
Bio-Enhancing Efficiency of Cow Urine and its Distillate Combination with Antibiotics against Bacterial Pathogens

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Abstract:

Cow urine has natural disinfectant and antiseptic qualities. In ayurveda it is suggested for improving general health. In our work we used urine as a bio-enhancer to increase the antimicrobial activity of some antibiotics such as ampicillin, chloramphenicol, erythromycin, streptomycin, rifampicin and norfloxacin. The test bacterial pathogens used in this study include *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Bacillus sp*, *Staphylococcus sp* and *Pseudomonas aeruginosa*. The antimicrobial activity was tested using disc diffusion technique. The plates were incubated at 37°C for 48 hours. After incubation period the zone of inhibition was measured and recorded.

Key Words: Cow urine, cow urine distillate, antibiotics, bacterial pathogens.

Introduction:

In India since ancient times the urine has been described as a substance with innumerable medicinal properties. The elements present in cow blood are also present in its urine¹. Cow urine has natural disinfectant and antiseptic qualities. Cow urine has amazing germicidal power to kill variety of microorganisms. The cow urine has ability to prevent chromosomal aberrations. Cow urine has been found to enhance the effect of antibiotics. A composition by Indian scientists, using cow urine distillate to enhance the antimicrobial effect of the antibiotic present in the formulation, has been granted a US patent. Use of the distillate could help reduce the dosage of antibiotics, drugs and anti-cancer agents while increasing the efficiency of absorption of antibiotics and other drugs². Cow urine provides immune power by increasing resistance against diseases, it activates macrophages. It contains many minerals especially copper, it compensates for bodily mineral deficiency. When cow urine distillate is mixed with antibiotics it helps to improve the efficiency of the drug³. The cow urine distillate enhances the transport of antibiotics such as rifampicin, tetracycline and ampicillin across the gut as well as artificial membrane⁴.

The bacterial pathogens used in this study include *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Bacillus sp*, *Staphylococcus sp* and

Pseudomonas aeruginosa. *Escherichia coli* is a predominant organism causing urinary tract infection⁵. Shigellosis is caused by *Shigella sp*⁶. It produces shiga toxin and is associated with serious complications⁷. *Pseudomonas aeruginosa* is of medical importance it causes nosocomial infection, the infection occurs from contaminated hospital equipments such as syringes, lumbar needle and catheters⁸. *Staphylococcus aureus* causes urinary tract infection in individuals with renal calculi after instrumentation⁹. Most common cause of nosocomial bacteremia clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) was recognized in the United States¹⁰. It causes pyogenic lesions, abscesses and suppurative lesions. *Salmonella typhi* causes typhoid fever it is resistant to environmental conditions, it causes fever head ache, abdominal pain and malaise⁵. *Enterobacter aerogenes* causes urinary tract infection and to less extend it also causes nosocomial infection¹¹.

The aim of the present study is the bio-enhancing efficiency of cow urine and its distillate in combination with antibiotics against bacterial pathogens.

Materials and Methods:

The cow urine sample was collected in a sterile conical flask and it was wrapped to avoid deterioration.

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Preparation of Cow Urine Distillate:

The urine distillate was collected using distillation process. The urine was heated in a distillation apparatus, the vapour which comes out from the device is condensed with cold water and was collected in a sterile conical flask. Some of the qualities of distilled urine are not same as that of the whole cow urine. The distilled urine is free from the characteristic odour.

Isolation of Bacterial Pathogens:

The test bacterial pathogens such as *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Bacillus sp*, *Staphylococcus sp* and *Pseudomonas aeruginosa* were isolated from patients. They were confirmed using biochemical tests.

Antibiotics:

The antibiotics used in this study include ampicillin, chloramphenicol, streptomycin, erythromycin, rifampicin and norfloxacin.

Preparation of Whatmann Filter Paper:

The whatmann filter paper No 1 was cut in the form of circle of about 1mm in diameter. Then the discs were sterilized in hot air oven at 70°C for 30 minutes. These discs were used for antibiotic susceptibility testing¹².

