IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 June, 2012; received in revised form, 26 February, 2013; accepted, 13 March, 2013

ANTIBACTERIAL ACTIVITY OF COW URINE AGAINST SOME PATHOGENIC AND NON-PATHOGENIC BACTERIA

S. Raad [1], D.V. Deshmukh*[2], S. N. Harke [2] and M.S. Kachole [1]

Department of Biochemistry, Dr. Babasaheb Amberdkar Marathwada University [1], Aurangabad, Maharashtra, India
MGM's, Institute of Biosciences and Technology [2], Auranagabad, Maharashtra, India

Keywords:

Cow urine, Antibacterial activity, Zone of inhibition, Antimicrobial Peptides

Correspondence to Author:

Dr. Devendra V. Deshmukh

Assistant Professor, MGM's, Institute of Biosciences and Technology, N-6, CIDCO, Aurangabad, India

E-mail: devcyano@gmail.com

ABSTRACT: Cow urine therapy and all traditional practices from Indian systems of medicine have a strong scientific base. The cow has proved to be a boon in the areas of agriculture, science and technology, industry, energy, medicine etc for the development of any nation, in addition being eco-friendly in nature. In the present study the antibacterial potentials of cow urine were investigated. Total 14 pathogenic and non-pathogenic bacterial cultures were used as test organism against 10 different cow urine samples. The highest zone of inhibition was shown by sample G against *P aeruginosa* NCIM 2945 (1.8cm) while the smallest zone of inhibition was shown against *E. coli* NCIM 2065(0.3 cm) by sample A. Based on cumulative effect against the test organism, the urine sample G was found to be the most efficient inhibiting all the 14 test cultures. The antibacterial activity reported by sample G was comparable with standard antibiotics. A higher zone of inhibition was observed by sample G against *P aeruginosa NCIM* 2945 as compared to that of Gentamicin, Oxacillin and vancomycin. Though the urine sample G showed a strong antibacterial activity against all the test organisms, but the activity was reported low against the entire Gram positive bacteria compared to Gram negative bacteria. The presence of proline which is considered as a major amino acid in antimicrobial peptides was also observed in the urine sample G.

INTRODUCTION: It is widely accepted among clinicians, medical researchers, microbiologists and pharmacologists, that antibiotic resistance will, in the very near future, leave healthcare professionals without effective therapies for bacterial infections.

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
www.ijpsr.com

As an example, it is now estimated that about half of all *Staphylococcus aureus* strains found in many medical institutions are resistant to antibiotics such as methicillin [1].

Presently we face a global public health crisis, as infectious diseases top the list for causes of death worldwide.

While it is likely that antibiotic resistance contributes significantly to this problem, data on consumption and resistance to antibiotics are limited for most countries [2] and the relationship of resistance to morbidity and mortality is quantitatively unclear.

Cow, *Bos indicus* is a most valuable animal in all community. The cow urine is useful in number of disease particularly in gulma, filaria, cancer ets. It is also used with herbs to cure diseases like fever, epilepsy, anemia, abdominal pain, constipation, etc by the traditional healers [3 4]. Immunomodulatory [5], hypoglycemic [6] and cardio-respiratory effects [7]. 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 are extensively searched and scientifically endorsed [8].

In the Present study the antibacterial potentials of the cow urine have been investigated against 14 different pathogenic and nonpathogenic bacteria.

MATERIALS AND METHODS:

Collection of the urine sample: 10 urine samples were collected from different cows from the farm; all the samples were collected from milking cows. Random sampling was a method of choice for collection of the samples. Samples were collected in sterile containers, 20 ml of middle stream urine was collected and brought to the laboratory and stored in fridge until further use. The samples were designated as sample A, B, C to Sample J.

Qualitative test for proteins: The qualitative test of protein was performed as according to Martin and Mittelman [9]. The urine Samples were centrifuged at 3000 rpm for 10 mins for the removal of sediments. After centrifugation the supernatant was collected and heat test for proteins was performed to observe the presence of protein.

