Biochemical Characteristics of Bacterial Isolates obtained from Bovine MastiticMilk Samples(Area of the study)

ABSTRACT

Aim: Bovine mastitis affects the milk production in milking cattle and the milk producer. The major causative organism of mastitis includes the bacteria. The present study is focused on the identification of bacterial isolates obtained from milk samples of bovine mastitis based on their biochemical activities.

Study design: The bacterial isolates were collected from Department of Veterinary Microbiology, Veterinary College, KVAFSU, Bengaluru, Karnataka and subjected to staining for morphology, Gram's reaction and other biochemical tests. Identification key for both Gram positive and Gram negative bacterial isolates were prepared and based on the activities they were identified up to species level.

Place and Duration of Study: The work was carried out in the Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bengaluru, Karnataka from Feb.2021 to Feb. 2022.

Methodology:Bacterial isolates obtained were subcultured, purified on plate count agar. The bacterial isolates were coded and subjected to simple staining, spore staining, Gram's staining, oxidase test, catalase test and other biochemical tests like indole, VP, methyl red, citrate tests by preparing specific sterile media. The results obtained for the tests conducted were compared with the standard identification key and declared the species of bacterial genera.

Result:In the present study, 45 isolates of bovine mastitis obtained were phenotyped through staining techniques and biochemical tests. Among 45 isolates, 40 Grampositive isolates and 5 Gram negative, which were identified as *streptococcus uberis* (10), *streptococcus agalactiae*(12); one each of *staphylococcus delphini*, *staphylococcus carnosus* and *staphylococcus gallanarium* while 6 eachof *staphylococcus equorum*; *Staphylococcus epidermis* and 3 *staphylococcus aureus*. Gram negative (5) isolates were identified as *Escherichia coli* (3), *Salmonella enterica* (1) and *Alcaligenes* sp. (1) based on their biochemical characteristics.

Conclusion:Among the 45 bacterial isolates of bovine mastitis, 40 were Gram positive and 5 Gram negative. Out of 40 Gram positive isolates, 22 were *Streptococcus* while 18 belonged to *Staphylococcus* genus. Species of *Escherichia, Salmonella* and *Alcaligenes* were the 3 Gram negative bacterial isolates noticed.

***Key words**: Bovine mastitis; Biochemical tests; Bacterial isolates; Gram positive; Gram negative*

1. INTRODUCTION

Bovine mastitis is defined as inflammation of the mammary gland and is most commonly caused by bacterial infection. Bovine mastitis occurs in two different clinical manifestations: subclinical and clinical mastitis that ranges from mild, moderate to severe cases. The California mastitis test (CMT) is one of the most common tests for the diagnosis of subclinical mastitis and an indirect indicator of qualitative measurement of the somatic cell count (SCC) in milk and can be adopted in dairy farm by milkers. Mastitis directly affects the technical characteristics and hygienic quality of milk, indirectly altering its intrinsic qualities[1, 2].Mastitis is caused by wide variety of microorganisms including bacteria, mycoplasma, yeasts, algae and among the etiological agents, bacteria play a major role in causing mastitis. Bovine mastitis is classified as: contagious mastitis, generally caused by bacteria presiding on the skin of the teat and inside the udder, transmitted from one cow to another during milking (e.g., *Staphylococcus aureus* or *Streptococcus agalactiae*) and environmental mastitis, caused by pathogens such as *Escherichia coli*, *Streptococcus uberis* and *Klebsiella* sp. normally found in shed surroundings such as bedding, manure, soil, and feed. Among 20,000 clinical mastitis cases in the Netherlands, 40 % were caused by *Streptococcus uberis* and *S. dysagalactiae*, 30 % by *Staphylococcus aureus* and 30 % by *Escherichia coli*[3,4].*Streptococcus agalactiae* is the most common Gram positive bacterium from clinical mastitis, followed by *Staphylococcus aureus* while *Klebsiella* sp. and *E. coli* were the most isolated Gram negative bacteria from clinical mastitis[5, 6]. The bacteria causing bovine mastitis having public health importance causing intoxication or infection in human beingsare *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli, Klebsiella pneumonia*[7,8].

