IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

IDENTIFICATION OF STAPHYLOCOCCUS CAPRAE FROM GOAT MILK

[1] Sumita Dasgupta [2] Snehal Singh

[1]Assistant Professor, [2] Post graduate student

[1]Biotechnology Department, Bhagwan Mahavir College of Science and Technology, Surat, Gujarat, India

Abstract: *Staphylococcus caprae*, reported as human pathogen was first described to be present in goat milk in 1983.The current study was undertaken to determine the presence of *S. caprae* in goat milk procured from different regions of Surat city located in South Gujarat. Five samples of goat milk were obtained from different dairies of Surat. Isolated bacterial strains were characterized on basis of colony morphology, gram reaction and motility. The gram positive, non-motile, cocci isolates were further tested for catalase and oxidase production. A total of 05 isolates found to be catalase-positive and oxidase-negative were provisionally identified as *Staphylococcus caprae.* Molecular identification using 16S rRNA sequence was carried out. The sequence was used to carry out BLAST with the database of NCBI GenBank. Based on maximum identity score first fifteen sequences were selected and aligned using multiple sequence alignment software programs. The strain was found to be Staphylococcus *caprae* based on nucleotide homology analysis.

***Key words - Staphylococcus caprae*, catalase, oxidase, 16S rRNA**

INTRODUCTION

Goat (*Capra hircus*), after cow milk, is one of the main sources of milk and milk products consumed. Goat milk represents an excellent source of food to human nutrition (Silva and Costa, 2019). Milk and other goat-derived products contain several bioactive compounds that might be useful in patients suffering from a variety of chronic diseases. Several peptides, fats, and oligosaccharides present in goat's milk can be potentially useful in cardiovascular disease, metabolic disorders, neurological degeneration, or in promoting intestinal health. They have also shown chemopreventive properties in cancer.In addition, the oligosaccharides present in goat's milk have immunomodulatory properties, prevent adhesion of pathogenic bacteria, and have prebiotic, probifidogenic effects. Due to its potential health benefits, goat milk is particularly recommended for infants, older adults, and convalescing people (Lima et.al. 2016). Even though goat milk has high nutritional content it was reported that *Staphylococcus caprae*, a human pathogenic bacteria to be present in goat milk. This organism was initially isolated from goat milk in 1983 and is known to colonize the skin and mammary glands of goats (Seng et.al., 2014; Mazur et.al. 2017).This bacterium has been reported in several cases as a human pathogen causing peritonitis, meningitis, urinary tract infections,endocarditis, endophthalmitis, prosthetic joint infections, recurrent sepsis, bacteraemia and osteomyelitis. Many risk factors for *S. caprae* have begun to emerge and include immunosuppression, diabetes, chronic renal failure,obesity, open or traumatic fractures and contact with sheep or goats (Hilliard et.al., 2017).The current study aims to determine the occurrence of *S. caprae* strains in goat milk obtained from different regions of Surat, located in South Gujarat.

Bacterial strains were isolated from samples of goat milk. The isolates were characterized morphologically, biochemically and at molecular level , based on 16S rRNA technique.

MATERIALS AND METHODS

Sample Collection

Five goat milk samples were collected from different dairies of Surat city in Gujarat. The samples were collected in sterile carriers and were kept in a refrigerator (around 4°C) till the analysis begins.

Isolation of the bacterial strains

Once delivered to the laboratory, they were taken to the procedure for isolation. Pour plate technique was used to isolate the organism. 10 milliliters of each milk samples were homogenized with sterilized peptone physiological saline solution (1% peptone, 0.9% NaCl) for about 1-3 minutes aseptically. Appropriate serial dilution (10^{-1} to 10^{-6}) was prepared for each sample using 1mililiters of homogenate. A volume of 0.1 milliliters of appropriate dilutions was spread plated on MRS (Man, Rogosa and Sharpe) agar media . Then the plates were incubated for 48hours in anaerobic jar at 37ºC (Wassie and Wassie,2016).

Identification of the isolated Bacteria

The bacterial colonies were selected and purified by streaking on fresh MRS agar plates. The isolates were examined on the basis of their colony morphology, gram reaction and motility. Gram positive non motile cocci were tested for Catalase and Oxidase production. The morphological, physiological and biochemical characteristics help in preliminary identification of the isolates.

Molecular Identification using 16s rRNA

DNA was extracted from strains that grew in MRS by a revised Cetyltrimethylammonium bromide (CTAB) method. Purified DNA template was diluted to 100 ng/µL for 16S rRNA gene amplification (Wang et.al., 2016). DNA was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA was observed. Fragment of 16s rRNA gene was amplified by PCR. A single discrete PCR amplicon band was observed when resolved on Agarose. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with 357F (5'-CTCCTACGGGAGGCAGCAG-3') and 1391R (5'-GACGGGCGGTGTGTRCA-3') primers using BDT v3.1 Cycle Sequencing Kit on ABI 3500xl Genetic Analyzer. To confirm the identity of the strains the sequence of the 16S rRNA gene was used to carry out BLAST program with the database of NCBI GenBank. Based on maximum identity score first fifteen sequences were selected and aligned using multiple sequence alignment software programs.

