



## Subject Area : Medical

# ANTIBACTERIAL ACTIVITY OF COW URINE ON ORAL CARIOGENIC BACTERIA-AN IN-VITRO STUDY

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ARTICLE INFO	ABSTRACT
<p>Article History:</p> <p>Received 11<sup>th</sup> January, 2024</p> <p>Received in revised form 24<sup>th</sup> January, 2024</p> <p>Accepted 14<sup>th</sup> February, 2025</p> <p>Published online 28<sup>th</sup> February, 2025</p>	<p><b>Background:</b> Dental caries is an ubiquitous disease that results from the interaction of specific bacteria and constituents of the diet within plaque formed on the tooth surfaces. Streptococcus mutans is considered as the main Cariogenic microorganism associated with dental caries. Cow urine acts as a most competent agent against a wide range of gram negative and gram-positive bacteria. Thus, the present study was undertaken to analyze the efficacy of Cow urine on Oral Cariogenic Bacteria in-vitro. <b>Objectives:</b> To evaluate the antimicrobial activity of Cow urine on salivary s.mutans counts in caries active individual (In-vitro). To compare the antimicrobial activity of Cow urine with commercially available mouth rinses i.e., Chlorhexidine. <b>Materials and method:</b> Distillates prepared from cow urine and Chlorhexidine samples were assessed for its antimicrobial activity against Streptococcus mutans using agar well diffusion method. Statistical analysis was performed with Analysis of Variance (ANOVA). <b>Results:</b> Both Cow urine and Chlorhexidine samples showed potent antimicrobial activity ranging from 2-5µg/ml against dental caries pathogens, S. mutans. A statistically significant (p&lt;0.05) zone of inhibition was observed for all samples against S. mutans. However, Cow urine resisted the micro-organisms effectively equivalent to Chlorhexidine. <b>Conclusion</b> Samples of cow urine and Chlorhexidine were found to be effective with therapeutic potential against dental caries pathogens. Use of cow urine can be of great importance in treating diseases, especially the microbial one.</p>
<p><b>Key words:</b></p>	
<p>Antibacterial effect, Cow urine, Dental Caries, <i>Streptococcus Mutans</i>.</p>	
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## INTRODUCTION

With pertinent increase in the incidence of antibiotics misuse and overuse, many studies are focusing towards the naturally available products to combat infections in human beings. Since ancient times, the use of natural products has been in practice in both eastern and western traditional medications.<sup>1</sup>

Nature is considered as the primary drug house with a very wide range of animals, plants and innumerable microorganisms acting as an infinite source for discovery, development and supply of various drugs to treat a large variety of clinical conditions.<sup>1</sup>

Cow, Bos indicus is a most valuable animal in all community. Urine is the most effective secretion of cow with various therapeutic uses,

having antimicrobial and antifungal properties. The ancient literature of cow urine has always focused on prevention of disease and maintaining the health and treatment of diseases. Cow urine acts like a magical potion for the treatment of the disease like cancer, asthma, chronic renal failure, hepatitis ABC, urological disorders, and respiratory diseases.<sup>2</sup>

Dental caries is a ubiquitous disease that results from the interaction of specific bacteria and constituents of the diet within plaque formed on the tooth surfaces. Streptococcus mutans are considered the main Cariogenic microorganism associated with dental caries. Amongst the various antimicrobial delivery systems, mouthwashes have been found to be one of the safest and effective vehicles, as they have the ability to deliver therapeutic ingredients to all accessible surfaces in the mouth including interproximal surfaces.<sup>3</sup>

Cow urine acts as a most competent agent against a wide range of gram negative and gram-positive bacteria. However, there is no enough relevant evidence to suggest the antimicrobial activity of Cow

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urine against streptococcus mutans. Thus, the present study was undertaken to analyze the efficacy of Cow urine on Oral Cariogenic Bacteria in-vitro.

Objectives:

- A. To evaluate the antimicrobial activity of Cow urine on salivary s. mutans counts in caries active individual (In-vitro).
- B. To compare the antimicrobial activity of Cow urine with commercially available mouth rinses i.e., Chlorhexidine.

## MATERIALS AND METHODS

The study was conducted on sixty subjects aged 18-30 years having one or more than one inter-proximal carious lesions, well into the dentin, visualized radiographically. The study was conducted in the Department of Oral Pathology, Navodaya Dental College and Central Research Lab, Navodaya Medical College, Raichur. All the necessary ethical guidelines were followed as prescribed by the Institutional Ethical Committee with the number SS013. Written and informed consent was also obtained from all the subjects.

**Collection of saliva sample:** All the subjects were instructed to have an early breakfast on the day of sample collection and made to sit in an erect position on the dental chair and given paraffin block to chew. Approximately 2–3 ml of the stimulated saliva was collected in sterile containers.

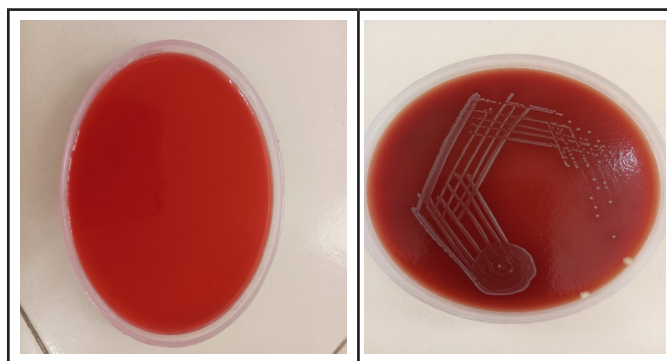
**Collection of Cow urine:** Cow urine was collected from the well-maintained Goshala of local area. Fresh cow urine was collected in a sterile container and is photo-activated by keeping it in sunlight for about 72 hours. All pure Syringe Filters with Hydrophobic Nylon Membrane Disc were used to filter the urine so as to remove any debris and microbial contamination, then stored at 40C.



