Chapter-1

Introduction

Staphylococcus aureus is a common commensal and pathogen of a large number of animal species, including humans. *Staphylococcus* is a Gram-positive coccal bacteria originated from Greek "staphyle" means "bunch of grapes" and "kokkos" meaning "granule". When observed under microscope the organisms display as grape-like appearance. In this genus, there are forty species, which includes *S. aureus, S. intermedius, S. hyicus, S. epidermidis, and S. saprophyticus* (Blood *et al*., 1989). *Staphylococcus* found as normal flora including skin, nose and mucous membranes of healthy humans and animals (Lozanoet al., 2016). In the healthy lower reproductive tract especially in vagina and vulva in female animals, urinogenital tract and various intra-abdominal organs, *S. aureus* is a normal inhabitant (Murray *et al*., 2008; Ateba *et al*., 2010). Generally, these bacteria create no problems or result in relatively minor skin infections. But it can cause life threatening problem if the bacteria enter into deeper body, entering bloodstream, joints, bones, lungs or heart.

S. aureus is a contagious pathogenic organism due to it is responsible for lots of infectious diseases like skin infections (pimples, impetigo, boils, cellulites, folliculitis, carbuncles, scalded skin syndrome), abscesses, pneumonia, myocarditis, pericarditis, encephalitis meningitis, osteomyelitis, septicemia, bacteremia, sepsis, gastroenteritis, endocarditis, toxic shock syndrome and certain food intoxications (Soomro *et al*., 2003; Ryan *et al*., 2004; Kateete *et al*., 2010). Most of the times this bacterium is spread by skin-to-skin contact. They can also expansion when individuals touch anything that has

contain the staph germ, such as clothing or a towel. According to (Lowy, 1998) *S. aureus* causes superficial skin and soft tissue infections to life-threatening endocarditis, toxic-shock syndrome, and necrotizing pneumonia (Lowy, 1998).

In addition, that *S. aureus* is of the major causative pathogen of clinical and subclinical mastitis of goat. According to (Guss *et al*., 1977) subclinical mastitis in goat is 15 to 40 times more prevalent than the clinical form. Therefore, milk of goat could also be a feasible source of Staphylococcal infection responsible for food intoxication and toxico-infection. Presence of *S. aureus* in milk or any food causing public health significance (Balaban and Rasooly,2000) and 50 % strain of this organism are capable to produce enterotoxins associated with food poisoning (Putturu,2013). Besides *S. aureus* causing food poisoning. Food poisoning is an intoxication due to ingestion of food that has been contaminated with enterotoxins, extracellular proteins which also have superantigen activity if released systemically (Fraser *et al*,2008). Eating contaminated food symptoms come on quickly, usually within hours or less time. Symptoms usually disappear rapidly, too, often lasting just half a day. According to (Kadariya et al., 2014), food borne diseases (FBD) causing by *S. aureus* in humans plays a significant public health concern in worldwide. According to (Bergdoll,1983) findings food stuffs it thought to be more resistant than in a laboratory culture medium. Therefore, this study was designed to form the following objectives:

Objectives:

- To learn the Isolation and identification of *Staphylococcus* bacteria from goat milk.
- Bacterial isolates in different bacteriological culture media and biochemical test.
- To know the prevalence of *S. aureus* from healthy goat milk.

Chapter-2

Materials And Methods

2.1: **Duration and Area of study**

The study was undertaken from October 1- October 19,2021 in Shahedul Alam Quadery Teaching Veterinary Hospital (SAQTVH) in Chittagong Veterinary and Animal Sciences University (CVASU) complaining with any disease but without any infection of udder.

2.2**: Study animal**

Individual Lactating doe is selected as a study unit.

2.3: **Sample Collection**

Ten sample were collected from healthy lactating goats in 15 ml falcon tubes and sent it to the Poultry Research and Training Centre (PRTC), CVASU for bacteriological analysis.

2.4**: Design of study**

Isolation and identification of the bacterial isolates from goat milk done by based on their cultural characteristics in different agar plates and characters on Gram's staining etc. Finally, characterization of the organisms was done by using catalase test.

