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Traditional Investigation of Bacterial Pathogens in Urine of Adult Cows

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ABSTRACT

Bacterial infections can have severe effects on the body systems of animals and humans, and reducing the growth rate and increasing the morbidity and mortality of cattle. Urinary tract infection (UTI) is one of the health problems that cows suffering from it in most countries, and consider as the second most common disease after respiratory tract infection. This study aims to identifying the pathogenic bacteria implicated in occurrence of UTIs in cattle of Wasit province (Iraq). Totally, 135 urine samples were collected from female cattle including 86 samples from those does not calving previously and 49 urine samples from cows calving for one time. The results showed there were 81.4% of positive isolates distributed among 39 first calving, and 71 non-calving cows. The percentage of types of bacterial infection were 31.8%, 18.2%, 14.5%, 12.7%, 12.7%, and 10% for Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus epidermidis, and Staphylococcus aureus, respectively. In conclusion, this variety of bacterial infections is important in treatment process, preserving the animal, reducing the risk of reproductive infections, and controlling UTIs in cows, especially before and after calving.

INTRODUCTION

Urinary tract infection (UTI) is one of the health problems that cattle suffering from it in most countries and consider as the second most common disease after respiratory tract infection (Ahamed *et al.*, 2015; Sahay, 2020). Females are more susceptible to urinary tract infections (UTIs) than males because the urethra is much closer to the anus in females, as well as the presence of prostate glands secreting bacteriostatic bacterial inhibitors in males (Al-Jubouri *et al.*, 2012). Generally, all female animals are susceptible to being affected by UTIs, especially older females, due to hormonal changes resulting from aging and general immunosuppression (Naber *et al.*, 2006; Mohammed *et al.*, 2018). Bacteria are considered the main cause of urinary tract infections at 95%, and most often bacteria that live in the digestive system, the vagina, and even around the urethra (urethra), where most of these bacteria are transmitted to the bladder and kidneys to cause the UTIs (Saleem *et al.*, 2021). The main cause of urinary tract disease in the world is the bacterium Uropathogenic *Escherichia coli* (UPEC) that constitutes 80-85% of UTI, while staphylococci constitute 5-10% of uncomplicated UTI (Ahamed *et al.*, 2015). There is a difference in the virulence of the types of bacteria that cause infection, as their gram-positive increases their pathogenicity directly or indirectly. These are called virulence factors, which combine the urease enzyme and work on hemolysin and produce bacteriocin and beta-lactamase to form biofilm and others (Hassan *et al.*, 2011). Resistance to antibiotics by the bacteria that cause UTIs has begun to increase recently due to the incorrect use of antibiotics (Madjeed *et al.*, 2022). Recently, studies in Iraq focused on bacterial isolation in UTIs by dvarious authors that showed the highest infection by different bacteria such as *Corynebacterium pyogenes*, and *Staphylococcus* spp. (Sawalha, 2019). Other studies investigated urinary bladder lesions among the slaughtered local calves demonstrating the high incidence of this infection suggesting the importance of additional research to reduce the economic damage resulting from the impact on the reproductive system of cattle and reduce abortion (Iso, 2020; Lilo and AL-Jasim 2020). Therefore, the current study was conducted to identify the pathogenic bacteria implicated in occurrence of UTIs in cattle of Wasit province (Iraq)

Material and method

Ethical approval

This study was licensed by the Scientific Committee of the College of Veterinary Medicine in the University of Wasit (Wasit, Iraq).

Samples

Totally, 135 urine samples were collected from female cattle including 86 samples from those does not calving previously and 49 urine samples from cows calving for one time. The urine samples were collected carefully to avoid the first drops of urine taking the average amount and keep them in special sterile

collection tubes. The samples were then transferred to the laboratory for cultivation and diagnosis, where they were planted in Petri dishes containing MacConkey agar and solid blood agar using the planning method. The dishes were incubated at a temperature of 37˚C for 18-24 hours to diagnose bacteria growth in the culture media (Hussein, 2019). For accuracy of diagnosis, colonies growing on Hoyles Media and Mannitol salt agar were examined. The isolates were then classified using biochemical tests and using the sugar fermentation test to characterize and classify bacteria (Sheet, 2018).

Statistical analysis

The *t*-test in the GrapPad Prism Software was applied to estimate significant differences between the obtained data at a significant variation level of $P<0.05$ (Gharban *et al.*, 2022).