RESULTS

In total 19 numbers of isolates were obtained from the five samples of goat milk collected from different diaries of Surat. 04 isolates were obtained from Sample 1, 05 isolates from sample 2, 02 isolates from sample 3, 04 isolates from sample 4 and sample 5. Of these 19 isolates,08 number of isolates (01 isolate from sample 1, 02 isolates from sample 2, 01 isolate from sample 3 and 02 isolates from each of sample 4 and sample 5) was found to be gram positive, non motile cocci and subsequent test for catalase and oxidase production showed them to be catalase-positive and oxidase-negative. These five isolates were provisionally identified as *Staphylococcus caprae*.

Molecular identification with BLAST (Figure 2) searches in the GenBank database using 16S rDNA sequences (Figure 1) revealed the strain to be *Staphylococcus caprae* based on nucleotide homology analysis. Phylogenetic tree analysis (Fig. 3 and Table 1) showed the relationship between the representative isolates with the known reference strains.

DISCUSSIONS

The discovery of human infections with a species of Staphylococcus originally isolated from goat milk indicted possibility of transmission of these bacteria from animals to humans (Vandenesch et.al., 1995). In the current investigation strains morphologically and physiologically characterized to be gram positive, non motile cocci were tested for catalase and oxidase reaction.

In a previous study by Vandenesch et.al. (1995) catalase-positive, oxidase-negative strains were identified as *Staphylococcus caprae*.

In the present study, strains presumptively identified strains to be *S. Caprae* were subjected to 16S rRNA gene sequences analysis for precise confirmation about the identification of these strains. Phylogenetic tree analysis revealed the strains to be *Staphylococcus caprae* (Table 1).The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.02032500 is shown (next to the branches). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 971 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et.al., 2016).

Sample : >RES-1891_357F

GCAATGGGCGGAGCCTGACGGAGCACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAA
GAACAAATGTGTAAGTAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTAAGTCTGATG
TGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCA
TGTGTAGCGGTGAAATGCGCAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTG
ATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
GGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTC
AAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAAT
CTTGACATCCTCTGACCCCTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGT
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGG
CACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGG
GCTACACACGTGCTACAATGGACAATACAAAGGGTGGCGAAACCGCGAGGTCAAGCAAATCCCCATAAAGTTGTTCT
CAGTTCGGATTAGTAGTCTGCAACTCGACTACATGAAGCTGGAAATCGCTAGTAATCGTGCATCAGCATGCCCACGGA
TGATTACGTTCCCCGGGCTCCTTGCTACACACCCCGGCCCCCGCGGTTCGTAAGCAG

Sample : >RES-1891_1391R

CGTCGTGCATGCTGATCCACGATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGA
ACAACTTTATGGGATTTGCTTGACCTCGCGGTTTCGCCACCCTTTGTATTGTCCATTGTAGCACGTGTGTAGCCCAAAT
CATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCAACTTAGAGTGCCCAACTT
AATGATGGCAACTAAGCTTAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAA
CCATGCACCACCTGTCACTCTGTCCCCGAAGGGGAAAACTCTATCTCTAGAGGGGTCAGAGGATGTCAAGATTTGGT
AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAA
CCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTA
GCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTT
ACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGCTACACATGGAATTCCACTT
TCCTCTTCTGCACTCAAGTTTCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAA
AACCGCCTACGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGT
AGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGACGTGCATAGTTACTTACACATTTTGTTCTTCCCTAATAACAGA
GTTTTTACGATCCGAAAACCTTCATCACTCACGCGGGCGTTGCTCCGTCAGCCTTCCGCCCATTTGCGGAGAATTCC
CCTTACTGCCTAGCTCTCCCCCCCGATAAAGGAAGAGAA

Sample : >RES-1891_consensus sequence

TGACGGAGCACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAG
TAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT
GGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTAAGTCTGATGTGAAAGCCCACGGCTC
AACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAAT
GCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGG
GGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTT
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTCTGACC
CCTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGA
TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTAC
AATGGACAATACAAAGGGTGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTC
TGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTGGATCAGCA

