Research article

Antibacterial properties of distilled cow's urine on bacterial species from clinical specimens

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ABSTRACT

Introduction and Aim: In Ayurveda, cow's urine has been reported to have many therapeutic values. The raising trend in antibacterial resistance is a cause of concern and demands an alternative therapeutic agent. Hence, the present study aims to know the antibacterial properties of urine from cows on clinical and reference strains of bacteria.

Materials and Methods: The present *in vitro* experimental study, used agar dilution method to know the Minimum Inhibitory Concentration (MIC) of cow's urine distillate (CUD) on few reference bacterial strains and *Enterococcus faecalis, Staphylococcus aureus, Salmonella* Typhi, *Escherichia coli*, and *S.* Paratyphi A isolated from clinical samples. Resistance and sensitivity of these isolates to routinely used antibiotics was studied by Kirby Bauer's disk diffusion method.

Results: MIC of CUD for different standard bacterial strains varied from 250 to 500µl/ml. Among the clinical isolates, all *Salmonella* Typhi, 85% of *S.* Paratyphi A, 60% of *E. coli*, 80% of the *S. aureus*, 64.28% *S. aureus* were resistant to methicillin (MRSA) and 24% of *E. faecalis* were killed by 500µl/ml of CUD. Clinical strains of bacteria, susceptible to routinely used antibiotics, were also found to be susceptible to CUD and showed a concentration-dependent inhibitory effect. Multi drug resistant strains of nine MRSA, one *E. faecalis* and two *E.coli* were also susceptible to 500µl/ml concentration of CUD.

Conclusion: CUD can be considered as an alternative anti-bacterial agent for multi-drug resistant bacterial pathogens as it showed concentration dependent inhibitory effect on both Gram-positive and Gram-negative pathogenic bacteria.

Keywords: Antibacterial effect; cows' urine; *Enterococcus* spp.,; *Salmonella* spp.,; *Staphylococcus* spp.,.

INTRODUCTION

Increasing prevalence of communicable diseases caused by multiple drug resistant pathogens and shortage of new antimicrobial agents is a significant health concern, which necessitated a quest for new antimicrobial agents (1). Nature has always been a source of bioactive compounds which have cured many human ailments. Ayurveda, the Indian system of medicine has been practiced as a natural alternative medicine since ancient times and is considered as a standard health care system in India (2). Cow urine (*gomutra*) has an exceptional place in Ayurvedic medicine. *'Sushruta Samhita'* and '*Ashtanga'* describes cows' urine as an effective secretion of animal with many beneficial healing effects. It is highly efficacious against stem borers and acts as a bioenhancer (3-5). The cow's urine distillate (CUD) is considered having activity enhancing and facilitator property for biologically dynamic molecules. CUD has been used as anti-infective and anti-cancer agent (6). It is known to possess immunomodulatory, anti-diabetic, antioxidant, and anti-fungal effects (7-10). Further, enhanced wound healing, delayed aging and curative effects on skin lesions have also been reported (11). Medicinal value of cow's urine was appreciated long ago as its efficacy and mechanism of antibacterial effect remain elusive, thorough scientific validation is required to prove its efficacy. Few studies from different Indian states like Madhya Pradesh, Kerala, Karnataka, Gujarat, and New Delhi, have reported the antibacterial effect of cow's urine (12-17). Interestingly, urine of most of the native cow exhibit antimicrobial properties but not the urine of buffalo, goat and crossbred cows (18). Thus, antimicrobial property of urine of cows is dependent on cow breed and geographical area. Anti-bacterial action of CUD on clinical bacterial isolates is not reported from Mangalore India. Moreover, rise in drug resistance demands for an alternative easily available cost-effective agent. Hence, we attempted to study the antibacterial effect of CUD on reference bacterial strains and common Gram-positive and Gram-negative bacteria isolated from patient samples.

