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Research Article

SUBCLINICAL URINARY TRACT INFECTION IN MURRAH BUFFALO HERD: POINT PREVALENCE, BACTERIAL ETIOLOGY AND ANTIBIOGRAM

SUNIL PUNIA*, ASHOK KUMAR, GAURAV CHARAYA and NITESH
Department of Veterinary Medicine,
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, Haryana, India

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ABSTRACT

A total of 46 murrah buffaloes were screened for subclinical urinary tract infections on the basis of examination of urine samples for physical, chemical, microscopic and bacteriological examination. Urine samples were collected by catheterization in a sterile container. Buffaloes having high numbers of pus cells (>5 per high power field) in urine samples and urine showing positivity for bacterial culture without showing any clinical signs were considered to be suffering from subclinical UTI. Preliminary identification of bacterial isolates was done on the basis of colony characteristics, Gram's staining, oxidase test and catalase test. Purified colonies were streaked on specific media for confirmation of Genus. All the isolates were subjected to *in vitro* antimicrobial sensitivity testing. Twelve buffaloes were found to be suffering from subclinical UTI, which was accounting for 26.08% point prevalence. For bacterial isolation and identification, samples were streaked on blood agar and Mac Conkey lactose agar. Mixed infections were seen in 58.33% samples and pure colony growth in 41.66% samples. Among the bacteria isolated, S*taphylococcus* spp. (31.81%) and *Micrococcus* spp.(31.81%) were the most prevalent microorganisms followed by *Escherichia coli* (13.63%), *Corynebacterium* spp. (9.09%), *Pseudomonas* spp, *Streptococcus* spp., and *Citrobacter* spp. (4.54%) each. Antibiogram of bacterial isolates were determined against 26 antimicrobials belonging to seven different classes. Overall, maximum sensitivity of isolates was found towards amoxicillin-clavulanic acid and amoxicillin-sulbactam (86.36%) and least towards kanamycin, norfloxacin and tobramycin (36.36%). *In vitro* sensitivity revealed a significant proportion of bacteria to be multidrug resistant.

Keywords: Antibiogram, Bacteria, Multidrug resistance, Subclinical UTI

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Urinary system plays important role in regulation and conservation of body fluid components and responsible for removal of toxic waste from the body (Divers and Peek, 2018). Urinary tract infection (UTI) affecting the integrity of system can affect functioning of other system. UTI refers to the microbial colonization of the urinary tract or of any urinary tract organ, except the distal urethra, which has a normal bacterial flora. Various microorganisms have been involved in the etiology of UTI in cattle and among them bacteria are mostly responsible for disease causation. *E. coli* is mostly associated with UTI in cattle (Abdullah and Mustafa, 2019). Other commonly isolated bacteria include *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas* spp. (Nikvand *et al.*, 2014). Microbiological culture combined with susceptibility testing is the cornerstone in diagnosis and treatment of UTI in buffaloes. Periodic monitoring of pathogens isolated from urinary tract and their susceptibility patterns help in the determining drug therapy and can also be used to monitor the presence of resistant bacteria.

In this paper, we have described the presence of different UTI infection causing pathogenic bacteria in an organized buffalo farm. In addition to that, the antimicrobial sensitivity, the susceptibility of pathogenic bacteria to different class of antibiotics and multidrug resistant pattern are described for better understanding of the drug sensitivity of these organisms and to device a therapeutic regimen to prevent the clinical infection.

*Corresponding author: sunilpunia62@gmail.com

MATERIALS AND METHODS

Sample size, collection and sample herd

Forty six Murrah buffaloes' urine samples were collected randomly from the animals which were neither pregnant nor in estrus from a herd of 150 buffaloes. Samples were collected by catheterization using sterile two way foley's 18F catheter. Samples were immediately processed for physical, chemical, microscopic and cultural examination to rule out urinary tract infection.

Ethical approval

Approval of Institutional Animal Ethics Committee was taken vide number VCC/IAEC/560-82 dated 18.03.2021 before the start of study.

