

Original Research Article

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Assessment of Antimicrobial Properties of Cow Urine Distillates

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ABSTRACT

The present study was planned to assess antimicrobial properties of cow urine distillate. A total of six cow urine distillates comprising of five commercially available and one prepared in the laboratory were used in present study. All these six cow urine distillates were coded as C1, C2, C3, C4, C5 and P. The phenol coefficient of all six cow urine distillates was evaluated against *S. aureus* and *E. coli*. The phenol coefficients of C4 and C5 against *S. aureus* and *E. coli* were 0.04 ± 0.00 and 0.03 ± 0.00 respectively. Agar well diffusion technique was utilized for the *in-vitro* antimicrobial property of the cow urine distillate against *S. aureus* and *E. coli*. The C4 distillate showed maximum zone of inhibition (18 ± 0.45 mm) against *S. aureus* and (15.21 ± 0.82 mm) against *E. coli*. The zones of inhibition of C5 distillate against *S. aureus* and *E. coli* were 10.51 ± 0.44 mm and 15.21 ± 1.27 mm respectively. The distillates C3, C4 and C5 were assessed for efficacy of them for their antimicrobial activity in three randomly selected poultry slaughter shops on platform used for poultry slaughter. The observations were made by calculating TVC, *S. aureus* and *E. coli* counts before and after treatment of cow urine distillates. It was interesting to note that all three distillate (C3, C4 and C5) showed highly significant ($p < 0.01$) lower TVC counts post treatment. Comparison of mean *S. aureus* counts of C3, C4 and C5 groups before and after treatment showed that C4 and C5 distillates highly significant ($p < 0.01$) lower the *S. aureus* counts. However, the effect of C3 on *S. aureus* counts was not seen. The C3 and C4 cow urine distillates exhibited highly significant ($p < 0.01$) reduction in *E. coli* counts whereas, C5 distillate significantly ($p < 0.05$) reduced the *E. coli* counts.

Keywords

Cow urine distillate,
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Introduction

Indian cow urine is of medicinal value as per ancient Ayurveda. The literature like Charaka Samhita, Sushruta Samhita, Atharva Veda etc. narrate details of its use. A lot of research has been conducted in Cow Urine Treatment and Research Centre, Indore. It has been reported

that cow urine is capable of curing many diseases like blood pressure, asthma, thyroid etc. In Ayurveda, cow urine used almost as broad spectrum antibiotic as therapeutic agent. It is also used in agriculture and sericulture. Cow urine is used as disinfectant and for purification. It has approximate shelf life of five years (Sai Kishore *et al.*, 2015).

A study of cow urine distillate against clinical pathogens like *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and fungus *Aspergillus niger* and *Aspergillus flavus* showed antimicrobial activity. Antimicrobial activity of cow urine distillate was tested using agar well method against microbes like *E. coli*, *S. aureus*, *Klebsiella pneumoniae*. Comparatively fresh cow urine was found to be more active than its distillate. The activity of fresh cow urine was comparable with ofloxacin. Cow urine distillate with extracts of herbs like *Azadirachta indica* showed antimicrobial activity against multidrug resistant clinical isolates of *E. coli* and *Klebsiella pneumoniae* (Kaushik *et al.*, 2016).

Various infectious agents of public health significance survive in the environment like *E. coli*, *S. aureus*, *Klebsiella*. The infectious agents are shed in excretions or secretions of animals or present in production like meat and remain viable for long periods in the environment. In poultry slaughter house building, water, utensils, personnels, platform etc. can be source of infectious agents in food chain. Disinfection is an essential part of hygiene control programme. Chemical disinfectants like sodium hypochlorite or heated strong solution of sodium hydroxide are being used. The most important decision in disinfection programme is the choice of disinfectant is based on superiority of particular disinfectant for the circumstances in which it is being used and infectious agents. To ensure success disinfection requires planning and thorough implementation. Many chemicals used as disinfectants are corrosive, toxic or hazardous. Selection of disinfectant requires knowledge of antimicrobial spectrum, cost, availability and safety (Koleci *et al.*, 2007).

For antimicrobial pharmaceutical composition of cow urine distillate granted U.S. patent. The patent was granted for bioenhancing

property of cow urine distillate for various antibiotics (Khanuja *et al.*, 2002).

Many herbal extracts in cow urine distillates have been found to be effective as antimicrobials. Rajapandiyan *et al.*, (2011) used cow urine extract of *Azadirachta indica* as a novel controlling agent towards clinically significant antimicrobial. Poornima *et al.*, (2012) used cow urine extracts of *Polyalthia longifolia* for estimation of antimicrobial activity. Farida *et al.*, (2014) used *Shatavari*, *tulsi*, *neem*, *guduchi* cow urine extracts to study their antimicrobial activity on human pathogens.

