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Antibiogram of bacteria associated with bacterial urinary tract infection in buffaloes

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Abstract
Bacterial urinary tract infection in buffaloes is one of the important problem leading to decrease in production and reproduction status of a herd. Diagnosis and treatment is the major step in limiting the condition and its ill effects. Therefore, the present study was to determine the bacteria involved in urinary tract infection and to determine their antibiogram which can be used in field condition for empirical treatment. Buffaloes presented to teaching veterinary clinical complex during OPD hours were screened for UTI on the basis of clinical and microscopic examination of urine samples for pus cells. Out of 72 buffaloes, samples from 11 buffaloes collected by catheterization were subjected to cultural examination and determining antibiogram of isolates obtained. Out of eleven samples, mixed growth was evident in 72.72% and pure growth of single colony in 27.27% with isolation of 23 isolates. *Staphylococcus* sp. were the most isolated bacteria followed by *Streptococcus* sp., *E. coli* and single isolate of *Klebsiella* sp. Overall antimicrobial sensitivity revealed higher percentage of resistance with all the isolates being multi drug resistant. Among multidrug resistant isolates, 60.86% were extreme drug resistant and 30.43% were found to be pan drug resistant. Complete resistance was recorded against oxytetracyclin and streptomycin irrespective of the isolates. Gram negative bacteria were found to be completely resistant towards penicillin. Chloramphenicol was found to be most effective antimicrobials against Gram positive bacteria. Results of current study clearly depicts the importance of determination of antibiogram associated with bacterial urinary tract infection before initiating the therapy as empirical treatment can result in treatment failure in view of such a large proportion of multidrug resistance prevalent in field condition.

Keywords: bacterial urinary tract infection, multidrug resistance, chloramphenicol, antibiogram

Introduction
Urinary system is one of the most important systems in the animal's body. Any defect in this system functioning affects the functions of other systems because it contributes to the regulation and conservation of body fluid components and responsible for removal of toxic waste from the body (Divers *et al*. 2018) [3]. Infection and inflammation of urinary tract is characterized clinically by frequent, painful urination (Pollakiuria and dysuria), hematuria, inflammatory cells, and bacteria in the urine. Clinically, loss of milk production, inappetance to anorexia, lower conception rate and weight loss has been described as signs of urinary tract infections (Yerhuam *et al*., 2006).
Diagnosis of UTI is done mainly based on clinical signs, haemato biochemical analysis and urine analysis. Routine urine analysis is done for evaluation of abnormal urine constituents based on test strips, microscopic and gross examination is mostly used as indicator of urinary tract infection. Bacterial isolation and treatment based on cultural sensitivity testing has been reviewed as the most important point in controlling the urinary tract infections. Most of the research in past has been either done in samples of slaughtered animals or in cattle mainly. Therefore, present study was proposed to determine the bacterial etiology associated with clinical urinary tract infection in buffaloes and develop antibiogram which can be used for empirical treatment in future.

Material and Methods
Sample collection
A total of 72 buffaloes were screened for possible urinary tract infections on the basis of clinical signs and microscopic examination. Urine samples from 11 buffaloes with pus cell greater than five were collected by catheterization and sent to laboratory for bacteriological examination and determination of antibiogram.

Bacterial isolation

Urine samples were inoculated in 0.01 ml volume on 5% sheep blood agar (BA) and MacConkey lactose agar (MLA) plates with the help of a 4 mm diameter platinum loop. The plates were incubated aerobically at 37 °C for 24-48 h. Subcultures of the resulting growth were made on BA for purification of isolates and identified on the basis of Gram's reaction, morphology, and colony characteristics. The Gram-positive cocci were subjected to catalase test to differentiate between staphylococci and streptococci. To rule out the possibility of micrococci, all the catalase-positive cocci were subjected to oxidase test and oxidation fermentation test. Other organisms such as *E. coli* and *Klebsiella* spp. were differentiated on the basis of growth on MLA and eosin methylene blue agar. Butyrous large colonies and no growth on eosin methylene blue were taken as *Klebsiella* spp., moreover, small shiny colonies with typical metallic sheen growth were taken as *E. coli* (Quinn *et al*., 2004) [10].

***In vitro* antibiotic sensitivity pattern**

Different strains of various organisms isolated from urine samples of the infected animals were subjected to *in vitro* drug sensitivity testing, using 19 antimicrobials belonging to seven different classes by the disc-diffusion method (Bauer *et al*., 1966) [2]. With the help of a platinum loop, a small amount of test culture was transferred into a tube of brain heart infusion broth and incubated for 2-5 h at 37 °C, to obtain turbidity. With the help of a sterile cotton swab, the broth culture was then evenly spread by smearing over the surface of BA/Mueller-Hinton agar plates. The antimicrobial discs were placed on the agar and gently pressed. These were then incubated at 37 °C for 24 h. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. To remain conservative in our estimates of resistance, isolates exhibiting intermediate zones of inhibition were interpreted as resistant.

Determination of multidrug-resistant (MDR) bacteria

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 were classified as MDR. Among MDR isolates, 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.

Result and Discussion

Urinary tract infection is one of the important ailments in dairy animals. In recent past, UTI in dairy animals have been shown to effect production and reproduction status (Yerhuam *et al*., 2006). Out of eleven samples, mixed growth was evident in 72.72% and pure growth of single colony in 27.27%. In contract to our study, higher isolation of pure culture have been reported by Karimi *et al*., (2006) [7], Floeck (2007) [5], Kushwaha *et al*., (2012) [8], Nikvand *et al*., (2014) [9], Hajikolaei *et al*., (2015) [6], Al-Iraqi *et al*., (2016) and Solomon *et al*., (2020).

