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THE PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *Staphylococcus aureus* ISOLATED FROM MASTITIS GOAT MILK ADMITTED AT SAQ TEACHING VETERINARY HOSPITAL, BANGLADESH

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Staphylococcus aureus is an infectious, opportunistic organism that causes clinical or subclinical mastitis in dairy goats. The genetic situations and antimicrobial resistance of isolates from dairy goats that are affected in clinical mastitis have not been studied in SAQ Teaching Veterinary Hospital (SAQTVH). The objective of this study was to characterize *S. aureus* from dairy goats that suffered clinical mastitis, by PCR, and by assessment of antimicrobial susceptibility. A total of 15 *S. aureus* isolates from intramammary infections were studied. Conventional PCR was used to detect nuc genes. Antimicrobial susceptibility was determined by the using disc diffusion method was carried out. In total 25 (78.12%) samples out of 32 were found Staphylococcus positive according to their cultural properties on Mannitol salt agar and Blood agar, a morphological appearance by Gram's staining, and 15 (46.87%) samples were found positive by Catalase test. Out of 15 (46.87%) Staphylococcus positive isolates, 3 (20%) samples were Coagulase positive. Among the Coagulase-positive Staphylococcus species, one was confirmed as *S. aureus* by nuc gene PCR. The remaining isolated Staphylococcus were not confirmed at the species level that was considered unclassified Staphylococcus species. Methicillin resistance was not found, but resistance to penicillin/ ampicillin (81.25%) and (75%) was very common. We found a high degree of relatedness among bovine *S. aureus* isolates in SAQTVH and the strains that are often resistant to commonly used antimicrobials. This highlights the need for effective preventive measures that aim at limiting the transmission of bacteria rather than using antimicrobials to control *S. aureus* mastitis.

Keywords: *Staphylococcus aureus*; nuc gene; antimicrobial resistance; mastitis.

1. INTRODUCTION

Staphylococcus is the genus of Gram-positive bacteria in the family Staphylococcaceae in the order Bacillales. When observed under the microscope the organisms display a grape-like form. *Staphylococcus sp* is a vital organism due to it is liable for many infectious diseases. The signs of Staphylococcal

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infection differ among animals. *Staphylococcus aureus* is the most effective pathogen that inhabits the world with its wide genetic variety and wide host range and different pathologies related to mild to severe infections. *S. aureus* is considered an important food borne pathogen [1, 2]. "Subclinical mastitis is 15 to 40 times more common in goats than clinical mastitis. The existence of *Staphylococcus spp* in milk or any food is very significant in public health. On heating at normal cooking temperature, the bacteria may be killed but the toxins remain active" [3]. "It is thought that it is more resistant in food than in laboratory culture media. About 50 % strain of this organism is able to produce enterotoxins associated with food poisoning" [4]. "Therefore, the milk of goats could also be a probable source of Staphylococcal infection accountable for food intoxication andtoxico-infection. Although these microorganisms are part of the normal flora and are generally considered to be of low virulence, they appeared to be implicated in the etiology of a variety of human and animal infections, and they have established themselves as important pathogens causing subclinical mastitis in goat" [5, 6]. "It is believed to be more resistant in foods than in laboratory culture medium.Few coagulase negative species, such as *S. cohnii*, *S. epidermidis*, *S. xylosusb*, and *S. haemolyticus* have been isolated from ewe's milk and proven to produce staphylococcal enterotoxins. *S. saprophyticus, S. warneri, S. chromogenes and S. lentus* isolated from healthy goat milk and dry-cured hams were found to have the enterotoxigenic potential" [7,8,9]. "Staphylococci often are present part of normal bacterial flora of the skin and mucosal surfaces of the respiratory such as nose and nostril, upper alimentary and urogenital tract of mammals" [10]. *S. aureus* is a normal inhabitant of the healthy lower reproductive tract especially in vagina and vulva in female animals, urogenital tract and various intra-abdominal organs [1]. "The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils" [11]. "Staphylococci are easily spread between animals and under certain conditions to humans as well as by skin to skin contact, but also by contact with an excretion that contains staphylococci, such as saliva, or aerosols released during sneezing and coughing but frequently cause severe infections" [12]. Teat, skin, muzzles, nostrils, and vagina, as well as infected udders, and teat lesions act as major reservoirs of the bacteria that cause mastitis, and the bacteria tend to spread to uninfected parts via teat cup liners, milker hand, wash cloths, and flies. Nowadays aerosols play a vital role in the dispatch of bacteria to uninfected parts. Generally, Staphylococci do not settle on healthy teat skin, but willingly colonize the teat canal if there are lesions exist. The organisms redouble in infected lesions and enter into the udder causing the demolition procedure to be more severe. Bacteria adjust and accumulate in the milk, being access to the upper part of the gland. It has been mentioned that bacteria cling to the ductular and alveolar epithelium in the gland and initiate production of toxins. These agglutinated bacteria roadster macrophage activation and the neutrophil passage from the blood into the milk (a situation turn out in an increment of the somatic cell count), inflammation of the mammary gland, loss of the host immune system, and epithelial cell damage. So that, bacteria touch the basal subepithelial cell layers, attach fibrinogen and other host receptor proteins, and finally introduce an infection. Overall, of view the importance of goats as an essential source of milk, and an efficient zoonotic threat, the current study was planned to dealing the following objectives: to predict the prevalence of *Staphylococcus sp*in healthy udder in the study population of goats. To describe the phenotypic characterization of bacterial isolates in different bacteriological culture media and biochemical tests, and to determine the antimicrobial resistance pattern of *S. aureus*.