The herbal disinfectants was found to be effective in controlling microbes, non-corrosive and safe to use on most surfaces, environment friendly and biodegradable. (Mandavgane *et al.*, 2005) developed cow urine based disinfectant. They used neem, ritha, pine oil extracts in cow urine.

The slaughter house is a critical stage in meat production as it presents opportunities for contamination. The manner in which poultry is slaughtered, eviscerated, dressed and stored can affect growth of pathogens and potential contamination of meat. The lack of hygiene practices by plant workers can also contaminate carcasses. Slaughter house is considered as a vehicle for human food-borne diseases. Although spectrum of meat-borne diseases of public health importance has change due to improved production system continuation of problem is well illustrated (Zailani *et al.*, 2016).

Keeping these facts in view, the present study was planned to study antimicrobial properties of commercially available cow urine distillates against *E. coli* and *Staphylococcus aureus* and to compare of efficacy of commercially and locally available cow urine distillates as disinfectant.

Materials and Methods

Cow urine distillate: A total of five commercially available cow urine distillates were procured from the market and cow urine distillate was also prepared in the laboratory. All these six cow urine distillates were given code as C1, C2, C3, C4, C5 and P, respectively.

Phenol coefficient method

Phenol coefficient was used for evaluation of disinfectant property of cow urine distillates using *S. aureus* (MTCC96) and *E. coli* (MTCC1652) cultures procured from Department of Veterinary Public Health and Epidemiology, Nagpur Veterinary College, Nagpur. The method used was United States FDA method of testing antiseptics and disinfectants described by Ruehle and Brewer, (1931). The highest dilution of that kills bacteria in 10 min was considered positive for calculation of phenol coefficient. Phenol coefficient was calculated by the formula,

Phenol coefficient of test compound=

$$\frac{\text{Dilution of test disinfectant}}{\text{Dilution of Dilution of phenol}}$$

Antimicrobial activity

Antimicrobial activity of cow urine distillates were evaluated by method described by Ahuja *et al.*, (2012). The test organisms used in the study were *Staphylococcus aureus* (MTCC96) and *Escherichia coli* (MTCC1652) cultures procured from Department of Veterinary Public Health and Epidemiology, Nagpur Veterinary College, Nagpur. The cultures were maintained at 4°C on nutrient agar (Himedia laboratories, Mumbai). The antibacterial activity of all cow urine distillates were performed using Agar Well Diffusion method.

Poultry slaughter shop

A total of 3 poultry slaughter houses were randomly selected for evaluation of antimicrobial property of cow urine distillates.

Swab collection from poultry slaughter house

Sterile swab samples were collected from selected poultry meat shops platform before and after cow urine distillate treatment by following standard procedures Patterson (1971).

Total Viable Count (TVC)

The TVC were determined by using Standard Plate Count Agar as per method described in AOAC (1997)

Isolation of organisms

Isolation and enumeration of *Staphylococcus aureus*, *E. coli* was done by using selective media as per method described in BAM (1998).

Recording and handling of data

The phenol coefficient of cow urine distillates were recorded and their means were compared amongst groups using CRD. The mean zones of inhibition of *S. aureus* and *E. coli* were calculated. The zones of inhibition were recorded as (Ømm). The mean zones of inhibition were compared amongst group using CRD. The data were analysed using WASP - Web Agri Stat Package 2.0 developed by ICAR Research complex for Goa.

Results and Discussion

Cow urine distillates

A total of five commercially available cow urine distillates were procured from the

market. Cow urine distillate was also prepared in the laboratory. All these six cow urine distillates were given coded as C1, C2, C3, C4 and C5 whereas the laboratory prepared cow urine distillate was given code as P. Cow urine distillates C2, C3, C4 and C5 contains extracts of various herbs in cow urine. A total of eight replicates of each cow urine distillate were made consisted of 48 samples for analysis. The details of sample collection are given in Table 1. Antimicrobial properties were tested including phenol coefficient of all 48 samples.

Poultry slaughter shop

After quality assessment, the cow urine distillates were evaluated for their efficiency in poultry slaughter shop for disinfection. The cow urine distillates showing antimicrobial property upon *in-vitro* analysis were used for efficacy assessment. A total of 3 poultry slaughter house were randomly selected from Parbhani city. All the poultry slaughter shop were given code names as S1, S2 and S3. The details of poultry slaughter shop used are given in Table 2. A total of three cow urine distillates were tested during experiment. A total of six replicates of each cow urine distillate were made. The cow urine distillates were analysed before and after treatment of platform in relation to total viable counts, total *Staphylococcal* and *E. Coli* counts.