EXPERIMENTAL DESIGN

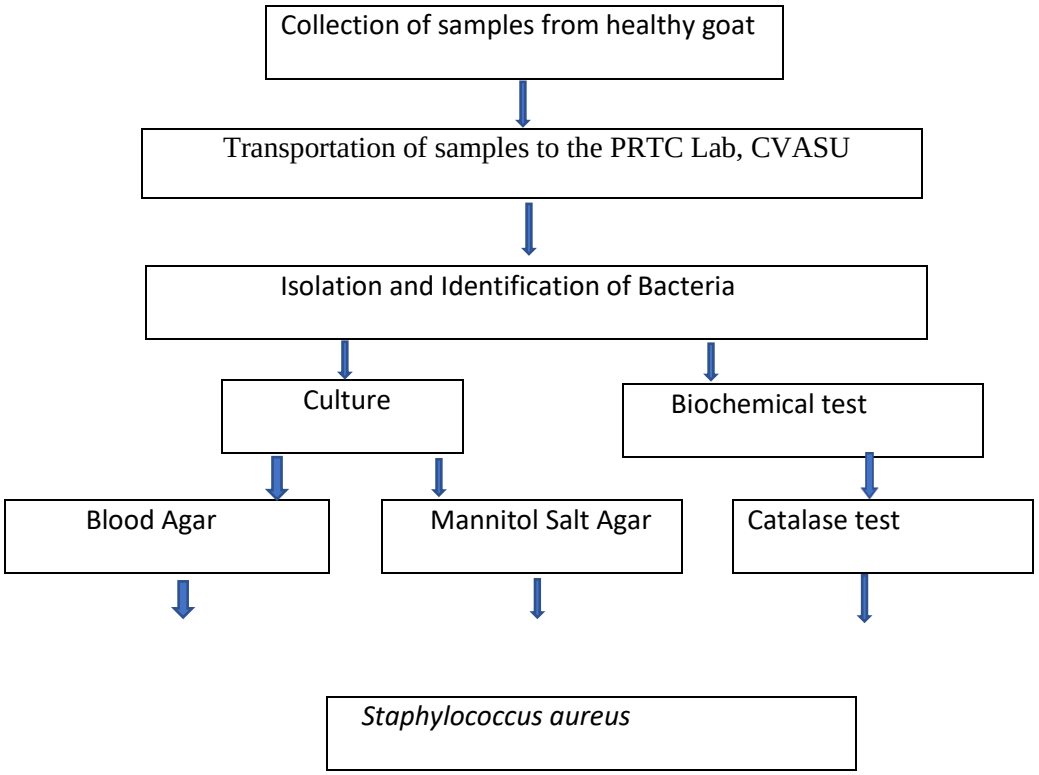


Fig: Outline of Isolation and identification of *S. aureus* bacteria from goat milk.

Bacteriological Investigation:

Both Mannitol salt agar (MSA) and Blood agar base were prepared according to the instructions of manufacturer (Oxoid Ltd, Basingstoke, Hampshire, UK).

Blood agar was prepared by adding 5% citrated-bovine blood in the blood agar base. A loop full of inoculum from enrichment were streaked on Blood Agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours for detection of hemolysis.

According to (Kateete *et al*., 2010) growth of yellow colonies on MSA (Oxoid Ltd, Basingstoke, Hampshire, UK) surrounded by yellow zones as a result of fermentation of mannitol after 24 hours of incubation at 37°C indicated a positive result.

The pure cultures were further subjected to **catalase tests for biochemical confirmation** of *Staphylococcus sp* (Monica, 1991).

Phenotypic-Characteristics:

Grams staining method was used for identification of morphology and staining characters of microorganisms. Suspected (*Staphylococcus sp*) was stained as described by manual of veterinary investigation laboratory Technique (OIE, 2000).

The procedure was as follows: A small colony was picked up with a bacteriological loop, smeared on clean grease free microscopic glass slide and fixed by gentle heating. Then we used crystal violet solution on smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine were then added to act as mordant for one minute and poured off excess fluid. Acetone alcohol was then added for few

seconds who act as a decolorizer. After washing with running water, safranin was added as counter stain on smear and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under microscope with high power objective (100X) using immersion oil. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes.