Isolation of mastitis causing bacteria from milk

Mastitis was studied by comparing to the gold standard of somatic cell count and California mastitis test and a combination of the two methods was found to be the best option. Microbiological evaluation, both by biochemical methods as well as by monoplex and multiplex polymerase chain reaction, revealed that coagulase-negative staphylococci were the most predominant (64.8 %) bacteria, followed by streptococci (18.1 %), *Escherichia coli* (9.8 %) and *Staphylococcus aureus* (7.3 %) among 190 bubaline quarter milk samples from 57 domesticated dairy buffaloes from two organized farms (n = 64; Dharwad district, Karnataka) and three unorganized sectors (n = 126; in and around Bengaluru district, Karnataka)[9]. Out of 58 CMT positive samples collected from Murraha buffalo farm of NDRI, ICAR, Karnal, 51 samples that showed bacterial colonies were streaked onto blood agar, mannitol salt agar and MacConkey agar media and examined macroscopically for colony morphology, characteristics hemolytic pattern, microscopically by Gram's stain method and further the identification of all isolates was done by using Histaph kit (KB004A HIMEDIA) and Histrept (KB005A) biochemical kits. The major agents involved in bacterial intramammary infection (IMI) isolated from mastitis milk samples were *Staphylococcus aureus, S. epidermidis.* The culturally examined and characterized samples revealed *Staphylococcus aureus* (35.29 %) as the predominant bacteria followed by *Streptococcus agalactiae* with an isolation rate of 25.49 %. The incidence of coliforms was not detected in any of the sample cultured and examined[10].A total of 94 bacterial isolates including 3 yeasts were recovered from 71 milk samples obtained from mastitis cases of Meerut region, Uttar Pradesh. The study indicated that the major prevalent pathogen associated with bovine mastitis was *Staphylococcus* spp. (42.55 %) followed with *E. coli* (21.28 %). Other commonly isolated pathogens were *Streptococcus* spp. (6.38 %), *Proteus* spp.

(8.51 %), mixed infection (Gram positive and Gram negative bacilli of 18.09 %) and *Candida* spp. (3.19 %)[11].

Out of the 83 samples collected from a total of 12 farms of small scale and government dairy farms in Asella town, Ethiopia and examined, all (100%) were positive for the cultural isolation of bacterial species. Among the 83 isolates, 32 (38.6%), 24 (28.9%), and 6 (7.2%) were *Staphylococcus aureus*, *Staphylococcus intermedius,* and *Staphylococcus hyicus,* respectively. Other bacteria like *Escherichia coli* 12 (14.5%) and *Streptococcus* species 2 (2.4%) were also isolated. *Bacillus* species 2 (2.4%), *Proteus* species 2 (2.4%), and 3 (3.6%) of them were mixed bacterial infections [12].

A total of 200 bovine milk samples from CMT confirmed clinical mastitis cases were collected from Kashmir valley and cultured on Nutrient agar, Blood agar, Mac-Conkey agar, Eosine Methylene Blue (EMB) agar, Mannitol salt agar and Edwards media for bacterial isolation. The isolates were then subjected to biochemical tests for identification. Upon microbiological testing and biochemical confirmation, the major mastitis causing bacteria isolated from clinical cases of Bovine mastitis were *Staphylococcus* Spp. showing highest prevalence of 46.4% followed by mixed infection with *Staphylococcus* and *Streptococcus* (20.8%), *Streptococci* Spp. (18.4%) and *Escherichia coli* (14.4%) [13].

The most common bacteria recovered from mastitis milk samples were *Staphylococci* spp. (34 %), followed by *Escherichia coli* (19 %), S*treptococci* spp. (9 %), and *Klebsiella* spp. (8 %) while minor bacteria included *Salmonella* spp. (2 %), *Proteus* spp. (1 %) and *Candida* spp. of 0.6 % [14].