2. METHODS AND MATERIALS

2.1 Study Area

The samples were collected from the household goats' farms in Akbarshah, Bandar, Bayejid, Halishahar, Kotwali, Khulshi, and Pahartali Thana under the Chittagong district and goats that were admitted to Shahedul Alam Quadery Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU) complaining of infection of the udder. A total of Thirty-two goats (N=32) were sampled during the study period.

2.2 Study Period

The study was carried out for a period of 6 months from January to June 2021.

2.3 Data Collection

A prepared questionnaire was used to collect the epidemiological data and clinical data that were related to the study. The questionnaire consisted of general information on individual goat breed, age, lactation period, udder condition, body temperature, bedding materials used, types of feed supply, other health status, history of antimicrobial therapy and other drugs.

2.4 Sample Collection

Milk samples were collected aseptically. Briefly, the does were restrained, and there after the teats were scrubbed with cotton wool saturated with 70% ethanol. The teats were dried using a towel and the first three streams of milk were discarded. The sample was collected from healthy goats in 15 ml falcon tubes and the sample was transferred to 4ml Muller Hinton broth (MHB) (Himedia laboratories private ltd, Mumbai, "Indai"). supplemented with 6.5% NaC1 respectively. Then Muller Hinton broths were transferred to the Department of Microbiology and Veterinary Public Health (DMVPH) and preserved at 4°C temperature in a refrigerator for further laboratory investigation.

2.5 Isolation and Identification of *S. aureus*

2.5.1 Isolation of *S. aureus*

Both Mannitol salt agar and Blood agar base were prepared according to instructions of the manufacturer (Himedia laboratories private Ltd, Mumbai, India). For isolation and identification of staphylococci, each Muller Hinton Broth was incubated at 37°C for 24 hours in an incubator for enriched culture. Thereafter by using a sterile inoculating sterile cotton swab, the enriched culture was streaked onto a Mannitol salt agar plate and incubated at 37°C for 24 hours. *S. aureus*was presumptively identified based on the colony characteristics on Mannitol salt agar. The *S. aureus* produced golden yellow-colored colonies. Again, for sub-cultured, the presumptive positive colonies on the Mannitol salt agar were sub-cultured onto the blood agar *and* incubated at 37°C for 24 hours to notice the hemolytic properties of *S. aureus* and *S. pseudintermedius*. Both positive *S. aureus* and *S. pseudintermedius* produced complete hemolysis on blood agar. After that, the isolated positive colonies were picked up and transferred to a tryptic soy broth with 50% glycerol containing a 2ml Eppendorf tubes and stored at -80°C until further investigation.

2.5.2 Identification of *S. aureus* by Gram's staining

Gram's staining was applied for the identification of morphology and staining characteristics of micro-organisms. Suspected colonies from the Blood agar of *S. aureus* were stained as described by the manual of veterinary investigation laboratory Technique [13]. A positive small colony and two drops of water were placed on a clean glass slide and a smear was prepared on the glass slide and dried by Slight heat. Then heat-fixed smear was flooded with crystal violet solution to stain for two minutes. The smear was washed with running water followed by adding mordant Gram's iodine for one minute. Then acetone was added for 5-6 sec and act as a decoloring agent. After washing with running water, Safranin was added as counterstains for one to two minutes. Then the slides were washed with running water, blotted and dried in the air, and then examined under a microscope with high power objective (100X) using immersion oil. Smear revealed Gram-positive, purple-colored, cocci shaper, arranged in clusters (grape-liked) *S. aureus* under the microscope.

2.5.3 Identification of *S. aureus* by Catalase test

The catalase test was performed by adding a drop of 2% H_2O_2 on a clean glass slide and then 2/3 of bacterial colonies were transferred on that glass slide by using a sterile loop and mixed properly. The positive result was observed within 5-10 sec by forming air bubbles. But the negative result was no bubbles [14, 15].