Biochemical examination

Biochemical tests were performed to confirm *Staphylococcus sp* using Catalase test.

Catalase Test:

The catalase test is used to detect presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water.

Procedure: Small amount of pure growth culture was transferred with a bacteriological loop from the Blood Agar into clean grease free microscopic glass slide, and then a drop of catalase reagent (3% H2O2) was added. The evolution of gas bubbles indicates a positive result described by (Hogan *et al*., 1999; Macfaddin, 2000)

Chapter-3

Results

For the isolation and identification of *Staphylococcus sp*, each sample was cultured on different culture media. The colonies which reflected various morphological characteristics were identified based on their staining, cultural, morphological and biochemical properties.

Blood agar:

Enriched media such as in blood agar colonies of *Staphylococcus sp* were
Smooth, small, circular raised with gray white or yellowish in color in **Figure B.**

Staining characteristic:

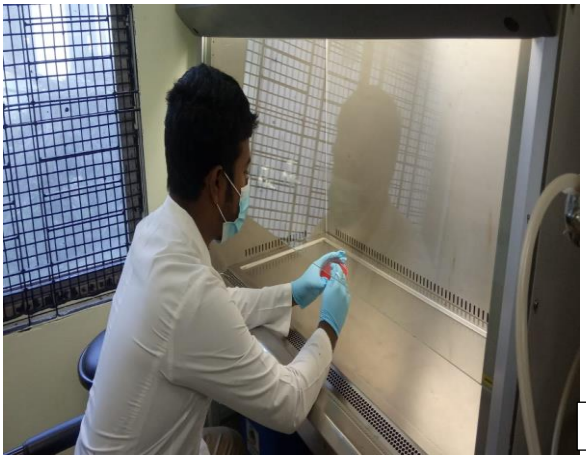
Following Gram's staining technique the smear of the slide that revealed as gram positive, cocci, spherical cells and irregularly arranged in grapes like cluster depicted as *Staphylococcus sp* shown in **Figure C.**

Catalase test:

Slide catalase test was performed to identify Staphylococci as biochemical test. Hydrogen peroxide was breakdown into water and oxygen. Production of oxygen was reflected by the bubble formation. Staphylococcal isolates were found to be positive in catalase tests in **figure D.**

Mannitol salt agar:

Staphylococcus sp showed different colonies on the mannitol salt agar (MSA).
S. aureus fermented MSA with the production of small to large yellowish colonies in **Figure E.**



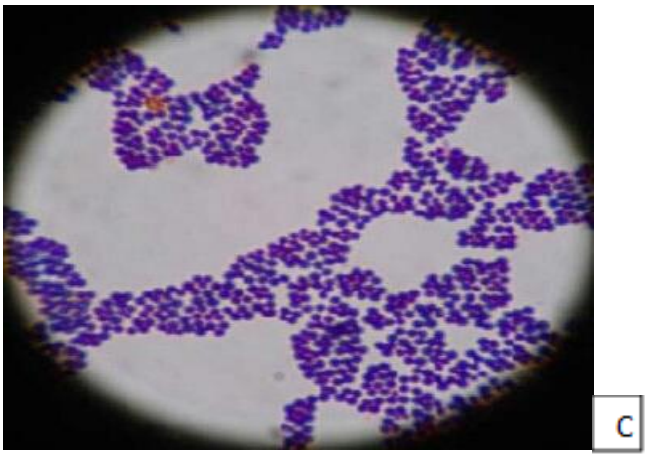
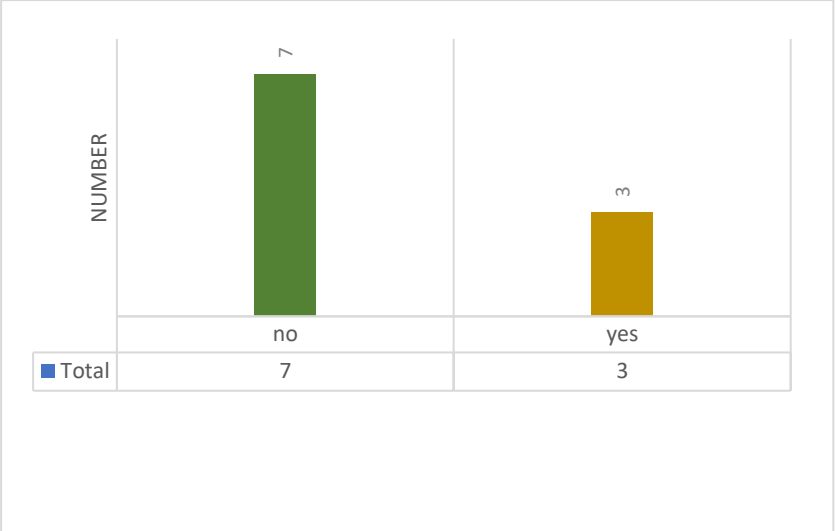