One hundred and one milk samples from mastitic cattle and buffaloes from farms in and around Ludhiana and mastitis cases presented in veterinary clinical complex, GADVASU, Ludhiana, Punjab, India were collected. Out of a total of 101 samples, 86 samples yielded bacterial growth. Out of these 86 samples, 76 (88.37%) samples had a single bacterial growth whereas rest 10 (11.63%) samples had more than one bacterial growth. It was observed after the organisms were subjected to cultural, morphological, biochemical tests and PCR that, a total of 33 (34.02%) *Staphylococcus aureus,* 24 (24.74%) *Escherichia coli,* 16 (16.49%) *Klebsiella pneumoniae,* 5 (5.15%) *Pseudomonas aeruginosa*, 2 (2.06%) *Streptococcus agalactiae,* 12 (12.37%) *Bacillus* spp. and *Serratia marcescens* 5 (5.15%) were isolated. It was observed that *Pseudomonas aeruginosa* showed resistance against most of the antibiotics and was sensitive only for ofloxacin (80%) and gentamicin (40%) [15].

A total of 300 milk samples were collected from mastitis dairy cows of organized and unorganized cattle farms irrespective of parity and stage of lactation, in Jammu region of Union territory of Jammu and Kashmir, India. 235 (78.33%) samples were culturally positive and yielded 1,100 bacterial isolates. Among 1100 bacterial isolates, 379 isolates of *S. aureus*, 235 isolates of *Escherichia coli*, 149 isolates of coagulase negative staphylococci, 115 isolates of *Streptococcus agalactiae*, 93 isolates of *Salmonella typhimurium*, 78 isolates of *Klebsiella pneumonia*, 18 isolates of *Enterococcus faecalis*, 15 isolates of *Proteus vulgaris*, 11 isolates of *Corynebacteriumdiptheriae*, and 7 isolates of *Bacillus cereus*) were obtained from 235 samples. *Staphylococcus aureus* was found to be the prime etiological agent involved in the mastitis of dairy cows, followed by *Escherichia coli* and other environmental pathogens[16].

Pathogenic bacteria were isolated and cultured from 208 clinical milk samples from cows with mastitis of four farms in the Jiangsu Province, China. The results show that pathogenic bacteria were positively detected in 186 milk samples with a detection rate of 89.42%. The total number of bacterial species was a total of 438 isolates of 16 pathogenic bacteria that were detected in milk samples, among which the majority were environmental pathogenic bacteria. *Klebsiella pneumoniae* (15.35%) accounted for the highest proportion, followed by *Escherichia coli* (13.70%), *Pseudomonas aeruginosa* (12.33%), and *Streptococcus agalactiae* (10.5%) compared to the other detected bacteria [17].

A total of 179 milk samples from cows with clinical mastitis (CM) and subclinical mastitis (SCM), as well as eight bulk tank milk (BTM) samples from 48 dairy farms of Bosnia and Herzegovina were analysed by standard bacteriological and mycological methods. A total of 88 (49.2%) mastitis samples were positive for known mastitis pathogens at 32 of 47 farms (68.1%). Mycoplasma bovis was a predominant pathogen (13.4%) in the majority of herds (29.2%) and accounted for 48.9% of positive CM samples. *Escherichia coli* was the second most dominant CM pathogen (34%), followed by *Streptococcus agalactiae* (10.6%), whereas *Staphylococcus aureus* and coagulase-negative staphylococci were the most common in SCM samples (17.1%). Other mastitis pathogens included *Candida* spp. and *Protothecazopfii*. Two BTM samples were positive for *M. bovis* only and one was positive for a mixed culture of *S. aureus* and *Streptococcus uberis*[18].