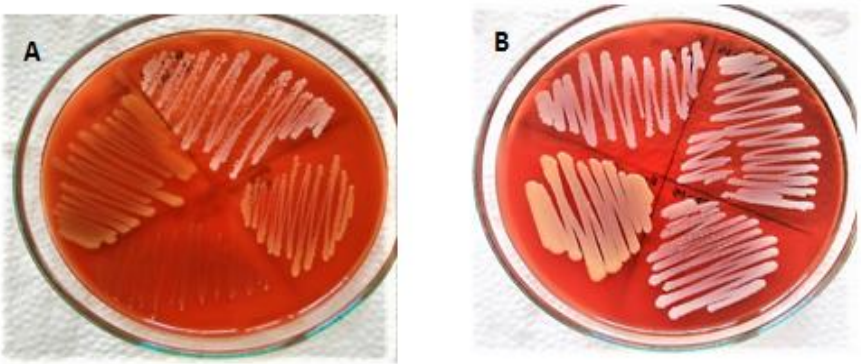

Fig. 1. Growth of *Staphylococcus sp*. in blood agar (A and B)

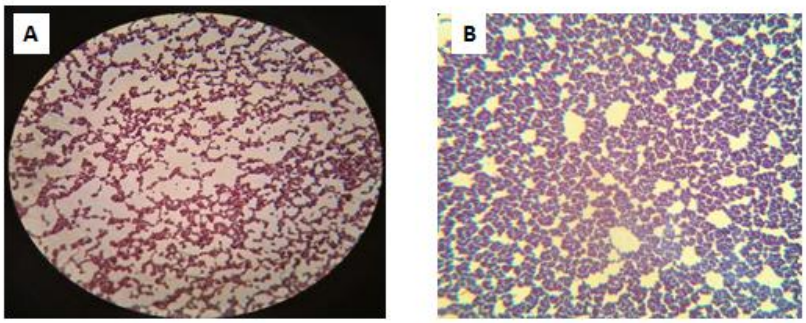

Fig. 2. Bunches of grapes' like arrangement of *Staphylococcus sp*. in Gram's stain smear (A&B)

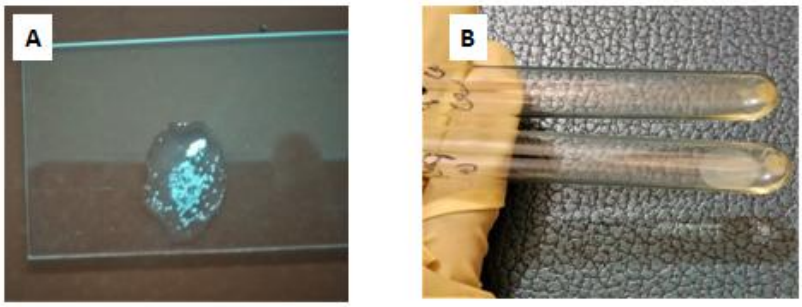

Fig. 3. Catalase (A) and Coagulase (B) test results of *Staphylococcus sp*

Table 1. Oligonucleotide primer sequences for *Staphylococcus species* conformation genes

Species	Gene	Primer	Sequence (5′3′)	Annealing temperature	Amplicon Size	References
S. aureus	**nuc**	au-F3 au-nucR	TCGCTTGCTATGATTGTGG GCCAATGTTCTACCATAGC	56℃	**359**	[3]

2.6 Identification of *S. aureus* by Coagulase Test

2.6.1 Collection of horse plasma

Whole blood was collected from a horse into a commercially available anticoagulant (citrated phosphate dextrose) treated blood bag to perform the coagulase test. The collected whole blood was centrifuged at 3000 rpm for 10 minutes using a centrifuge machine. Then the resulting supernatant plasma was transferred into a 10 ml sterile tube by using a sterile micropipette. Then the collected plasma was stored at -20°C for further use.

2.6.2 Tube coagulase test

One to three well-isolated colonies from Blood agar were transferred into 0.5 ml horse plasma in a sterile glass tube. Then the tube was rotated gently to mix the contents properly and incubated for 37°C 24 hours. A positive test, clotting of plasma occurred within 24 hours indicates the presence of *S. aureus*. But the negative result, no clotting of plasma occurred within 24 hours.

2.7 Molecular Identification of *S.aureus* by Polymerase Chain Reaction (PCR)

The coagulase-positive isolates were selected for the molecular detection of *S. aureus* by targeting species-specific gene nuc and pse [3].

2.8 DNA Extraction for PCR Test from Bacterial Culture

For bacterial DNA extraction boiling method was followed. Using a sterile inoculating loop, a loop full of fresh colonies (2-3) was picked up from each of Blood agar the plates and transferred to 1.5 ml Eppendorf tubes containing 200ul deionized water.