Figure: Laboratory activity **(A),** Smooth, small, circular raised with gray white Colonies in Blood Agar Plate **(B),** Grape's like cluster under microscope in Gram's staining **(C)** Bubble formation in Catalase test **(D),** Small to large yellowish colonies in MSA Plates **(E).**

Prevalence of ***Staphylococcus aureus*:**



Graph :*S. aureus* in goat milk

This graph illustrates a total of 3 samples were found positive out of 10 Collected samples from healthy goat samples for *S. aureus*. The numbers were confirmed by their colonial growth characteristics on blood agar mannitol salt agar and biochemical test. The samples were further characterized by catalase-test. In Catalase test, 3 samples were found positive out of 10 samples that were positive to mannitol salt agar and blood agar test.

Chapter-4

Discussion

On the basis of bacteriological culture 3 (30%) out of 10 samples were found positive for *S. aureus* in the study population. This finding is agreed with the findings of (Adamu *et al*., 2010) that showed 30% prevalence in found goat population. Besides (Fotou et al., 2011) found 24% prevalence of *S. aureus* was detected in the tested raw ovine milk samples in Greece that is slightly low of my findings.

This type of variation due to geographic location of the region from where the samples were collected, mixed bacterial population in animals, variation of the techniques adopted by different laboratories for conducting the experiments.

S. aureus is major causative pathogen of clinical and subclinical mastitis of goat. According to (Samad et al.,2008) mastitis is an inflammatory condition of the mammary glands, a common disease affecting dairy goats which is accompanied by physical, chemical, bacteriological changes in milk and pathological changes in glandular tissue of the udder.

According to CDC, people who ingest *S. aureus* contaminate food, and don't wash their hands before touching contaminated food, it's can make people ill due to production of toxin. Staph bacteria are killed by cooking, but the toxins are not destroyed and will still be able to cause illness.

Proper milking procedure, maintaining biosecurity, Hygiene, overall good farm management reduction of this bacterial agent in farm which causes many diseases and food borne infection.

Chapter-5

Limitation

The study was conducted in a small scale, area, short time period which might not be the representative of actual scenario. Further bacteriological investigation needs for future study.

Chapter-6

Conclusion

The isolation and identification of *Staphylococcus sp* from milk samples of goat indicates that the prevalence or presence of the organism in goat is common. *S. aureus* organisms act as a reservoir both clinical mastitis and subclinical Mastitis. Although it is a threat to human health, its presence instigates the steps required to control such a disease of zoonotic potential, which may lead to serious matter in community. In this study 30% samples were carried out *S. aureus* organism out of 10 samples.

Chapter-7

References

Ateba CN, Mbewe M, Moneoang MS, Bezuidenhout CC. Antibiotic-resistant Staphylococcus aureus isolated from milk in the Mafikeng Area, North West province, South Africa. South African Journal of Science. 2010 Nov 1;106(11):1-6.

Adamu JY, Raufu AI, Chimaroke FC, Ameh JA. Antimicrobial susceptibility testing of Staphylococcus aureus isolated from apparently healthy humans and animals in Maiduguri, Nigeria. International Journal of Biomedical and Health Sciences. 2021 Jun 4;6(4).

