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# Bacterial profile and comparative antimicrobial efficacy of fresh urine of cows, buffaloes and humans

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***Author contributions***

Bhoj R Singh designed the study, wrote the manuscript, analyzed bacteriological and data. Himani Agri, Dhayananth Balusamy, Varsha Jayakumar collected sample, awrote the manuscript, analyzed bacteriological and data.

***Competing interests***

The authors declare no conflicts of interest.

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***Abbreviations***

MH, Mueller Hinton; MHB, Mueller Hinton broth; UTI, urinary tract infections.

***Citation***

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***Abstract***

**Background:** Holy cow (*Bos indicus*) urine is often considered a valuable therapeutic agent in India for treatment of many illnesses including infectious diseases. This study was conducted to assess the presence of potentially pathogenic bacteria and to determine antibacterial activity of cow urine vis a vis human and buffalo urine using standard bacteriological methods. **Method:** The study on 73 urine samples (Murra buffalo, *Bubalus bubalis* 13; Sahiwal cattle, *Bos indicus* 11; Tharparkar cattle, *Bos indicus* 18; Vrindavani cattle, a cross bred strain of *Bos indicus* and *Bos taurus* 12, and 19 humans), were collected aseptically and bacteriological analysis was done using standard clinical bacteriological methods. To determine antibacterial activity of filter-sterilized urine agar well diffusion and microplate broth dilution methods, against a battery of 18 strains, were used. **Results:** Revealed presence of at least 14 types of potentially harmful bacteria in fresh urine samples, *Escherichia coli*, was the most common detected in 13 samples followed by *Hafnia alvei* (11), *Staphylococcus epidermidis* (8), *Bacillus mycoides* (7), *Proteus mirabilis* (5), *Enterococcus faecium* (4), *Acinetobacter calcoaceticus* (3), *Enterococcus faecalis* (3), *Paenibacillus pantothenticus* (3), *Salmonella enterica* ssp. *enterica* Ser Enteritidis (3), *Klebsiella pneumoniae* ssp. *pneumoniae* (2), *Pantoea agglomerans* (2), *Erwinia rhapontici* (1), and *Providencia rettgeri* (1). The analysis for antimicrobial activity of filter-sterilized urine samples (73) against 18 test strains revealed that none of the urine could inhibit bacterial growth in agar well diffusion assay and 45 samples failed to inhibit any of the 18 bacteria with broth dilution assay. Those 28 urine samples which inhibited growth of one or more test bacteria were not bactericidal. For most of the test strains, there was no significant ($P > 0.05$) difference in antibacterial potential of different urines irrespective of species, breed and sex of urine donors. However, *S. epidermidis* was inhibited by significantly ($P < 0.05$) more human male urine samples than human female urine samples. Buffalo urine samples were more often inhibitory to *A. xyloxidans* than urine of human and all three types of cattle ($P < 0.05$). Similarly, urine of buffaloes was significantly ($P < 0.05$) more effective on *E. rhapaontici* and *S. epidermidis* than urine from humans, Tharparkar and Vrindavani cattle. There was no significant ($P > 0.05$) difference in antibacterial activity of urines of three different breeds of cattle (Sahiwal, Tharparkar and Vrindavani). **Conclusion:** The study concluded that a sizeable proportion of urine samples from apparently healthy individuals carry potentially pathogenic bacteria. The urine of some individuals irrespective of sex and breed or species might be inhibitory to a select group of bacteria but the belief, that holy cow urine is antibacterial, can't be generalized. In no case, fresh urine can be recommended for human consumption.

