19]. An attempt was made in the present work to study the *In -vitro* drug sensitivity pattern of bacteria isolated from clinical mastitis in goats.

A total of eight antibiotics were selected and *in vitro* drug sensitivity was tested against the 20 whole milk cultures in mastitis-affected goats. The efficacy pattern in the 20 whole milk cultures revealed 95 percent sensitivity to Cefoperazone and Ceftriaxone followed by Amoxyclav acid (85%), Enrofloxacin (70%), Gentamicin (60%), Doxycycline (45%) and Ampicillin (35%).(Figure-4 &Table-2)

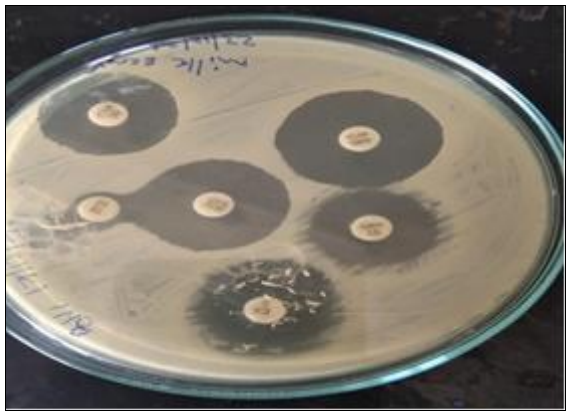

Fig 4: Antibiogram of whole milk culture showing more sensitivity to Ceftriaxone and Cefoperazin

Table 2: Pattern of antibiotic sensitivity of whole milk cultures in mastitis affected goats

S. No.	Name of the antibiotic sensitivity disc	Symbol	Concentration	No. of samples tested	Results		No. of samples resistant	% Resistance
					No. of samples sensitive	% sensitivity		
1.	Cefoperazone	CPZ	75 mcg	20	19	95	1	5
2.	Ceftriaxone	CTR	30 mcg	20	19	95	1	5
3.	Amoxyclav	AMC	30 mcg	20	17	85	3	15
4.	Enrofloxacin	Ex	5 mcg	20	14	70	6	30
5.	Gentamicin	G	10 mcg	20	12	60	8	40
6.	Doxycycline	DO	10 mcg	20	9	45	11	55
7.	Ampicillin	AMP	10 mcg	20	7	35	13	65
8.	Penicillin G	P	10 units	20	0	0	20	100

All (100%) whole milk cultures were resistant to penicillin G. However, previous researchers have recorded varied patterns of sensitivity. *In vitro* sensitivity tests observed in the present investigation was in close accordance with [14] who reported that, the bacteria identified were sensitive to Gentamicin, Amoxicillin, Cloxacilin, and Chlortetracycline. While, they were resistant to Streptomycin and Penicillin. Antibiogram on bacterial isolates of mastitis milk in goats revealed that the isolates were highly sensitive to the Ceftriaxone or Amoxicillin [20]. The difference in sensitivity patterns of microbes to various antimicrobials observed could be ascribed to various seasonal change, ecologies, un identical microbial patterns, multiple drug resistance and area specificity [13]. While, bacteria isolated were more sensitive to Cefoperazone, Enrofloxacin, and Ceftiofur and the isolates were resistant to Tetracycline, Amoxicillin and Ampicillin,. The bacterial isolates sensitivity towards antibiotics generally changes among different countries [21]. In the current study, found that bacteria have become more resistant to the Penicillin G of antibiotics, may be due to the long-term and widespread use of the penicillin group of antibiotics in the management of mastitis [22].

Conclusion

Diagnosis of clinical mastitis in goats was based on cultural examination and In-vitro drug sensitivity tests revealed 95 percent sensitivity to Cefoperazone and Ceftriaxone.

Acknowledgment

The authors are thankful to the Department of veterinary medicine, College of veterinary science, Rajendranagar, P.V. Narsimha Rao Telangana Veterinary university Hyderabad for providing necessary facilities for research work.

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