MATERIALS AND METHODS

This is an *in vitro* experimental study, where antibacterial action of CUD was tested on reference bacterial strains and common Gram-positive and

Gram-negative bacteria cultured from patient samples. Institutional Ethics committee clearance was obtained before commencing the study (Reference No: IEC KMC MLR, 02-14/66).

Source of distilled cow's urine

Distillate of cow's urine was procured from a specific local breed (Kapila) of healthy cow, confined to Southern Karnataka, reared at a cow yard located at Surabhivana, Kompadavu, Mangalore.

Bacterial strains used in the study

Standard reference strains of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were obtained from departmental stock. Clinical isolates (n=120) comprised of Gram-positive cocci: *S. aureus* (n=25), *E. faecalis* (n= 25) and Gram-negative bacilli: *E. coli* (n=25), *S.* Typhi (n=25) and *S*. Paratyphi A (n=20). The culture media and chemicals utilised in our study were obtained from Hi-Media Laboratories Pvt Ltd. Mumbai, India. Standard biochemical tests were performed to identify the bacterial isolates (19).

Sterility check of distillate of cow's urine

Sterility check was performed by inoculating 9 ml of Brain Heart Infusion (BHI) broth with one millilitre of CUD and incubating the same at 37^0C for four weeks. Subculture of BHI broth was done on blood agar, MacConkey's agar and Sabouraud's dextrose agar on 2^{nd}, 4^{th}, 7^{th}, 14^{th}, 21^{st} and 30^{th} day of incubation. Inoculated culture media were incubated at 37^0C for 48h and observed for any growth (19). Only those distilled cow's urine samples that showed no detectable bacterial or fungal growth were used in the study.

MIC detection of cow's urine on reference and clinical bacterial strains

Agar dilution technique was used to study the MIC of sterile CUD on reference strains of *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and bacterial isolates from clinical samples (20). Different concentrations of CUD ranging from 200 to 500µl/ml were added to molten sterile Muller Hinton agar (MHA) and allowed to solidify. Reference and clinical strains were grown in Muller Hinton broth (MHB) for 6 hrs. Turbidity of culture was maintained at 0.5 Mac Farland standard (10^6 CFU/ml). Two microliters of this turbidity adjusted culture was used to inoculate MHA with varying concentration of CUD (200 to 500µl/ml) and MHA without CUD. The culture plates were incubated at 37°C for 24hr. Testing was repeated twice, and reproducible results were obtained. The maximum dilution of the CUD that did not show noticeable growth on MHA plates with CUD was considered as MIC. MHA without CUD acted as the growth control.

Antibiotic susceptibility testing of clinical isolates

S. aureus (n=25) isolated from pus, *E. faecalis* and *E.coli* (n=25 each) isolated from urine samples, S. Typhi (n=25) and S. Paratyphi A (n=20) cultured from patient's blood sample were identified by Vitek-2 system. The Kirby Bauer disk diffusion technique was used to check for the antimicrobial resistance of the bacterial strains to routinely used antibiotics and interpreted as per CLSI guidelines (21, 22). Different antibiotic discs (Himedia laboratories Ltd, Mumbai, India), as mentioned in Tables (2, 4-6) were used in the study. *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853 were used as control strains.

RESULTS

Sterility of CUD

BHI inoculated with CUD did not show any observable growth of bacteria or fungi during the prolonged incubation period of 30 days. This sterile CUD was used in all our experiments.

Effect CUD on standard bacterial strains

The standard reference strains of *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853 were treated with varying concentration of CUD to know the MIC and the outcome is depicted in Table 1.