Balaban N, Rasooly A. Staphylococcal enterotoxins. International journal of food microbiology. 2000 Oct 1;61(1):1-0.

Blood DC, Henderson JA, Radostits OM. Veterinary medicine 4th edition. London, Baillière Tindall. 1974.

Bergdoll MS. Enterotoxins, p 559–598. Staphylococci and staphylococcal infections. Academic Press, London, United Kingdom. 1983.

Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. Immunological reviews. 2008 Oct;225(1):226-43.

Fotou K, Tzora A, Voidarou C, Alexopoulos A, Plessas S, Avgeris I, Bezirtzoglou E, Akrida-Demertzi K, Demertzis PG. Isolation of microbial pathogens of subclinical mastitis from raw sheep's milk of Epirus (Greece) and their role in its hygiene. Anaerobe. 2011 Dec 1;17(6):315-9.

Guss SB. Management and diseases of dairy goats. Dairy Goat Journal Publishing Corporation.; 1977.

Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, Smith KL. Laboratory handbook on bovine mastitis. National Mastitis Council, Madison, WI. 1999;78(7):485-8.

Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FC. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of clinical microbiology and antimicrobials. 2010 Dec;9(1):1-7.

Kadariya J, Smith TC, Thapaliya D. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed research international. 2014 Oct;2014.

Lowy FD. Staphylococcus aureus infections. New England journal of medicine. 1998 Aug 20;339(8):520-32.

Lozano C, Gharsa H, Ben Slama K, Zarazaga M, Torres C. Staphylococcus aureus in animals and food: methicillin resistance, prevalence and population structure. A review in the African continent. Microorganisms. 2016 Mar;4(1):12.

Murray PR, Baron EJ, Pfaller MA, Jorgensen JH, Yolken RH, Nachmakin I. Campylobacter and Arcobacter in Manual of Clinical Microbiology. Washington, DC: American Society for Microbiology Press. 2003;5:902-14.

Monica C. Medical Laboratory manual for Tropical countries. VOL. 1991;11:60-3.

MacFaddin JF. Biochemical tests for identification of medical bacteria.

Manual OI. Manual of standards for diagnostic tests and vaccines. Office International Des Epizooties. 2000.

Putturu R, Thirtham M, Eevuri TR. Antimicrobial sensitivity and resistance of Salmonella enteritidis isolated from natural samples. Veterinary world. 2013 Apr 1;6(4).

Ryan KJ, Ray CG. Medical microbiology. McGraw Hill. 2004;4:370.

Samad MA. Animal Husbanry and Veterinary Science, volume II, LEP pub no. 11, Bangladesh Agricultural University campus.

Soomro AH, Arain MA, Khashkeli M, Bhutto B, Memon AQ. Isolation of Staphylococcus aureus from milk products sold at sweet meat shops of Hyderabad. On Line Journal of Biological Sciences. 2003;3(1):91-4.

Singh P, Prakash A. *Isolatin of Escherichia coli, Staphylococcus aureus and Listeria monoctogenes from milk products sold under market conditions at Agra Region. Acta Agri. Solv. 2008; 92(1): 83-88.*

Appendix

Composition of Blood Agar

Composition	%
Peptone	0.5
Beef extract	0.3
Agar	1.5
NaCl	0.5
Sheep Blood	5

Composition of Mannitol Salt Agar

Composition	gm/lt
Casein	5
Animal tissue	5
Beef extract	1
D mannitol	10
Sodium chloride	75
Phenol red	0.025
Agar	15

Biography

I am Kazi Shams Al Arefin, son of Kazi Nurul Abser and Rahima Begum. I passed my Secondary School Certificate (SSC) examination from Nasirabad Gov't High School in 2013 (G.P.A-5.00) and Higher Secondary Certificate (HSC) examination from Gov't City College, Chattogram in 2015 (G.P.A-4.42). Now I am an intern veterinarian under the Faculty of Veterinary Medicine in Chattogram Veterinary and Animal Sciences University, Bangladesh in 2015-16 session. In the future I would like to be a good veterinarian.