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Incidence, Bacterial causes and Antibiotic Resistance Patterns of Urinary Tract Infection in Pet Animals

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ABSTRACT

The primary goal of the study was to determine the prevalence and various bacterial risks of lower urinary tract infections (UTI) in diseased and seemingly healthy pet animals with and without urine retention whether they were catheterized or not. The bacterial isolates were in vitro tested for their antibiotic resistance and antibiotic resistance genes were investigated. Between October 2020 and January 2022, 128 urine samples were randomly collected from pets recruited to veterinary hospitals and clinics in Cairo and Giza. Samples were cultivated for bacteriological isolation. *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Klebsiella* spp. were found to be the most common bacterial causes of urinary tract infections in pets, with prevalence rates of 32.9%, 28%, and 19.5%, respectively followed by *Proteus mirabilis (P. mirabilis)* and *Pseudomonas aeruginosa (P. aeruginosa)* with incidences of 18.2% and 1.2%, respectively. Based on bacterial types and their virulence genes, antibiotic resistance and multi-drug resistance (MDR) behaviour varied. Epidemiology, diagnosis, and control of the urinary tract infection would benefit from the identification and characterization of isolated bacterial species.

Keywords: Catheterization, E. *coli*, *K. pneumoniae*, Pet animals, *P. mirabilis*, *S. aureus.*

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INTRODUCTION

The importance of urinary tract infections (UTIs) in pet animals cannot be overstated because they are one of the most common diseases that affect cats and dogs and contribute to the development of antibiotic resistance. As a result, owners suffer financial losses due to lost productivity and the expense of medical care **(Nicolle, *et al.*, 2005).**

The feline urinary tract has a variety of natural defence mechanisms, including resident microbiota, urinary tract physiology, systemic immunity, antibacterial characteristics of urine, and full voiding **(Litster *et al.*, 2011).** When those defence mechanisms are compromised, the virulent pathogen that causes UTIs finds its way to invade **(Olin and Bartges, 2015).**

Cats that have chronic diseases are more likely to develop UTIs **(Freitag *et al.*, 2006).** UTIs are also connected to abnormally frequent urination, structural abnormalities, urothelial alterations, antibacterial features of urine, waning immunity, length of inpatient stay, and usage of antibiotics **(Lekcharoensuk, *et al.*, 2001).**

In terms of gender prevalence, feline urinary tract disease is one of the most prevalent issues that affect cats. It has been estimated that 11.5% of cats have UTIs; however, males are more prone than females to developing the condition **(Saevik, *et al.*, 2011**; **Hostutler, *et al.*, 2005).**

Similar to dogs, it has been estimated that bacterial urinary tract infections (UTIs) affect up to 18.9% of cats **(Dorosch, *et al.*, 2014),** ranging from 1% to 12% **(Eggertsdottir, *et al.*, 2007, Saevik, *et al.*, 2011** and **Martinez-Ruzafa, *et al.*, 2012).** Up to 4.5 % of dogs who visited veterinary facilities had UTIs **(Norris *et al.*, 2000)**, while the canine UTI occurrence is up to 14% **(Hall *et al.*, 2013).**

In dogs and cats, E. coli was the most common bacterial cause of UTI **(Norris *et al.*, 2000; Litster *et al.*, 2007; Hall *et al.*, 2013)**.However, other Gram-negative bacterial causes such as Proetus and Klebsiella, in addition to Staphylococcus, are also blamed as causes for UTIs in dogs and cats **(Ling, *et al.*, 2001; Litster, *et al.*, 2007).**

MATERIALS AND METHODS

Sampling

One hundred-tenty eight (128) urine samples were collected in sterile tubes, primarily through catheterization, to collect urine in cases of urine retention in pet animals, particularly cats. However, in apparently healthy animals, free-voiding urine collection was used. Samples were transferred, while cold, to the laboratory with minimal delay **(Radostits, *et al.*, 2000)**.