Table 1: MIC of CUD on standard bacterial strains as determined by agar dilution technique

Bacterial strain	Minimum inhibitory concentration (MIC v/v)
S. aureus ATCC 25923,	500 µl/ml
E. faecalis ATCC 29212	450 µl/ml
E. coli ATCC 25922	300 µl/ml
P. aeruginosa ATCC 27853	250 µl/ml

Susceptibility of Salmonella isolates to routinely used antibiotics and CUD

Isolates of *S.* Typhi (n=25) and *S.* Paratyphi *A* (n=20) obtained from the blood culture of patients suffering from enteric fever were found to be susceptible to chloramphenicol, cefuroxime, ceftriaxone, cotrimoxazole, ciprofloxacin, and ofloxacin, which are the routinely used antibiotics for treating the patients. However, nalidixic acid resistance was around 80% in *S.* Typhi and 85% in *S.* Paratyphi A (Table 2). 100% of the *S.* Typhi and 85% of the *S.* Paratyphi A tested were inhibited by 500µl/ml of CUD. It was also observed that CUD had concentration-dependent or dose-dependent action as shown in Table 3.

Table 2: Antibiogram of *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A to routinely used antibiotics by disk diffusion method

Antibiotics Tested (µg)	***Salmonella* Typhi (n =25)**		***Salmonella* Paratyphi A (n=20)**	
	Susceptible N (%)	Resistant N (%)	Susceptible N (%)	Resistant N (%)
Ampicillin (10µg)	22 (88)	3 (12)	20 (100)	0 (0)
Chloramphenicol (30µg)	25 (100)	0 (0)	20 (100)	0 (0)
Cefuroxime (30µg)	25 (100)	0 (0)	20 (100)	0 (0)
Ceftriaxone (30µg)	25 (100)	0 (0)	20 (100)	0 (0)
Co-Trimoxazole (23.75+1.25µg)	25 (100)	0 (0)	20 (100)	0 (0)
Ciprofloxacin (5µg)	25 (100)	0 (0)	20 (100)	0 (0)
Ofloxacin (5µg)	25 (100)	0 (0)	20 (100)	0 (0)
Nalidixic Acid (30µg)	05 (20)	20 (80)	03 (15)	17 (85)

Table 3: Susceptibility of bacterial isolates to varying concentrations of CUD by agar dilution method

Concentration of cow's urine distillate (µl/ml)	**Different bacterial isolates tested** (n=120)					**Total N (%)**
	***S.aureus* N (%)**	***E.faecalis* N (%)**	***E. coli* N (%)**	***S.* Typhi N (%)**	***S.* Paratyphi A N (%)**	
200	0 (0)	3 (12)	0 (0)	1 (4)	0 (0)	04 (3.4)
250	01 (4)	3 (12)	0 (0)	3 (12)	0 (0)	07 (5.8
300	05 (20)	4 (16)	0 (0)	3 (12)	0 (0)	12 (10.0)
350	07 (28)	5 (20)	0 (0)	8 (32)	0 (0)	20 (16.8)
400	09 (36)	6 (24)	0 (0)	13 (52)	7 (35)	35 (29.2)
450	14 (56)	5 (20)	12 (48)	16 (64)	17 (85)	64 (53.4)
500	20 (80)	6 (24)	15 (60)	25 (100)	17 (85)	83 (69.2)
≥550 (No effect)	05 (20)	19 (76)	10 (40)	0 (0)	3 (15)	37 (30.8)
Total	**25 (20.8)**	**25 (20.8)**	**25 (20.8)**	**25 (20.8)**	**20 (16.8)**	**120 (100)**

Table 4: Antibiogram of *Escherichia coli* (n=25) to routinely used antibiotics by disk diffusion method

Antibiotics Tested	**Susceptible N (%)**	**Resistant N (%)**
Ampicillin (10µg)	09 (36)	16 (64)
Ampicillin + Sulbactam (10/10µg)	19 (76)	06 (24)
Amoxiclav (20/10µg)	18 (72)	07 (28)
Gentamicin (10 µg)	17 (68)	08 (32)
Ceftazidime (30 µg)	15 (60)	10 (40)
Cefotaxime (30 µg)	20 (80)	05 (20)
Piperacillin (100 µg)	14 (56)	11 (44)
Nitrofurantoin (300µg)	15 (60)	10 (40)
Nalidixic Acid (30µg)	13 (52)	12 (48)
Norfloxacin (10µg)	17 (68)	08 (32)
Ofloxacin (5µg)	21 (84)	04 (16)
Ceftriaxone (10µg)	19 (76)	06 (24)
Ticarcillin+Clavulanic Acid (75/10µg)	21 (84)	04 (16)
Cefazolin (30µg)	24 (96)	01 (04)
Netillin (30µg)	23 (92)	02 (08)
Co-Trimoxazole (23.75+1.25µg)	13 (52)	12 (48)
Ciprofloxacin (5 µg)	23 (92)	02 (08)

