The Haryana Veterinarian (June, 2024) **63(1),** 97-101 ISSN 0033-4359 (P) **Short Communication**

ANTIBIOGRAM STUDIES ON URO-GENITAL INFECTIONS IN BUFFALOES

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Received: 26.04.2023; Accepted: 15.06.2023

SUMMARY

At the Veterinary Clinical Complex, LUVAS, Hisar, a total of one hundred and five dairy buffaloes underwent screening for urinary tract infections and post-partum genital infections based on their history, clinical signs, and microbiological examination. Among these, forty-five buffaloes (42.85%) tested positive for uro-genital infections through cultural examination of urine and genital samples. Out of these forty-five, fifteen had urinary tract infections (33.33%), while thirty were diagnosed with post-partum genital infections (66.66%). Additionally, fourteen of the thirty buffaloes had concurrent urinary tract and genital infections. Single and mixed bacterial infections were observed in urinary tract and genital infections, respectively, with *E. coli* being the most common isolate found in uro-genital samples. Antimicrobial sensitivity testing revealed a high degree of resistance among different isolates, with the maximum antibiotic sensitivity observed for chloramphenicol, followed by kanamycin. Among multi-drug resistant isolates, 43.04% exhibited extreme drug resistance, while 16.45% and 11.39% showed multi-drug resistance and pan-drug resistance, respectively.

Keywords: Antibiogram, Buffalo, Multi-drug resistant, Urogenital infections

How to cite: Deepika, Agnihotri, D., Charaya, G., Jhambh, R. and Singh, Y. (2024). Antibiogram studies on uro-genital infections in buffaloes. *The Haryana Veterinarian* **63(1)**: 97-101.

The urinary system plays a vital role in maintaining the balance and regulation of bodily fluids and eliminating harmful wastes from the body (Divers and Peek, 2018). Urinary tract infections (UTIs) typically occur due to bacterial colonization in the urethra, which can then ascend to the bladder and kidneys, leading to infection. In ruminants, conditions such as cystitis, urethritis, and pyelonephritis often stem from bacterial infections ascending the urinary tract, commonly caused by *Corynebacterium renale* or *Escherichia Coli* (Constable *et al.*, 2017). Around seventy-five percent of UTIs, particularly pyelonephritis, are observed following events like abortion, dystocia, or puerperal infection (Braun *et al.*, 2006). Uterine bacterial contamination is almost inevitable after parturition in dairy cows, and the postpartum uterine health significantly impacts fertility rates (Kasimanickam *et al.*, 2004). Metritis, a common uterine infection in dairy animals, leads to infertility and economic losses, with commonly isolated bacteria including *Trueperella pyogenes*, *E. Coli*, *S. aureus* and *Streptococcus* spp. from vaginal discharge in cows with clinical metritis (Serdal *et al.*, 2019). Past attempts to treat uterine infections with antibiotics have shown varying degrees of success, and the efficacy of antibiotics has been periodically evaluated due to the continuous emergence of drug-resistant bacterial strains (Barman *et al.*, 2009). Considering these factors, an antibiogram study of bacteria isolated from uro-genital samples from buffaloes suffering from uro-genital infections was undertaken.

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MATERIALS AND METHODS

Screening and sample collection

One hundred and five dairy buffaloes showing clinical signs of UTI with or without the history of infertility were screened for a period of six months from April, 2022 to September, 2022. Urine samples were aseptically collected using two way Foley's catheters while genital samples were collected using cytobrush assembly.

Culture examination of uro-genital samples

After aseptic collection of samples from the urinary and genital tracts of buffaloes, urine samples (0.01 ml) and genital samples were individually inoculated onto 5% sheep blood agar (BA) and MacConkey's lactose agar (MLA) plates using a 4 mm diameter platinum loop. The inoculated plates were then aerobically incubated at 37°C for 24-48 hours. Bacterial isolation and identification relied on characteristic morphology, colonial traits, and biochemical tests such as catalase, oxidase, and IMViC tests.