Figure 1. The 16s rRNA sequence

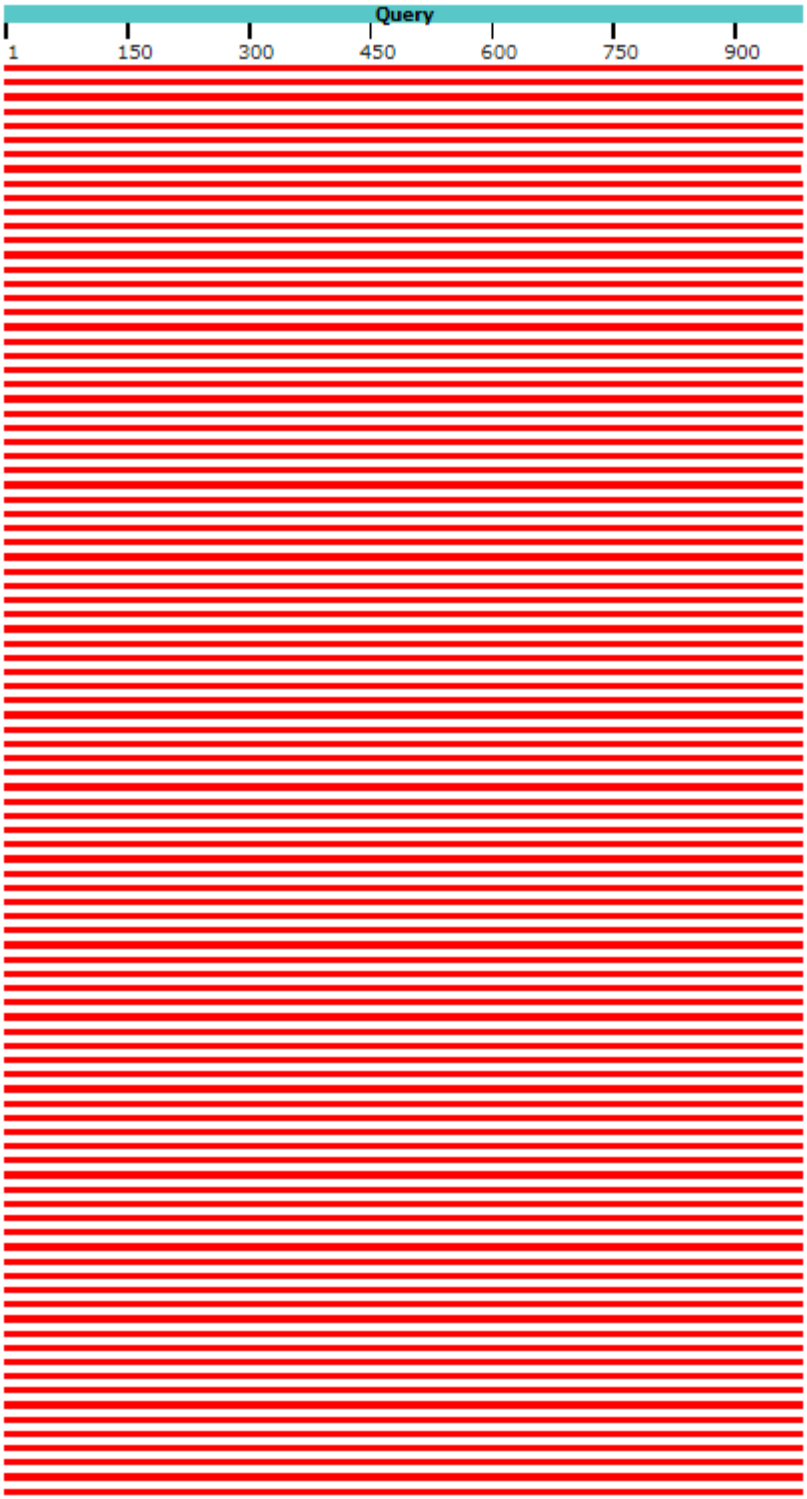

Figure 2 Distribution of the top 100 Blast Hits on 100 subject sequences

Table 1 Sequences producing significant alignments

Description	Max score	Total score	Query cover	Evalue	Ident	Accession
Staphylococcus caprae strain DSM 20608 16S ribosomal RNA, partial sequence	1797	1797	100%	0	99.80%	NR_119252.1
Staphylococcus capitis subsp. urealyticus strain MAW 8436 16S ribosomal RNA, partial sequence	1786	1786	100%	0	99.59%	NR_027519.1
Staphylococcus epidermidis strain NBRC 100911 16S ribosomal RNA, partial sequence	1777	1777	100%	0	99.39%	NR_113957.1
Staphylococcus capitis strain JCM 2420 16S ribosomal RNA, partial sequence	1775	1775	100%	0	99.39%	NR_113348.1
Staphylococcus caprae strain ATCC 35538 16S ribosomal RNA, partial sequence	1775	1775	100%	0	99.39%	NR_024665.1
Staphylococcus epidermidis strain Fussel 16S ribosomal RNA, partial sequence	1775	1775	100%	0	99.39%	NR_036904.1
Staphylococcus capitis strain LK 499 16S ribosomal RNA, partial sequence	1775	1775	100%	0	99.39%	NR_036775.1
Staphylococcus capitis strain ATCC 27840 16S ribosomal RNA, partial sequence	1768	1768	99%	0	99.39%	NR_117006.1
Staphylococcus saccharolyticus strain JCM 1768 16S ribosomal RNA, partial sequence	1764	1764	100%	0	99.18%	NR_113405.1
Staphylococcus saccharolyticus strain S 1 16S ribosomal RNA, partial sequence	1764	1764	100%	0	99.18%	NR_029158.1
Staphylococcus petrasii strain CCM 8418 16S ribosomal RNA, partial sequence	1748	1748	100%	0	98.88%	NR_118450.1
Staphylococcus hominis subsp. novobiosepticus strain GTC 1228 16S ribosomal RNA, partial sequence	1742	1742	100%	0	98.78%	NR_041323.1
Staphylococcus petrasii subsp. jettensis strain SEQ110 16S ribosomal RNA, partial sequence	1740	1740	100%	0	98.67%	NR_118248.1
Staphylococcus haemolyticus strain JCM 2416 16S ribosomal RNA, partial sequence	1738	1738	100%	0	98.67%	NR_113345.1
Staphylococcus petrasii subsp. pragensis strain CCM 8529 16S ribosomal RNA, partial sequence	1736	1736	100%	0	98.67%	NR_136463.1

Figure 3 Phylogenetic tress depicting evolutionary relationships of Taxa

Conclusion

In the current study *S. caprae* was found to be present in all the samples of goat milk procured from different regions of Surat city. *S caprae* has been recognized as human pathogen. It is a matter of concern for human health as raw milk are used for manufacturing many dairy products and goat milk products are considered dairy products with greatest marketing potential (Yadav et.al., 2016). Raw goat milk does not undergo any pathogen elimination or reduction step,therefore any pathogenic contamination, regardless of origin, may pose a risk to public health.Based on the reports obtained in the present investigation, *pasteurizing goat milk* is recommended for human consumption to avoid serious health hazards. However a more detailed study with a larger number of isolates of *S. caprae* is required for a greater understanding of the bacterium isolated from goat milk.