Estimation of Phenol Coefficient

Varley and Reddish (1936) recommended Phenol coefficient test developed by Rideal and Walker in 1903 adopted by U.S. FDA for evaluation of disinfectants using *S. aureus* and *E. coli* with a exposure time of 5 minutes interval upto 20 minutes. In India, Food Safety and Standard Regulations,(2011) stipulated microbial standards for poultry slaughter houses (Pradeep Kumar *et al.*, 2014). Various workers developed cow urine

based disinfectants. Mandavgane *et al.*, (2005) used neem, tulsi, ritha and pine oil for development of cow urine based disinfectant. The phenol coefficient of it against *S. typhi* and *S. aureus*. The cow urine based disinfectant was found to be useful in cleaning surfaces.

In present study all six cow urine distillates were evaluated against *S. aureus* and *E. coli* for a period of 20 minutes.

Against *Staphylococcus aureus*

All six cow urine distillates were evaluated for estimation of phenol coefficient against *S. aureus*. The results are shown in Table 3. It is interesting to note that cow urine distillate C4 and C5 could only inactivate *S. aureus* within 5 minutes at 1:5 concentrations. The phenol coefficients recorded of C4 and C5 are 0.04 ± 0.00 and 0.03 ± 0.00 respectively. The comparison of them revealed non-significant effect amongst them. Earlier, Randhawa (2010) reported use of cow urine distillate as bioenhancer of antimicrobial property of drugs. The cow urine disinfectants are prepared by using various herbal extracts were found to be effective against *S. aureus* (Mandavgane *et al.*, 2005).

In present study effectiveness of cow urine distillate C4 and C5 against *S. aureus* (Table 3) may be due to its bioenhancing property to boost antimicrobial activity of herbal extracts than other cow urine distillates as reported earlier.

Against *Escherichia coli*

The results of estimation of phenol coefficient of all cow urine distillates are given in Table 4. The cow urine distillates C4 and C5 showed phenol coefficients of 0.04 ± 0.00 and 0.03 ± 0.00 respectively against *E. coli*. The average phenol coefficients of both the

disinfectants (C4 and C5) are found to be at par with each other. The other cow urine distillates C1, C2, C3 and P were found to be ineffective against *E. coli*.

The efficacy of disinfectant used in food industry against *E. coli* by using phenol coefficient test was compared by Taylor *et al.*, (1999). In present study also phenol coefficient test has been successfully exploited to evaluate cow urine distillates against *E. coli*.

In-vitro antimicrobial analysis of cow urine distillates

Cow urine is having antimicrobial property in addition to its disinfectant property (Chauhan and Dhama, 2010). The antimicrobial activity is due to presence of various components like urea, phenol, carboxylic acids, calcium, manganese in addition to certain amino acids and urinary peptides (Gulhane *et al.*, 2017).

Sathasivam *et al.*, (2010) analysed antimicrobial activity of cow urine distillate *in-vitro* against clinical pathogenic micro-organism by using agar well diffusion test. Goswami and Kaushik (2017) also successfully used disc diffusion assay for comparing cow urine distillate with antibiotics.

In present study *in-vitro* antimicrobial analysis of all six cow urine distillates was made. The results are given in Table 5. The zones of inhibition were recorded against *S. aureus* and *E. coli*. The mean zones of inhibition were compared between and amongst groups. The cow urine distillates C1, C2 and P did not show any antimicrobial activity against *S. aureus* and *E. coli*.

The distillate C4 showed maximum zone of inhibition against *S. aureus* (18.55 ± 0.45 mm) and *E. coli* (15.21 ± 0.82 mm). The C5

distillate showed zone of inhibition of 10.51 ± 0.44 mm against *S. aureus* and 15.21 ± 1.27 mm against *E. coli*. Statistically C4 cow urine distillate showed highly significant ($p < 0.01$) increased zone of inhibition against *S. aureus* and significant ($p < 0.05$) increase against *E. coli*. The cow urine distillate C5 showed significant ($p < 0.05$) increase against both *S. aureus* and *E. coli*.