In vitro antibiotic sensitivity pattern

In vitro drug sensitivity testing involved the use of twenty-three antimicrobials categorized into seven antibiotic groups, following the disc-diffusion method (Bauer *et al.*, 1966). A small portion of the test culture was transferred into a tube of BHI broth using a platinum loop and then incubated for 2-5 hours at 37°C until achieving turbidity equivalent to 0.5 McFarland standards. Using a sterile cotton swab, the broth culture was evenly spread by smearing it over the surface of MHA plates. Antimicrobial discs were then gently pressed onto the MHA surface. Subsequently, the plates were incubated at 37°C for 24 hours, and sensitivity was assessed based on zone size interpretation charts provided by the manufacturer (Hi media). Various degrees of antimicrobial resistance among different isolates were determined. Isolates resistant to three or more antibiotics from different antimicrobial categories were classified as multi-drug resistant (MDR). Among MDR isolates, those susceptible to only two or fewer antibiotics from different groups were deemed extreme drug resistant, while isolates resistant to all antibiotics were classified as pan drug resistant (PDR) (Magiorakos *et al.*, 2012).

RESULTS AND DISCUSSION

Among the twenty-nine buffaloes identified as positive for urinary tract infections (including fourteen with concurrent infections), a predominance of single bacterial infections (65.52%) was observed, consistent with earlier studies by Hajikolaei *et al.* (2015), Al-Iraqi *et al.* (2016), and Solomon *et al.* (2020). Among the total of forty bacterial isolates from UTIs, *E. Coli* was the most frequently encountered isolate (Fig. 1), followed by *Staphylococci* spp., *Streptococci* spp., *Pasteurella* spp., *Corynebacterium* spp., *Micrococci* spp., and *Klebsiella* spp. These findings align with the results reported by El-Naser *et al.* (2011) and Abdullah and Mustafa (2019). *E. Coli* is the resident flora of gut and uro-genital tract. Frequent faecal contamination of uro-genital tract often leads to ascending bacterial infection in urinary system along with its ability of adherence to epithelial cells of urinary bladder with the means of pili (Yeruham *et al.*, 2004; El-Deeb and Elmoslemany, 2016 and Solomon *et al.*, 2020). In a similar study, single bacterial infection was found more prevalent in UTI affected buffaloes where *Staphylococcus* spp. (31.6%) was identified as the more prevalent cause of UTI (Nikvand *et al.* (2014). The heightened occurrence of *Staphylococci* spp. rather than *E. Coli* in UTI-affected bovines in the current investigation could stem from its capability to survive intra-cellularly post-attachment to epithelial cells of the urinary bladder and its propensity to develop antibiotic resistance due to limited penetration. Punia (2021) also observed *Staphylococci* spp. as the most prevalent isolate in buffaloes suffering from sub-clinical urinary tract infection. In contrast to the findings of this study, Braun *et al.* (2006) and Al-Iraqi *et al.* (2016) reported the isolation of *Corynebacterium renale* from the urine samples of affected buffaloes.

Isolates from the buffaloes affected with post-partum reproductive disorders such as metritis, endometritis, pyometra

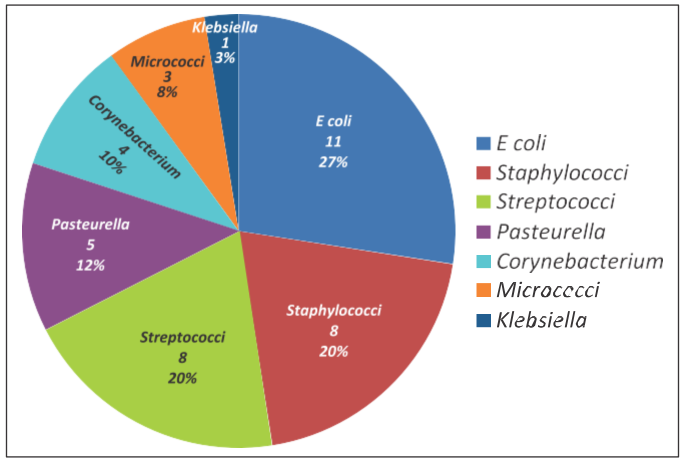

Fig. 1. Bacterial isolations from urine samples of buffaloes suffering from UTI (n=29)