Results and discussions

The presence of bacteria causing urinary tract infections was detected in 135 samples urinary samples, as the number of isolates that gave a positive result for bacterial culture was 110 (81.4%) isolates, with 17 samples of females having their first calving and 39 of females not first calving (Table 1); while the number of isolates that gave a negative result was 25 (18.6%). This result is close to the results of Ibrahim *et al.* (2020) and Alwan *et al.* (2023). The percentages were 78.6% and 78.2%, respectively. The results differed from the results of Kubaisy (2013), where the percentage was 32.6%, as well as to Ondari (2020), where the percentage was 3.83%. The reason may be due to differences in the size, environment, and nature of the sample because the animals were given antibiotics before taking the samples, which may have led to the absence of bacterial growth in the sample (Al-Abdali, 2010). This may be the result of a non-bacterial infection that may be viruses, parasites, fungi, or anaerobic bacteria (Brooks *et al.*, 2007).

Table (1): Prevalence of UTIs between first and non-first calving

Type of study cows	No. of sample		Positive culture		Negative culture	
	No.	%	No.	%	No.	%
Non-calving	86	63.7	71	82.5	15	17.4
First calving	49	36.3	39	79.6	10	20.4
Total	135	100	110	81.4	25	18.6

This study also showed that the percentage of cows with non-first calving infected with UTIs was 6.82%, while in females with first calving was 6.79%. This agrees with the findings of Abedin *et al.* (2022) who detect the infection rate at 66% in cows of multiple births, and this is due to the reason for high rate of hormonal and differences in anatomical (Raka *et al.*, 2010) The detection and isolating bacteria in this study showed the isolation of six types of Gram-positive and Gram-negative bacteria (Figures 1, 2).