2. MATERIAL AND METHODS

2.1Collection of isolates of bovine mastitic pathogens

The pathogens of bovine mastitis from California Mastitis Test (CMT) positive milk samples, selected in and around Bangalore were procured from Veterinary Microbiology Department, Veterinary college, Bangalore in sterile nutrient broth [19] and subjected for purification and characterization.

2.2 Purification and maintenance of isolates of bovine mastitis

The collected pathogens were purified by streaking technique on to poured plates of using the nutrient agar medium sterile inoculation loop and the plates were incubated for 37 ˚C/24 h. After incubation, the obtained single pure colony was selected and transferred to nutrient agar slants and incubated for 37 ˚C/ 24 h. and preserved as stock culture on nutrient agar slant in the refrigerator [19].

The stock culture was subcultured freshly once in a month while working culture was sub cultured whenever required or once a week.

2.3 Characterization of bacterial isolates of bovine mastitis

The **o**btained purified isolates were subjected for Gram staining, Catalase test, Oxidase test. Gram positive through standard procedures [19] and Gram negative isolates were further subjected to specific biochemical tests and based on the identification key placed in genus and species level.

2.3.1 Identification of Gram positive isolates

The isolates were subjected to preliminary test to confirm the genus using Grams staining, catalase test, oxidase test.Gram positive isolates showing coccus shaped, based on chain or bunch arrangement considered as streptococci or staphylococci. Further specific tests [19] like Oxidation- fermentation test, Voges-Proskauer, urease, esculin hydrolysis, arginine hydrolysissugar fermentation like mannitol, arabinose, maltose, and sucrose placed the isolates under either streptococci or staphylococci. The identification key for species of *Streptococcus*[20] and *Staphylococcus* [21] were prepared to help in phenotypic identification of the isolates of streptococci and staphylococci.

Tests	**Identity**				
	S. anginous	*S. mitis*	*S. uberis*	*S. agalactiae*	*S. constellatus*
Gram Staining	+				
Catalase	-				
Oxidase					
Oxidative Fermentative	O^+F^+(oxidative and fermentative)				
Voges Proskauer's	+	-	-	+	+
Esculin Hydrolysis	+	+	+	-	+
Arginine Utilization	+	-	+	+	+
Sucrose	+	+	+	+	-

2.3.1.1 Identification key for *Streptococcus* species

Tests	**Identity**					
	S.delphini	*S.gallinarium*	*S.equorum*	*S.aureus*	*S.carnosus*	*S.epidermidis*

~~**SL No**~~	~~**Tests**~~	~~**Identity**~~				
		~~*S. anginous*~~	~~*S. mitis*~~	~~*S. uberis*~~	~~*S. agalatiae*~~	~~*S. constellatus*~~
~~1~~	~~Gram Staining~~	~~+~~				
~~2~~	~~Catalase~~	~~-~~				
~~3~~	~~Oxidase~~					
~~4~~	~~Oxidative Fermentative~~	~~O^+F^+~~				
~~5~~	~~Voges Proskauer's~~	~~+~~	~~-~~	~~-~~	~~+~~	~~+~~
~~6~~	~~Esculin Hydrolysis~~	~~+~~	~~+~~	~~+~~	~~-~~	~~+~~
~~7~~	~~Arginine Utilization~~	~~+~~	~~-~~	~~+~~	~~+~~	~~+~~
~~8~~	~~Sucrose~~	~~+~~	~~+~~	~~+~~	~~+~~	~~-~~

2.3.1.2 Identification key for *Staphylococcus* species

Gram staining	+					
Catalase	+					
Oxidase	-					
Oxidative fermentative	O^+F^+ (oxidative and fermentative)					
Voges Proskauer's	-	-	-	+	+	+
Urease	+	+	+	+	-	+
Mannitol	+	-	+	+	+	-
Arabinose	-	+	+	-	-	-
Maltose	+	+	-	+	-	+