Then the tubes were vortexed to produce a homogenous bacterial cell suspension. Then the tubes with bacterial cell suspension were boiled at 96℃ for 10 minutes in a hot water bath. After boiling in a hot water bath, immediately the tubes were placed in refrigerator at -20℃ for 5 minutes for rapid cooling. The process of boiling at high temperature and rapid cooling permitted the bacterial cell wall to breakdown thus releasing DNA. Then 100 µl of bacterial DNA containing supernatant from each Eppendorf tube was transferred into another sterile Eppendorf tube and preserved at - 20℃ until further testing.

2.9 Polymerase Chain Reaction

PCR tests were performed using primers [3].The primer sequences used for the PCR are shown inthe Table. 2. PCR reactions were performed with a 50 µl reaction volume. Proportions of different reagents that were used for PCR for *Staphylococcus sp* are given previously identified strains of *S. aureus* (Obtained from the University of Copenhagen) were used as positive control and Nuclease-free water was used as the negative control.PCR amplification reaction was carried out in a thermocycler (Applied Biosystem. 2720 thermal cycler. Singapore). The cycling conditions were 95°C for 2 mins (Initial denaturation), 95°C for 30 secs (final denaturation), 56°C for 35secs (Annealing), 72°C for 1-2 mins (extension) followed by 30 cycle.

2.10 Visualization of PCR Products by Agar Gel Electrophoresis

Agarose gel (1%) was prepared for electrophoresis of PCR- amplification products**.** A gel tray was assembled by setting a proper teeth-sized comb in the tray**.** Then1 % agarose gel solution was prepared by dissolving 0.5 gm of agarose powder properly (Seakem LE agarosc -Lonza) in 50 ml of 1x TAE buffer in a sterile conical flask. Then the microwave was done for boiling of solution for 2 minutes until the agarose powder completely dissolved. Then melted agarose was cooled down about 50°C in water for a bath for 5 minutes having added 1 drop of ethidium bromide at a concentration of 5 pg per ml. Finally, agarose gel was poured into the gel tray and allowed about 20 minutes to stand for solidification of the gel at room temperature. Then the gel was shipped into an electrophoresis tank. Then 5 µl of each of the PCR products was loaded up into the gel holes. One hole was loaded with a DNA marker (Thermo Scientific O' Gene Rular 1 kb plus) to compare the amplicon size of the gene product. For negative and positive control one hole was loaded up with 5µl of distilled water and one hole was loaded up with known Staphylococcus species. Electrophoresis was done at NO volts and 80 amps for 20 minutes. Finally, the gel was examined under an I Viral Willuminaior (I3DA digital, biometra GmbH. Germany).

2.11 Antimicrobial Susceptibility Testing

Penicillin, tetracycline, Erythromycin, sulfa methoxazole-trimethoprim, ciprofloxacin, cefoxitin, nalidixic acid, Ampicillin, Ceftriaxone, Gentamycin, and Amoxicillin were used in antimicrobial susceptibility testing using the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute. 1 to 2 colonies were extracted from blood agar and placed in a clean fresh test tube containing 3 ml PBS saline and a matching o.5 McFarland standard in just eye levels for antimicrobial susceptibility testing. McFarland standard was prepared by adding 1% (1.175 g/L) $BaCl_2.2H_2O$ to 99.5ml of 1 % (0.36 N) H_2SO_4 [15]. After matching bacterial turbidity, a sterile cotton swab was dipped into bacterial colonies containing PBS saline and rotated against the side of the tube with firm pressure. After removing the excess fluid from the swab then the swab streaked over the entire dried surface four times of the Muller Hinton ager plate (MH) (Himedia laboratories private Ltd. Mumbai, India.) and rotating the plates approximately at 60 degrees for each time to ensure an even distribution of the agar plate.MH agar was prepared according to the manufacturer's instruction (Himedia laboratories private Ltd. Mumbai, India). After a few minutes, the antibiotics were applied on the surface of the inoculated plate with sterile forceps. Within 30 minutes of the application of antimicrobial discs (Oxoid Ltd, Basingstoke, Hampshire, UK), the plat was incubated at 37°C for 24 hours. After 24 hours of incubation the plates were examined and the zone of inhibition around each antimicrobial agent was measured and categorized into Susceptible (S), intermediate (I) or resistant (R) based on recommendations from a document for veterinary pathogens (CLSI).

3. RESULTS

A total of 32 samples were obtained from 32 goats. The milk samples were collected from each clinical mastitis goat.