Susceptibility of *E. coli* isolates to routinely used antibiotics and CUD

E. coli (n=25) obtained from patients with urinary tract infection were found to be resistant to most of the routinely used antibiotics. 92% of the isolates were susceptible to netillin and ciprofloxacin whereas 96% of the *E. coli* isolates were sensitive to cefazolin. (Table 4). Fifteen strains (60%) of *E. coli* were inhibited by 500µl/ml concentrate of CUD. However, 10 (40%) of the isolates were resistant to CUD as depicted in Table 3.

Susceptibility of *S. aureus* to routinely used antibiotics and CUD

Antibiotic susceptibilities of *S. aureus* (n=25) got from pus samples of patients with wound infection is shown in Table 5. All *S. aureus* tested were sensitive to vancomycin, linezolid, teicoplanin, netillin, and chloramphenicol, whereas 56% of the *S. aureus* isolates were MRSA. 20% of the strains were resistant to CUD. However, 80% of the strains were inhibited by 500µl/ml concentration of CUD as shown in Table 3.

Table 5: Antibiogram of *S. aureus* (n=25) to routinely used antibiotics by disk diffusion method

Antibiotics tested (µg)	Susceptible N (%)	Resistant N (%)
Ampicillin (10µg)	0 (0)	25 (100)
Cefoxitin (30µg)	11 (44)	14 (56)
Gentamicin (10µg)	11 (44)	14 (56)
Cefoperazone (75µg)	19 (76)	06 (24)
Clindamycin (2µg)	20 (80)	05 (20)
Linezolid (10µg)	25 (100)	0 (0)
Teicoplanin (30µg)	25 (100)	0 (0)
Vancomycin (30µg)	25 (100)	0 (0)
Chloramphenicol(30µg)	25 (100)	0 (0)
Rifampicin (5µg)	24 (96)	01 (04)
Netillin (30µg)	25 (100)	0 (0)
Erythromycin (15µg)	11 (44)	14 (56)

Susceptibility of *E. faecalis* isolates to routinely used antibiotics and CUD

Antibiotic susceptibility pattern *of E. faecalis* (n=25) isolated from pus and urine samples of patients is shown table 6. All the isolates were found to be susceptible to teicoplanin as shown in Table 2. 12% of the strains were suppressed by 200µl/ml concentration of CUD, and 24% of the strains were suppressed by 500µl/ml concentration of CUD as shown in Table 3.

Table 6: Antibiogram of *E. faecalis* (n=25) to routinely used antibiotics by disk diffusion method

Antibiotics tested	Susceptible N (%)	Resistant N (%)
Ampicillin (10µg)	20 (80)	05 (20)
Amikacin (30µg)	8 (32)	17 (68)
Amoxicillin/ Clavulanic acid ((20/10µg)	19 (76)	06 (24)
Cefotaxime (30µg)	14 (56)	11 (44)
Clindamycin (2µg)	19 (76)	06 (24)
Teicoplanin (30µg)	25 (100)	0 (0)
Vancomycin (30µg)	23 (92)	2 (8)
Ticarcillin+Clavulanic Acid (75/10µg)	16 (64)	9 (36)
Erythromycin (10µg)	2 (8)	23 (92)
Penicillin (10 units)	8 (32)	17 (68)
Piperacillin+Tazobactam (100/10 µg)	23 (92)	2 (8)
Ciprofloxacin (5 µg)	13 (52)	12 (48)