Tests	**Identity**		
	Escherichia coli	*Salmonella enterica*	*Alcaligenes* sp.
Gram staining	-		
Catalase	+		
Oxidase	-		
Oxidative fermentative	O^-F^+ (not oxidative, only fermentative)		O^-F^- (not oxidative and not fermentative)
TSI-H_2S	Acid butt, Acid slant, Gas production	Acid butt, Alkaline slant, H_2S production	Alkaline butt, Alkaline slant, No gas or H_2S

2.3.2 Identification key for Gram negative isolates

Gram staining, catalase, oxidase, OF test, urease test were followed for Gram negative isolates along with TSI-H_2S test, Indole test, Methyl-red test, Voges Proskauer test and Citrate utilization test. The identification key for Gram negative rods was prepared by referring to Bergey's Manual of Systematic Bacteriology [22].

2.3.2.1 Identification of Gram negative isolates

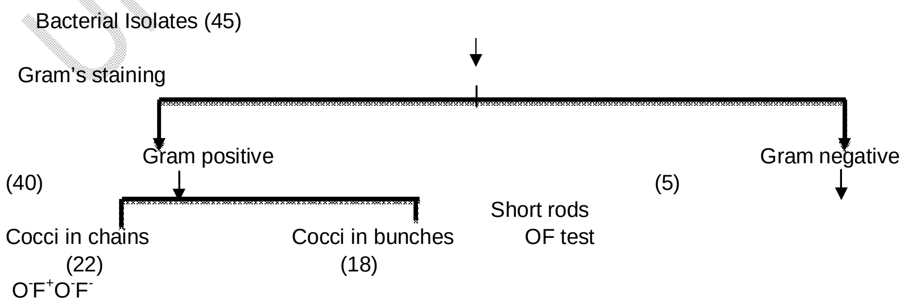
			production
Indole	+	-	-
Methyl red	+	+	-
Voges-Proskauer	-	-	+
Citrate	-	+	+
Urease	-	-	-

3. RESULTS AND DISCUSSION

Result obtained in the present study are explained below and later the obtained results are discussed with the results of other authors

3.1 Genus level identification of the isolates of bovine mastitis

The cell morphology and cell arrangement of the isolates grouped them as Gram positive streptococci, staphylococci and Gram negative short rods. Few of the preliminary identification tests like oxidase, catalase and oxidative-fermentative tests, TSI H_2S (for Gram negative rods) were conducted to place the isolates under specific genus. Out of 40 Gram positive cocci, 22 were streptococci due to cocci in chains, both oxidase & catalase negative and had fermentative characteristics while another 18 isolates of cocci in bunches were staphylococci as they showed oxidase positive, catalase negative, both oxidative and fermentative properties. Gram negative short rods with oxidase negative, catalase positive, only fermentative with gas production placed them under Genus *Escherichia or Enterobacter*; similarly acid butt, alkaline slant with H_2S production may be genus *Salmonella*. A Gram negative rod, both oxidase & catalase negative as well as no reaction on TSI H_2S was guessed as *Alcaligenes* sp. (Fig. 1).



Catalase Oxidase Catalase Oxidase Enterobacteriaceae *Alcaligenes sp.*
negativenegative positive negative (4) (1)

	Isolate codes

TSI-H_2S

O^-
F^+O
$^+F^+$
Acid
Acid
Alka
line
Stre

ptococcus spp.*Staphylococcus* spp. butt/slant, butt /Alkaline butt/slant
(22) (18)Gas production slant, H_2S
production

Escherichia sp. *Salmonella* sp.
Enterobacter sp. (1)
(3)

Fig 1: Overview of identity of isolates of bovine mastitis up to genus level

3.2Identification of streptococcal isolates of bovine mastitis

Isolates of streptococci were subjected to Voges Proskauer's, esculin hydrolysis, arginine utilization, sucrose fermentation to identify the species. A total of 10 isolates (M4, M6, M9, M12, M13, M22, M39, M40, M44, M45) were identified based on positive results for Voges-Proskauer's, esculin hydrolysis, arginine utilization, sucrose fermentation as *Streptococcus uberis*after comparing with identification key. Similarly, another 12 streptococcal isolates matched with identification of *S. agalactiae* (M2, M3, M5, M15, M17, M18, M19, M26, M30, M31, M34, M36) based on specific tests where in the isolates hydrolyzed esculin and fermented sucrose where as VP test arginine tests were negative. Isolates of streptococci of 22 numbers when subjected to specific tests like Voges-Proskauer's, esculin hydrolysis, arginine utilization, sucrose fermentation, 10 were identified as *Streptococcus agalactiae* while 12 were placed under *Streptococcus uberis*based on identification key (Table 1).