Totally 5 category of discs were prepared

1. Cow urine
2. Cow urine distillate
3. Antibiotics and distilled water.
4. Antibiotics and cow urine, and
5. Antibiotic and cow urine distillate

Each organism is tested against the above categories of discs. The discs were loaded into each of the above categories of urine and antibiotics and then dried on oven at 60°C to avoid contamination. The concentration of antibiotics were reduced half of amount in combination with cow urine and its distillate as shown in the table below

Table.1:

S. No	Antibiotics	Stock Concentration Of Antibiotics (In µl)	Cow Urine (In µl)	Cow Urine Distillate (In µl)	Antibiotic+ Distilled Water (In µl)		Cow Urine +Antibiotics (In µl)		Cow Urine Distillate +Antibiotics (In µl)	
					AB	D.W	CU	AB	CUD	AB
1	Ampicillin	1mg/10ml	10	10	10	10	10	5	10	5
2	Chloramphenicol	1mg/10ml	10	10	10	10	10	5	10	5
3	Erythromycin	1mg/10ml	10	10	10	10	10	5	10	5
4	Streptomycin	1mg/10ml	10	10	10	10	10	5	10	5
5	Rifampicin	1mg/10ml	10	10	10	10	10	5	10	5
6	Norfloxacin	1mg/10ml	10	10	10	10	10	5	10	5

AB-Antibiotic; CU-Cow Urine; CUD-Cow Urine Distillate; DW-Distilled Water

Antimicrobial Testing:

The drug sensitivity of microorganisms was assessed using Kirby Bauer method. This method allows the rapid determination of the bio enhancing activity of cow urine/distillate in combination with antibiotics by measuring the diameter of zone of inhibition. The cultures were swabbed onto the Muller Hinton agar plates to form a lawn. The dried discs were aseptically placed by using sterile forceps on Muller

Hinton agar plates. The plates were incubated at 37°C for 24 hours.

After incubation the plates were examined for the clear zone around each disc. The diameter of zone of inhibition of antibiotics, cow urine, antibiotics and cow urine were measured and noted.

Results:**Combination of Cow Urine Distillate (CUD) and antibiotics:**

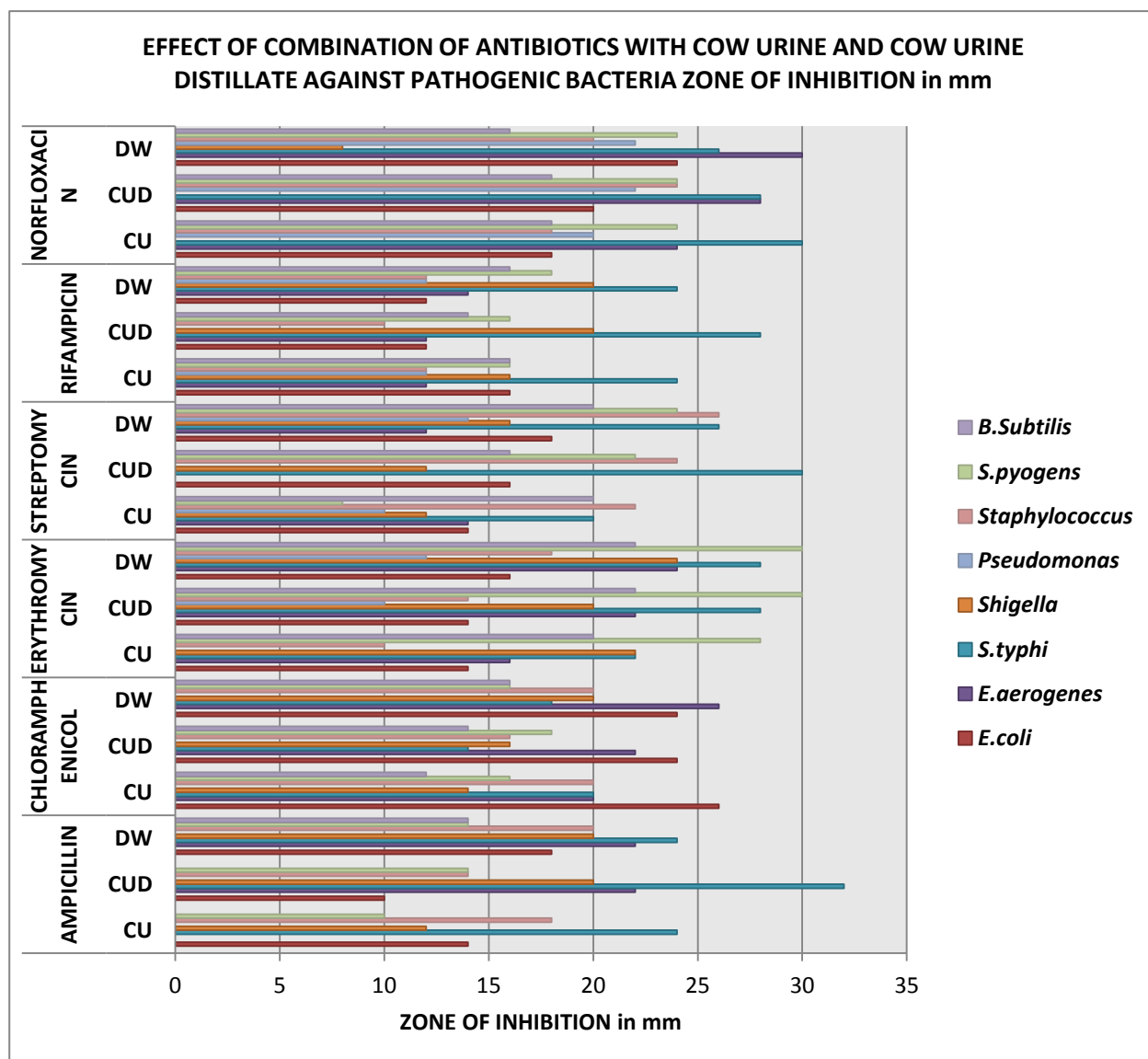
The table 2 shows that the cow urine distillate (CUD) actively enhanced the activity of antibiotics such as ampicillin which was active against *Enterobacter aerogenes*, *Salmonella typhi*, *Shigella* and *Streptococcus pyogenes*. The antibiotic erythromycin and CUD was active against *E. aerogenes*, *S. typhi*, *Shigella*, *Pseudomonas aeruginosa*, *Staphylococcus* sp and *Bacillus subtilis*. The antibiotic