3.1 Prevalence of *S. aureus* and Other Staphylococcus Species

Among investigated 32 goats, 3 (9.37%) goats carried Coagulase positive *S. aureus* and other Staphylococcus species. and 22 (68.75%) dogs only carried other *Staphylococcus sp* and *E. coli* (12.5%),

Bacillus sp (9.37%). In total 25 (78.12%) samples out of 32 were found Staphylococcus positive according to their cultural properties on Mannitol salt agar and Blood agar, a morphological appearance by Gram's staining, and 15 (46.87%) samples were found positive by Catalase test. Out of 15 (46.87%) Staphylococcus positive isolates, 3 (20%) samples were Coagulase positive.

3.2 Analysis of Risk Practices Associated with the Frequency of Presence of *S. aureus*

A total number of 15 samples were found *Staphylococcus aureus* positive out of 32 collected clinical mastitis goat samples. The numbers were confirmed by their colonial growth characteristics on blood agar mannitol salt agar and biochemical test. The samples were further characterized by a coagulase test. In the Coagulase test 3, samples were found positive out of 15 samples that were positive forthe mannitol salt agar and blood agar test. In case of breed variation, the frequency of *Staphylococcus aureus* was respectively in Black bangle 75% and Jumunapari 28.12%. The estimated frequency of *S. aureus* in brick type, concrete type, and muddy type flooring systems of farmhouses were respectively 18.75%, 31.25%, and 50% where as the estimated frequency of *Staphylococcus aureus* in concrete type flooring systems (26.32%) was higher than others.1 time daily, 2 times daily, the cleaning frequency of floor, and the frequency of *Staphylococcus aureus* were respectively 9.37% and 90.62%. The prevalence of *S. aureus* was 12.73%, 31.43%, and 18.75% respectively when the CMT score was1, 2, and 3 where the CMT score was 2 (31.43%) had a higher prevalence than others. The frequency of occurrence of clinical mastitis in case of age variation respectively >1.5 years, 1.5-3 years,and 3> years is 28.12 %, 65.62 %, and 6.25 %.But in case of lactation stage 1, 2 and 3 it varies respectively 37.5%, 46.87% and 15.62 %. The udder condition hard and firm respectively 28.12% and 71.87 % and the color of the udder black, reddish, and both greenish and brownish is respectively 12.5%, 81.25%, 3.12% but swollen and pain in the udder and the CMT test is positive all of the cases. Depending on the use or not useof the bedding materials the frequency of occurrence of mastitis is respectively 28.11% and 71.87%. Having drainage facility or not and the floor cleaning once or two times and the disinfectant use or not use is respectively 21.87%, 78.12%, and 90.62%, 9.37% and 9.37 %, 90.62 %. The supply of concentrate and concentrate roughage both and the supply vitamin-mineral or not supply is respectively 65.62%, 34.37% and 37.5%, 65.5%.During clinical mastitis, the antibiotics used named Amcox, Amcox, and Sp-vet, Hicomox and SP-vet both and Hicomox respectively 62.5%, 50%, 18.75% but in the case of vaccination the frequency of occurrence of mastitis is respectively 9.37% and 90.62 %.

3.3 Detection of Antimicrobial Susceptibility Pattern of Isolated coagulase-positive *S. aureus*

After performing coagulase tests all four-coagulase positive *S. aureus* isolates were subjected to an antimicrobial susceptibility test. Antibiotic resistance of *S. aureus* was identified by accessing the zone of inhibition. For this reason, 13 different antimicrobial discs were used to know their susceptibility and resistance to antibiotics.

The highest resistance was observed in both Nalidixic acid, Ampicillin, and penicillin is 81.25%.On the other hand, Cefoxitin, Erythromycin, Streptomycin, and Tetracycline were observed same resistance 75%, and other antibiotics such as Sulfamethoxazole-Trimethoprim, Ciprofloxacin, Ceftriaxone and both Gentamycin and Amoxicillin are respectively 68.75%, 18.75%,62.5%, and 25%. The highest susceptibility was observed in Ciprofloxacin (68.75%) followed by and others are Gentamycin (62.5%), Amoxicillin (56.25%), Erythromycin

Table 2. Concentrations and Diffusion zone for resistance against antimicrobials standard for isolated *Staphylococcus* species

Antimicrobial class	Antimicrobial agent	Disc content in µg.	Diameter of zone of inhibition to nearest mm		
			Susceptible	Intermediate	Resistant
Tetracycline	Tetracycline	30	≥19	15-18	≤14
β-lactam	Penicillin	10 units	≥29	-	≤28
antibiotics	Cefoxitin	10 units	≥18	-	≤14
Quinolones	Nalidixic acid	10	≥23	20-22	≤19
Fluroquinolone	Ciprofloxacin	5	≥21	16-20	≤15
	Fleroxacin	5	≥19	16-18	≤15
Macrolides	Erythromycin	15	≥23	14-22	≤13
Sulfonamides	Sulfamethoxazole-Trimethoprim	23.75+1.25	≥16	11-15	≤10

and Tetracycline both(12.5%), Ceftriaxone (18.75%), Penicillin and Nalidixic acid both (6.25%), but Streptomycin, Amoxicillin, and Ceffloxacin did show any sensitivity. The highest intermediate resistance was observed for both Cefoxitin and Streptomycin at 25% but the "Sulfamethoxazole" did not show the intermediate pattern.