Isolation and Identification

Upon arrival to the laboratory, samples were centrifuged at 3000 rpm for 10 minutes and the supernatant fluid was discarded **(Reine and Langston, 2005).** A loop from the sediment was then cultivated into nourishment broth (specify) and incubated at 37°C for 24 hours. After that, a loopful of broth was streaked onto blood agar, MacConkey agar, nutrient agar, Eosin Methylene Blue agar (EMB) and Mannitol Salt Agar (MSA) plates **(Eggertsdottir, *et al.*, 2007)** and incubated under aerobic conditions **(Luts, *et al.*, 2019).**

Identification of colonies was conducted using the diagnostic colony growing characters on selective media, colonial morphology and Gram staining, followed by biochemical identification. Biochemical tests were catalase, coagulase, oxidase, urease, TSI and IMViC tests **(Cruickshank *et al.*, 1975).** In catheterization, bacterial infection was suggested when the causative agent count was $\geq 10^3$cfu/mL in cats and $\geq 10^4$ and $\geq 10^5$ cfu/mL in male and female dogs, respectively. **(Weese *et al*., 2019)**

Antibiotic sensitivity test (AST)

An antibiotic sensitivity test was applied by the Kirby-Bauer method using Muller-Hinton agar plates The interpretation was carried out following the guidelines of **NCCLS (2002)** and **CLSI (2018)**. Bacteria were classified as MDR if they had resistance to three or more different drug classes (Amphaiphan et al., 2021).

Polymerase Chain Reaction (PCR)

Using nine pairs of primers supplied by Metabion (Germany) and Biobasic (Canada), representative bacterial isolates were tested using PCR for detection of virulence genes. Five *E. coli isolates* were tested for the *iss*, *omp*A, *lux*S, *qnr*S and *bla*TEM virulence genes. Two isolates of Proetus were tested for the detection of the *rsb*A, *ure*C, *qnr*S and *bla*TEM genes. Three isolates of *Staphylococcus spp*. were tested for *nor*A, *blaZ*, *clf*A and *seb* genes and only one isolate of pseudomonas was tested for 16 S rRNA, arr, mexR, qnrS and blaTEM) and finally, two isolates of *Klebsiella* were tested for detection of *mag*A, *mp*A and *mrk*A virulence genes. The primer sequence and amplicon size are shown in table 1.

Table 1: Oligonucleotide primers sequences and amplicon sizes of the PCR targeted genes

Bacteria	Gene	Primer sequence (5'-3')	Amplicon size	Reference
Staph	*norA*	TTCACCAAGCCATCAAAAAG	620 bp	Pourmand *et al.*, 2014
		CTTGCCTTTCTCCAGCAATA		
	blaZ	TACAACTGTAATATCGGAGGG	833 bp	*Bagcigil et al.*, 2012
		CATTACACTCTTGGCGGTTTC		
	clfA	GCAAAATCCAGCACAACAGGAAACGA	638 bp	Mason *et al.*, 2001
		CTTGATCTCCAGCCATAATTGGTGG		
	Seb	GTATGGTGGTGTAACTGAGC	164 bp	Mehrotra *et al.*, 2000
		CCAAATAGTGACGAGTTAGG		
Klebsiella	*magA*	GGTGCTCTTTACATCATTGC	1282 bp	Yeh *et al.*, 2007
		GCAATGGCCATTTGCGTTAG		
	rmpA	ACTGGGCTACCTCTGCTTCA	535 bp	
		CTTGCATGAGCCATCTTTCA		
	mrkA	CGGTAAAGTTACCGACGTATCTTGTACTG	475 bp	Alcántar-Curiel *et al.*, 2018
		GCTGTTAACCACACCGGTGGTAAC		
Pseudomonas	*16S rDNA*	GACGGGTGAGTAATGCCTA	618 bp	Spilker *et al.*, 2004
		CACTGGTGTTCCTTCCTATA		
	Arr	AGCGCATCACCCCCAGCAAC	685 bp	Jones *et al.*, 2013
		CGCCAAGTGCGAGCCACTGA		
	mexR	GCGCCATGGCCCATATTCAG	637 bp	Sánchez *et al.*, 2002
		GGCATTCGCCAGTAAGCGG		
Proteus	*rsbA*	TTGAAGGACGCGATCAGACC	467 bp	Pathirana *et al.*, 2018
		ACTCTGCTGTCCTGTGGGTA		

	ureC	GTTATTCGTGATGGTATGGG	317 bp	
		ATAAAGGTGGTTACGCCAGA		
E. coli	*Iss*	ATGTTATTTTCTGCCGCTCTG	266 bp	Yaguchi *et al.*, 2007
		CTATTGTGAGCAATATACCC		
	ompA	AGCTATCGCGATTGCAGTG	919 bp	Ewers *et al.*, 2007
		GGTGTTGCCAGTAACCGG		
	luxS	ATGCCGTTGTTAGATAGCTTCA	513 bp	Wang *et al.*, 2016
		GATGTGCAGTTCCTGCAACTTC		
Gram negative bacteria	*qnrS*	ACGACATTCGTCAACTGCAA	417 bp	Robicsek *et al.*, 2006
		TAAATTGGCACCCTGTAGGC		
	blaTEM	ATCAGCAATAAACCAGC	516 bp	Colom *et al.*, 2003
		CCCCGAAGAACGTTTTC		