Effect of CUD on multidrug-resistant (MDR) clinical isolates

Effects of different concentrations of CUD on isolates got from patient samples [*S. aureus*, *E. coli*, and *E. faecalis*] that were resistant to more than three routinely used antibiotics, were studied. It is interesting to note that there were 9 MDR *E. coli*, 14 MRSA, 7 MDR *Enterococcus*. Out of these MDR strains two *E. coli*, nine MRSA and one *E. faecalis* were susceptible to a 500µl/ml concentration of CUD. Effect of CUD on bacteria that are sensitive to all routinely used antibiotics, as well as MDR bacteria is shown in Table 7.

Table 7: Effect of CUD on multidrug resistant (MDR) and multidrug susceptible (MDS) clinical bacterial isolates.

Clinical isolates tested	No. of strains studied (n)	No. of MDS strains	No. of MDS* strains inhibited by 50% CUD concentration	No. of MDR# strains	No. of MDR strains inhibited by 50% CUD concentration
Escherichia coli	25	3	2	9	2
Staphylococcus aureus	25	5	5	14	9
Enterococcus spp.	25	0	0	7	1
Salmonella Typhi	25	5	5	0	0
Salmonella Paratyphi A	20	3	3	0	0

*MDS: Bacterial strains susceptible to all the antibiotics tested by disc diffusion.

#MDR: Bacterial strains resistant to three or more antibiotics tested by disc diffusion

DISCUSSION

The study was undertaken to know the effect of CUD on clinical bacterial isolates. We selected four different bacterial species, which are the common cause of human infections in this part of the country. Distilled cow's urine was initially tested for sterility and was found to be sterile as observed in the earlier studies (16,17). Sterile CUD was tested on the standard strain of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, to know the MIC. It was surprising to note that standard strains of Gram-negative bacilli were more sensitive (lower MIC) to CUD than the standard strains of Gram-positive cocci (Table 1). Our findings indicate that CUD has a higher antibacterial effect on standard strains of Gram-negative bacteria.

Further, we studied the effect of CUD on 25 strains each of *S. aureus*, *E. coli*, *E. faecalis*, *S. Typhi* and 20 strains of *S. Paratyphi* A. The effect of CUD on these clinical isolates are shown in Tables 3 and 7. It is interesting to note that 500µl/ml concentration of CUD could kill 100% of *S. Typhi*, 85% of *S. Paratyphi* A, and 60% of *E. coli* (Table 3). Our results highlight the fact that CUD has concentration-dependent action on clinical isolates of bacteria. Similar effect was observed for *Candida* spp. isolated from clinical samples (23). Our study shows CUD to be very effective against *S. Typhi* when compared to *E. coli*. Our observation of maximum strains of *S. Typhi* being sensitive to CUD is in line with a study conducted by Muthaiya Research Foundation in Tamil Nadu, India (10). However, they have studied the effect of CUD only on standard strains of bacteria and not on the clinical bacterial isolates.

Among the Gram-positive clinical strains studied 80% of the *S.aureus*, and only 24% of *E. faecalis* were killed by 500µl/ml concentration of CUD (Table 3). Further, we also observed that most of the strains which were susceptible to routinely used antibiotics (100% strains of *S. aureus* and *Salmonella* and 66.67% of *E. coli*) were also susceptible to CUD (Tables 2-5 and 7). In an earlier study, standard strains of *S. aureus* and *E. coli* which were sensitive to ofloxacin were also sensitive to CUD, and *S. aureus* was more susceptible than *E. coli* (12). However, their study has not checked the effect of CUD on clinical isolates as has been done in our study.