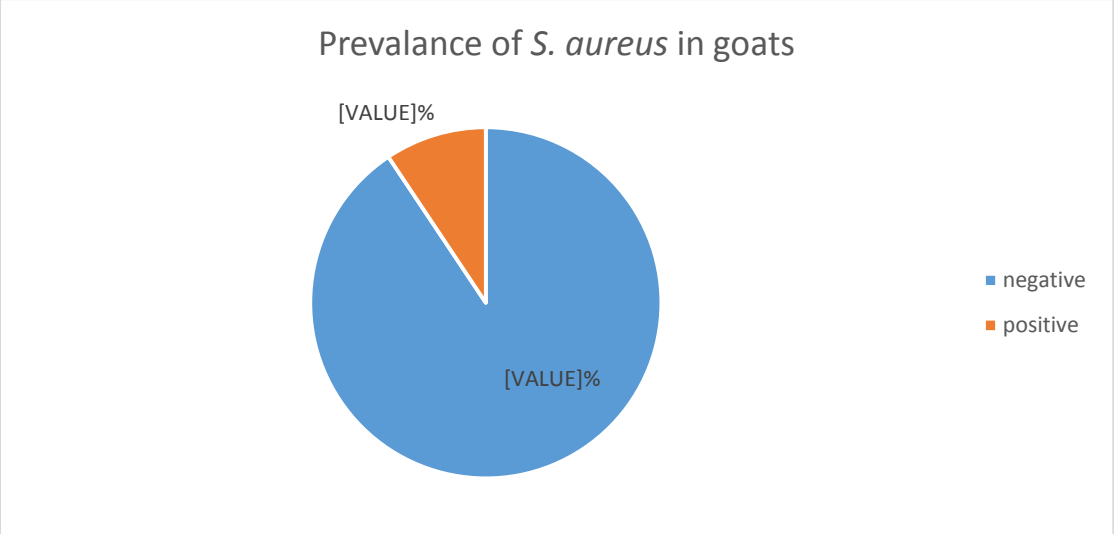

Fig. 4. Prevalence of *S. aureus* in goat

Table 3. The frequency of clinical mastitis and analysis

Variables	Co-variables	Number of samples (Percentages)
Breed	Black Bengal	24 (75%)
	Jamunapari	9 (28.125%)
Age	>1.5 years (Early stage)	21 (65.62%)
	1.5-3 years (Middle stage)	9 (28.15%)
	>3	2 (6.25%)
Lactation stage	1	15 (46.87%)
	2	12 (37.5%)
	3	5 (15.625%)
Udder Condition	Hard	9 (28.125%)
	Firm	23 (71.875%)
Udder color	Black	4 (12.5%)
	Reddish	26 (81.25%)
	Green	1(3.125%)
	Brownish	1 (3.125%)
Udder	Swollen	32 (100%)
Udder	Pain	32 (100%)
CMT result	Positive	32 (100%)
Floor-type	Brick	6 (18.75%)
	Muddy	16 (50 %)
	Concrete	10 (31.25%)
Bedding materials	Used	9 (28.125)
	Not used	23 (71.875)
Isolation shed	No	32 (100%)
Quarantine shed	No	32 (100%)
Drainage facility	Yes	7 (21.87%)
	No	10 (78.12%)
Floor cleaning frequency	once	29 (90.62%)

Variables	Co-variables	Number of samples (Percentages)
	two	3 (9.37%)
Disinfectant used	Yes	3 (9.375%)
	No	29 (90.625%)
Feed supply	concentrate	21 (65.625%)
	Concentrate, Roughage	11 (34.375%)
Frequency of feed supply	Two times	32 (100%)
Vitamin –mineral supply	Yes	12 (37.5%)
	No	20 (62.5%)
Probiotic supply	Yes	32 (100%)
Number of clinical mastitis	Yes	32(100%)
Number of clinical mastitis goats last 6 months	Yes	1 (3.125%)
	No	31(96.875%)
Number of subclinical mastitis last 6 months	No	32 (100%)
Treatment provided	Yes	31 (100%)
	No	1 (3.125%)
Drug used	Antibiotic	32 (100%)
Treatment	Amcox	20 (62.5%)
	Amcox, sp vet	2 (50%)
	Hicomox, sp-vet	2 (50%)
	Hicomox	6 (18.75%)
	Sp-vet	2 (50%)
Vaccination	Yes	3 (9.375)
	No	29 (90.625%)
Herbal Treatment provided	No	32 (100%)
Number of culled mastitis goat	No	32 (100%)
Number of dead mastitis goat	No	32 (100%)