Table 1: Identification of streptococcal isolates obtained from bovine mastitis up to species level

Fig 1: Overview of genus identification of isolates of bovine mastitis

Biochemical tests conducted	M4, M6, M9, M12, M13, M22, M39, M40, M44, M45 (10)	M2, M3, M5, M15, M17, M18, M19, M26, M30, M31, M34, M36 (12)
Voges - Proskauer	-	+
Esculin Hydrolysis	+	-
Arginine Utilization	+	+
Sucrose fermentation	+	+
Identity	***Streptococcus uberis*** **(10)**	***Streptococcus agalactiae*** **(12)**

Biochemical tests **Isolate codes**

Note:

- All the 22 isolates were Gram positive cocci in chains with catalase andoxidase negative, non-oxidative but fermentative (O^-F^+)

3.2 Identification of species of *Staphylococcus*obtained from bovine mastitis

All the 18 staphylococcal isolates after conducting specific test for speciation such as Voges-Proskauer's, urease production, utilization of mannitol, arabinose and maltose, the isolates belonged to *Staphylococcus delphini*(1); *S. gallinarium*(1),*S. carnosus* (1); *S. equorum*(6); *S. aureus* (3) and *S. epidermis* (6). This indicated predominance of both the species *S. equorum*and *S. epidermis* followed by *S. aureus* in causing mastitis among the herd.

Table 2: Identification of staphylococcal isolates obtained from bovine mastitis up to species level

	M1	M16	M7, M10, M23, M24, M37, M31	M35, M28, M21	M20	M8, M11, M25, M29, M32, M43
Staining and	**(1)**	**(1)**	**(6)**	**Isolate code** **(3)**	**(1)**	**(6)**
Voges - Proskauer's	-	-	-	+	+	+
Urease	+	+	+	+	-	+
Mannitol	+	-	+	+	+	-
Arabinose	-	+	+	-	-	-
Maltose	+	+	-	+	-	+
Identity	***Staph. delphini*** **(1)**	***Staph. gallinarum*** **(1)**	***Staph. equorum*** **(6)**	***Staph. aureus*** **(3)**	***Staph. carnosus*** **(1)**	***Staph. epidermis*** **(6)**

Note:

- All the 18 isolates were Gram positive cocci in bunches with catalase positive, oxidase negative, both oxidative and fermentative (O^+F^+)

3.3 Identification of Gram negative isolates of bovine mastitis

Gram negativeisolates of 5 numbers were subjected to Indole, methyl red, Voges-Proskauer's and citrate utilization tests. As per the results obtained for these biochemical tests, the 3 isolates (M14, M33, M38) belonged to *Escherichia* genus with species *E. coli*; while one (M27) was identified as *Salmonella enterica* and last one of Gram-negative isolate (M42) matched with *Alcaligenes* sp. (Table 3). As per the results obtained for the biochemical tests, like IMViC for 5 isolates of Gram negative rods catalase positive and oxidase negative with fermentative nature, the 3 isolates were *E*. *coli*, while one *Salmonella enterica* and one may be *Alcaligenes* sp.