Routine urine analysis

Physical examination of urine was done at the time of collection of samples. Chemical urine examination for estimating quantitative level of RBC, WBC, Bilirubin, Ketone bodies, Urobilinogen, Protein, Nitrite and Glucose was performed by dipstick method. Microscopic examination was performed after centrifugation of 10 ml of urine at 3000 rpm and visualizing the sediments under microscope.

Cultural examination of urine samples

Urine samples (0.01 ml) were inoculated on 5%

sheep blood agar (BA) and MacConkey lactose agar (MLA) plates. The plates were incubated aerobically at 37°C for 24h and extended period of incubation in case, no growth was seen after 24 h. Bacteria were identified on the basis of colony characteristics, Gram's reaction, morphology, catalase test, oxidase test, OF test and IMViC test using KB001 HiMViC biochemical test kit. For confirmation, bacteria were grown on specific media.

***In vitro* antibiotic sensitivity**

Isolates were subjected to In vitro drug sensitivity testing using 26 antimicrobials belonging to seven groups of antibiotics as mentioned in table I by disc-diffusion method (Bauer *et al.*, 1966). To remain conservative in our estimates of resistance, isolates exhibiting intermediate zones of inhibition were interpreted as resistant. On the basis of sensitivity pattern, isolates were categorized into MDR, extreme drug resistant (XDR), and pandrug-resistant. Isolates resistant to three or more antibiotics belonging to different groups of antimicrobials were classified as MDR. Among MDR isolates, the isolates susceptible to only two antibiotics belonging to two different groups were considered XDR, while resistance to all the antibiotics was considered as pandrug-resistant.

RESULTS AND DISCUSSION

Point prevalence

The urine samples showing growth on different media and having >5 pus cells per high power field (40X) under microscope were taken as positive sample. Point prevalence rate of UTI on the basis of routine urine analysis and bacterial culture was 26.08% (12/46). Kushwaha *et al.* (2012) found 58.06% (18 out of 31) urine samples collected from buffalo calves suffering from urolithiasis positive on bacterial culture. Comparatively higher percentage reported by Kushwaha *et al.* (2012) could be due to considering only urolithiasis cases showing clinical signs whereas in present study screening of samples was done before considering any urinary tract ailments. The prevalence study carried out at slaughter houses by Somvanshi *et al.* (2012) reported acute (18.64%) and chronic (31.35%) cystitis on pathological studies in 236 slaughtered cow and buffaloes. The prevalence rate in our study was comparatively less due to high class management and hygienic conditions.

Colour of the urine samples varied from pale to dark yellow with clear to turbid in appearance. Urine was found turbid in 41.66% of affected cases. Specific gravity of the urine samples was on lower side ranging from 1.005 to 1.030. The specific gravity of healthy animals (1.024 ± 0.0108) varied significantly from that of affected animals

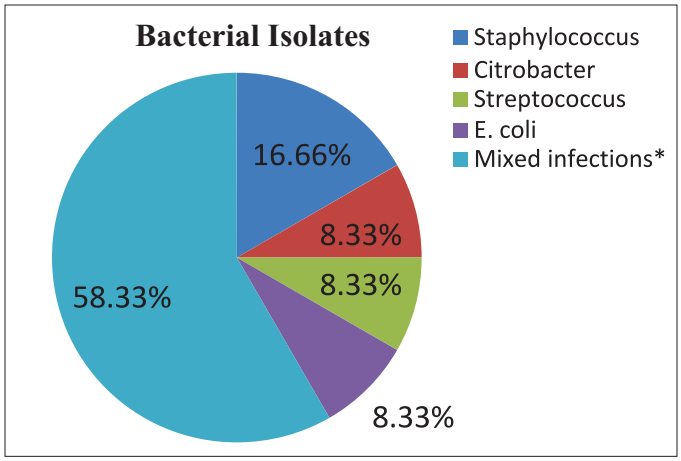

Fig. 1. Relative frequency of organisms isolated from urine samples of buffaloes suffering from urinary tract infections
**Staphylococcus* spp.+*Micrococcus* spp.(2), *Staphylococcus* spp.+*Micrococcus* spp.+*Corynebacterium* spp.(2), *Micrococcus* spp.+*Pseudomonas* spp.+E. coli(1), *Micrococcus* spp.+*E. coli.*(1), *Streptococcus* spp.+ *Micrococcus* spp.(1)