Quantitative estimation of Protein: The Folin Lowry method was a method of choice for estimation of protein. Aliquots of protein standard solution were pipetted out as into a series of tubes as 0.1, 0.2….1.0 ml and the total volume was made to 4 ml with distilled water. To each tube 5.5 ml of alkaline mix (reagent C) was pipetted out, mixed well and allowed to stand for 15 min, at room temperature. 0.5 ml of FC reagent was pipetted out into each tube, mixed thoroughly and kept in dark for 30 min. The blue color formed was measured at 650 nm against a proper blank. The same was conducted for the samples [10].

Antimicrobial activity:

Test bacterial cultures: Fourteen bacterial cultures from laboratory repository viz. *Escherichia coli NCIM 2345, Escherichia coli NCIM 2065, Escherichia coli NCIM 2310, Bacillus subtilis NCIM 2113, Bacillus licheniformis NCIM 2015, Bacillus megaterium NCIM 2083, Staphylococcus aureus NCIM 2124, Staphylococcus aureus NCIM 2079, Staphylococcus aureus NCIM 2125, Pseudomonas aeruginosa NCIM 2945, Pseudomonas aeruginosa NCIM 2053, Proteus vulgaris NCIM 2857, Kebshella pneumonie NCIM 2957* and *Salmonella typhimurium NCIM 2501* were used in the study. Freshly grown 12 h old cultures in nutrient broth were used as the inoculum in antibacterial assays.

Disc Preparation: Paper disc of filter paper Whattman No. 1 were prepared. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. The sterile discs were kept in a presetrilized container until further use.

Disc diffusion assay: Antibacterial activity of urine samples against the test organisms was done by disc diffusion assay [11]. Petri plate containing 15 ml of solidified nutrient agar was spread inoculated with 100 µl of 12 h old test bacterial cultures. Presterilized Whatman No.1 paper discs (6 mm) were saturated with 50 µl of urine and dried to be used in assays. The plates were kept at 4^{o}C for 10 min before they were incubated at 37^{o}C for 24 h. Anti-bacterial was assessed by measuring the diameter of growth inhibition zone around the discs. Sensitivity of test organisms was also checked against commercial discs (Hi Media, India) containing standard antibiotics.

Paper chromatography: The urine sample showing highest protein content and antibacterial activity was analyzed for the presence of amino acids using paper chromatography technique . A strip of wattman's filter paper No. 1. was used, approximately 1 cm from one end of the length a line was drawn with the help of a pencil. At the centre of the line a tiny spot of the sample was placed. The spot was allowed to dry and then placed in the chamber containing the saturated solvent system (Butanol : Acetic acid : Water, 4 : 1 : 5). The chromatogram was allowed to run upto ¾ th the paper and then taken out and dried in an oven and then spayed with locating reagent

(Ninhydrine). The Rf value of the spot that appeared was calculated.

RESULTS:

Qualitative test for proteins: All the urine samples tested for the presence of protein gave a positive result. All the test tubes contain the urine sample showed cloudiness with granules which gave a positive test for protein in the urine sample.

Protein estimation by Lowry method: The qualitative estimation of protein gave mixed results. Sample G gave the highest protein content 520 µgm/ml while sample J showed the lowest concentration of protein (**Table 1**).

Antibacterial assay: Antibacterial activities of all the urine samples were tested using disc diffusion method. On the basis of cumulative antibacterial effect against all cultures under test, sample G appeared as most effective. A highest cumulative inhibition against all the fourteen bacterial cultures was 15 cm for the urine sample G while the lowest effect was shown by sample A (**Table 2**).