streptomycin and CUD inhibited the growth of *Escherichia coli*, *S. typhi*, *Staphylococcus* sp and *S. pyogenes*. The antibiotic rifampicin and CUD arrested the growth of *E. aerogenes*, *S. typhi*, *Shigella* and *S. pyogenes*. The antibiotic norfloxacin and CUD inhibited the growth of *E. aerogenes*, *P. aeruginosa*, *Staphylococcus* sp and *S. pyogenes*.

Table .2: Effect of Combination of Antibiotics with Cow Urine and Cow Urine Distillate against Pathogenic Bacteria (Zone Of Inhibition in mm)

Bacteria	Ampicillin			Chloramphenicol			Erythromycin			Streptomycin			Rifampicin			Norfloxacin		
	C U	CU D	D W	CU	CU D	D W	CU	CU D	D W	C U	CU D	D W	C U	CU D	D W	C U	CU D	D W
	ZONE OF INHIBITION (in mm)																	
<i>E. coli</i>	14	10	18	26	24	24	14	14	16	14	16	18	16	12	12	18	20	24
<i>E. aerogenes</i>	-	22	22	20	22	26	16	22	24	14	R	12	12	12	14	24	28	30
<i>S. typhi</i>	24	32	24	20	14	18	22	28	28	20	30	26	24	28	24	30	28	26
<i>Shigella</i>	12	20	20	14	16	20	22	20	24	12	12	16	16	20	20	R	R	8
<i>P. aeruginosa</i>	R	R	R	R	R	R	R	10	12	10	R	14	12	R	12	20	22	22
<i>Staphylococcus</i>	18	14	20	20	16	20	10	14	18	22	24	26	12	10	12	18	24	20
<i>S. pyogenes</i>	10	14	14	16	18	16	28	30	30	8	22	24	16	16	18	24	24	24
<i>B. Subtilis</i>	-	-	14	12	14	16	20	22	22	20	16	20	16	14	16	18	18	16

CU-Cow Urine; CUD-Cow Urine Distillate; DW-Distilled Water.

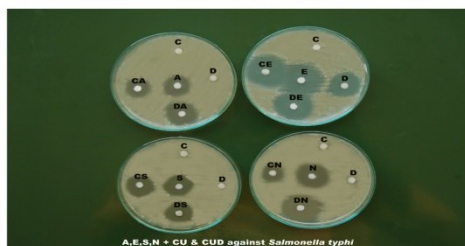


Combination of Cow Urine (CU) and antibiotics:

The table 2 shows that the antibiotics in combination with Cow Urine (CU) show some excellent results against some pathogens. The chloramphenicol and CU was active against *Escherichia coli*, *Enterobacter aerogenes*, and *Staphylococcus*. The antibiotic erythromycin as active against *S. pyogens*, *S. typhi*, and *Shigella*. The antibiotic norfloxacin and CU