A total of 23 isolates was recovered from the positive samples as shown in table 1. Among the bacteria isolates, *Staphylococcus* sp. was found to be most prevalent (39.13%) followed by *Streptococcus* sp. (34.78%), *E. coli* (21.73%) and *Klebsiella* sp. in 4.34%. In current study, *Corneybacterium* sp. In conjuction with the present study, *Staphylococcus* spp was reported to be most prevalent by Nikvand *et al*., (2014) [9] and Hajikolaei *et al*., (2015) [6]. Whereas El-Deeb *et al*., (2016) and Solomon *et al*., (2020) [1] reported *E. coli* as major cause of UTI in cattle. In contrast to results of present study with zero percent isolation of *Corneybacterium* sp., Al-Iraqi *et al*., (2016) reported it to be main cause of UTI.

Table 1: Percentage of organism isolated from urine samples of dogs suffering from urinary tract infections.

Bacteria	Number of isolates (23)	Percent isolates
Staphylococcus sp.	09	39.13%
Streptococcus sp.	08	34.78%
Escherichia coli	05	21.73%
*Klebsiella*sp.	01	4.34%

Overall antimicrobial sensitivity revealed very high percent of resistance pattern with sensitivity in a range of 9.09% to 36.36% as shown in table 2. Indiscriminate use of antibiotics, irregular doses of antibiotics or under dosing of antibiotics can be attributed as the probable reason. Hundred percent resistance has been recorded towards oxytetracyline and streptomycin and maximum sensitivity towards chloramphenicol (36.36%). This clearly correlates with the usage of these antibiotics in field practice as both oxytetracyclin and streptomycin is widely used. Cephalosporins and pencillin have been found to be effective against 9.09 to 18.18% isolates. Among fluroquinolones, enrofloxacillin and moxifloxacillin were found to be effective against 27.27% isolates. All the isolates were found to be multidrug resistant as shown in table 4. Among them 60.86% were extreme drug resistant and 30.43% were found to be pan drug resistant.

Table 2: Overall antimicrobial sensitivity pattern of different bacterial isolates recovered from clinical cases of urinary tract in buffaloes

Class of Antimicrobials	Antimicrobials used	Sensitivity (%)
Tetracyclines	Oxytetracycline	0
Penicillins	Ampicillin	09.09
	Amoxicillin	11.11
	Penicillin	09.09
	Cloxacillin	09.09
Fluoroquinolones	Enrofloxacin	27.27
	Ciprofloxacin	20.00
	Moxifloxacin	11.11
	Levofloxacin	27.27
Aminoglycosides	Gentamicin	09.09
	Amikacin	27.27
	Neomycin	18.18
	Streptomycin	0
Cephalosporins	Ceftriaxone	09.09
	Cefoperazone	18.18
	Cephalexin	09.09
Macrolides	Chloramphenicol	36.36
	Erythromycin	22.22
Sulfonamides	Septran	11.11

Antibiotic sensitivity against various microorganisms isolated has been depicted in Table-3. *E. coli* and *Klebsiella* sp. was found to be Gram negative bacteria isolated from urine samples. No *In vitro* activity of penicillin was recorded against Gram negative bacteria. A single isolate of *Klebsiella* sp. was isolated which was found to be pan drug resistant. *E. coli* was found to be equally sensitive (20%) to enrofloxacin, levofloxacin, amikacin, neomycin, cefoperazone and

chloramphenicol. Maximum sensitivity of *Staphylococcus* spp. was observed for chloramphenicol followed by ciprofloxacin, enrofloxacin, levofloxacin, neomycin, amikacin, erythromycin, moxifloxacin, septran, amoxicillin, ampicillin, penicillin, cloxacillin, gentamicin, ceftriaxone, cefoperazone, and cephalexin and complete resistant towards oxytetracyclin and streptomycin. Comparatively higher sensitivity of gentamicin as compare to chloramphenicol has been reported by Kushwaha *et al.* 2012 [8] which is in contrast to result of present study.

Table 3: *In vitro* antibiotic sensitivity pattern of different bacterial isolates

Class of antimicrobials	Antimicrobials used	Sensitivity (%)			
		***Staphylococci* sp.**	**Streptococci sp.**	***E. coli***	***Klebsiella* sp.**
Tetracyclines	Oxytetracycline	0	0	0	0
Penicillins	Ampicillin	11.11	12.50	0	0
	Amoxicillin	14.28	14.28	0	0
	Penicillin	11.11	12.50	0	0
	Cloxacillin	11.11	12.50	0	0
Fluoroquinolones	Enrofloxacin	22.22	25.00	20.00	0
	Ciprofloxacin	25.00	14.28	0	0
	Moxifloxacin	14.28	14.28	0	0
	Levofloxacin	22.22	25.00	20.00	0
Aminoglycosides	Gentamicin	11.11	12.50	0	0
	Amikacin	22.22	25.00	20.00	0
	Neomycin	22.22	25.00	20.00	0
	Streptomycin	0	0	0	0
Cephalosporins	Ceftriaxone	11.11	12.50	0	0
	Cefoperazone	11.11	25.00	20.00	0
	Cephalexin	11.11	0	0	0
Macrolides	Chloramphenicol	33.33	37.50	20.00	0
	Erythromycin	22.22	25.00	0	0
Sulfonamides	Septran	12.50	14.28	0	0

Variation recorded in terms of prevalence of different isolates and difference in sensitivity among different researchers can be attributed to variation in the empirical treatment being undertaken in different area. Results of current study clearly depicts the importance of determination of antibiogram associated with bacterial urinary tract infection before initiating the therapy as empirical treatment can result in treatment failure.

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13. Ethical Matter: As the samples were taken from clinical cases and as per IAEC permission was not required.