TABLE 1: PROTEIN ESTIMATED FROM ALL THE 10 URINE SAMPLES USING FOLIN LOWRY METHOD

Sample	Absorbance at 660 nm	Concentration of protein in µgm/ml
A	0.4420	250
B	0.7230	420
C	0.6830	400
D	0.7055	410
E	0.5882	330
F	0.8063	460
G	0.9490	550
H	0.8641	510
I	0.4990	290
J	0.4412	250

TABLE 2: ZONE OF INHIBITIONS (IN CM) OBSERVED AGAINST 14 BACTERIAL CULTURES FROM 10 DIFFERENT COW URINE SAMPLES

Bacterial cultures	Urine samples									
	A	B	C	D	E	F	G	H	I	J
E. coli NCIM 2345	0.5*(0.25)	1.2(0.30)	R	R	1.0(0.15)	1.5(0.5)	1.5(0.30)	1.0 (0.45)	1.5 (0.5)	0.5(0.12)
E. coli NCIM 2065	0.3 (0.5)	1.0(0.45)	0.5(0.15)	1.5(0.15)	0.9(0.12)	0.5(0.15)	1.2(0.27)	1.0(0.5)	1.0(0.12)	1.2(0.35)
E. coli NCIM 2015	0.8 (0.30)	0.7(0.15)	1.5(0.20)	0.5(0.20)	0.7(0.20)	0.5(0.75)	0.8(0.36)	0.7 (0.30)	0.7 (0.55)	R
B subtilis NCIM 2113	R	0.5(1.0)	0.8(0.30)	R	0.5(0.15)	0.7(0.30)	1.0(0.15)	0.5 (0.12)	0.5 (0.35)	R
B licheniformis NCIM 2015	0.5 (1.0)	R	0.8(0.15)	R	R	0.7(0.12)	1.2(0.30)	0.5 (0.5)	1.0 (0.36)	0.5
B megaterium NCIM 2083	R	R	0.9 (0.30)	R	R	1.0 (0.5)	1.1 (0.5)	R	0.5 (0.34)	0.3
S aureus NCIM 2124	R	R	R	R	R	0.5 (0.75)	0.9(0.11)	R	R	R
S aureus NCIM 2125	R	R	R	R	0.6(0.12)	R	0.8(0.25)	R	R	R
S aureus NCIM 2079	R	R	R	R	R	R	0.5(0.45)	R	0.6 (0.22)	0.6
P aeruginosa NCIM 2945	R	1.0(0.30)	1.2(0.15)	0.6(0.12)	R	1.0(0.15)	1.8(0.12)	1.0 (0.15)	1.0 (0.45)	0.7
P aeruginosa NCIM 2053	0.4(0.4)	0.5(0.12)	R	0.8(0.30)	0.5(0.5)	0.7(0.12)	1.0(0.36)	0.7 (0.25)	0.5 (0.12)	1.0
P vulgaris NCIM 2857	0.8 (0.75)	R	1.1(0.25)	1.0(0.12)	1.0(0.12)	1.5 (0.5)	0.8(0.47)	0.6 (0.12)	R	0.4
K pneumonie NCIM 2957	0.5 (1.0)	R	0.5(0.30)	0.5 (0.5)	1.1 (0.5)	0.8(0.30)	0.9(0.12)	R	0.5 (0.45)	1.2
S typhimurium NCIM 2501	1.0 (0.5)	R	R	0.5(0.15)	0.5(0.12)	0.6(0.25)	1.5(0.12)	R	R	0.6
Cumulative Inhibition	4.8	4.9	7.3	5.4	6.8	10	15	6	7.8	7

* Zone of inhibitions in centimeters, Values in the parenthesis is standard deviations, R- Resistant.

