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Teat Health and Mastitis in Buffalo

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ABSTRACT

Background: With a focus on the teat end microbiota and their molecular characterisation, the goal of the current study is to determine how teat health characteristics affect the development of this condition.

Methods: Total 100 lactating dairy buffaloes of different parity were screened for mastitis. A battery of tests, including the California mastitis test (CMT), white side teat (WST), pH and somatic cell count (SCC), were used to screen buffaloes for mastitis. Samples that showed a strong positive reaction were chosen. Teat-end swabs collected for bacterial examination and analyse for molecular characterization and phylogenetic analysis.

Result: Significant correlation (P<0.01) was established between teat end score and all other teat parameters namely teat skin color score, teat roughness, teat chapping and teat condition score. The amplification of 16S r DNA by PCR with universal primer 27F-1492R which span nearly full length of 16 SrRNA gene with expected amplicon of 144-1500bp. If farmers are to be properly prevented, awareness among them must be raised. It was anticipated that if the microbial load were to decrease, so would the likelihood of contracting an infection. The udder health kit, which was made with locally created pre- and post-milking dips and cleaning chemicals, decreased the bacterial load, enhanced the teat health score and improved the milk profile and could thus be replicated for use in the field.

Key words: Bacterial examination, Buffalo, Mastitis, Molecular characterization, Somatic cell count, Teat score.

INTRODUCTION

Mastitis is the most commonly encountered and discussed problem in dairy animals that ensues when microorganisms overcome a three-tiered defense of udder immune system comprising of physical barriers and non-specific and specific immune responses to initiate inflammatory process (Sordillo *et al*., 2002). Physical barriers of the udder comprises of anatomic features of the teat and associated structures namely the teat skin, teat sphincter muscle and keratin plug that block the invading bacteria at the teat sphincter, the point of entry. The causative pathogen may be present near the opening of the teat canal, either through dirty and wet conditions at the teat end, through teat end lesions or colonization, on contaminated surfaces of milking units (liners or claws), or cow prep procedures. Teat health thus plays an important role in prevention of mastitis. When these pathogens enter mammary glands through teat canal, they colonize, proliferate and release toxins, damaging the mammary gland cells (Quirk *et al*., 2012) and initiating inflammatory reaction. Teat health or mastitis play important role in dairy farming it causes huge economic loss to the farmers. Therefore teat health is an important predisposing factor for mastitis and can be addressed for the prevention and control of mastitis. The present work was therefore designed to study the effect of teat health attributes as a predisposing factor in development of this disease with special emphasis on teat end microbiota and their molecular characterization.

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MATERIALS AND METHODS

Screening of buffaloes for sub clinical/clinical mastitis

Total 100 lactating dairy buffaloes of different parity were screened for mastitis. The animals were selected randomly

and grouped on basis of Teat condition score. Thorough examination of udder was performed for the detection of any abnormality in the udder *viz.* presence of any lesion, pain, heat and swelling. Milk from each quarter was striped out for detecting the abnormality in the milk like colour and consistency. Screening of buffaloes for mastitis was performed with the help of battery of tests namely: California Mastitis Test (CMT), White side teat (WST), pH and Somatic Cell Count (SCC) to detect mastitis in and samples showing strong positive reaction were selected. These examinations were continued until final selection of buffaloes (Radostits *et al*., 2010).

Teat health assessment

Teat health was assessed by scoring the teats for teat end condition, teat skin color, teat dryness, teat skin chapping and teat end bacterial analysis

Teat end condition was scored on a 1 to 4 scale, corresponding to the visibility of a hyperkeratosis ring. A score of 1 indicated no visible ring, 2 indicated moderate visibility, 3 indicated complete visibility and 4 indicated extreme ring thickness (NMC, 2007).

Teat skin color was evaluated by examining any change in color tint or pigment. A score of 1 indicated a teat with normal coloring, 2 indicated a hint of blue or red coloring and a score of 3 indicated complete reddening of teat skin (NMC, 2007).

Teat dryness/roughness was evaluated using a friction test examining resistance upon touch determined by the scorer (Reeves *et al*., 2017). A score of 1 indicated a smooth surface with no frictional resistance involved, 2 indicated slight frictional resistance and slight roughness and a 3 indicated extreme roughness and a high frictional resistance level (NMC, 2007).

