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Research Article

Antibacterial Properties of Cow Urine Collected from a Badri Calf

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ABSTRACT

Background: This study investigates the antibacterial potential of Badri cow urine against various pathogenic bacteria, including *Pseudomonas aeruginosa, Aeromonas salmonicida, Escherichia coli, Listeria monocytogenes*, and *Xanthomonas campestris*. **Materials and Methods:** The urine was collected aseptically from Badri cattle, filtered, and tested using the tube dilution method to determine the Minimum Inhibitory Concentration (MIC) and the agar well diffusion assay to assess the zone of inhibition. **Results:** MIC values ranged from 15.62 to 125 µL/mL, with *E. coli* (MTCC 724) showing the lowest MIC at 15.62 µL/µ, indicating high sensitivity and followed by 31.2562 µL/mL, *Xanthomonas campestris* (ITCCBH0006). The agar well diffusion assay revealed inhibition zones ranging from 7.00 to 24.00 mm, with *E. coli* (MTCC 40) and *Xanthomonas campestris* (ITCCBH0006) exhibiting the largest zones at 20.00 mm and 19.00 mm, respectively. **Conclusion:** Notably, cow urine demonstrated significant antibacterial efficacy against *Xanthomonas campestris*, a plant pathogen resistant to the positive control, ampicillin. These findings align with previous studies and highlight cow urine's potential as a natural antimicrobial agent, suggesting its use as an alternative therapeutic strategy to combat antibiotic resistance and support sustainable disease management.

Keywords: Antibacterial, Badri Cow, Urine, MIC, well Diffusion.

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INTRODUCTION

Cow urine is cited in Ayurveda for treating several diseases and regarded as an immunomodulator and elixir.[1] The intake of cow urine has been regularly practiced for many years and is regarded as the premier natural remedy globally.[2] Cow urine distillate, or cow urine ark, is fully distilled cow urine utilized to treat ailments caused by diverse pathogenic bacteria, opportunistic fungi, and parasitic helminths.[3] Cow urine distillate has been granted a patent for its usefulness as a bio-activity enhancer and bio-availability facilitator for bioactive compounds that have anti-cancer and anti-infective properties.[4] Oxidative stress, resulting from an imbalance between free radicals and antioxidants in the human body, contributes to serious diseases such as cancer, diabetes, cardiovascular disorders, and liver cirrhosis.[5] Cow urine has also been used as a stabilizing and reducing agent through a biogenic method for synthesizing ZnO nanoparticles. It is also helpful in calcite precipitation through a reaction involving hydrolysis of urea.[6]

Ayurvedic texts characterize cow urine as a highly beneficial and potent medicinal agent for therapeutic applications. In recent years, numerous products have been developed from cow urine, including soap, shampoo, face wash, natural fragrance, and oil. These products have garnered a significant amount of popularity in the consumer market. Cow urine is an essential component in cosmetic goods that are manufactured in India since it contains allantoin.[7] Cow urine has good medicinal potential for the discipline of anti-microbial, antioxidant, anti-anthelmintic, anti-cancer, and biosensors, which play an essential role in biotechnology. Research into cow urine has shown promising results in treating a wide range of medical conditions, including arthritis, hypertension, diabetes, heart disease, cancer, thyroid, asthma, psoriasis, skin inflammation, headaches, ulcers, gynecological disorders, and more.[8] Fresh urine comprises protein, urea, uric acid, and creatinine. Additionally, it encompasses phenol, aromatic acids, and various enzymes such as acid phosphatase, alkaline phosphatase, amylase, along with vitamins. The components found in cow urine may serve as an effective reducing agent in the synthesis of gold nanoparticles.[9]

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Cow's urine has traditionally been promoted for medical and spiritual uses, either on its own or in combination with other substances. The cow is regarded as a home for all gods. The spiritual, medical, and traditional values of Gomutra are significant. Traditionally, Gomutra is dispersed in courtyards and residences for its sacred roles: it bestows happiness, purity, prosperity, wellness, and wealth.[10] Antibiotic Growth Promoters (AGPs) were administered to animal feed at sub-therapeutic doses during the growing phase, accounting for around 42% of all veterinary antibiotics. The goal was to increase growth rate and feed conversion efficiency.[11] In the present investigation the Badri cow's urine was evaluate for its potential against the selected pathogens. The Badri cow represents the first registered cattle breed of Uttarakhand, having received certification from the National Bureau of Animal Genetic Resources (NBAGR).[12]

MATERIALS AND METHODS

Sample Collection and Preparation

The first Badri cow calf urine flow was collected from Badri cattle in a sterile bottle using aseptic precautions and brought to the laboratory. The Collected urine sample was aliquoted and filtered by Whatman No.1 filter paper to remove the debris and precipitates.