TABLE 3: ZONE OF INHIBITION SHOWN BY 14 BACTERIAL CULTUTRES AGAINST STANDARD ANTIBIOTICS

Bacterial cultures	Standard Antibiotics			
	Methicillin (5mcg/disc).	Gentamicin (10 mcg/disc)	Oxacillin (5mcg/disc)	Vancomycin (30mcg/disc)
E. coli NCIM 2345	1.5*	1.2	1.5	1.0
E. coli NCIM 2065	1.3	1.5	1.2	1.5
E. coli NCIM 2015	2.8	1.6	1.5	1.5
B subtilis NCIM 2113	2.0	2.5	2.4	1.8
B licheniformis NCIM 2015	1.5	1.0	2.0	1.3
B megaterium NCIM 2083	1.5	2.8	1.7	1.7
S aureus NCIM 2124	1.3	1.5	1.5	1.3
S aureus NCIM 2125	1.4	1.4	1.9	2.0
S aureus NCIM 2079	1.9	1.8	1.7	2.3
P aeruginosa NCIM 2945	2.6	1.4	1.5	1.5
P aeruginosa NCIM 2053	2.3	1.5	1.8	1.5
P vulgaris NCIM 2857	1.8	2.0	2.5	1.0
K pneumonie NCIM 2957	1.5	1.8	2.0	1.7
S typhimurium NCIM 2501	1.0	2.8	2.7	1.5

Paper Chromatography: The chromatogram after development was observed for the presence of spot and the Rf value of the spot reveled the amino acid present in the urine sample. From the calculated Rf value it was clear that amino acid proline was prominent amino acid and was confirmed with the Rf value of standard proline.

DISCUSSION: Commonly, antibiotics are widely as conservative treatment in various microbial infections and diseases [12]. Considering the enormous quantity of antibiotics used, the situation should have been that there would be no infectious diseases. But, the fact is that the problems of infectious diseases are increasing day-by-day. Some of the major hindrances are that bacteria have genetic ability to transmit and acquire resistance towards the drugs [13] and there are also adverse effects of drugs on the host.[14] Therefore to combat such problems many natural products have been explored. The nature is an almost infinite resource for drug development and discovery. It has endowed with a complete repository of remedies to cure all ailments of mankind, as it has always been a first rate drug store with enormous range of plants, micro organisms and animals.[15]

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, respiratory diseases and also plays its part as antimicrobial against disease like Eczema, Psoriasis, acne vulgaris, scabies and other various kinds of allergies. Urine contains volatile salts which are beneficial to the human body because these salts destroy acidity and get rid of pain in kidney, intestine, and womb; furthermore urine, a natural tonic, eliminates giddiness, tension in nerves, lazy feeling, hemicrama, paralysis, common cold, diseases of brain, nerves and joints.

In the present study the antibacterial potential of 10 different urine samples from cows at the MGM's farm house was revealed. The variation in the color of the urine samples may be due to the amount and type of fodder consumed and the protein content in them.

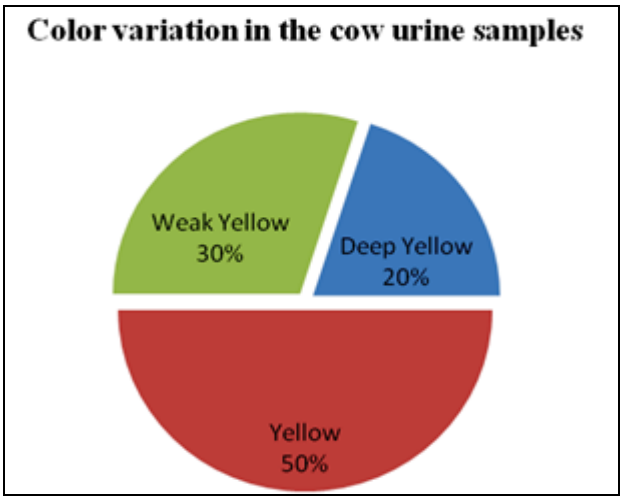

FIGURE 1: COLOR VARIATION IN THE 10 URINE SAMPLES TAKEN FROM COWS.

According to **Figure 3,** 50% showed yellow color , weak yellow for 30% of the urine samples, while 20% of the urine samples showed deep yellow coloration.

Cow urine contains different constituents; it is rich in potassium, chloride, calcium, estrogen, phosphorous, urinary proteins [16]. Various research have also found different components like urea, uric acid, nitrogen, sulfur, copper, iron, sodium, other salts, carbolic acid, ammonia, sugar lactose, Vitamin-A,B,C,D,E, gonadotropin, phenols and also some anticancer substances.

All the cow urine samples showed the presence of protein. Vats and Kanupriya [17] has reported that the components of cow urine are responsible for showing antimicrobial activity.