**Keywords:** holy cow; urine; antimicrobia; *Escherichia coli*; *Salmonella*; *Acinetobacter*; *Klebsiella pneumoniae*

### *Introduction*

Cow, a sacred animal in India is under threat of survival due to one or other reasons [1, 2]. Livestock owners who used to be proud of having cows in their herds are now leaving those to stray due to several socio-political and judicial reasons [2]. To save the Holy cow various organizations are coming up with novel ideas to protect the cow as an invaluable mother-cow, like advocating medicinal value s of cow wastes like urine and dung. Though cow urine along with urine of other animals and humans has been claimed as a valuable medicine in various Ayurvedic scriptures including Ashtanga Sangrah, Bhav Prakash Nighantu, and Sushruta Samhita. The utility of cow urine (Gaumutra) is exaggerated through targeted studies for the treatment of AIDS, cancer, diabetes, diarrhea, oedema, jaundice and tuberculosis [3-5] without comparing with the urine of other animals and humans for the same purpose. It is often claimed that creatinine, phenols, certain urinary peptides, and urea are responsible for the antimicrobial properties of cow urine [6, 7]. However, similar components are present to more or a less extent in the urines of all mammals. Therefore, the present study was undertaken to detect bacteria in apparently healthy subjects and evaluates the antimicrobial activity in fresh urine of male (young non-breeding bulls) and females (heifers) of three different types of cows (Tharparkar, Sahiwal, and Vrindavani), buffaloes of Murra breed and humans.

### *Materials and methods*

#### Urine samples

From September 2022 to October 2022; from local organized dairy farms urine was aseptically collected in the morning hours from seven Murra buffalo (*Bubalus bubalis,* Figure 1) heifers and six young non-breeding bulls; six Sahiwal (*Bos indicus,* Figure 2) heifers and five young non-breeding bulls; 12 Tharparkar (*Bos indicus,* Figure 3) heifers and six young non-breeding bulls; six each of heifers and young non-breeding bulls of Vrindavani (Figure 4), a synthetic cow strain containing blood from both *Bos indicus* and *Bos taurus* [8, 9]; 11 male and eight non-pregnant female postgraduate students of the institute. All urine samples were filter-sterilized within an hour of collection through a 0.2 µm membrane filter. Filtered urines were tested for sterility after transferring 1 mL of filtered urine into 10 mL of sterile thioglycollate medium (Difco-BBL, USA) and incubating for 24 h at 32 ℃ [10]. All filter-sterilized urine samples were tested for antimicrobial activity within 48 h of collection of urine.

All urine samples were inoculated on blood agar and MacConkey agar medium before sterilizing as per standard protocol for detection of any microbe in urine [11] and all isolates were identified based on their growth, morphological, staining, and biochemical characteristics [12].

According to Institute Animal Ethics Committee (IAEC), this study is not needed to collect urine samples as it is a non-invasive process and collection of excretion (waste). So, the authors do not need any permission from IAEC. In addition, all the participants signed informed consent form.

#### Determination of the antimicrobial activity of filter sterilized fresh urine

A total of 18 strains of bacteria revived from the repository of the Division of Epidemiology including *Achromobacter xyloxidans* (1, HB242WS), *Bacillus cereus* (1, HB173), *Enterobacter cloacae* (1, HB263P1), *Klebsiella pneumoniae* ssp. *pneumoniae* (3, HB273PM, HB134PM, HB145PM), *Aeromonas trota* (1, HB124P), *Eschericha coli* (5, ATCC2469, ATCC25922, ATCC35218, KDESP1, HB292NHY), *Raoultella terrigena* (1, HB293PPM), *Providencia rettgeri* (1, HB294SM), *Erwinia rhapontici* (1, HB191W), *Staphylococcus epidermidis* (1, HB265PS), *Salmonella enterica* ssp. *enterica* Ser Enteritidis (1, VF6NH), and *Staphylococcus aureus* (1, ATCC43300), and were confirmed for purity and identity [11, 12]. All revived strains were stored on nutrient agar (Difco, USA) slants for the period of the study. For testing the susceptibility of bacteria to filter sterilized urines, two methods were used, 1) agar well diffusion assay, and 2) Micro-titer plate assay (broth dilution test). For both of the tests, test bacteria were grown overnight at 37 ℃ and then stored on ice after adjusting their optical density to 0.1 at 595 nm with sterile NSS.

**Figure 1 A Murra buffalo (*Bubalus bubalis*) heifer aged 28 months**

**Figure 2 A Sahiwal cow (*Bos indicus*) heifer aged 26 months**

**Figure 3 A Tharparkar cow (*Bos indicus*) heifer aged 36 months**

**Figure 4 Vindavani cows (Cross-bred strain of *Bos indicus* and *Bos taurus*)**

**Agar-well diffusion assay**

On Mueller Hinton (MH) agar (Difco, USA) plates, pretested for sterility, eight 6 mm wells were cut at equal distances, 2 cm inside to periphery, and one well was cut in the centre. The bottoms of the wells were sealed with 10 µL molten MH agar. Thereafter, plates were swab inoculated with a test culture, and the periphery wells were filled with (50 µL) of the test filter sterilized urines while the central well was kept as a negative control, filled with 50 µL of sterilized normal saline solution. Plates were incubated for 4 h without inversion (for adsorption of the fluid in wells), and then for 18 h after inversion at 37 ℃. After a total of 24 h incubation plates were observed for any zone of growth inhibition around the wells. Any clear inhibition zone was measured in mm to conclude the antibacterial activity of the test sample. All tests were repeated twice for conformity.