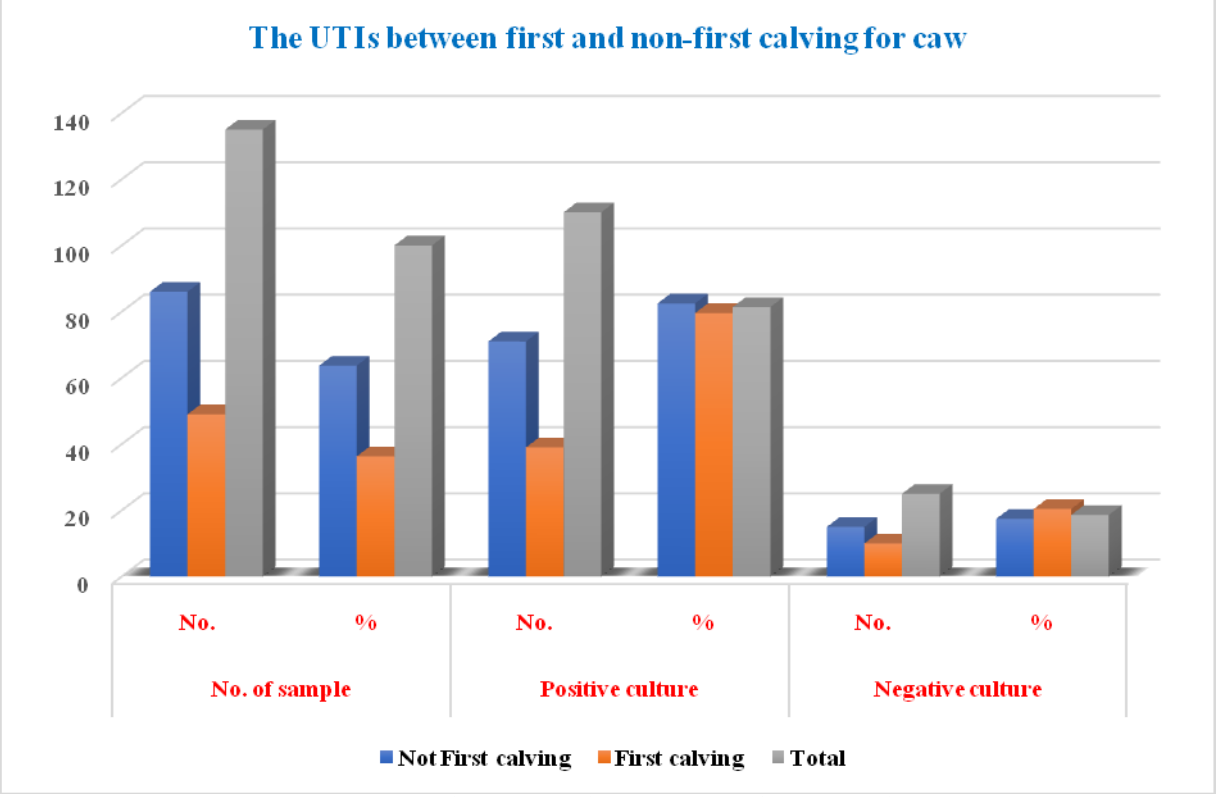

Figure (1): Prevalence of UTIs between first and non-first calving

Whilst, the Gram-staining bacteria were initially identified on blood agar and MacConkey agar, depending on specific tinctures diagnostic and differential to each type of bacteria detection the *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. penumonia*, *S. aureus*, and finally *S. epidermidis*. After isolating and fragmenting them from urine samples, based on the microscopic characteristics of the bacterial cells, the morphological and cultural characteristics, including the size of the colonies, their color, their edges, and their height, and the biochemical tests that are used to diagnose each bacterium and conform to what is stated in the approved diagnostic system (Cowan and Steels, 2009; Al-Saffar, 2019). The bacteria were identified on blood culture media and MacConkey culture and based on some of the differentiating characteristics of each type of bacteria, the different types of bacteria that cause UTIs.

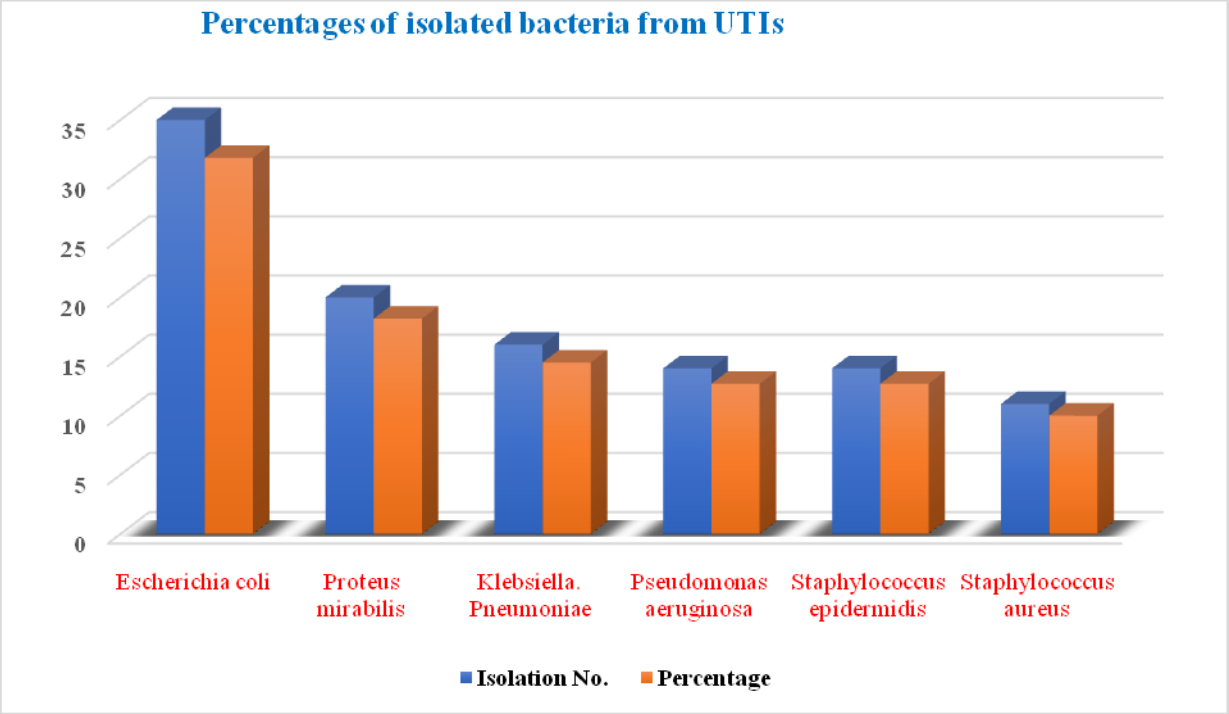

Figure (2): Results of isolated bacteria from UTIs

After isolating and diagnosing the bacteria in this study and after isolating separate Gram-positive and negative bacteria (Table 2). The results showed the isolation of 35 *Escherichia coli* at a rate of 31.8%, the isolate of *Proteus mirabilis* at a rate of 18.2%, and the isolate of *Klebsiella pneumoniae* at a rate of 14.5% and 14%. isolate of *Pseudomonas aeruginosa* at a rate of 12.7%, as well as 14 isolates of *Staphylococcus epidermidis* at a rate of 12.7%, and finally 11 isolates of *Staphylococcus aureus* at a rate of 10%.

