International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 1065-1068 www.ijprajournal.com ISSN: 2249-7781

"Isolation and Identification of Bacteria Fromurine of Indian Cow to evaluate its Antagonistic Potential"

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Submitted: 25-01-2024 Accepted: 03-02-2024

ABSTRACT
It's unfortunate to see that lifestyle diseases such as heart disease, stroke, obesity, type II diabetes, and lung cancer are becoming more prevalent in our modern era. Additionally, the irrational use of antibiotics is contributing to the rise of antibiotic-resistant infectious diseases, which is concerning.The classical texts of Ayurveda have a wealth of information on various topics, including healthcare. Charaka, Sushruta and Vagbhatta Samhita are some of the most well-known texts in this field. One interesting topic that these texts cover is the Ashta Urine or eight types of urine. These texts describe the properties, indications, and formulations of each type of urine. This shows how Ayurveda has been used to understand the human body and its functions for centuries.Cow urine, or Gomutra, has been used in Ayurveda for centuries for its medicinal properties. In recent years, scientific research has also been conducted to study its potential health benefits. One of the most significant findings is that cow urine has immunomodulatory properties, meaning it can help regulate the immune system. Additionally, Gomutra has bacteriostatic effects, which means it can inhibit the growth of bacteria. These properties make cow urine a potentially useful substance for treating various health conditions. Various studies have been conducted to understand its activities and research, and a summary of these findings is available in this article.
The present study is to isolate the bacteria from cow urine, & see their antimicrobial effect against different microorganism.

I. INTRODUCTION

As per traditional beliefs, cow urine is considered to have therapeutic properties and is used in various drug formulations. It is primarily used as a disinfectant and for purification purposes.Biochemical studies of cow urine have identified many powerful elements that are almost physically required by our body.Cow urine has been considered sacred in many cultures, including in India, where it is also used for medicinal purposes. In fact, cow-based treatment is called Panchagavya therapy or Cowpathy. This therapy involves using five cow-derived products, which are cow urine, milk, ghee, curd, and cow dung, for their therapeutic properties. Cow urine is one of the key ingredients in Panchagavya therapy and has been used for various health conditions, including liver disorders, arthritis, and cancer. Its antimicrobial and anti-inflammatory properties make it useful in treating infections and inflammation. Additionally, because of its immunomodulatory properties, cow urine may also help boost the immune system. Overall, cow urine and other cow-derived products have important medicinal uses and are an important part of Ayurvedic medicine.

Recently the cow urine has been granted U.S. Patents (No. 6,896,907 and 6,410,059) for its medicinal properties, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers. Medicinal usage of cow urine is extensively searched and scientifically endorsed.

II. MATERIAL AND METHODS

Cow urine collection

Different samples (Desi Cow) of cow urine were collected from different regions of Agra, aseptically in sterile, wide mouthed container with screw cap tops. All the samples were labelled and transported to the biotechnology laboratory, RBS College, Agra for further processing. The urine samples were processed on same day. If there is any delay (before or after delivery to the lab) in initiating culture, urine samples must be refrigerated.

Isolation of Bacteria

For isolation of bacteria, a fresh cow urine sample of Desi Cow weighing 1g was mixed with 9ml of distilled water in a test tube and shaken well with a vortex mixer for 2 hours to create a stock culture. A volume of 1 ml was then transferred aseptically from the stock culture to a tube containing 9 ml of distilled water and mixed well.

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From this tube, 1 ml of liquid was again transferred to another tube containing 9 ml of distilled water to make a 10/100 dilution factor. Dilutions up to 10^{-8} were made using the serial dilution technique for the cow urine samples. Agar plates were then prepared for the culture of urine microbes, and 1ml of liquid from each dilution was added to agar plates by streaking method with the help of a sterilized inoculation loop, for enumerating bacteria. One plate of each dilution was maintained and incubated at 37 Degrees Celsius for 24 hours.