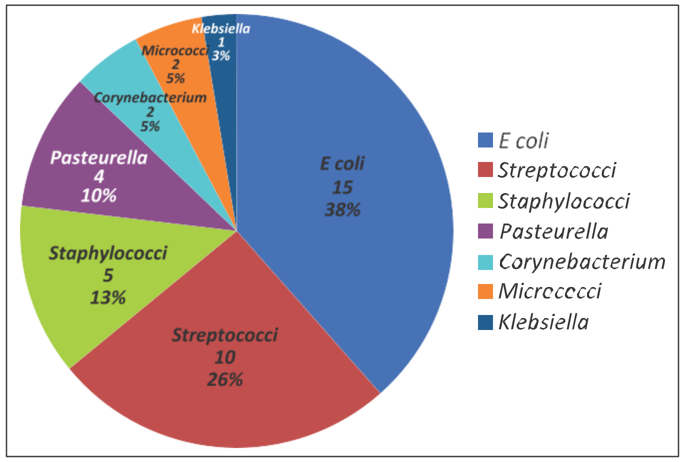

Fig. 2. Bacterial isolations from genital tract of buffaloes suffering from infertility (n=22)

and vaginitis in the current study primarily showed mixed bacterial infections (54.54%). These results contrast with the earlier findings of Bajaj *et al.*, 2018, where they identified single bacterial infection as the prevailing cause of endometritis in buffaloes. Out of a total of 39 bacterial isolates from buffaloes with reproductive disorders in the current study, *E. Coli* was most commonly isolated (Fig. 2), followed by *Streptococci* spp., *Staphylococci* spp., *Pasteurella* spp., *Corynebacterium* spp., *Micrococci* spp. and *Klebsiella* spp. In a similar study, *E. Coli* was reported to be the most prevalent cause of post-partum uterine disorders (Udhayavel *et al.*, 2013; Bajaj *et al.*, 2018; Shafique *et al.*, 2021).

The *in-vitro* antimicrobial sensitivity pattern of urine samples from UTI-affected buffaloes (Table 1) revealed that most isolated bacteria were susceptible to antibiotics such as chloramphenicol (65.71%), followed by kanamycin (60%), ceftiofur (45.71%) and amikacin (37.14%), while ceftriaxone and gentamicin showed sensitivity to only 31.43% of bacteria. Notably, amoxicillin demonstrated resistance to most of the isolated and tested

Table 1. The antimicrobial sensitivity profile of bacterial isolates obtained from uro-genital samples of buffaloes afflicted with urinary tract infections (UTI) and infertility

S.No.	Antimicrobials Group	Sensitivity % Antibiotics	Sensitivity %	
			Bacterial isolates from urine samples (n=35)	Bacterial isolates from cytobrush samples (n=36)
1.	Tetracyclines	Tetracycline	10 (28.57%)	7 (19.44%)
		Oxytetracycline	8 (22.86%)	10 (27.78%)
2.	Penicillins	Penicillin	3 (8.57%)	8 (22.22%)
		Ampicillin + Cloxacillin	3 (8.57%)	7 (19.44%)
		Amoxicillin	0 (0%)	4 (11.11%)
		Amoxicillin/clavulanic acid	3 (8.57%)	5 (13.89%)
		Amoxicillin/sulbactam	5 (14.28%)	6 (16.67%)
3.	Fluoroquinolones	Enrofloxacin	8 (22.86%)	8 (22.22%)
		Ciprofloxacin	9 (25.71%)	8 (22.22%)
		Levofloxacin	5 (14.28%)	8 (22.22%)
		Moxifloxacin	4 (11.43%)	6 (16.67%)
		Norfloxacin	8 (22.86%)	9 (25%)
		Ofloxacin	9 (25.71%)	8 (22.22%)
4.	Aminoglycosides	Gentamicin	11 (31.43%)	9 (25%)
		Amikacin	13 (37.14%)	6 (16.67%)
		Tobramycin	9 (25.71%)	7 (19.44%)
		Kanamycin	21 (60%)	23 (63.89%)
5.	Cephalosporins	Ceftriaxone	11 (31.43%)	8 (22.22%)
		Cefoperazone/sulbactam	4 (11.43%)	7 (19.44%)
		Ceftiofur	16 (45.71%)	9 (25%)
		Cefpodoxime	7 (20%)	6 (16.67%)
6.	Macrolides	Erythromycin	8 (22.86%)	6 (16.67%)
7.	Amphenicols	Chloramphenicol	23 (65.71%)	24 (69.44%)

organisms. These findings partially align with the study conducted by Punia (2021), which reported high sensitivity to chloramphenicol (81.81%) and low sensitivity to kanamycin (36.36%) in sub-clinical UTI in buffaloes. Additionally, Kushawaha *et al.* (2012) reported that cephalosporin and fluoroquinolone antibiotics were more effective against *Escherichia Coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Klebsiella* spp. causing UTI in calves. Armanullah *et al.* (2018) found that among *E. Coli* isolated from bovine clinical samples, 35.5% showed maximum sensitivity to amikacin (87.93%) and chloramphenicol (84.48%), while the least sensitivity was observed towards penicillin (1.72%).