**Micro-titer plate assay (broth dilution test)**

All urine samples were diluted two-fold in Mueller Hinton broth (MHB) medium (Difco, USA), dispended in 100 µL volume to all wells of row A to row G, and wells of row H were dispended with 100 µL of MHB medium. The cultures of test strains were further diluted 1:10 with MHB and 2 µL of a diluted culture of test strains were transferred to all the well of column 1-11 only. All urine samples and all cultures were tested in duplicate to avoid any errors. Thereafter, 96-well plates were incubated at 37 ℃ for 24 h and observed for the growth of bacteria indicated by the haziness of the medium and measuring opacity change using microplate reader (iMark, Biorad, Germany) at 595 nm in comparison to wells in the $12^{th}$ column. Any growth in the wells of the $12^{th}$ column or no growth in wells of the H row of 1-11 columns warranted the retest.

**Determination of bactericidal activity of the filtered urine samples**

Contents of the wells inoculated with bacteria in Micro-titer plate assay showing growth inhibition of the test strain were transferred to tubes containing 5 mL of sterile thioglycollate broth and incubated at 37 ℃ for 72 h, any visible growth in the tube after incubation indicated the bacteriostatic activity and no growth indicated bactericidal activity of the filtered urine.

**Statistical analysis**

Data of susceptibility of test strains to different urine samples entered in an Excel sheet were analyzed using the Chi-square test to understand the significance of the difference in antibacterial activity of the urines obtained from humans and different animals.

***Results***

**Bacteria detected in the urine of animals and humans**

Of the 73 urine samples collected from apparently healthy animals (54) and humans (19), a total of 57 samples had one or more types of bacteria (Table 1). Of the 19 human urine samples, 10 (52.63%) samples had bacteria while of the 54 urine samples from cattle and buffaloes 47 (87.04%) had bacteria. Sixty-six bacterial isolates were identified from 57 urine samples belonging to 14 species, the most common being *Escherichia coli*, which was detected in 13 samples followed by *Hafnia alvei* (11), *Staphylococcus epidermidis* (8), *Bacillus mycoides* (7), *Proteus mirabilis* (5), *Enterococcus faecium* (4), *Acinetobacter calcoaceticus* (3), *Enterococcus faecalis* (3), *Paenibacillus pantothenticus* (3), *Salmonella enterica* ssp. *enterica* Ser Enteritidis (3), *Klebsiella pneumoniae* ssp. *pneumoniae* (2), *Pantoea agglomerans* (2), *Erwinia rhapontici* (1), and *Providencia rettgeri* (1).

**Antimicrobial activity in filter-sterilized urine of animals and humans using agar-well diffusion assay**

None of the 73 urine filtrates had any detectible bacteria as no growth was evident in thioglycollate medium after 24 h of incubation and thereafter up to 7 days of incubation at 32 ℃. None of the filtrates of 73 urine samples showed inhibition of any of the 18 strains tested for their susceptibility to 50 µL of urine filled in the agar well (Figure 5).