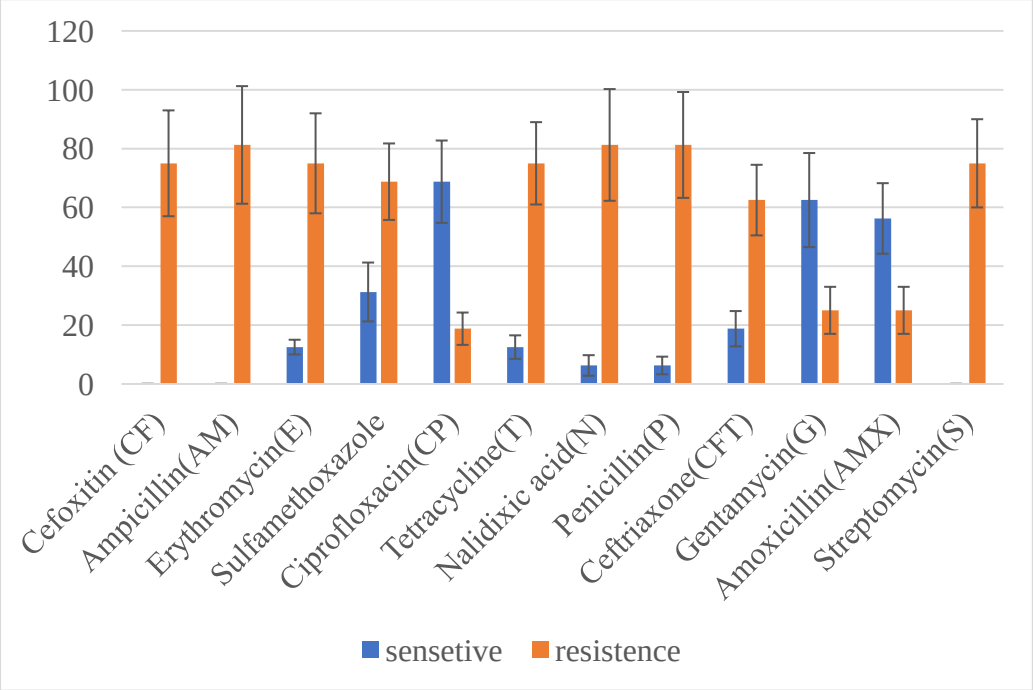

Fig. 5. Antimicrobial susceptibility and resistance of Coagulase positive isolates of S. aureus, P= penicillin, CF=Cefoxitin,T=Tetracycline,N=nalidixic acid, CP=ciprofloxacin. E=erythromycin, ST=sulfamethoxazole, AM=Amoxicillin, G=Gentamycin

Table 4. Percentage of Susceptibility and Resistant of isolated *S. aureus* against different antibiotics

Antimicrobials	Antimicrobial resistance pattern		
	Sensitive %	Intermediate %	Resistant %
Cefoxitin (CF)	0	25	75
Ampicillin(AM)	0	18.75	81.25
Erythromycin(E)	12.5	12.5	75
Sulfamethoxazole-	31.25	0	68.75
Ciprofloxacin(CP)	68.75	12.5	18.75
Tetracycline(T)	12.5	12.5	75
Nalidixic acid(N)	6.25	12.5	81.25
Penicillin(P)	6.25	12.5	81.25
Ceftriaxone(CFT)	18.75	18.75	62.5
Gentamycin(G)	62.5	12.5	25
Amoxicillin(AMX)	56.25	18.75	25
Streptomycin(S)	0	25	75