(1.014±0.007). The specific gravity of normal urine ranges from 1.020-1.040. Similar findings have been reported by Yeruham *et al.*, 2004; Braun *et al.*, 2008 and Al-Iraqi *et al.*, 2016. The lower urine specific gravity values of UTI affected animals in the present study might be the result of dysfunction of urinary system and loss of concentrating ability of animal kidneys when compared with that of healthy animals.

Chemical alteration recorded in urine samples in present study has been highlighted in Table 1. Major observations recorded on chemical test using dipstick urinalysis were haematuria in 66.66% affected animals, proteinuria in 33.33 %, pyuria (severe in 33.33% and moderate in 25% cases) and bilirubinuria was observed in only 8.33%. Nitrite was present in 33.33%. No sample was positive for glucose and ketone bodies. Similarly Floeck (2007) and Braun *et al.* (2008) reported an increased amount of protein, blood, leukocytes, glucose, ketones and nitrite in UTI affected animals by urine strip test. Yeruham *et al.*, 2004 also found proteinuria,bacteriuria and inflammatory cells in UTI infected cattle.

Presence of intact RBCs, epithelial cells, pus cells, cylindiuria and crystalluria were the major findings on microscopic examination of urine. Out of 12 buffaloes with UTI, 41.66% were positive for >10 RBCs/HPF and 58.33% for >10 WBCs/HPF. Number of pus cells was always less than 5 in unaffected animals while in affected animals mean number of pus cells was 12.33±6.91 per HPF. Casts and epithelial cells were observed in 91.66% and 41.66% cases, respectively in positive cases of UTI. Triple phosphate crystals (magnesium ammonium phosphate crystals) were more prevalent as 75% followed

Table 1

Antimicrobial sensitivity pattern of different bacterial isolates (n=22) from urine of buffaloes affected with UTI

Sr. No.	Antimicrobials used		Sensitivity
	Group	Antibiotics	
1	Tetracyclines	Tetracycline (30 mcg)	16 (72.72%)
2		Oxytetracycline (30 mcg)	16 (72.72%)
3	Penicillins	Ampicillin (25 mcg)	14 (63.63%)
4		Ampicillin + Cloxacillin (10 mcg)	14 (63.63%)
5		Amoxicillin (10 mcg)	18 (81.81%)
6		Amoxicillin/clavulanic acid (50/10 mcg)	19 (86.36%)
7		Amoxicillin/sulbactam (30/15 mcg)	19 (86.36%)
8	Fluoroquinolones	Enrofloxacin (10 mcg)	13 (59.09%)
9		Ciprofloxacin (10 mcg)	11 (50%)
10		Levofloxacin (5 mcg)	16 (72.72%)
11		Moxifloxacin (5 mcg)	15 (68.18%)
12		Norfloxacin (5 mcg)	8 (36.36%)
13		Ofloxacin (5 mcg)	15 (68.18%)
14	Aminoglycosides	Gentamicin (30 mcg)	18 (81.81%)
15		Amikacin (10 mcg)	11 (50%)
16		Tobramycin (10 mcg)	8 (36.36%)
17		Kanamycin (30 mcg)	8 (36.36%)
18	Cephalosporins	Ceftriaxone (10 mcg)	14 (63.63%)
19		Cefoperazone/sulbactam (50/50 mcg)	9 (40.90%)
20		Ceftriaxone/sulbactam (30/15 mcg)	14 63.63%)
21		Ceftizoxime (30 mcg)	12 (54.54%)
22		Cefpodoxime (10 mcg)	14 (63.63%)
23		Ceftixime/clavulanic acid (5/10 mcg)	13 (59.09%)
24	Macrolides	Erythromycin (5 mcg)	13 (59.09%)
25		Azithromycin (30 mcg)	11 (50%)
26	Amphenicols	Chloramphenicol (30 mcg)	18 (81.81%)

by oxalate crystals as 16.66%.