**Table 1 Bacteria detected in urine samples of apparently healthy humans, cattle and buffaloes**

| Source of urine (number of samples) | Bacteria detected in urine samples, number of samples positive | Urine samples with no detectible bacteria |
|---|---|---|
| Human male (11) | *Pantoea agglomerans, 2; Escherichia coli, 2; Enterococcus faecium, 2; Staphylococcus epidermidis, 1* | 6 |
| Human females (8) | *Escherichia coli ,3; Enterococcus faecium, 2; Staphylococcus epidermidis 3* | 3 |
| Murra buffalo heifers (7) | *Escherichia coli, 2; Hafnia alvei, 2; Staphylococcus epidermidis, 1; Proteus mirabilis, 1* | 2 |
| Murra buffalo male (6) | *Escherichia coli, 3; Hafnia alvei, 2; Staphylococcus epidermidis, 2; Proteus mirabilis, 2* | 0 |
| Sahiwal heifers (6) | *Bacillus mycoides, 2; Escherichia coli, 1; Enterococcus faecalis, 1; Hafnia alvei, 1* | 1 |
| Sahiwal males (5) | *Bacillus mycoides, 2; Escherichia coli, 2; Enterococcus faecalis, 1; Hafnia alvei, 1* | 0 |
| Tharparkar heifers (12) | *Paenibacillus pantothenticus, 3; Hafnia alvei, 2; Staphylococcus epidermidis, 1; Bacillus mycoides, 2; Providencia rettgeri, 1; Erwinia rhapontici, 1* | 1 |
| Tharparkar males (6) | *Hafnia alvei, 2; Proteus mirabilis, 2; Klebsiella pneumoniae* ssp. *pneumoniae, 2* | 0 |
| Vrindavani heifers (6) | *Salmonella enterica* ssp. *enterica* Ser Enteritidis, 2; *Acinetobacter calcoaceticus, 1* | 3 |
| Vrindavani males (6) | *Salmonella enterica* ssp. *enterica* Ser Enteritidis, *1; Acinetobacter calcoaceticus, 2; Bacillus mycoides, 1; Enterococcus faecalis,1; Hafnia alvei,1* | 0 |

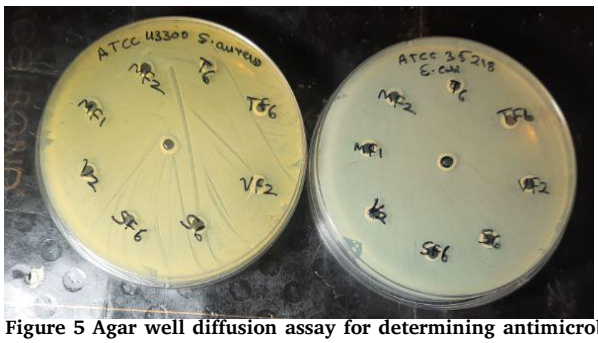


**Figure 5 Agar well diffusion assay for determining antimicrobial activity of filter sterilized urine of Murra buffalo (*Bubalus bubalis*) and cows (Tharparkar, Sahiwal, Vrindavani) against two reference strains (ATCC 43300 *Staphylococcus aureus* and ATCC35218 *Escherichia coli*)**

**Antimicrobial activity in urine filtrates of animals and humans using micro-titer plate assay**

Of the filtrates of 73 urine sample 45 (human females 8, males 6; buffalo bull 1, Sahiwal heifers 3, bulls 5; Tharparkar heifers 9, bulls 5; Vrindavani heifer 3, bulls 5) showed no inhibition of any of the 18 strains tested. Fourteen urine filtrates (human males 5, buffalo bulls 2, buffalo heifer 1, Tharparkar bull 1, Tharparkar heifers 2, Vrindavani heifers 3) inhibited the growth of only one strain of bacteria (1 *A. xyloxidans*, 12. *S. epidermidis*, 1 *E. rhapontici*) and filtrates of urine from 10 animals (buffalo heifers 4, buffalo bulls 2, Sahiwal heifers 3, Vrindavani heifer 1) inhibited the growth of two strains of bacteria. Only two samples of urine, one each from a buffalo heifer and a buffalo bull inhibited the growth of three test bacteria each. One filtrate each of buffalo heifer urine and Tharparkar heifer urine inhibited the growth of 10 and 12 bacterial strains, respectively.

Results of urine filtrates' antibacterial activity tested against 18 strains of bacteria (Table 2) revealed that none of the 73 urine filtrates could inhibit the growth of 4 *E. coli* and *S. enterica* ser Enteritidis strains used in the study. *Bacillus cereus* and *E. cloacae* were inhibited by the filtrate of one Tharparkar heifer's urine while all three *K. pneumoniae* strains were inhibited by one each of the Tharparkar and buffalo heifers' urine filtrtaes. Eight urine filtrates (5 buffalo heifers', two buffalo bulls' and one Tharparkar heifer's) inhibited *A. xyloxidans* and 12 urine filtrates (4 buffalo heifers, 3 buffalo bulls, 3 heifers, 1 Tharparkar heifer, and one Vrindavani bull) effectively inhibited growth of *E. rhapontici* strain. The most sensitive strain for urine filtrates was of *S. epidermidis* (HB215PS), it was inhibited by 26 urine samples (5 human males, 6 buffalo heifers, 4 buffalo bulls, 3 Sahiwal heifers, 3 Tharparkar heifers, 1 Tharparkar bull, 3 Vrindavani heifers, 1 Vrindavani bull).