Teat skin chapping was evaluated by observing cracks on the skin surface. A score of 1 indicated a smooth surface with no visible cracking, 2 indicated minor appearances of skin cracks and 3 indicated extensive and irritated skin cracking (NMC, 2007).

Teat-end swabs collection and analysis of bacterial count

Two teat-end swabs from each buffalo were collected individually from both hind quarters by rotating a moistened cotton swab, covering an area of 2 cm^2 outside the teat orifice. The first swab was taken immediately after udder sanitization whereas the second swab was performed on another quarter after applying the udder wash followed with teat spray solution. Teats were sprayed with the oil and allowed contacting buffalo's teat skin for 30 s and subsequently dried using a dry and single use sterile towel to remove the surplus oil. The teat-end swabs were placed in a separate test tube containing 0.1% peptone water and stored in ice container until analyzed following all aseptic measures. All teat-end swabs were analyzed for bacterial counts, *i.e.* total bacterial count (TBC), Staphylococcal count (STA), Streptococcal count (STR) and Coliform count (COL). The swab tubes were vigorously shaken using vortex mixer for 30s to extract the bacteria from the cotton swab and the cotton tips were then removed.

Molecular characterization of teat end swabs

Molecular characterization and PCR were carried out by Cytogene lab, Lucknow using Sanger's Method.

Sequence alignment and data analyses

The obtained forward and reverse sequences were aligned using online pairwise alignment tool BioEdit. The query sequences were identified considering *E* value a $<1 \times 10^{-5}$ and maximum hits (99 or 100%) with a species in the reference database NCBI. In addition to BLAST, MEGA X was used for phylogenetic tree analysis employing Maximum Likelihood (ML) method.

Stastical analysis

Statistical analysis of data was done by using SPSS 20 software.

RESULTS AND DISCUSSION

Influence of teat health on prevalence of mastitis

Teat end condition

Prevalence was studied for bufflaoes with different teat end scores. The prevalence in buffaloes with teat end score 1 was 45.94% (Sub clinical: 43.24% and clinical: 2.70%) which was 23.28% lower than buffaloes with teat end score 2 *i.e.* 69.23% (Sub clinical: 46.15% and clinical: 23.08%). The prevalence of clinical mastitis was much higher in animals with teat end score 2. A significant correlation ($P<0.05$) was established between teat end score and prevalence as well as with somatic cell count. Significant correlation ($P<0.01$) was established between teat end score and all other teat parameters namely teat skin color score, teat roughness, teat chapping and teat condition score.

Teat skin chapping

Teat skin chapping was evaluated by observing cracks on the skin surface with increased score referring to more visible cracks (Fig 1). Highest prevalence was recorded in buffaloes with teat chapping score 3 (80%) with prevalence of clinical mastitis also highest in this group of buffaloes (20%). The prevalence of subclinical mastitis was also highest in group 3 (60%). Cracks on the skin harbor the dirt/milk after milking which in turn increases the microbial load, a predominant cause of mastitis. No significant correlation could however be established between teat chapping and status of mastitis. Teat chapping however had significant correlation ($P<0.01$) with teat roughness, teat skin color score, teat end score and teat condition score.

Teat roughness score

Teat roughness had a significant role in prevalence with buffaloes with teat roughness score 3 having highest overall

prevalence (87.5%) followed by teat roughness score 2 buffaloes (58.82%) and 36% in buffaloes with score 1. Maximum number of clinically affected buffaloes were from score 3 (37.5%). The prevalence of subclinical mastitis was maximum in score 2 (52.94%), 2.94% higher that score 3 (50%). This increase can be attributed to more number of buffaloes screened with score 2 (17) in comparison to score 3 (8). Group 1 had least prevalence as the skin surface was smooth and healthy. A significant correlation ($P<0.05$) was established between teat roughness score and prevalence as well as with somatic cell count. Significant correlation ($P<0.01$) could also be established between teat roughness and all other teat parameters namely teat skin color score, teat end score, teat chapping and teat condition score.