References:

Silva, V.B. and Costa M. P., 2019. Influence of Processing on Rheological and Textural Characteristics of Goat and Sheep Milk Beverages and Methods of Analysis. Processing and Sustainability of Beverages. *Woodhead Publishing.,* 373-412.

Lima, M.J., Teixeira-Lemos, E., Oliveira, J., Teixeira-Lemos, L. P., Monteiro, A., and Costa, J., 2018. Nutritional and Health Profile of Goat Products: Focus on Health Benefits of Goat Milk. *Intechopen*.70321 189-232.

Seng,P.,Barbe,M.,Pinelli,P.O.,Gouriet, F.,Drancourt,M., Minebois, A.,Cellier, N.,Lechiche,C., Asencio,G., Lavigne, J.P., Sotto,A., Stein, A. 2014.Staphylococcus caprae bone and joint infections: a re-emerging infection?. Clin Microbiol Infect., 20:1052-1058.

Mazur, E., Zychowski, P., Juda, M., Korona-Glowniak, I., Niedzielska G, Anna, M., Koziol-Montewka, M. 2017. First report of a Staphylococcus caprae isolated from middle ear fluid of an infant with recurrent acute otitis media. Ann Agric Environ Med., 24(3):357-359.

Hilliard, C.A., Masri, J.E. and Goto, M. 2017.Staphylococcus caprae bacteraemia and native bone infection complicated by therapeutic failure and elevated MIC: a case report. JMM Case Reports, 4(9):1-4.

Wassie, M. and Wassie, T. 2016. Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk. Int. J. Adv. Res. Biol. Sci.,3(8): 44-49.

Wang, D., Liu, W., Ren, Y., De, L., Zhang, D., Yang,Y., Qiuhua Bao, Q., Zhang, H. and Menghe, B. (2016). Isolation and Identification of Lactic Acid Bacteria from Traditional Dairy Products in Baotou and Bayannur of Midwestern Inner Mongolia and q-PCR Analysis of Predominant Species. Korean J. Food Sci., 36(4): 499-507.

Vandenesch, F., Eykyn, S.J., Bes, M., Meugnier,H. Fleurette, J.and Etienne, J. (1995). Identification and Ribotypes of *Staphylococcus caprae* Isolates Isolated as Human Pathogens and from Goat Milk. Journal of Clinical Microbiology,33(4): 888–892.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425.

Nei, M. and Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.
Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Yadav, A.K., Singh, J., Yadav, S.K. (2016). Composition, nutritional and therapeutic values of goat milk: A review. Asian Journal of Dairy and Food Research,35(2):96-102.