A research study from Gujarat, India by Shah *et al.*, concluded that owing to the variation in the cell wall structure, cows' urine has better bactericidal effect on Gram-positive bacteria when equated to Gram-negative bacteria (16). However, the present study does not support these findings, as *S.* Typhi was 100% inhibited by CUD, followed by *S. Paratyphi A*, *S. aureus* then *E. coli* and lastly *Enterococcus* (Table 3). Difference in the findings of our study may be attributed to the fact that we have used agar dilution method and studied the effect of CUD on standard and clinical bacterial isolates whereas, earlier study from Gujarat have examined the antibacterial effect by disk diffusion method on the standard strains only. Alternatively, there could also be differences in the genotype of clinical isolates and the nature of cow's urine which is dependent on the cow's breed and their feeding habits. Sharma *et al.,* have detected the antimicrobial peptides belonging to defensin family in cows' urine using Mass Spectrometry (24). Further studies are necessary to know whether excretion of these antimicrobial peptides vary in different breeds and depends on the diet of the cattle. Additional studies are also necessary to identify the best suited method for MIC determination of CUD on clinical isolates of bacteria.

In the present study clinical isolates of *Enterococcus* spp. were found to be more resistant to CUD than other clinical bacterial isolates (Tables 3 and 6). Earlier workers have observed invitro antibacterial effect of photo activated cow's urine on standard strains of *E. faecalis* inoculated into root canals of teeth (25). However, to compare our results, studies focusing on the effect of CUD on clinical isolates of *Enterococcus* spp. are lacking.

Another notable observation was the antibacterial effect of CUD on MDR bacterial isolates. Nine strains of MRSA were susceptible to 500µl/ml concentration of CUD (Table 7). We did not come across any report on the effect of CUD on MRSA. Hence, in the era of emerging MRSA, cow's urine might show some promise in treating infections caused by MRSA after performing a clinical trial. Further, one MDR *E. faecalis* and two MDR *E.coli* were also susceptible to 500µl/ml concentration of CUD. Additional studies involving large number of these MDR *Enterococcus* and *E. coli* strains are necessary to draw any inference.

It has been reported that urea, aurum hydroxide, creatinine, phenols, calcium, present in CUD impart antimicrobial property to it. Further, bioenhancer property of CUD is more than cow urine and hence CUD enhances the antimicrobial effect of drugs (9). Recent study has identified antimicrobial peptides in cows' urine using mass spectrometry (24). Thus, remarkable inhibition of pathogenic clinical bacterial isolates by CUD provides an incentive to perform more studies to discover the exact constituent and deduce its mechanism of action which can further be exploited to reduce increasing dependence on antibiotics. Cow's urine offers potential to be explored as an antibacterial for the broader spectrum of bacterial strains thereby establishing scientifically its long-known healing power.

CONCLUSION

The present study highlights the remarkable dose-dependent effects of cow's urine distillate as an

antibacterial agent, exhibited maximally for *S. Typhi* and minimally for *E. faecalis*. CUD was shown to inhibit the growth of most bacterial strains which were either sensitive or resistant to routinely used antibiotics, indicating that the antibacterial effect is almost comparable to antibiotics. Further studies are necessary to characterize the different antimicrobial peptides present in CUD of different breed of cattle and their antibacterial action on different clinical bacterial isolates. Thus, antimicrobial peptides in CUD when characterized fully may be a promising alternative to treat drug resistant bugs.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