Teat skin color score

Color was evaluated by examining any change in color tint or pigment (Fig 1). In the present study maximum buffaloes had a score of 1 having normal coloring followed by score 2 buffaloes and score 3 buffaloes. All the buffaloes with score 3 had only clinical mastitis (100%) followed by score 2 buffaloes (overall prevalence 73.33%, Sub clinical mastitis 46.67% and Clinical mastitis 26.67%) and score 1 buffaloes that had overall prevalence 37.5% with no cases of clinical mastitis. Red discoloration is an indication of genesis of inflammation and thus score 3 buffaloes were clinically affected buffaloes. A significant correlation ($P<0.01$) was established between teat skin color score, teat end score, teat roughness, teat chapping, teat condition score, status of mastitis as well as with somatic cell count.

Teat condition score

Buffaloes with teat condition score 3 had highest overall prevalence (71.43%) with highest prevalence of clinical mastitis (42.86%). Sub clinical mastitis was highest in score 2 buffaloes with prevalence 55.56% and overall prevalence of 61.11%. Teat condition score had a significant correlation ($P<0.01$) with all teat parameters discussed, status of mastitis as well as with somatic cell count (Table 1).

All teat-end swabs were analyzed for bacterial counts, *i.e.* total bacterial count (TBC), staphylococcal count (STA), streptococcal count (STR) and coliform count (COL). The total bacterial count declined significantly ($P<0.01$) after spraying with oil from $\log_{10}$TBC of 8.55 to 4.29 after 15 minutes

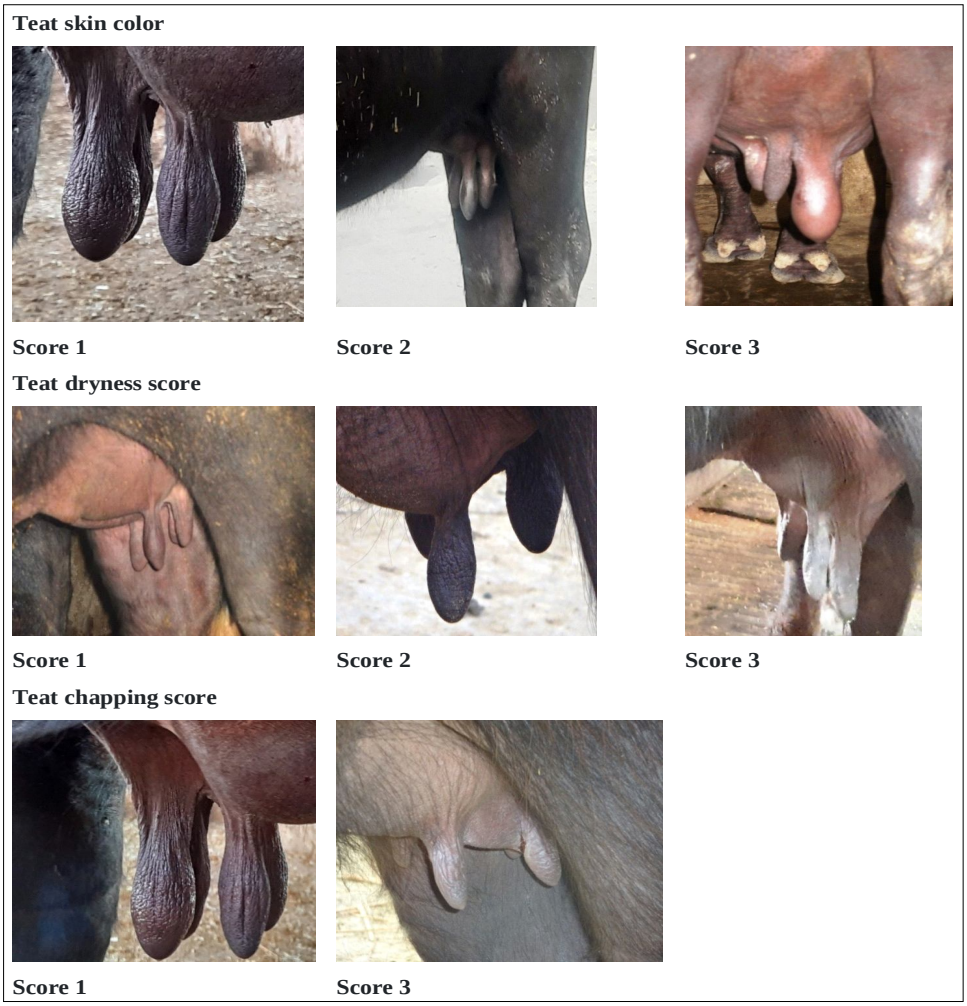

Fig 1: Showing teat skin color score, teat dryness score, teat chapping score.