Table (2): Results of isolated bacteria from UTIs

Bactria type	Isolation No.	Percentage (%)
Escherichia coli	35	31.8 *
Proteus mirabilis	20	18.2
Klebsiella. Pneumoniae	16	14.5
Pseudomonas aeruginosa	14	12.7
Staphylococcus epidermidis	14	12.7
Staphylococcus aureus	11	10
Significance * (P<0.05)		

When comparing the current research results with previous researchers, the results showed the presence of bacteria isolated from UTIs, with a percentage of isolation of 31.8%. This result agreed with Ondari (2020) who found 37.87%, as well as the Nabbugodi (2013) who reported 40%, while this percentage disagreed with Ahmed *et al*. (2019), who reported 70.49%. The high rate of *Escherichiacoli* bacteria may be isolated The cause of urinary tract infection compared to the rest of the intestinal bacteria is due to the presence of these normal flora bacteria in huge numbers in the human gastrointestinal tract as a natural flora, in addition to the possession of multiple harmful factors such as the production of the hemolysin enzyme, as well as the ability to form a biofilm, helps to cause and sustain the infection (Alsamarai and Abdulaziz, 2016).

The *P. mirabilis* bacteria is in second with UTIs at 18.2%, and these results agree with other researchers (Abedin *et al.*, 2022; Salami *et al* 2022; Shati *et al.*, 2022), who detected different infection rates; while disagree with the results of Khorshid (2005) in Kirkuk, as well as Tabasi *et al*. (2015). This reason is due to the presence of *E. coli* bacteria in the first place, followed by *P. mirabilis* bacteria due to kidney problems and the formation of kidney stones (Saeed and Mansoor, 2015).

The percentage of infection by *K. pneumoniae* bacteria was 14.5%, and the results agreed with Abdullah *et al*. (2010) who detected 17.6%, as well as Ondari (2020) who reported 13.6%, and the current results showed disagreement with Ali *et al*. (2018) and Haider *et al*. (2010). The difference in the rates of results is because the strains of *K. pneumoniae* bacteria depend on their ability to adhere to mucous surfaces, and this is the first step for infection to occur, without adherence, the bacteria lose a large part of its virulence (Huang *et al.*, 2012). Regarding the *P. aeruginosa* bacteria, the percentage in this research was 12.7%; and these results agree with Abedin *et al*. (2022) found 9.8% and disagree with Lilo and AL-Jasim (2020) who identified 41.3%. These conditions may be due to the number of samples, the difference in health conditions of animals during talk sampling, and some development of resistance in some strains of bacteria that cause UTIs (Sabir *et al.*, 2014).

While the Gram-positive bacteria *Staphylococcus spp*. represented 12.7% of the Staph type isolates, *S. epidermidis*. The isolation of *S. aureus* was 10%, and these results are agreed with Smita (2020) who found that the results of *S. epidermidis*, *S. aureus* by 13.44% and 5.38%, respectively. Razgar and Raoof (2018) recorded a rate of 19.35% for *S. aureus* and with Kazemier (2014) who recorded a rate of infection at 19.35%. These results disagree with the results in current research, and this difference in results is attributed to both *S. aureus* and *S. epidermis* caused by contaminated skin found on the female urinary tract when samples were taken (Wistrom *et al.*, 2014).

CONCLUSION

Finally, the results showed a difference in the incidence of UTIs between the non-first calving of cows and the extent of their impact on pregnant cows. At a lower rate than what is found in the first calving of cows, through the most common types of bacteria, The current study also showed the types of bacteria that are Gram-positive and negative, which are *E. coli*, followed by *M. Proteus*, then *K. pneumonia* and then *P. aeruginosa*, *S. aureus* and *S. epidermidis*. Therefore, this recommends research

further studying the relationship the antibiotic resistance to the formation of multiple virulence factors for bacteria in the urinary system and the detection of bacteria causing UTIs using PCR, from a genetic standpoint, to determine the epidemiology of bacteria at the molecular level, to limit their spread, and diagnose the appropriate treatment for them.

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