Sathasivam *et al.*, (2010) reported zone of inhibition of 7.6 ± 0.04 mm of cow urine distillate against *B. Subtilis* and 13.6 ± 0.07 mm against *S. typhi*. Kekuda *et al.*, (2010) also used disc diffusion method for evaluation of antimicrobial activity of cow urine distillate using *S. aureus* and *E. coli*. The zone of inhibition observed against *S. aureus* was 16 mm and 14mm against *E. coli*. Rajapandiyani *et al.*, (2011) used cow urine distillate with herbal preparation for evaluation of antimicrobial activity using *E. coli* by disc diffusion method. A zone of inhibition of 12.68 mm was observed against *E. coli*.

The results of present study are on the similar lines as reported earlier. However, absence of antimicrobial activity in cow urine distillates C1, C2 and P might be due to absence of herbal extracts in the same. Whereas in cow urine distillate C3, C4 and C5 the bioenhancer activity of herbal extracts and other additives is clearly evident. Randhawa (2010) also reported bioenhancer property of cow urine distillate in antimicrobial properties. The results of present study are in confirmation of the same.

In-vivo Antimicrobial quality assessment of cow urine distillates

Khanuja *et al.*, (2002) successfully filed U.S. patent of cow urine distillate. The invention relates to novel use of cow urine distillate as activity enhancer of antimicrobial agents.

Poultry slaughter house is an important source of entry of bacteria of public health significance in food chain. The meat contact surfaces of slaughter house are important and needs hygienic maintenance (Zailani *et al.*, 2016). In India retail meat shops are important source of bacteria of public health significance. The Food Safety and Standards Regulations, (2011) requires hygienic maintenance at poultry slaughter houses (Pradeep Kumar *et al.*, 2014). The Food industry requires use of disinfectants which are hazard free for maintenance of public health (Taylor *et al.*, 1999). Various methods are used for microbiological quality assessment of surfaces like swab, rinse, agar contact and direct plating. However, swab method was found to be useful in quality assessment of surfaces (Patterson, 1971).

In present study, cow urine distillates C3, C4 and C5 which were found to be having antimicrobial and disinfectant property used in poultry slaughter shop for quality assessment of platform.

Evaluation of TVC counts

Estimation of TVC counts of platform used for poultry slaughter before and after use of disinfectant gives an idea of efficacy of disinfectant and antimicrobial agent

(Patterson, 1971; Pradeep Kumar, 2014).

In present study, TVC counts estimation has been successfully exploited for assessment of efficacy of cow urine distillates. The results are given in Table 6.

Perusal of Table 6 clearly indicate that highly significant reduction in TVC counts of platform was found. After treatment in all three cow urine distillates (C3, C4 and C5) when compared to pre-treatment. The reduction in TVC counts of C3 was $7.49 \pm 0.03 \log_{10} \text{ cfu/cm}^2$ from $7.62 \pm 0.03 \log_{10} \text{ cfu/cm}^2$, in C4 it was $7.23 \pm 0.04 \log_{10} \text{ cfu/cm}^2$ from $7.62 \pm 0.002 \log_{10} \text{ cfu/cm}^2$ and $7.33 \pm 0.03 \log_{10} \text{ cfu/cm}^2$ from $7.62 \pm 0.03 \log_{10} \text{ cfu/cm}^2$ in C5.

Earlier Chauhan and Dhama, (2010) described use of cow urine distillate for antimicrobial activities. Diversified uses of cow urine distillates as antimicrobial and disinfectant have also been reported by Mohanty *et al.*, (2014). Use of cow urine in purification and detoxification processes was also been reported by Gulhane *et al.*, (2017). In present study reduction in total TVC counts of platform used for poultry slaughter indicate possibility of using cow urine distillate based disinfectants for maintainence of public health.

Table.1 Details of cow urine distillates used in the experiment

Sr. No.	Code	Source	Contents	No. of Replicates
1	C1	Commercial	Cow urine distillate	8
2	C2	Commercial	Gulvel goarka	8
3	C3	Commercial	Neem gomutra	8
4	C4	Commercial	Neem gomutra	8
5	C5	Commercial	Lemon grass, Eucalyptus oil, Pine oil	8
6	P	Lab prepared	Cow urine distillate	8
Total				48

Table.2 Details of poultry shops used in the experiment

Sr. No.	Code	No. of cow urine distillate tested	No. of Replicates
1	S 1	3	18
2	S 2	3	18
3	S 3	3	18
	Total	9	54

Table.3 Results of Phenol coefficient test of cow urine distillates against *Staphylococcus aureus*

Sr. No.	Sample Code	Phenol Coefficient Mean \pm SE (n=8)
1	C1	NA
2	C2	NA
3	C3	NA
4	C4	0.04 \pm 0.00
5	C5	0.03 \pm 0.00
6	P	NA

Table.4 Results of Phenol coefficient test of cow urine distillates against *Escherichia coli*