of application of oil.in control untreated quarter no decline in the TBC was recorded. The *E. coli* count also declined significantly (P<0.01) from log_{10}COL 8.26 to 4.88 in O-4 treated group. In control group the log_{10}COL increased from 8.27 to 8.46. Significant decline of Log_{10} *S. aureus* count and *Streptococcal* count was also recorded after application of oil from 7.01 to 4.83 and 7.13 to 4.91.

Molecular characterization of Teat end surface microflora

Teat end swabs were collected asceptically to isolate the common micro organisms present and to assess the total bacterial count. The common bacteria isolated were *E. coli, S. aureus* and *Streptococcus* sp. The log_{10}TBC was 8.55. The log_{10} *E. coli* count (log_{10}COL) was 8.26, Log_{10} *S. aureus* count and *Streptococcal* count was 7.01 and 7.13 respectively.

Bacterial counts

The isolates (06) were subjected for molecular characterization to identify the strains present in this area. The amplification of 16S r DNA by PCR with universal primer 27F-1492 R which span nearly full length of 16 SrRNA gene with expected amplicon of 144-1500 bp, the band was excised from gel and purified for sequencing (Fig 2). Phylogenetic tree was constructed and five different organisms were identified with highest percentage identity (Fig 3, 4, 5, 6, 7, 8).

The possible association between teat morphometric traits and subclinical mastitis (SCM) in dairy buffaloes was studied by Kaur *et al.* (2018). They concluded that teat morphometric traits seem to be associated with indicators of udder health in buffaloes, thus, their inclusion in breeding programmes for selection against undesirable dairy type traits may be of value in reducing susceptibility to intramammary infections in Indian buffaloes. Fox and Norell, (1994) reported that teat and udder skin should be healthy before milking and free of sores, wounds, or chapping where *S. aureus* could colonize the teat end and surrounding skin, one of the most predominant cause of mastitis.

Fox (1992) and Fox *et al.* (1991) demonstrated a correlation between teat skin condition and colonization of skin by *Staphylococcus aureus* and stated that rough or chapped skin will provide more places for bacteria to attach and survive. An impact on udder health and mastitis can be anticipated. McKinzie and Hemling (1996) showed an impact of teat skin condition on milk yield and milk out time. In the present study the use of pre milking wash and post milking essential oil combination spray significantly reduced the bacterial counts and it is therefore hypothesized to be of potent use in prevention of mastitis and its efficacy was further assessed changes in milk profile and teat health. Zecconi *et al.* (2006), found a significant increase in new intramammary infections with an increase of 5% in the thickness of the edges of teats. Santos and Fonseca (2007) opined that mainting the teat ends in good conditions is of primary importance because its sphincter muscle plays a

Table 1: Correlation between teat health score, somatic cell count and status of mastitis.

		Teat condition score	Teat end	Teat chapping	Teat roughness	Teat skin color	SCC	Status
Teat condition score	Pearson Correlation	1	.649**	.707**	.772**	.677**	.401**	.386**
	Sig. (2-tailed)		.000	.000	.000	.000	.004	.006
	N	50	50	50	50	50	50	50
Teat end score	Pearson Correlation	.649**	1	.524**	.825**	.756**	.356*	.324*
	Sig. (2-tailed)	.000		.000	.000	.000	.011	.022
	N	50	50	50	50	50	50	50
Teat chapping	Pearson Correlation	.707**	.524**	1	.703**	.467**	.250	.113
	Sig. (2-tailed)	.000	.000		.000	.001	.080	.436
	N	50	50	50	50	50	50	50
Teat roughness	Pearson Correlation	.772**	.825**	.703**	1	.711**	.296*	.305*
	Sig. (2-tailed)	.000	.000	.000		.000	.037	.031
	N	50	50	50	50	50	50	50
Teat skin	Pearson Correlation	.677**	.756**	.467**	.711**	1	.382**	.377**
	Sig. (2-tailed)	.000	.000	.001	.000		.006	.007
	N	50	50	50	50	50	50	50
SCC	Pearson Correlation	.401**	.356*	.250	.296*	.382**	1	.869**
	Sig. (2-tailed)	.004	.011	.080	.037	.006		.000
	N	50	50	50	50	50	50	50
Status	Pearson Correlation	.386**	.324*	.113	.305*	.377**	.869**	1
	Sig. (2-tailed)	.006	.022	.436	.031	.007	.000	
	N	50	50	50	50	50	50	50