The presence of protein in all the samples was clear evidence that all the samples do contain the presence of bioactive compounds. Marshall and Arenas [18] pointed out the use of the importance of naturally occurring peptides and their use as an alternative to chemical antibiotics and their role as antimicrobials.

The antibacterial potentials of the cow urine was tested against some pathogenic and non pathogenic bacteria (Table 2). The highest zone of inhibition was shown by sample G against *P aeruginosa* NCIM 2945 (1.8cm) while the smallest zone of inhibiton was shown against *E. coli* NCIM 2065(0.3 cm) by sample A.

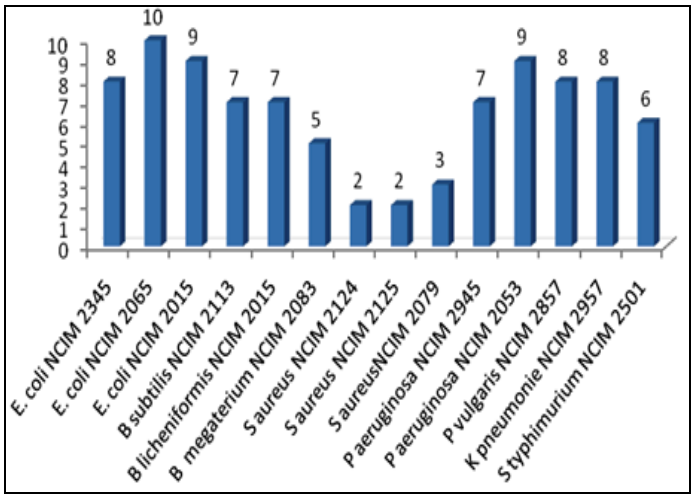

FIGURE 2: SENSITIVITY PATTERN OF THE TEST CULTURES AGAINST 10 URINE SAMPLES

All the samples showed the presence of antibacterial activity. From the **Figure 2**, it was observed that the maximum activity of all the 10 urine samples was against Gram negative bacteria than gram positive bacteria. Similar results were obtained by Edwin *et al* [19]. Where they have reported the antibacterial effect of cow urine against gram negative and gram positive bacteria.

The gram negative bacteria were more efficiently inhibited than gram positive bacteria. Sathasivam *et al* [20] has also reported the antibacterial activity of the cow urine distillate against 4 gram negative bacteria.

A synergistic effect of *Azadirachta indica* and cow urine against some gram negative bacteria and yeast was observed by Vats and Miglan [17]. Though all the urine samples showed the antibacterial activity, sample G was a promising candidate showing antibacterial activity against all given test organisms.

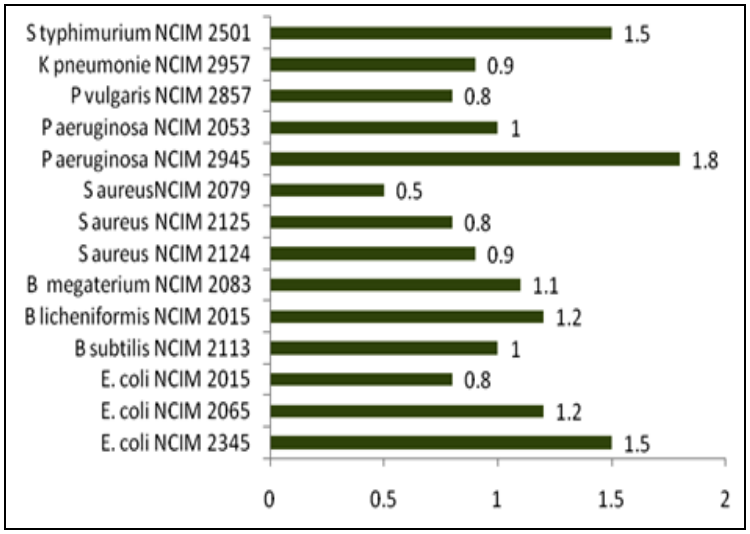

FIGURE 3: ZONE OF INHIBITION (in cm) RECORDED BY SAMPLE G AGAINST ALL THE 14 BACTERIAL CULTURES

As according to **figure 3**, the sample G gave the highest zone of inhibition against *P aeruginosa NCIM 2945*(1.8 cm) followed by inhibition against *E.coli* NCIM 2345 and *S typhimurium* NCIM 2501 (1.5). The antibacterial activity shown by the urine sample G was comparable with the antibacterial activity by standard antibiotics.