4. DISCUSSION

Changes in mastitis prevalence have been linked to differences in host and management factors that influence intra-mammary infection in goats in several studies. Mastitis is complex, with evident interactions between the host, the agent, and the environment. The differences in the prevalence of studies undertaken in SAQTVH could be due to differences in management and climate of the various regions. Further, the high incidence of clinical mastitis may be attributed to poor hygiene, lack of standard milking procedures, lack of proper pre-and-post under washing, and nonu sage of teat dips. In this study, Black goats are more prone to clinical mastitis, 24(75%). In case of age found the highest prevalence of clinical mastitis, was in >1.5 years 21(65.62%). On the other hand, clinical mastitis was found in the first lactation highest level, 15 (46.87%).The left udder had a greater infection rate than the right udder in a previous study. It has been proposed that this is because the udder that is milked first is more susceptible to infection than the udder that is milked later. This occurs over a long time relying on right or left-handedness. As many people in the population are right-handed, there attends to be more infection on the left udder as this is milked first. The most frequently isolated bacteria pathogen was coagulase-negative *Staphylococci* (CNS). "Members of the genus Staphylococci are the most common mastitis-causing bacteria seen in all types of mastitis in goats and other ruminants. Staphylococci are the most common cause of culling in domestic ruminants, causing all types of mastitis from asymptomatic to clinical to acute to gangrenous. This is because Staphylococci can be found on the skin of the udder, inside the teat canal, and in the mammary glands, and they are spread through unsanitary milking practices. In the current study, a significant proportion of clinical mastitis was due to *S. aureus*. Enterotoxin-secreting *S. aureus* intramammary infections are associated with mastitis in dairy ruminants" [16]. "Subclinical mastitis caused by these bacteria, apart from reducing milk yield, can develop into the clinical form and is the most isolated pathogen in clinical mastitis of small ruminants such as goats" [17]. This is due to the fact that Staphylococci can be found on the skin of the udder, inside the teat canal, and in the mammary glands, and are spread by unsanitary milking practices. In addition, coliforms such as Bacillus sp. and E. coli were often isolated microorganisms in this investigation. This is in line with previous research that found coliform bacteria to be the primary cause of environmental mastitis in domestic ruminants. Environmental mastitis was also found to account for more than half of the bacteria identified in the milk of mastitis-affected goats in previous investigations. "Coliforms thrive in unsanitary housing and living conditions of the dairy animals which were highly prevalent in the study area. In the present study, the majority of CMT-positive milk samples yielded growth in bacterial culture. High sensitivity (99%) of CMT in diagnosis has been reported" [18]. A link has also been found between CMT and the prevalence of mastitis germs in CMT-positive milk samples. This means that CMT is a reliable screening tool in the detection of both clinical and subclinical mastitis and can be used to investigate subclinical mastitis in dairy goat farms. The test can be used by farmers to screen for subclinical mastitis since it is a simple, field-based test that is less costly and is easy to be carried out even by the farmers themselves.

In this study, there was a significantly higher prevalence of mastitis in does whose houses were cleaned every two weeks compared to those which were cleaned more frequently. These results are in agreement with those of others. Environmental mastitis caused by organisms such as coliforms

oversees to thrive in dirty environments and hence quick cleaning of goat houses is recommended.

"In the present study, parity was found to be a risk factor for clinical mastitis. This is in agreement with studies undertaken by others" [19]. The use of antibiotics is popular in our daily life. The random and improper use of these remarkable drugs is the common cause of the emergence of drug-resistant bacterial strains. So, isolation, and identification of bacterial strains a very important factors to solve this situation. In this study, the *S. aureus* is identified by Gram's staining, colony morphological character, catalase, and coagulase test. Finally, the coagulase-positive sample 3(9.37%) from 32 samples. The coagulase-positive sample was next subjected to PCR. In clinical mastitis, PCR can be performed to confirm the presence of bacterial products. The nuc gene was used for *S. aureus* PCR amplification.

"Most bacteria in the current study were sensitive to Streptomycin, Gentamycin. A study in Nigeria [20] reported a high resistance of bacterial isolates to Streptomycin. This could have been causing the overuse of antibiotics in the treatment of goat diseases in the study area. The sensitivity of bacterial isolates in this study to Streptomycin maybe because the antibiotic is seldom used in mastitis treatment in the study area." "Studies in Bangladesh reported high resistance of *S. aureus* to Streptomycin" [21]. "In another study in Ethiopia, [22] varying degrees of resistance of bacteria to Chloramphenicol, Gentamycin and Streptomycin were reported." These studies show that there are differences in sensitivity to antibiotics founded on the region and the usage of a particular antibiotic.

"The resistance to beta-lactams and tetracyclines is due to the fact that these antibiotics are commonly used in the treatment of various bacterial diseases in dairy animals"[23]. "Results of the current study also found that most bacteria are still susceptible to antibiotics as reported by others" [24]. Hence, they can still be used in the treatment of subclinical mastitis. The present study showed the significant prevalence of Subclinical mastitis which has a great influenceon the production of small ruminants. This, they do require good outlook and management practices to control or prevent the occurrence of the disease. The proper isolation and identification of the etiological organism play a significant role in the prevention and control of the disease. Further and detailed epidemiological studies should be conducted to identify the prevalence of the disease at regional and national levels. Additionally, studies on antibiotic susceptibility tests should be done to identify the effective drug that can be used for the successful treatment of the disease.

5. CONCLUSION

The identification of *Staphylococcus sp* in milk samples of goats from field conditions indicates that the prevalence of the organism in goats is common. These organisms act as an assortment for future clinical mastitis. Though it is silent intimidation to human health, its presence instigates the steps required to control such a disease of zoonotic potential, which may lead to dire consequences if not addressed. A further extensive experiment is required for the detection of possible risk factors of the organism which will help in taking the prevention and control strategies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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