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

crucial role in teat canal contraction keeping it closed between milkings, thereby preventing the entry of pathogens into the mam-mary gland. This action is aided by mature keratin cells present in the teat canal and together they represent the primary resis-tance barrier to mastitis. Kaur *et al.* (2018) thus were of the view that teat morphometric traits seem to be associated with indicators of udder health in buffaloes, thus, their inclusion in breeding programmes

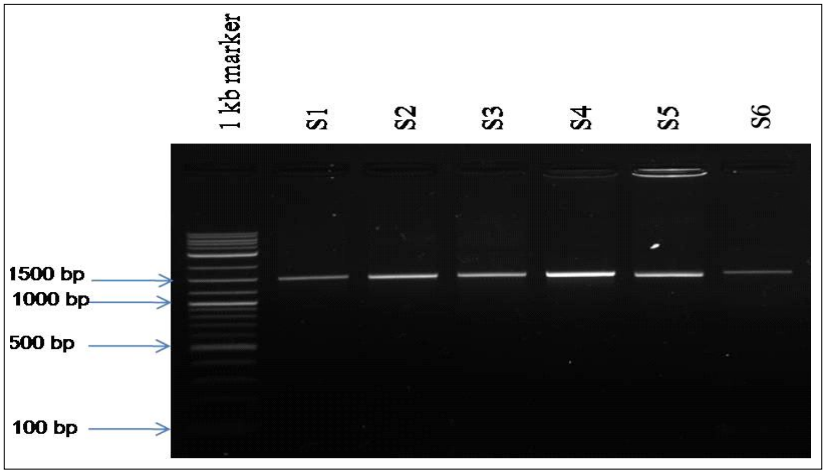

Fig 2: PCR (1.2% Gel); Lane 1- 1 kb ladder; lane 2- S1; lane 3- S2; lane 4- S3; lane 5- S4; lane 6- S5; lane 7- S6 (Approx size: 1.2-1.4 kb).

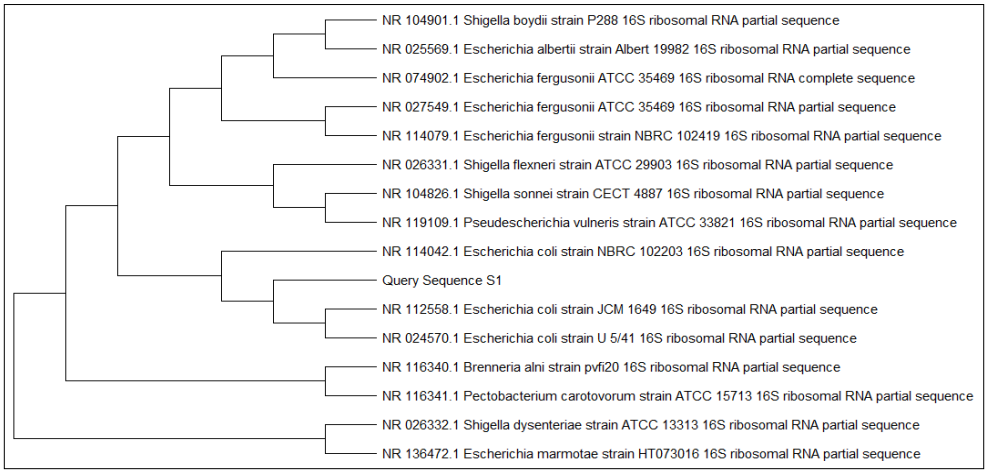

Fig 3: Phylogenetic tree of S1 identified as *E. coli* with highest percentage identity with it.