None of the urine filtrate showing inhibition of different strains of the bacteria had bactericidal activity as growth was evident in thioglycollate broth after inoculation with the contents of the well showing no growth of bacteria in micro-titre plates.

Statistical analysis revealed that for most of the test strains there was no significant ($P > 0.05$) difference in the antibacterial potential of urine from different species/ breeds and sex of donors. However, *S. epidermidis* was inhibited by significantly ($P < 0.05$) more human male urine samples than human female urine samples. Buffalo urine samples were more often inhibitory to *A. xyloxidans* than the urine of humans and all three types of cattle ($P < 0.05$). Similarly, urine of buffaloes was significantly ($P < 0.05$) more effective on *E. rhapaontici* and *S. epidermidis* than urine from humans, Tharparkar and Vrindavani cattle. There was no significant difference in the cumulative antibacterial activity of urines of three different breeds of cattle (Sahiwal, Tharparkar, and Vrindavani).

***Discussion***

Though antibacterial activity in cow urine has been reported earlier against many types of bacteria [3-7]. The authenticity of the claims is always questioned due to rampant urinary tract infections by bacteria in almost all mammals and even the isolation of bacteria from the urine of apparently healthy cattle. In the present study, from urines of apparently healthy animals and humans *E. coli, Hafnia alvei*, *Staphylococcus epidermidis*, *Bacillus mycoides*, *Proteus mirabilis*, *Enterococcus faecium*, *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, *Paenibacillus pantothenticus*, *Salmonella enterica* ssp. *enterica* Ser Enteritidis, *Klebsiella pneumoniae* ssp. *pneumoniae*, *Pantoea agglomerans*, *Erwinia rhapontici*, and *Providencia rettgeri* were detected (Table 1) from many of the samples. Besides *Klebsiella pneumoniae, Proteus mirabilis, Enterococcus* spp. *Enterobacter* spp. *Staphylococcus* spp. and *Pseudomonas* spp. strains, *E. coli,* isolated from 17.8% of urines in the present study, is reported as the most common cause of urinary tract infections (UTIs) in bovines [13-15] and humans [16-18]. A similar profile of bacteria has been reported in the urine of cattle affected with uroepithelial tumor without any direct implication of those bacteria in tumor causation [19]. The bacteria causing UTIs are often opportunistic pathogens and can be found in the urine of apparently healthy humans and animals [16], similar to the finding of the present study. The presence of bacteria like *S. enterica* ser Enteritidis, zoonotic *Salmonella* [20], detected in the urine of two Vrindavani female and one male cattle may be a public health threat as it may contaminate the environment and infect individuals consuming cow urine for one or other purpose. Though *S. enterica* ser Enteritidis is rarely reported to cause UTI, it is potentially pathogenic and may be important similar to other serovars of *Salmonella* reported either in urines of healthy animals [17] or causing UTIs [21, 22].

*Hafnia alvei,* isolated from 11 urine samples, is not a novel finding and this member of Enterobacteriaceae has often been reported to colonize the intestinal tract and urinary tract of healthy humans and sometimes cause UTIs too [23-25]. Though it is reported to cause several infections in cattle, it has rarely been reported in urine and UTI cases in cattle and buffalo [26]. Another organism, *A. calcoaceticus* isolated from the urine of Vrindavani cattle is a known UTI-causing pathogen in humans, dogs, and cats [27] but has rarely been reported in cattle urine indicating its adaptation to newer hosts and/or existence in other animals. Several *Bacillus* spp. strains have been isolated from cow dung [28] and urine [29], *B. mycoides* isolated from Sahiwal cattle urine, and *P. pantothenticus* (earlier known as *Bacillus pantothenticus*) from Tharparkar cattle in the present study have rarely been reported earlier in urine of cattle. The two bacilli (*B. mycoides* and *P. pantothenticus*) detected in the urine of cattle may be important in enhancing the composting process as these are identified as important bacteria in the process of composting (Patent, https://patents.google.com/patent/WO2009139443A1/en).