A higher zone of inhibition was observed by sample G against *P aeruginosa NCIM* 2945 as compared to that of Gentamicin, Oxacillin and vancomycin. Though the urine sample G showed a strong antibacterial activity against all the test organisms, but the activity was reported low against all the *S aureus* cultures, specifically *S aureus* NCIM 2079, (0.5 cm).

The chromatography of the sample revealed the presence of proline. The amino acid proline is considered as a major amino acid in antimicrobial peptides [21].

CONCLUSION: The Antibacterial property of the cow urine was reveiled using biological assay. Total 10 urine samples were tested against 14 different strains of bacteria. The ability of the cow urine sample was more to inhibit the gram negative bacteria than that of gram positive bacteria. The highest zone of inhibition that was observed was 1.8cm by sample G. Thus sample G was the only one which has inhibited the growth of all the test organism and when compared with standard antibiotic proved to be more promising. The presence of amino acid proline in the sample G has proved its potential similar to peptide antibiotics.

REFERENCES:

1. Roder BL, Wandall DA, Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT. Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. *Archives Internal Medicine,* 1999; 159(5):462-469.
2. Col NF, O'Connor RW. Estimating worldwide current antibiotic usage: report of Task Force 1. Rev Infect Dis 1987; 9: S232–243.
3. Pathak ML, Kumar A. Cow praising and importance of anchyagavya as medicine. Sachitra Ayurveda 2003, 5: 56-59.
4. Krishnamurthi, K, Dutta D, Devi SS, Chakrabarti T. Protective effect of diatillate and redistillate of cow,s urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. Biomed. Environ. Sci 2001; 17: 57-66.
5. Chauhan RS, Singh BP, Singhal LK. Immunomodulation with kamdhenu Ark in mice. J. Immunol. Immunopathol 2001; 71: 89-92.
6. Ojewole JA, Olusi SO. Effects of cow's urine concoction on plasma glucose concentration in fasted rats R. Soc. Trop. Med. Hyg 1976; 241-245.
7. Elegbe RA, Oyebola DDO. Cow's urine poisoning in Nigeria: the cardiotoxic effects of cow's urine in dogs. Trans. R. Soc. Trop. Med. Hyg 1976; 127-132.
8. Somvanshi R. Veterinary medicine and animal keeping in ancient India. *Asian Agri-History* 2006 ;10(2) :133–146.
9. Martin AJP , Mittelman R Quantitative Micro-analysis of Amino-acid Mixtures on Paper Partition Chromatograms' 1948. Biochem ; 43: 353- 358.
10. Setsuro M, Nobuko I, Yuki N. Colorimetric estimation of amino acids and peptides with folin phenol reagent, Analytical Biochemistry 1996; 16(2): 365-371.
11. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. *American. Journal of Clinical. Pathology* 1966; 45: 493-496.
12. Daniel B, Alexander R, Ehud IA. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. Orbit; 2005: 24 (2): 95-98.
13. Cohen ML. Epidemiology of drug resistance: implications for a post-antimicrobial era, Science 1992; 257: 1050-1055.
14. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62: 183-193.
15. Sujata MB, Charles HB. New agents for Gram-positive bacteria. Current Opinion in Microbiology. 2000; 3(5): 528-534.

How to cite this article:
Raad S, Deshmukh DV, Harke SN and Kachole MS: Antibacterial activity of Cow urine against some Pathogenic and Non-pathogenic Bacteria. *Int J Pharm Sci Res* 2013; 4(4); 1534-1539.