In-vitro antimicrobial sensitivity testing of samples from the genital tract of buffaloes suffering from metritis, endometritis, pyometra, and vaginitis in the current study revealed maximum sensitivity of organisms to chloramphenicol (69.44%), followed by kanamycin (63.89%). Conversely, oxytetracycline (27.78%), gentamicin, ceftiofur and norfloxacin (25% each) and amoxicillin (11.11%) exhibited below-average sensitivity towards the isolated organisms. In contrast, Udhayavel *et al.* (2013) noted that ceftriaxone displayed high sensitivity as an antibiotic for treating endometritis in cows against *E. Coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa* and *Clostridium* spp. Ingale *et al.* (2016) isolated *E. Coli* as the highly prevalent organism causing endometritis in buffaloes and found it to be 100% sensitive to tetracycline and cotrimoxazole, followed by gentamicin (90%) and chloramphenicol (88%). Additionally, Bala (2017) and Kumar and Singh (2022) reported fluoroquinolones as highly sensitive antibiotics for bacteria causing endometritis. Bajaj *et al.* (2018) found that bacterial isolates from uterine lavage samples exhibited a sensitivity of 93.10% to the ceftriaxone and sulbactam combination, followed by sensitivity to levofloxacin (86.20%), ceftriaxone (79.31%), ciprofloxacin (72.41%), enrofloxacin (55.17%), and gentamicin (55.17%). In another study by Serdal *et al.* (2019), *E. Coli* (35.29%), *T. pyogenes* (29.41%), *Streptococcus* spp. (17.65%), and *Staphylococcus aureus* (17.65%) were isolated from the vaginal discharge of cows suffering from metritis.

The phenotypic *in vitro* sensitivity pattern of

Table 2. The *in vitro* antimicrobial sensitivity pattern of various bacterial isolates derived from the uro-genital tract of buffaloes experiencing urinary tract infections (UTI) and infertility.

S. No.	Antimicrobials Group	Antibiotics	Sensitivity %						
			E. coli (n=24)	*Pasteurella* spp. (n=9)	*Corynebacterium* spp. (n=6)	*Staphylococcus* spp. (n=9)	*Streptococcus* spp. (n=13)	*Micrococcus* spp. (n=3)	*Klebsiella* spp. (n=2)
1.	Tetracyclines	Tetracycline	1 (4.17%)	1 (11.11%)	3 (50%)	4 (44.44%)	4 (30.77%)	2 (66.67%)	0
		Oxytetracycline	1 (4.17%)	2 (22.22%)	1 (16.67%)	5 (55.55%)	5 (38.46%)	2 (66.67%)	0
2.	Penicillins	Penicillin	0	0	2 (33.33%)	2 (22.22%)	3 (23.08%)	2 (66.67%)	0
		Ampicillin + Cloxacillin	0	1 (11.11%)	1 (16.67%)	2 (22.22%)	2 (15.38%)	2 (66.67%)	0
		Amoxicillin	0	0	1 (16.67%)	1 (11.11%)	2 (15.38%)	0	0
		Amoxicillin/ clavulanic acid	0	0	3 (50%)	2 (22.22%)	2 (15.38%)	1 (33.33%)	0
		Amoxicillin/ sulbactam	0	1 (11.11%)	3 (50%)	2 (22.22%)	4 (30.77%)	1 (33.33%)	0
3.	Fluoroquinolones	Enrofloxacin	0	0	4 (66.67%)	4 (44.44%)	4 (30.77%)	2 (66.67%)	0
		Ciprofloxacin	0	1 (11.11%)	4 (66.67%)	4 (44.44%)	4 (30.77%)	2 (66.67%)	0
		Levofloxacin	0	1 (11.11%)	3 (50%)	2 (22.22%)	2 (15.38%)	2 (66.67%)	0
		Moxifloxacin	0	1 (11.11%)	2 (33.33%)	1 (11.11%)	2 (15.38%)	2 (66.67%)	0
		Norfloxacin	0	0	4 (66.67%)	3 (33.33%)	5 (38.46%)	2 (66.67%)	0
		Ofloxacin	1 (4.17%)	1 (11.11%)	3 (50%)	3 (33.33%)	3 (23.08%)	2 (66.67%)	0
4.	Aminoglycosides	Gentamicin	0	1 (11.11%)	3 (50%)	5 (55.55%)	5 (38.46%)	2 (66.67%)	0
		Amikacin	1 (4.17%)	0	4 (66.67%)	4 (44.44%)	4 (30.77%)	2 (66.67%)	0
		Tobramycin	0	2 (22.22%)	3 (50%)	5 (55.55%)	5 (38.46%)	2 (66.67%)	0
		Kanamycin	20(83.33%)	8 (88.89%)	4 (66.67%)	4 (44.44%)	4 (30.77%)	1 (33.33%)	1 (50%)
5.	Cephalosporins	Ceftriaxone	0	1 (11.11%)	3 (50%)	5 (55.55%)	5 (38.46%)	2 (66.67%)	0
		Cefoperazone/ sulbactam	0	0	2 (33.33%)	2 (22.22%)	2 (15.38%)	2 (66.67%)	0
		Cefpodoxime	0	0	1 (16.67%)	3 (33.33%)	5 (38.46%)	2 (66.67%)	0
		Ceftiofur	3 (12.50%)	2 (22.22%)	4 (66.67%)	6 (66.67%)	6 (46.15%)	2 (66.67%)	0
6.	Macrolides	Erythromycin	0	0	2 (33.33%)	5 (55.55%)	6 (46.15%)	1 (33.33%)	0
7.	Amphenicols	Chloramphenicol	12 (50%)	6 (66.67%)	5 (83.33%)	9 (100%)	9 (69.23%)	3 (100%)	1 (50%)