A total of 22 bacterial isolates were obtained. *Staphylococcus* spp. and *Micrococcus* spp. were found to be predominant in 31.81% followed by *E. coli*, *Corynebacterium* spp., *Pseudomonas* spp., *Citrobacter* spp. and *Streptococcus* spp. Out of 12 samples positive for UTI, 5 (41.66%) samples yielded pure culture whereas mixed growth was observed in 7 (58.33%) samples as shown in Fig. 1. Higher frequency of mixed infection in present study is in conjunction with study conducted by Yeruham *et al.* (2006) whereas higher isolation of pure culture have been reported by Karimi *et al.* (2006), Floeck (2007), Kushwaha *et al.* (2012), Nikvand *et al.* (2014), Hajikolaei *et al.* (2015), Al-Iraqi *et al.* (2016) and Solomon *et al.* (2020). In conjuction with the present study, *Staphylococcus* spp. was reported to be most prevalent by Herenda *et al.* (1990) and Nikvand *et al.* (2014). Herenda *et al.* (1990),Yeruham *et al.* (2004), Rosenbaum *et al.*

Table 2

Chemical urinalysis of buffaloes suffering from UTI using dipstick method

Sr. No.	Parameter	Severity level	UTI (n=12)
1	RBC	Mild (5-10 Ery/µl)	3 (25%)
		Moderate (10-50 Ery/µl)	2 (16.66%)
		Severe (50-250 Ery/µl)	3 (25%)
2	Pus cells	Mild (10-25 Leu/µl)	5 (41.66%)
		Moderate (25-75 Leu/µl)	3 (25%)
		Severe (75-500 Leu/µl)	4 (33.33%)
3	Bilirubin	Present	1 (8.33%)
4	Ketone bodies	Present	-
5	Urobillinogen	Present	-
6	Protein	Mild	2 (16.66%)
		Moderate	2 (16.66%)
		Severe	-
7	Nitrite	Present	4 (33.33%)
8	Glucose	Present	-
9	Specific gravity	Normal range is 1.020-1.040	1.014