The cultures were sub-cultured and purified using the streak plate method on a Nutrient agar medium. Using a sterilized inoculating loop, slightly picked up the colony from the spread plate and dragged the loop over the surface of another plate in a zig-zag motion. Sterilized the loop over the flame, and dragged the loop over the area streaked before in a similar manner.sterilized loop was sterilized over the flame and the same process was repeated again, all the plates were incubated for 24 hours. The heaviest growth was observed in the first plate, while the isolated colonies were found in the third plate. The process was repeated multiple times until purified colonies were obtained.kept the purified bacterial cultures on Nutrient agar slant.

Characterisation and identification of Microorganisms: After the pure culturing method, these isolated colonies of microorganisms for their colony morphology including colour, shape, size, surface, and motility. These cultures were further identified using Gram's staining method and biochemical tests. For the present study,obtained a total of 14 isolates from the cow urine sample through morphological and biochemical tests.

Microscopic examination

The slide of cow urine samples which were gram stained were observed in a compound microscope lens under animmersion oil at 100X

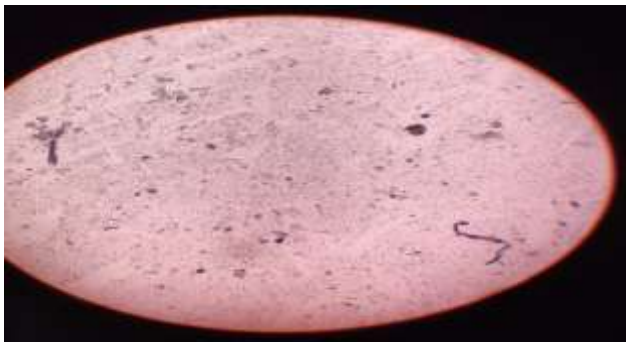

Fig: staining of bacteria

Table – 1: Morphological characteristics of isolates from cow Urine

Isolates	Gram stain reaction	Shape	Form of colony	opacity	Surface of colon
A	-	Coccus	circular	opaque	Smooth
B	-	Rod	Circular	opaque	Smooth
C	+	Rod	Irregular	Clear	Rough
D	+	Spiral	Irregular	Clear	Smooth
E	-	Bacillus	Regular	Opaque	Rough
F	-	Rod	Circular	clear	Smooth
G	-	Rod	Circular	Opaque	Smooth
H	-	Spiral	Irregular	Opaque	Rough
I	+	Coccus	Regular	clear	Rough
J	+	Rod	Circular	opaque	Smooth
K	-	Spiral	Regular	clear	Rough
L	+	Bacillus	Regular	clear	Rough
M	+	Coccus	Irregular	opaque	Smooth
N	-	Rod	Circular	opaque	Smooth

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Screening of isolates for antimicrobial sensitivity

To determine antimicrobial sensitivity, all 14 isolates were screened for activity against 4 test organisms: Klebsiella pneumoniae (MTCC 109),Candida albicans (MTCC 227), Staphylococcus aureus (MTCC 3160), and Enterococcus faecium (MTCC 5695).

To perform the test, you need to spread the test organisms on the entire surface of Muller-Hinton agar plates using a sterile cotton swab. Then, leave the plates for incubation for 15 minutes. Next, make three dilutions of each isolate using the serial dilution method. After that, use lab-prepared filter paper discs containing 0.4µg/disc of antibiotic with a diameter of 5mm. After the antimicrobial compound (antibiotic) is gently pressed onto the microbe at the centre of the plate, each dilution disc is positioned 15mm away from the edge and 25mm apart from each other. To complete the process, a disc of distilled water (negative control) is added and the plate is incubated overnight at 37°C for a duration of 18-24 hours.To determine the susceptibility or resistance to an agent in each disc, the diameter of the zone of bacterial growth inhibition (ZOI) around each disc is measured in millimeters. This can be done using a measuring scale.

Result

In the present study, we collected samples of cow urine (Desi cow) from a goshala, from which we isolated 14 isolates (I). Out of these, only four bacterial isolates (A, B, C, and D), obtained from the urine samples of Desi Cow, showed antimicrobial activity against all the test organisms.We observed the isolated microbes under an electron microscope, and the gram staining result indicated that the isolated bacteria could be identified as Klebsiella, E. coli, and Bacillus. There are claims that cow urine provides health benefits due to these microorganisms.