Sr. No.	Sample Code	Phenol Coefficient Mean \pm SE (n=8)
1	C1	NA
2	C2	NA
3	C3	NA
4	C4	0.04 \pm 0.00
5	C5	0.03 \pm 0.00
6	P	NA

NA- No Activity

Table.5 Results of *in-vitro* antimicrobial analysis of cow urine distillates

Sr. No.	Cow urine distillate code	Zone of inhibition (\emptyset mm) Mean \pm SE (n=6)	
		<i>S. aureus</i>	<i>Escherichia coli</i>
1	C1	ND	ND
2	C2	ND	ND
3	C3	8.58 \pm 0.53	3 \pm 1.91
4	C4	18.55 \pm 0.45 ^a	15.21 \pm 0.82 ^b
5	C5	10.51 \pm 0.44 ^b	15.21 \pm 1.27 ^b
6	P	ND	ND

a - p < 0.01

b - p < 0.05

ND – Not detected

Table.6 Evaluation of TVC counts of platform of poultry slaughter shop

Sr. No.	Sample Code	Pre-treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²	Post Treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²
1	C3	7.65 ± 0.03	7.49 ± 0.03 ^a
2	C4	7.62 ± 0.02	7.23 ± 0.04 ^a
3	C5	7.62 ± 0.02	7.33 ± 0.03 ^a

a - p < 0.01

Table.7 Estimation of *S. aureus* counts of platform of poultry slaughter shop

Sr. No.	Sample Code	Pre-treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²	Post Treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²
1	C3	7.00 ± 0.09	6.82 ± 0.10
2	C4	7.10 ± 0.08	6.63 ± 0.13 ^b
3	C5	7.20 ± 0.03	6.91 ± 0.04 ^b

b - p < 0.05

Table.8 Estimation of *E. coli* counts of platform of poultry slaughter shop

Sr. No.	Sample Code	Pre-treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²	Post Treatment (Average ± SE) (n=18) Log ₁₀ cfu/cm ²
1	C3	7.64±0.03	7.49±0.03 ^a
2	C4	7.62±0.02	7.24±0.03 ^a
3	C5	7.59±0.02	7.32±0.04 ^b

a - p < 0.01

b- p < 0.05

Estimation of *S. aureus* counts

The *S. aureus* counts of poultry slaughter platform were estimated by using VJ agar. The results are depicted in Table 7. All the three cow urine distillates were used in present experiment. The distillate C3 did not show any effect on *S. aureus* counts. The *S. aureus* are fairly resistant bacteria. The antimicrobial activity of the agent is

dependent upon concentration and exposure time. A significant (p<0.05) reduction in *S. aureus* counts was observed in distillate C4 after treatment from 7.10 ± 0.08 log₁₀ cfu/cm² to 6.63 ± 0.13 log₁₀ cfu/cm², the reduction was from 7.20 ± 0.03 log₁₀ cfu/cm² to 6.91 ± 0.04 log₁₀ cfu/cm² in distillate C5. The results are in agreement with earlier workers (Jarald *et al.*, 2008; Kalorey *et al.*, 2009; Goswami and Kaushik, 2017).

Estimation of *E. coli* counts

The *E. coli* counts were enumerated using EMB agar. The results are shown in Table 8. The distillate C3 pre-treatment *E. coli* counts were $7.63 \pm 0.03 \log_{10}$ cfu/cm² pre-treatment reduced significantly ($p < 0.01$) to $7.49 \pm 0.03 \log_{10}$ cfu/cm². A highly significant ($p < 0.01$) reduction in *E. coli* counts was observed after treatment of distillate C4. The pre-treatment *E. coli* counts were $7.62 \pm 0.02 \log_{10}$ cfu/cm² which were reduced to $7.24 \pm 0.03 \log_{10}$ cfu/cm² in C4 treated group. In C5 treated group also highly significant ($p < 0.05$) reduction of *E. coli* counts was observed.

Earlier many workers reported reduction of *E. coli* counts after treatment of cow urine distillates (Kekuda *et al.*, 2010; Rajapandiyan *et al.*, 2011; Ahuja *et al.*, 2012). The results of present study are in agreement with earlier work.

Cow urine distillate (C3, C4 and C5) acts as bioenhancer of herbal extracts used as antimicrobial. Commercial cow urine distillates (C3, C4 and C5) inactivate *S. aureus* and *E. coli in-vitro* and *in-vivo*. Cow urine distillates (C3, C4 and C5) can be used as inactivating agent for maintenance of hygiene of slaughter platform in poultry slaughter house.

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