### Isolation of Streptococcus mutans

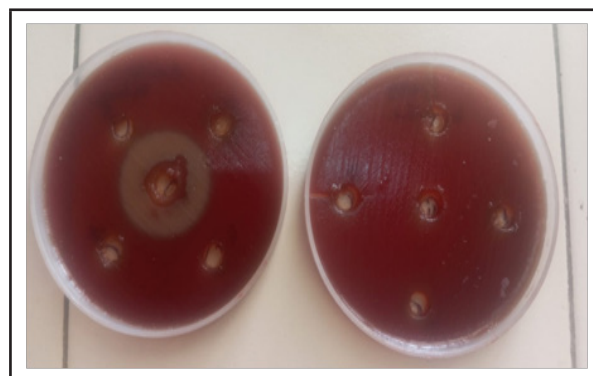
Saliva collected was incubated in sheep agar plates. Colonies of streptococcus were isolated and streaked using Metal Loop SS2 in Mitis-Salivarius agar which is the selective media for S. mutans.

The plates will be incubated at 37 °C for 48 h under 5–20% CO<sub>2</sub>. Streptococcus mutans colonies were identified. Confirmation of mutans streptococci was performed under light microscope.



### Screening for Antimicrobial Activity

The distillates of cow urine and Chlorhexidine were used for the antimicrobial screening using the agar well diffusion method. The media was punched with 7mm diameter wells and were filled with various concentrations of these samples at 2µg/ml, 5µg/ml, 10µg/ml, 25µg/ml and 50µg/ml. The plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition for each extract was measured in millimeters using vernier calipers.



### Statistical analysis

Level of significance “p” value will be kept at 0.05.  $p > 0.05$  not significant,  $p < 0.05$  significant. Mean values, ANOVA test was used for evaluating the data.

## RESULTS

Antimicrobial activity of cow urine and Chlorhexidine was seen against dental caries pathogen, S. mutans. Cow urine was found to be effective against S. mutans at the lower concentration (10µg/ml). However, Chlorhexidine was found to be more effective and showed a statistically significant mean zone of inhibition ( $6.3 \pm 0.46$ ) at a lower concentration of 5µg/ml ( $p=0.00$ ) (Table 1, Graph 1 & 2).

**Table 1** Mean zone of inhibition (mm) of cow urine and Chlorhexidine on Streptococcus Mutans.

CONCENTRATIONS (MEAN±S.D)						P- Value
S. Mutans	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	2µg/ml	
Cow Urine	12.7±0.45	10±0.35	8.2±0.27	6.5±0.38	R	0.05
Chlorhexidine	15±0.35	12.4±0.42	11.3±0.45	8.3±0.44	6.3±0.46	0.00

Test applied – ANOVA. \* $P \leq 0.05$  statistically significant, R- Resistant

## DISCUSSION

The increasing prevalence of antibiotic resistance in infectious bacteria, has raised the demand for the scientific community to search for new anti-bacterial components. Therefore, the focus is now slowly shifting towards natural products.<sup>4</sup>

The literature on cow urine and goat urine has always focused on prevention of disease and maintaining the health and treatment of diseases. In India specially, as well as various other parts of the world, the medicinal properties of cow urine are well recognized and used since centuries to cure various ailments of the human body.<sup>5</sup>

In the present study, Cow urine was found to be effective against *S. mutans* at the lower concentration of 10 µg/ml. However, Chlorhexidine was found to be more effective and showed a zone of inhibition at a lower concentration of 5 µg/ml. The assessment of the antimicrobial activity of the samples against *S. mutans* in the present study showed that at lower concentration (10 µg/ml), Cow urine was effective against this facultative anaerobic, gram-positive bacterium mainly due to the presence of high quantity of nitrogen and phenols in the urine.

Similar results were obtained by Dave et al. Their results obtained showed that cow urine samples were capable of inhibiting the dental caries causing bacteria at different concentrations thus proving their potential to be an excellent alternative as a natural alternative to antibiotics. They are capable of inhibiting the bacteria that causes initiation of dental caries i.e. *S. mutans* as well as the one which causes progression i.e. *L. acidophilus*.<sup>3</sup>

Siddharth Vats et al., conducted a study on many micro-organisms. Their results noted that Cow urine showed a strong anti-microbial activity towards *E. coli*, *S. aureus* and *C. albicans* but not against *Streptococcus mutans*.<sup>6</sup>

According to reports, cow urine's antibacterial properties are attributed to the presence of 2-hydroxycinnamic acid, ferulic acid, gallic acid, cinnamic acid, phenol, carbolic acid, and allantoin. The peptides and derivatives in cow urine increase bacterial cell surface hydrophobicity, resulting in an impressive bactericidal effect. Cow urine is also known to boost the phagocytic activity of macrophages. It was also claimed that cow urine has the ability to prevent the development of antibacterial resistance by blocking the R-factor, which is a component of the plasmid genome in bacteria. Hence it is suggested that it may be an effective solution to the problem of antibacterial resistance.<sup>7-10</sup>

## CONCLUSION

The present study showed that anti-microbial effect of cow urine against dental caries causing pathogens, *S. Mutans* was almost equivalent to Chlorhexidine. Thus, Cow urine has the potential to

become promising natural antimicrobial agent in pharmaceutical industry for controlling the oral pathogenic bacteria. However, more and more research on natural products to set a primary platform for further biological and pharmacologic in vivo studies are needed to find an agent against dental caries pathogens.

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