Table 3: Identification of Gram negative bacterial isolates of bovine mastitis

Biochemical tests conducted	**M14, M33, M38 (3)**	**M27 (1)**	**M42 (1)**
Gram staining	-		
Catalase	+		
Oxidase	-		
Oxidative Fermentative	O^-F^+ (not oxidative , only fermentative)		O^-F^- (not oxidative and not fermentative)
TSI-H_2S	Acid butt, Acid slant, Gas production	Acid butt, Alkaline slant, H_2S production	Alkaline butt, Alkaline slant, No gas production
Indole	+	-	-
Methyl red	+	+	-
Voges-Proskauer's	-	-	+
Citrate	-	+	+
Urease	-	-	-
Identity	***Escherichia coli* (3)**	***Salmonella enterica* (1)**	***Alcaligenes* sp. (1)**

3.4 Percent occurrence of species of mastitic causing bacteria

Out of 45 isolates (100%) of bovine mastitis, 26.7% (12) *streptococcus agalactiae*,22.2% *Streptococcus uberis* (10); 13.3% *staphylococcus equorum* (6), 13.3% *Staphylococcus epidermis* (6), 6.7% *Staphylococcus aureus* (3) and 2.2% each of *Staphylococcus delphini*(1), *Staphylococcus carnosus*(1)and *Staphylococcus gallinarum*(1) were found.Among Gram negative isolates,6.7% *Escherichia coli* (3), 2.2% *Salmonella enterica* (1) and 2.2% *Alcaligenes* sp. (1) were identified based on their biochemical characteristics.

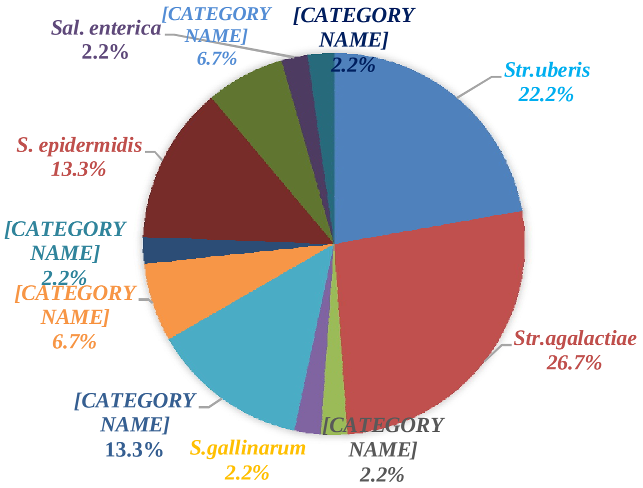


Fig 2: Per cent Occurrence of bacterial species obtained from bovine mastitic milk samples

Discussion:

In comparison to the present study where predominance of species of *Streptococcus* followed by *Staphylococcus* and Gram negative isolates were noticed. But on the contrary, among 2,767 positive clinical mastitis milk samples, *E. coli* was most frequently isolated (14.4%), followed by *Klebsiella* spp. (13.0%), Coagulase negative Staphylococci (11.3%), *Strep. dysgalactiae* (10.5 %), *Staph aureus* (10.2%) and other streptococci (8.0%). *Enterobacter* spp., *Strep. agalactiae* and *Strep. uberis* were isolated in 5.5, 2.8 and 2.1% of samples, respectively[12]. A high frequency of *S. aureus* (18*), S. agalactias* (13), *S. epidermis* (7), *S. saprophyticus* (3) while in combination was *S. aureus* + *S. uberis*(8), *S. agalactiae* + *S. saprophyticus* +*S. uberis*(1), *S. uberis* + *S. agalactiae* (1),in the milk collected from 51 buffaloes suffering from mastitis in livestock research farm, Haryana [10]. Even major prevalence of *Staphylococcus* spp. (42.55 %) followed by *E. coli* (21.28%) was noticedin mastitic milk samples collected in and around Meerut. Among the other commonly isolated pathogens like *Streptococcus* spp. (6.38 %), *Proteus* spp. (8.51 %), mixed infection (Gram positive and Gram-negative bacilli of 18.09 %) and *Candida* spp. (3.19 %) were also reported [11]. The major mastitis causing bacteria isolated from clinical cases of bovine mastitis (200 CMT positive milk samples) from Kashmir valley were *Staphylococcus* spp. showing highest prevalence of 46.4% followed by mixed infection with *Staphylococcus* and *Streptococcus* (20.8%), *Streptococc*us spp. (18.4%) and *Escherichia coli* (14.4%) [13]. In comparison to the present study where *Streptococcus* spp. Was more prevalent, but coagulase positive *Staphylococcus* species of 74.7 % with 38.6 % as *Staphylococcus aureus*, 28.9 % as *Staphylococcus intermedius*and 67.2 % as *Staphylococcus hyicus.* As well as other bacteria like *E. coli* 14.5 % and *Streptococcus* sp. 2.4 % were also isolated from milk samples positive for clinical mastitis of north-west Pakistan [12]. The present study had lower occurrence of Staphylococci and *E.coli* but in astudy were mastitic milk was collected from north west Pakistan, the most common bacteria recovered were *Staphylococci* spp. (34 %), followed by *Escherichia coli* (19 %), *Streptococci* spp. (9 %), and *Klebsiella* spp. (8 %) while minor bacteria included *Salmonella* spp. (2 %), *Proteus* spp. (1 %) and *Candida* spp. of 0.6 % [14]. Almost the same trend of mastitic bacteria were observed in 86 bovine mastitic milk samples from farms of Ludhiana when subjected to cultural, morphological, biochemical tests and PCR. The results revealed 33 (34.02%) *Staphylococcus aureus,* 24 (24.74%) *Escherichia coli,* 16 (16.49%) *Klebsiella pneumoniae,* 5 (5.15%) *Pseudomonas aeruginosa*, 2 (2.06%) *Streptococcus agalactiae,* 12 (12.37%) *Bacillus* spp. and *Serratia marcescens* 5 (5.15%) [15].*Staphylococcus aureus* was found to be the prime etiological agent involved in the mastitic milk samples of dairy cows of Jammu region, accounting for 379 (34%) out of 1100 bacterial isolates followed by *Escherichia coli* (235 numbers with 21%) and other environmental pathogen*Streptococcus agalactiae* (115 numbers- 10%) [16]. Gram negative bacterial isolates like *Klebsiella pneumoniae* (15.35%) accounted for the highest proportion, followed by *Escherichia coli* (13.70%), *Pseudomonas aeruginosa* (12.33%), while Gram positive *Streptococcus agalactiae* (10.5%) was lower in 208 clinical milk samples from cows with mastitis of four farms in the Jiangsu Province, China [17].*Staphylococcus aureus* and coagulase-negative staphylococci were the most common in subclinical mastitis milk samples (17.1%) while *Mycoplasma bovis* was a predominant pathogen (13.4%) in the majority of herds (29.2%) and accounted for 48.9% of positive Clinical mastitis milk samples of dairy farms of Bosnia and Herzegovina followed by *Escherichia coli* (34%) and *Streptococcus agalactiae* (10.6%) [18].

4. CONCLUSION

Bacterial isolates of 45 numbers from California Mastitis Test (CMT) positive milk samples in and around Bangalore obtained from the Dept of Veterinary Microbiology, Veterinary College, KVAFSU, Bengaluru, 22isolates were streptococci and when subjected to battery of specific identification tests found that 10 isolates as *Streptococcus uberis*, another 12 as *Streptococcus agalactiae*. Among 18 staphylococcal isolates, based on specific biochemical tests, the species were *S. delphini*(1); *S. gallinarium*(1),*S. carnosus* (1); *S. equorum*(6); *S. aureus* ssp. *aureus* (3) and *S. epidermis* (6). Gram negative isolates of 5 numbers when subjected to indole, methyl red, Voges-Proskauer tests and citrate utilization, urease test, 3 isolates belonged to genus *Escherichia* with species *E. coli*; while one was identified as *Salmonella enterica* and last one of Gram-negative isolate matched with *Alcaligenes* sp.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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