Table 3

***In vitro* antibiotic sensitivity pattern of different bacterial isolates (n=22)**

Sr. No.	Antimicrobials used		Sensitivity (%)						
	Group	Antibiotics	*Staphylococcus* spp. (n=6)	*Streptococcus* spp. (n=2)	*Micrococcus* spp. (n=7)	*E. coli* (n=3)	*Corynebacterium* spp. (n=2)	*Pseudomonas* spp. (n=1)	*Citrobacter* spp. (n=1)
1	Tetracyclines	Tetracycline	4 (66.66%)	2 (100%)	6 (85.71%)	1 (33.33%)	2 (100%)	0 (0%)	1 (100%)
2		Oxytetracycline	4 (66.66%)	2 (100%)	6 (85.71%)	1 (33.33%)	2 (100%)	0 (0%)	1 (100%)
3	Penicillins	Ampicillin	5 (83.33%)	1 (50%)	2 (28.57%)	2 (66.66%)	2 (100%)	1 (100%)	1 (100%)
4		Ampicillin + Cloxacillin	5 (83.33%)	1 (50%)	2 (28.57%)	2 (66.66%)	2 (100%)	1 (100%)	1 (100%)
5		Amoxicillin	4 (66.66%)	2 (100%)	5 (71.42%)	3 (100%)	2 (100%)	1 (100%)	1 (100%)
6		Amoxicillin/ clavulanic acid	4 (66.66%)	2 (100%)	6 (85.71%)	3 (100%)	2 (100%)	1 (100%)	1 (100%)
7		Amoxicillin/ sulbactam	5 (83.33%)	2 (100%)	5 (71.42%)	3 (100%)	2 (100%)	1 (100%)	1 (100%)
8	Fluoroquinolones	Enrofloxacin	5 (83.33%)	1 (50%)	5 (71.42%)	1 (33.33%)	1 (50%)	0 (0%)	1 (100%)
9		Ciprofloxacin	3 (50%)	1 (50%)	3 (42.85%)	2 (66.66%)	1 (50%)	1 (100%)	0 (0%)
10		Levofloxacin	6 (100%)	1 (50%)	6 (85.71%)	0 (0%)	2 (100%)	0 (0%)	1 (100%)
11		Moxifloxacin	5 (83.33%)	1 (50%)	6 (85.71%)	1 (33.33%)	2 (100%)	0 (0%)	1 (100%)
12		Norfloxacin	4 (66.66%)	0 (0%)	2 (28.57%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)
13		Ofloxacin	6 (100%)	1 (50%)	6 (85.71%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)
14	Aminoglycosides	Gentamicin	5 (83.33%)	1 (50%)	6 (85.71%)	2 (66.66%)	2 (100%)	1 (100%)	1 (100%)
15		Amikacin	4 (66.66%)	0 (0%)	5 (71.42%)	1 (33.33%)	1 (50%)	0 (0%)	0 (0%)
16		Tobramycin	3 (50%)	1 (50%)	3 (42.85%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)
17		Kanamycin	3 (50%)	1 (50%)	2 (28.57%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)
18	Cephalosporins	Ceftriaxone	4 (66.66%)	1 (50%)	4 (57.14%)	2 (66.66%)	2 (100%)	0 (0%)	1 (100%)
19		Cefoperazone/ sulbactam	3 (50%)	0 (0%)	2 (28.57%)	1 (33.33%)	1 (50%)	1 (100%)	1 (100%)
20		Ceftriaxone/ sulbactam	5 (83.33%)	0 (0%)	5 (71.42%)	1 (33.33%)	2 (100%)	0 (0%)	1 (100%)
21		Ceftizoxime	5 (83.33%)	1 (50%)	4 (57.14%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)
22		Cefpodoxime	5 (83.33%)	0 (0%)	4 (57.14%)	1 (33.33%)	2 (100%)	1 (100%)	1 (100%)
23		Ceftixime/ clavulanic acid	4 (66.66%)	1 (50%)	4 (57.14%)	2 (66.66%)	2 (100%)	0 (0%)	0 (0%)
24	Macrolides	Erythromycin	4 (66.66%)	0 (0%)	4 (57.14%)	2 (66.66%)	2 (100%)	1 (100%)	0 (0%)
25		Azithromycin	4 (66.66%)	0 (0%)	2 (28.57%)	1 (33.33%)	2 (100%)	1 (100%)	1 (100%)
26	Amphenicols	Chloramph-enicol	6 (100%)	1 (50%)	7 (100%)	1 (33.33%)	2 (100%)	0 (0%)	1 (100%)

(2005), Karimi *et al.* (2006),Yeruham *et al.* (2006), and Solomon *et al.* (2020) have reported *E. coli* as major cause of UTI in cattle. *Corynebacterium* spp. was reported as main cause of UTI by Floeck (2007), Braun *et al.* (2008), Al-Iraqi *et al.* (2016) and El-Deeb and Elmoslemany (2016). Comparatively lesser isolation of *Corynebacterium* spp. when compared to that reported by other researchers could be attributed to use of beta-lactam antibiotics for treatment of uterine infections in these UTI affected animals in the present study.

Overall antimicrobial sensitivity pattern of isolates is shown in Table 1. Maximum sensitivity of isolates was found towards amoxicillin-clavulanic acid and amoxicillin-sulbactam (86.36%) and least towards kanamycin, norfloxacin and tobramycin (36.36%). Isolate wise sensitivity is shown in Table 3. In case of *Staphylococcus* spp., maximum sensitivity was observed for ofloxacin, levofloxacin and chloramphenicol (100%) and least for cefoperazone-sulbactam, ciprofloxacin, kanamycin and tobramycin (50%). Almost similar pattern of sensitivity has been reported by Kushwaha *et al.* (2012). *Micrococcus* spp. showed maximum sensitivity towards chloramphenicol and least sensitivity towards kanamycin, gentamicin, ampicillin-cloxacillin and ampicillin, cefoperazone-sulbactam, azithromycin and norfloxacin (28.57%). *Pseudomonas* spp. isolate was found to be sensitive towards pencillins, aminoglycosides and cephalosporins.