0.5 McFarland standard

The 0.5 McFarland standard was made by mixing a 0.5-mL aliquot of a 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \bullet 2H_2O$) into 99.5 mL of 0.18 mol/L H_2SO_4 (1% vol/vol) while continuously stirring in order to maintain a solution. By measuring absorbance with a spectrophotometer equipped with a 1-cm light path and a matching cuvette, the correct turbidity was confirmed. The absorbance at 625 nm must range from 0.08 to 0.13 for the 0.5 McFarland standard. The barium sulfate suspension was dispensed in 4- to 6-mL aliquots into screw-cap tubes identical in size to those utilized for the bacterial inocula. The tightly sealed tubes were kept at room temperature for further work.

Tested Organisms

The bacterial strains used for this study were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, and the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi. The tested organisms include *Pseudomonas aeruginosa* (MTCC15024), a Gram-negative opportunistic pathogen frequently implicated in healthcare-associated infections and immunocompromised individuals due to its adaptability and reservoir diversity. It is commonly found in hospital environments and natural settings, with infection-related mortality rates reaching up to 40%.[13] *Aeromonas salmonicida* (MTCC1522), a Gram-negative aquatic pathogen responsible for furunculosis in fish, possesses multiple siderophore systems and a rich arsenal of antibiotic-resistant genes, making it relevant for alternative antibacterial studies.[14] Although traditionally associated with salmon, it can also infect humans, birds, and other mammals.[15] *Escherichia coli* (MTCC724), a common facultative anaerobe of the gut, includes pathogenic strains such as enteropathogenic *E. coli*, a major diarrheal agent in infants, and extraintestinal pathogenic *E. coli*, a leading cause of urinary tract infections and sepsis in adults.[16,17] *Listeria monocytogenes* (MTCC1143) is a Gram-positive, facultative intracellular rod-shaped bacterium that contaminates food products and thrives in a wide range of environments, including refrigeration. It causes severe illnesses such as septicemia and meningitis, particularly in vulnerable populations like the elderly, pregnant women, and neonates.[18] Lastly, *Xanthomonas campestris* (ITCCBH0006), a 1Gram-negative phytopathogen, affects a wide range of crops and is one of the most significant pathogens in plant pathology, especially known for causing black rot in cruciferous vegetables.[19] Its high host range and global distribution make it a critical organism for agricultural research.

Antibacterial potential test of Badri cow urine using the well diffusion method

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) test was conducted following the methodology described by.[5] The Minimum Inhibitory Concentration (MIC) is the lowest concentration of a chemical that prevents bacterial growth. The tube dilution method assessed the Minimum Inhibitory Concentration (MIC). Various amounts of cow urine (ranging from 2000 to 3.90 µL/mL) were created using a process of dilution in sterile nutrient broth within test tubes. 100 µL of bacterial cultures that had been developed for 24 hr were added to test tubes containing nutrient broth. The test tubes were then incubated at 37°C for 24 hr. Following incubation, the test tubes were inspected for any visible cloudiness or turbidity. The MIC refers to the lowest concentration of cow urine that effectively prevents the growth of tested microorganisms.

Agar well diffusion assay

The agar well diffusion assay was conducted using the protocol described by.[20] The bacterial strains were evenly spread on the surface of the Nutrient Agar Medium (NAM) plates using a sterile cotton swab. The plates were then left to dry. Using a sterile cork-borer, wells with a diameter of 6 mm were created. Subsequently, 50 µL of urine sample was added to each well. Thereafter, all plates were incubated at 37°C for 24 hr, after which the area of growth inhibition was measured. The promising fraction was selected for further analysis based on its antibacterial properties. Ampicillin (30 µg/mL) was used as a positive control.

RESULTS AND DISCUSSION

Determination of Minimum Inhibitory Concentration (MIC)

The MIC concentrations ranged from 2000 to 3.90 µL/mL (2000<1000<500<250<125<62.50<31.25<15.62<7.81<3.90). The MIC values ranged from 15.62 to 125 µL/mL. *E. coli* (MTCC 724) showed the least MIC value, i.e., 15.62 µL/mL, followed by *E. coli* (MTCC 40), i.e, 31.2562 µL/mL, *Xanthomonas campestris* (ITCCBH0006) which was again 31.2562 µL/mL, for *Aeromonas salmonicida* (MTCC 1522) and *Pseudomonas aerogenosa* (MTCC 15024), the MIC was 62.50 µL/mL and *Listeria monocytogenes* (MTCC 1143) showed the highest MIC value of 125 µL/mL.