1. Dall, C. WHO report highlights shortage of new antibiotics. CIDRAP News Apr 15, 2021. https://www.cidrap.umn.edu/news-perspective/2021/04/who-report-highlights-shortage-new-antibiotics [last accessed 29-Dec-2021]
2. Vaidya, A.D.B., Devasagayam, T.P.A. Current Status of Herbal Drugs in India: an overview. J. of Clin Biochem and Nutr. 2007; 41:1-11.
3. Garg, N., Kumar, A., Chauhan, R.S. Effect of indigenous cow urine on the nutrient utilization of white leghorn layers. International J Cow Sci. 2005;1: 36-38.
4. Gupta, G., Yadav, S. Cow Urine Efficacy Against Stem Borers and Cost Benefit in Soybean Production. International J Cow Sci. 2006; 2:15-17.
5. Randhawa, G.K. Cow urine distillate as bioenhancer. J Ayurveda Integr Med. 2010; 1:240-241.
6. Khanuja, S.P.S. Pharmaceutical composition containing cow urine distillate and an antibiotic.2002. US Patent No US6410059 B1/2002.
7. Chauhan, R.S., Singh, B.P., Singhal, L.K. Immunomodulation with kamdhenu arka in mice. J Immunol Immunopathol. 2001;71: 89-92.
8. Ojewole, J. A., Olusi, S.O. Effects of cow's urine concoction on plasma glucose concentration in fasted rats. Trans R Soc Trop Med Hyg. 1976;71: 241-245.
9. Krishnamurthi, K., Dutta, D., Sivanesan, S.D., Chakrabarti, T. Protective effect of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. Biomed Environ Sci. 2004;17: 57-66.
10. Sathasivam, A.K., Muthuselvam, M., Rajendran, R. Antimicrobial activities of cow urine distillate against some clinical pathogens, Global Journal of Pharmacology. 2010; 4:41-44.
11. Sanganal, J.S., Jayakumar, K., Jayaramu, G.M., Tikare, V.P., Paniraj, K.L., Swetha, R. Effect of cow urine on wound healing property in Wistar Albino Rats. Veterinary World. 2011;4: 317-321.
12. Jerald, E., Edwin, S., Tiwari, V., Garg, R., Toppo, E. Antioxidant and antimicrobial activities of cow urine. Global Journal of Pharmacology. 2008;2: 20-22.
13. Kumar, S. Analysis of cow's urine for detection of lipase activity and anti-microbial properties. IOSR Journal of Pharmacy and Biological Sciences. 2013; 7:1-8.
14. Rana, R., De, D. *In vitro* antimicrobial screening of cow urine-a potential natural antimicrobial agent. International Journal of Bioassays. 2013;2: 436-439.
15. Upadhyay, R.K., Dwivedi, P., Ahmad, S. Antimicrobial activity of photo-activated cow urine against certain pathogenic bacterial strains. African Journal of Biotechnology. 2010;9: 518-522.
16. Shah, P.C., Patel, D.M., Dhamil, P.D., Kakadia, J., Bhavsar, D., Vachhani, U.D., *et al., In vitro* screening of antibacterial activity of cow urine against pathogenic Human bacterial strains. Int J Curr Pharm Res. 2011;3: 91-92.
17. Mohanvel, S.K., Rajasekharn, S.K., Kandhari, T., Gopal Doss, B.P.K., Thambidurai, Y. Cow urine distillate as a bioenhancer for antimicrobial & antiproliferative activity and redistilled cow urine distillate as an anticlastogen agent. Asian Journal of Pharmaceutical and Clinical Research. 2017;10: 273-277.
18. Bangla, R.K., Singhal, L.K., Chauhan, R.S. Cow urine and immunomodulation: An update on cowpathy. International J Cow Sci. 2005;1: 26-29.
19. Mackie, W., Mccartney, L. Practical medical microbiology,13th ed. London: Churchill Living stone. 1989.
20. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07, 11th edn., Wayne, PA: Clinical and Laboratory Standard Institute, 2018.
21. Bauer, A.W., Kirby, W.M., Truck, M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Path. 1966;45: 493-496.
22. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Approved standard M100-S27 27th edn. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
23. Hoh, J.M., Dhanashree, B. Antifungal effect of cow's urine distillate on Candida species. J Ayurveda Integr Med. 2017;8: 233-237.
24. Sharma, A., Nigam, R., Kumar, A., Singh, S. Mass Spectrometry-Based Identification of Urinary Antimicrobial Peptides in Dairy Cows. Protein Pept Lett. 2020;27(3):225-235.
25. Rao, A.S., Lakkireddy, S., Rao, N.B., Rao, V.G. *In vitro* antimicrobial efficacy of photoactivated cow urine against enterococcus faecalis, Indian Journal of Conservative and Endodontics, 2016;1:13-16.