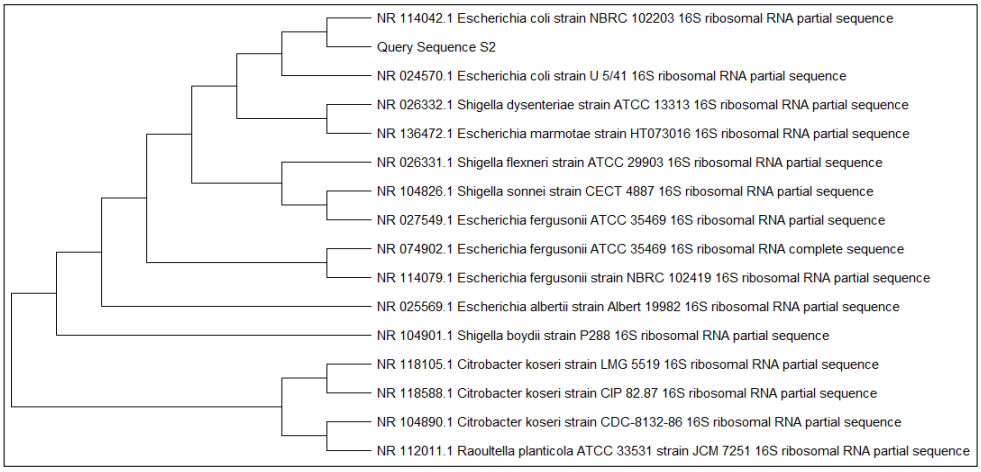

Fig 4: Phylogenetic tree of S2 identified as *E. coli* with highest percentage identity with it.

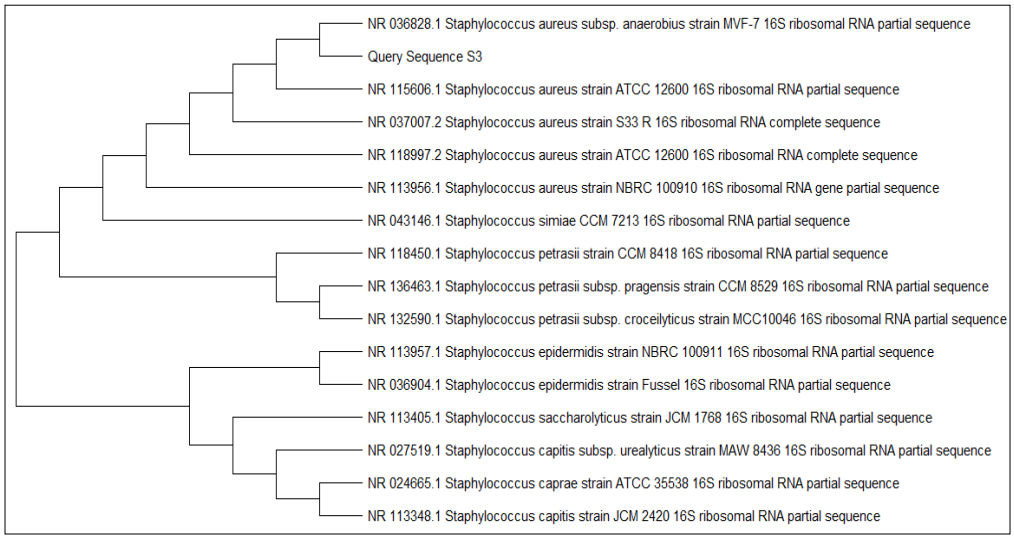

Fig 5: Phylogenetic tree of S3 identified as *S. aureus* with highest percentage identity with it.