Table 4

Determination of multidrug-resistant isolates using *in vitro* sensitivity pattern

Group	Antibiotics/Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Tetracyclines	Tetracycline			■		■							■				■		■			■	
	Oxytetracycline			■		■							■				■		■			■	
Penicillins	Ampicillin			■				■		■	■		■		■	■	■	■					
	Ampicillin+Cloxacillin			■				■		■	■		■		■	■	■						
	Amoxicillin			■		■					■		■										
	Amoxicillin/clavulanic acid			■		■							■										
	Amoxicillin/sulbactam					■					■		■										
Fluoroqunolones	Enrofloxacin	■							■	■				■			■		■		■	■	■
	Ciprofloxacin	■		■	■				■		■		■	■	■		■				■		■
	Levofloxacin								■						■		■	■	■			■	
	Moxifloxacin			■					■						■		■		■			■	■
	Norfloxacin	■				■		■	■	■			■	■	■	■	■	■	■		■	■	
	Ofloxacin								■						■		■	■	■			■	■
Aminoglycosides	Gentamicin					■			■				■				■						
	Amikacin	■		■				■	■					■		■	■		■		■	■	■
	Tobramycin	■		■		■			■		■		■	■	■		■		■		■	■	■
	Kanamycin	■		■		■			■	■	■		■	■	■		■	■	■		■	■	
Cephalosporins	Ceftriaxone			■		■		■		■						■		■	■			■	
	Cefoperazone/sulbactam	■		■	■			■	■	■	■					■	■	■			■		
	Ceftriaxone/sulbactam			■				■	■	■						■	■	■	■			■	
	Ceftizoxime	■							■				■	■	■		■	■	■		■		
	Cefpodoxime			■				■	■	■	■					■	■	■					
	Ceftixime/clavulanic acid			■	■			■		■	■					■			■			■	■
Macrolides	Erythromycin			■		■		■	■	■			■			■	■						■
	Azithromycin			■		■		■	■	■	■		■		■	■	■	■					
Amphenicols	Chloramphenicol								■								■		■			■	

Corynebacterium spp. isolates were found sensitive towards ofloxacin, ceftixime-clavulanic acid, erythromycin, amoxicillin-clavulanic acid, amoxicillin-sulbactam, amoxicillin, ampicillin-cloxacillin, ampicillin, gentamicin, azithromycin, cefpodoxime, chloramphenicol, levofloxacin, moxifloxacin, tetracycline, oxytetracycline, ceftriaxone-sulbactam and ceftriaxone. Single isolate of *Citrobacer* spp. was obtained in present study and found to be resistant to ciprofloxacin, ofloxacin, amikacin, tobramycin, ceftixime-clavulanic acid and erythromycin. On the phenotypic *in vitro* sensitivity pattern, we found 17 isolates to be multidrug resistant. No isolate was found to be extreme drug resistant or pan drug resistant isolate as shown in Table 4. Indiscriminate use of antibiotics, irregular doses of antibiotics or under dosing of antibiotics may lead to resistant mutants. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in a particular area; therefore, antimicrobial sensitivity is suggested before institution of treatment.

CONCLUSIONS

The present study indicated considerable resistance in pathogens associated with UTI in buffaloes. *Staphylococcus* spp. and *Micrococcus* spp. were the most prevalent bacteria isolated from urinary tract of affected buffaloes of the LUVAS farm. *In vitro* antimicrobial sensitivity testing revealed potentiated penicillins and amphenicols group to be the most effective group of antibiotics.

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