Although cow urine has been reported to inhibit the growth of *E. coli, Salmonella enterica* ser Typhi, *Proteus vulgaris*, *S. aureus, Bacillus cereus*, *S. epidermidis, K. pneumoniae, Pseudomonas aeruginosa, P. fragi, Streptococcus agalactiae, S. pyogenes, Enterobacter aerogenes, Aeromonas hydrophila, Micrococcus luteus, Streptomyces aureofaciens, Lactobacillus acidophilus* and *Bacillus subtilis*, and *Leishmania donovani* [5], in the present study the most effective cow urine from a Tharparkar heifer inhibited 12 bacterial strains under study and but had no activity against any of the three references and two other strains of *E. coli, Salmonella,* and *S. aureus*. Besides, there were other 9 Tharparkar heifers, 3 Sahiwal heifers, and 3 Vrindavani heifers without any detectable antibacterial activity in their urine. The study indicated that antibacterial activity in the urine of cows may be an individual trait, but not the trait of a breed of Holy Cow (*B. indicus*) or Unholy cow (*B. taurus*). On the other hand, there were Murra buffalo heifers; five of the seven heifers had antibacterial activity in their urines against 1 to 10 bacterial strains. The statistical analysis proved that antibacterial activity in buffalo urines was superior to cow urines. The analysis further indicated no significant difference in antibacterial activity of urine of males and females of cattle and buffalo but 5 of 11 human male urines were inhibitory to *S. epidermidis* but to none of the other 17 bacteria tested while none of the 8 urine samples from human females revealed any antibacterial activity against 18 test strains.

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**Table 2 Antibacterial activity of filter-sterilized samples of urine from different aniamls and humans determined using micro-titre plate assay**

| Bacteria | Strain number | Strain of bacteria inhibited by filter sterilized urine samples in micro-titer plate assay (number of urine donors) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Human male (11) | Human females (8) | Murra buffalo heifers (7) | Murra buffalo male (6) | Sahiwal heifers (6) | Sahiwal males (5) | Tharparkar heifers (12) | Tharparkar males (6) | Vrinda-vani heifers (6) | Vrinda-vani males (6) | All males (34) | All females (39) |
| *Achromobacter xyloxidans* | HB242WS | 0 | 0 | 5 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 6 |
| *Bacillus cereus* | HB173 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Enterobacter cloacae* | HB263P1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Klebsiella pneumoniae* ssp. *pneumoniae* | HB273PM | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Aeromonas trota* | HB124P | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Eschericha coli* | HB292NHY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Raoultella terrigena* | HB293PPM | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Klebsiella pneumoniae* ssp. *pneumoniae* | HB134PM | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Providencia rettgeri* | HB294SM | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Klebsiella pneumoniae* ssp. *pneumoniae* | HB145PM | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Erwinia rhaponticii* | HB191W | 0 | 0 | 4 | 3 | 3 | 0 | 1 | 0 | 0 | 1 | 4 | 8 |
| *Staphylococcus epidermidis* | HB265PS | 5 | 0 | 6 | 4 | 3 | 0 | 3 | 1 | 3 | 1 | 11 | 15 |
| *Salmonella enterica* ssp. *Enterica* Ser Enteritidis | VF6NH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Escherichia coli* | KDESP1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eschericha coli* | ATCC2469 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eschericha coli* | ATCC25922 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eschericha coli* | ATCC35218 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* | ATCC43300 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |

None of the urine samples had any detectible antibacterial activity against any of the five *E. coli* strains, the most common cause of UTI in humans and animals [13-18], indicated that claims of broad-spectrum antimicrobial potential of cow urine [3-7] maybe not true. Out of 23 urine samples from bulls 16 (69.56%) and 15 (48.39%) of the 31 urine samples of heifers failed to show any antibacterial activity against a panel of 18 bacteria in the study, the difference was statistically insignificant ($P > 0.1$) indicating that there is no strong evidence to suggest the use of female cattle urine and avoid male cattle urine for antimicrobial activity. The observations are in contrast to earlier reports [3-7] but the reasons are not explicit, it may be variations in susceptibility of the bacterial strains used in different studies or differences in the antibacterial potential of urine of different individuals/animals. The study concluded that urine of some individuals irrespective of sex and breed or species might be inhibitory to a select group of bacteria but the concept (urine has antibacterial activity) can't be generalized.

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