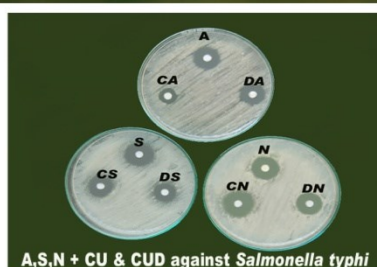
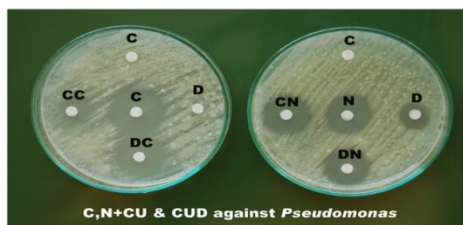
inhibited the growth of *S. typhi*, *E. aerogenes*, and *S. pyogens*. The antibiotic ampicillin and CU inhibited the growth of *S. typhi*, *Staphylococcus* sp and *E. coli*. The antibiotic streptomycin and CU was active against *Staphylococcus* sp, *Bacillus subtilis* and *S. typhi*. The antibiotic rifampicin inhibited the growth of *S. typhi*, *E. aerogenes*, and *S. pyogens*.

**BIOENHANCING EFFICIENCY OF COW URINE AND
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ANTIBIOTICS AGAINST BACTERIAL PATHOGENS**



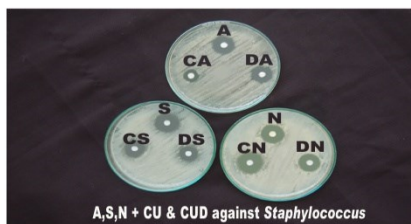
**C-COW URINE
D-COW URINE DISTILLATE
A-AMPICILLIN
E-ERYTHROMYCIN
S-STREPTOMYCIN
R-RIFAMPICIN
N-NORFLOXACIN**

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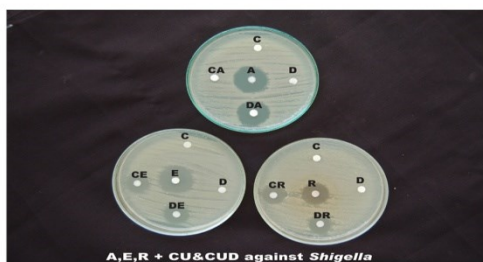
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C- COW URINE
D- COW URINE DISTILLATE
A- AMPICILLIN
E- ERYTHROMYCIN
N- NORFLOXACIN
R- RIFAMPICIN

Discussion:

According to Indian Ayurvedic Script, cow, Kamdhenu is a mobile hospital and cow urine has therapeutic uses in India¹³. The pharmaceutical composition containing antibiotics and cow urine distillate as a bio enhancer was granted a US patent¹⁴.

Cow urine possess anti-cancer activity in combination with drugs and fight against cancers¹⁵. As cow urine distillate and the fraction both shows enhanced membrane permeability by enhancing the antibiotics across the semi permeable gut membrane, it can be used for enhancing intestinal transport of molecules across membrane, it can be used for enhancing intestinal transport and transport of molecules across membrane of various biological function⁴.

In this study, cow urine and cow urine distillate are the effective bio enhancers for antibiotics. (Table 1). Totally 8 bacterial pathogens were tested in this study. Six antibiotics were taken against each pathogens. Out of 8 pathogens, majority were sensitive to the mentioned antibiotics with CU and CUD. In contrast, the organisms *Pseudomonas* was resistant to the antibiotics such as ampicillin,

chloramphenicol when combined both cow urine and cow urine distillate. But the same pathogen was inhibited by Rifampicin and streptomycin; with cow urine it showed the limited zone formation. The antibiotic Norfloxacin with cow urine distillate was efficiently inhibiting the growth of *Pseudomonas* sp (Table 2).

Urinary tract infection are caused by a widely variety of pathogens such as *Escherchia coli*, *Pseudomonas aerogenosa*, *Klebsiella*, *Proteus* and gram positive bacteria *Staphylococcus epidermis*, *Enterococci*, and *Staphylococcus aureus*. *Pseudomonas aeroginosa* strains are generally isolated pathogen in this type of infection and in 6% of primary nosocomial bacteremias¹⁶.

Conclusion:

Cow urine and its distillate drastically reduces the dosage of antibiotics while increasing the efficiency of adsorption of bio-active molecule thereby reducing the cost and toxicity of treatment. The concentration of antibiotics were reduced half of amount in combination with cow urine and its distillate.

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