Agar well diffusion assay

The tested Badri cow urine sample showed promising results. The inhibition zone ranged from 7.00 to 24.00 mm for different tested bacteria, i.e., *Aeromonas salmonicida* (MTCC 1522), *Pseudomonas aerogenosa* (MTCC 15024), *E. coli* (MTCC 40), *Listeria monocytogenes* (MTCC 1143), *Xanthomonas campestris* (ITCCBH0006) and *E. coli* (MTCC 724) as shown in Table 1. Badri cow urine was found to be the most effective against *E. coli* (MTCC 40), followed *Xanthomonas campestris* (ITCCBH0006) in the well diffusion test (Figure 1).

Previously, Badri cow urine sample has been fractionated and tested against *Listeria monocytogenes* (MTCC657), *Staphylococcus aureus* (MTCC7443), *Pseudomonas aeruginosa* (MTCC424), *Klebsiella pneumoniae* (MTCC432) and *Salmonella typhi* (MTCC733) and auspicious results were obtained by.[20] Nautiyal and Dubey[5] tested the Badri cow urine potential against *Listeria monocytogenes, Escherichia coli* and *Pseudomonas aeruginosa* and have found results in line with the present study.

The bacterium *Xanthomonas campestris* (ITCC BH0006) is a Gram-negative plant pathogen that can infect approximately 124 monocotyledonous and 268 dicotyledonous plant species. This includes various significant crops, leading to substantial losses in agricultural production.[21] *Xanthomonas oryzae* and *Xanthomonas campestris* are two of the most significant phytopathogens in the study of plant molecular pathology.[22] *Xanthomonas campestris* pv. *campestris* (Xcc) is a pathogenic bacterium that invades cruciferous plants, leading to black rot disease. This disease has a global distribution and affects plants regularly.[23]

In the current investigation, the bacterial strain exhibited an apparent resistance against the antibiotic Ampicillin, serving as a positive control within the experimental framework. However, noteworthy findings emerged as cow urine demonstrated a remarkable antibacterial efficacy against *Xanthomonas campestris* pv. *campestris*. This substantiates the premise that cow urine possesses inherent anti-microbial properties, warranting thorough exploration in subsequent research endeavours. The observed potency suggests that cow urine may serve as a promising candidate for development as an alternative therapeutic agent to conventional antibiotic treatments. This opens avenues for novel therapeutic strategies that harness the innate bioactivity of

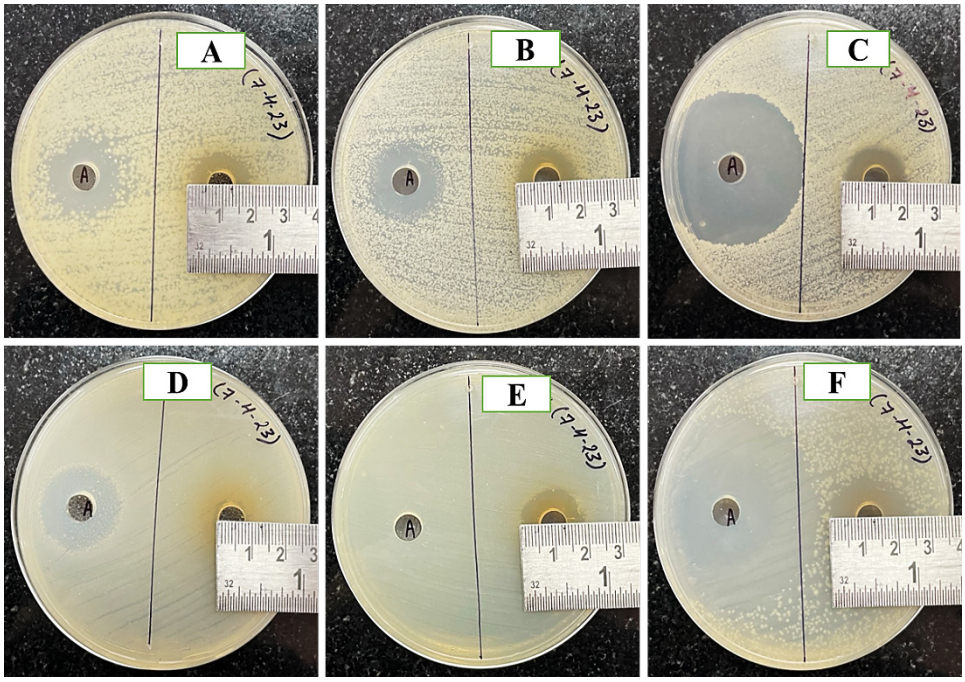

Figure 1: Well Diffusion Anti-microbial Susceptibility Test of Badri Cow Urine Aginst A- *Aeromonas salmonicida* (MTCC 1522); B- *Pseudomonas aerogenosa* (MTCC 15024); C- *E. coli* (MTCC 40); D- *Listeria monocytogenes* (MTCC 1143); E- *Xanthomonas phaseoli* (ITCCBH0006); F- *E. coli* (MTCC 724); the left well (without scale) in plates A to F represents a zone of inhibition by an Ampicillin (positive Control).