Table 3. Determination of multi-drug resistance (MDR) of different bacterial isolates from buffaloes with uro-genital affections

S. No.	Isolates	MDR	XDR	PDR
1.	*E. Coli* (n=24)	2 (8.33%)	20 (8.33%)	2 (8.33%)
2.	*Streptococci* spp. (n=13)	3 (23.08%)	4 (30.77%)	4 (30.77%)
3.	*Corynebacterium* spp. (n=6)	1 (16.67%)	2 (33.33%)	0
4.	*Staphylococci* spp. (n=10)	3 (30%)	2 (20%)	2 (20%)
5.	*Pasteurella* spp. (n=9)	4 (44.44%)	5 (55.56%)	0
6.	*Klebsiella* spp. (n=2)	0	1 (50%)	1 (50%)

bacterial isolates from uro-genital samples of dairy buffaloes exhibited varying degrees of antimicrobial resistance. Among a total of 79 isolates, 34 were classified as extreme drug resistant (XDR), meaning they were sensitive to only two or fewer antibiotics from different antibiotic groups; 13 isolates were identified as multi-drug resistant (MDR), indicating resistance to three or more antibiotics from different antibiotic groups; and nine isolates demonstrated pan drug resistance (PDR), showing resistance to all antibiotics. Notably, all Gram-negative bacterial isolates, including *E. Coli*, *Pasteurella* spp. and *Klebsiella* spp., displayed multi-drug resistance (Table 3). Amongthetwentyfour *E. Coli* isolates, extremedrug resistance was observed in 20 (83.33%), while 2 (8.33%) showed MDR and 2 (8.33%) displayed PDR. Five (55.56%) and four (44.44%) *Pasteurella* spp. isolates exhibited XDR and MDR, respectively. Additionally, one isolate each of *Klebsiella* showed XDR (50%) and PDR (50%). The indiscriminate use of antibiotics, irregular dosing or under-dosing may lead to the development of resistant mutants. Punia (2021) also reported that 77.27% of isolates from buffaloes with UTIs were multi-drug resistant. In another study by Kaur *et al.* (2021), all isolates from UTI-affected buffaloes were found to be multi-drug resistant, with 60.86% classified as extreme drug resistant and 30.43% as pan drug resistant. This study underscores the importance of conducting antimicrobial sensitivity testing before initiating treatment in affected animals to avoid treatment failure due to underlying bacterial drug resistance. Furthermore, the present study suggests the rotational use of antibiotics and a focus on identifying multiple bacterial etiologies to address complicated cases effectively.

CONCLUSION

Bacterial isolates of uro-genital infections in dairy buffaloes showed varying degree of antimicrobial resistance which is a matter of great concern. Out of 79 bacterial isolates from uro-genital disorders, 34 isolates were extreme drug resistant, 13 isolates showed multi-drug resistance and nine isolates showed pan-drug resistance. In general, the isolates exhibited the highest sensitivity to chloramphenicol, with kanamycin following closely behind. These antibiotics could potentially serve as effective antimicrobial agents for managing uro-genital infections in dairy buffaloes.

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