Table -2: Zone of inhibition (in mm) of isolates against Klebsiella pneumonie

	0.5	1.0	1.5	(-) control (DW)	(+) control (Antibiotic)
A	0	8mm	0	0	34 mm
B	6mm	9mm	10mm	0	32 mm
C	0	12mm	8mm	0	30 mm
D	0	0	15mm	0	34 mm

Fig.: Isolates A,B,C & D showing antimicrobial activityagainstKlebsiella pneumonie

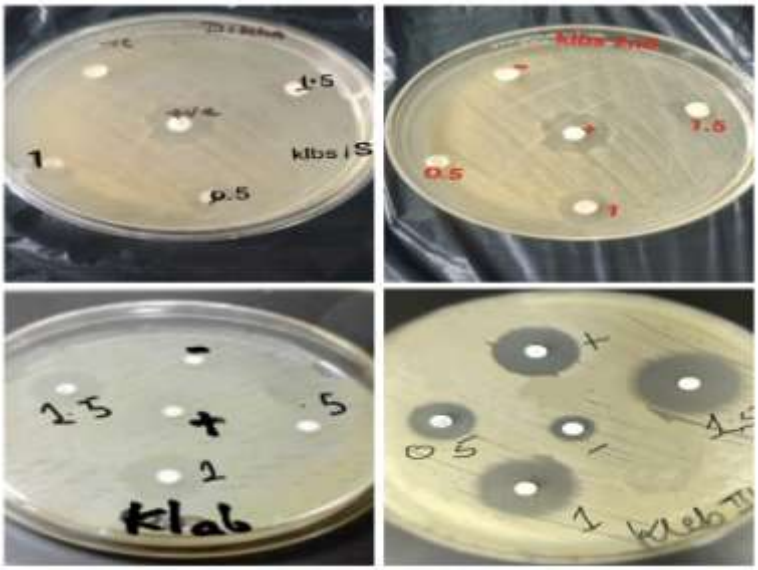

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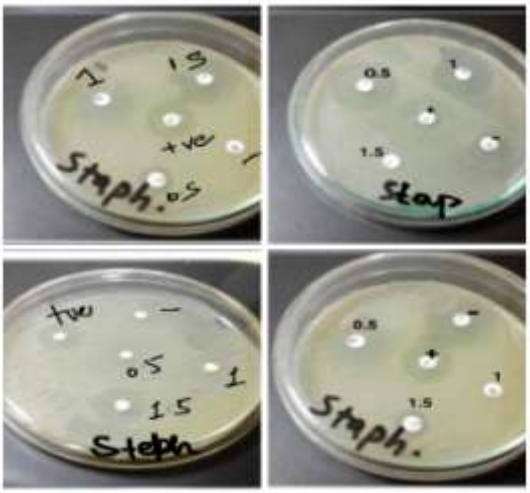
Table -3: Zone of inhibition (in mm) of isolates against Staphylococcus aureus

	0.5	1.0	1.5	(-) control (DW)	(+) control (Antibiotic)
A	0	22mm	30mm	0	36mm
B	26mm	20mm	0	0	30mm
C	6mm	10mm	0	0	24mm
D	24mm	8mm	5mm	0	28mm

Fig.: Isolates A, B, C & D showing antimicrobial activityagainst Staphylococcus aureus



III. CONCLUSION

In the present study, a total of 14 isolates were obtained. Out of these, 08 isolates were identified as Gram-negative while the remaining isolates were identified as Gram-positive through morphological examination (Gram Staining) and biochemical tests. Isolates C, D, I, J, L, and M could be Bacillus, Diplococcus, Enterococcus, etc. On the other hand, A, B, E, F, G, H, K, and N could be Pseudomonas, E. coli, Klebsiella, etc. Therefore, all the isolates have thepotential to be used on an industrial level and the produced antimicrobial agent should be further researched for its possibility of being used as a therapeutic agent.

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