Table 1: Antibacterial activity of Badri cow urine sample against the tested bacteria.

Plate	Bacterial Strain	Zone of Inhibition (mm)	Positive Control (mm)
A	*Aeromonas salmonicida* (MTCC 1522)	13.00±0.33	17.00±0.33
B	*Pseudomonas aerogenosa* (MTCC 15024)	12.00±0.33	17.00±0.33
C	*E. coli* (MTCC 40)	20.00±0.57	40.00±0.57
D	*Listeria monocytogenes* (MTCC 1143)	7.00±0.33	16.00±0.33
E	*Xanthomonas campestris* (ITCCBH0006)	19.00±0.00	00.00±0.33
F	*E. coli* (MTCC 724)	24.00±0.33	42.00±0.33

natural substances, potentially mitigating issues such as antibiotic resistance while offering sustainable and eco-friendly alternatives in the realm of infectious disease management.

CONCLUSION

This investigation revealed the substantial antibacterial efficacy of Badri cow urine against many clinically and agriculturally significant bacterial pathogens. The urine sample shown significant efficacy, particularly against *Escherichia coli* (MTCC 724 and 40) and *Xanthomonas campestris* (ITCCBH0006), as evidenced by minimum inhibitory concentration (MIC) and agar well diffusion experiments, with inhibition zones reaching 24.00 mm. The urine sample exhibits superior action compared to Ampicillin against *X. campestris*, a significant phytopathogen, underscoring its potential in plant disease control. The MIC values varied from 15.62 to 125 µL/mL, highlighting the sample's differential efficacy against several bacterial species. These findings corroborate previous research on the bioactivity of Badri cow urine and further underscore its potential as a natural, economical substitute for manufactured antibiotics. Given the increasing antimicrobial resistance and the necessity for sustainable treatments, the findings support the continued investigation and standardization of cow urine-derived bioformulations for therapeutic and agricultural purposes.

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ABBREVIATIONS

MIC: Minimum Inhibitory Concentration; **ZnO:** Zinc oxide; **AGPs:** Antibiotic Growth Promoters; **NBAGR:** National Bureau of Animal Genetic Resources; **$BaCl_2$:** Barium chloride; **H_2O:** Water; **H_2SO_4:** Sulfuric acid; **vol/vol:** Volume/Volume; **mol/L:** Mol/Liter (Molar Concentration); **w/v:** weight by Volume; **mL:** Milliliter; **nm:** Nano meter; **MTCC:** Microbial Type Culture Collection; **ITCC:** Indian Type Culture Collection; **°C:** Degree Celcius; **µL/mL:** Micro liter per milliliter; **µL:** Micro liter; **NAM:** Nutrient Agar Medium; **µg/mL:** Microgram per milliliter.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Mansi Arya collected all the samples and conceptualized the work. Nagma Parveen conducted experiments and helped in writing the manuscript. Shikha Bora provided substantial assistance in the field and laboratory work. Gaurav Rawat and Netra Pal Sharma contributed to the experimental design and the writing of the manuscript. Satpal Singh Bisht provided guidance throughout the entire experimental process and edited the manuscript.

SUMMARY

The study assesses Badri cow urine's antibacterial activity against pathogens like *Pseudomonas aeruginosa, Escherichia coli, Xanthomonas campestris* etc. Aseptically collected urine was tested via tube dilution for Minimum Inhibitory Concentration (MIC) and agar well diffusion for inhibition zones. MIC ranged from 15.62 to 125 µL/mL, with *E. coli* (MTCC 724) showing the lowest (15.62 µL/mL). Inhibition zones spanned 7.00 to 24.00 mm, with *E. coli* (MTCC 40) and *Xanthomonas campestris* highly susceptible. Cow urine outperformed Ampicillin against *Xanthomonas campestris*, a plant pathogen. Results confirm cow urine's antimicrobial potential, suggesting its use as a natural alternative to antibiotics. Further research is advised.

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