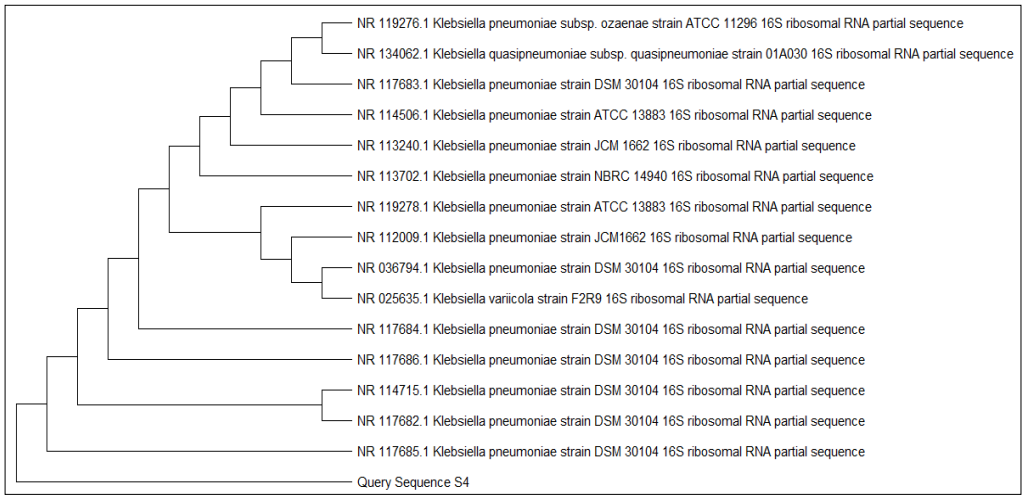

Fig 6: Phylogenetic tree of S4 identified as *K. pneumoniae* with highest percentage identity with it.

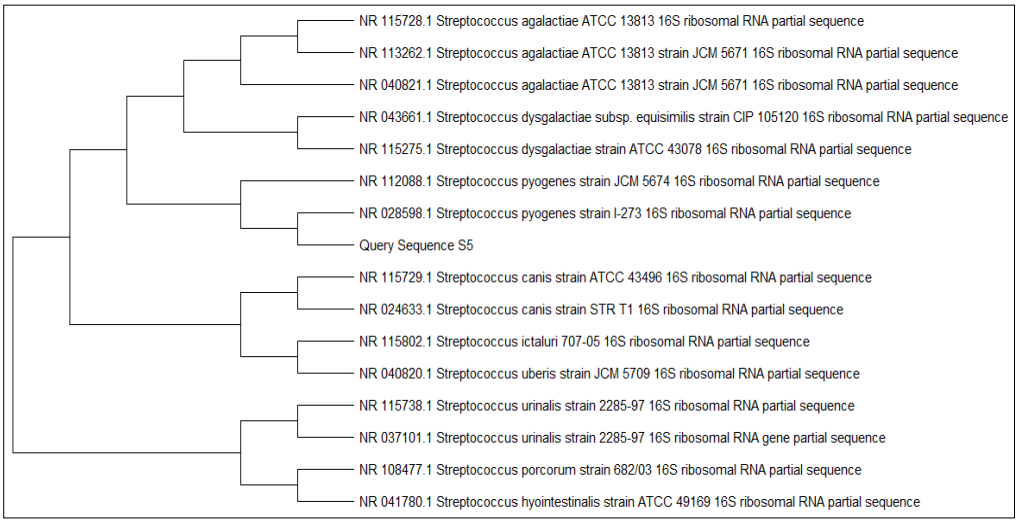

Fig 7: Phylogenetic tree of S5 identified as *S. pyogenes* with highest percentage identity with it.

Fig 8: Phylogenetic tree of S6 identified as *S. agalactiae* with highest percentage identity with it.

for selection against undesirable dairy type traits may be of value in reducing susceptibility to intramammary infections in Indian buffaloes. Prior to its use awareness campaigns were conducted among dairy owners screening their buffaloes milk and briefing about the ill effects of mastitis. Demonstration of the best package of practices and of the developed kit was done to sensitize the villagers and the farmers got convinced the kit was distributed which becomes popularize day by day among them.

CONCLUSION

The greatest way to prevent mastitis is to make changes to the environment, host and microorganisms on all three levels. As a result of the interaction between these elements, illness results. Farmers needed to be made more aware of the disease's development and prevention because it was more prevalent in animals kept under poor management techniques. Awareness amongst farmers needs to be created if effective prevention is desired. It was assumed that if the microbial load is reduced, the chances of gaining infection too decreases especially if the host and environment factors especially udder hygiene are also taken care. The udder health kit prepared with indigenously developed pre and post milking dips and cleaning products reduced the bacterial load, improved teat health score and milk profile and can thus be propagated for use in field condition.

Contribution of authors

SV: Researcher executed the experiment, JP and Vibha Yadav: Designed the research, Dinesh Yadav, RK: Analyzed the samples, DP: Critically revised the manuscript, Dinesh Yadav, Rakesh Gupta: Analysed the data.

Conflict of interest: None.

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