RESULTS

The total incidence of the targeted bacterial agents is depicted in table 2. Out of the 128 tested urine samples, 82 produced bacterial isolates (64.1%). Samples are allocated at 63.7% (72/113) from cats and 66.6% (10/15) from dogs. Regarding gender, the prevalence of infection was 62.3% in the urine of male cats (63/101) and 75% in the urine of female cats (9/12). Similarly, male dogs had a prevalence of 62.5% (5/8) and females had a prevalence of 71.4% (5/7).

Table 2: The overall incidences of bacterial isolates from urine samples of dogs and cats

Animal spp	Sex	NO.	Cath.	Non cath	Growth	positive samples	Percent to growth samples				
							E. coli	*S. aureus*	*K. pneumonia*	*P. miribalis*	*P. aeruginosa*
Cat	Male	101	78	23	63	62.3%					
	Female	12	0	12	9	75%					
Total		113	78	35	72	63.7%	23 (31.9%)	17 (23.6%)	16 (22.2%)	15 (20.8%)	1 (1.3%)
Dogs	Male	8	4	4	5	62.5%					
	Female	7	0	7	5	71.4%					
Total		15	4	11	10	66.6%	4 (40%)	6 (60%)			
Total		128	82	46	82	64%	27 (32.9%)	23 (28%)	16 (19.5%)	15 (18.2%)	1 (1.2%)

Table (3) shows that the highest bacterial incidence was recorded as *E. coli*, which was isolated from 27 urine samples (32.9 %) of the total positive samples and 21.1% of the total tested samples. *S. aureus* was recovered from 23 samples with an incidence of 28% from the total positive samples and 17.9% from the total tested samples, followed by *K. pneumoniae* in 16 samples with an incidence of 19.5% and 12.5% from the total positive samples and from the total tested samples, respectively. *Proteus mirabilis* was isolated from 15 samples with an incidence of 18.2% and 11.7% from the total positive samples and from the total tested samples, respectively. The lowest incidences were found for *Pseudomonas aeruginosa*, which was recovered from only one sample (1.2% and 0.7% from the total positive samples and from the total tested samples, respectively). The bacterial incidence was 64% in the catheterized group, either 82 out of 128 or 53 out of 82. In contrast, in the non-catheterized group, the incidence was 35.9% (46 out of 128) and 35.3% (29 out of 82), respectively.

Table 3: Prevalence of bacterial UTIs catheterized and noncatheterized cats and dogs

Bacteria					Cats			Dogs		
	Cath.	Non cath	Positive isolation	Bacterial % to total number of positive samples	Cath	Non cath	Total cat	Cath	Non cath	Total dog
E. coli	18	9	27	32.90 %	16	7	23	2	2	4
S. aureus	13	10	23	28 %	11	6	17	2	4	6
K. pnumoinea	11	5	16	19.50 %	11	5				
P. miribalis	10	5	15	18.20 %	10	5	15			
P. aeruginosa	1		1	1.20 %	1					
Total	53	29	82		49	23	55	4	6	10

Incidence of bacterial infection in catheterized and non-catheterized animals

The bacterial incidence in catheterized animals was recorded against the number of positive samples. Incidences in catheterized were *E.coli* 21.9% (18/82), *S.aureus*15.8% (13/82), *Klebsiella pneumoniae*13.4% (11/82), *Proteus mirbalis* 12.1% (10/82) and *Pseudomonas aeruginosa* 1.2% (1/82). In the non-catheterized animals, the bacterial incidences were 10.9% for *E.coli* (9 out of 82), *Aureus* 12.1% (10/82), *Klebsiella pneumoniae* and *Proteus mirbalis* 6% (5/82) each.

Incidences of bacteria regarding the animal species

Out of 113 cats and 15 dogs tested, the bacterial incidences in both species were calculated. *E. coli* was prevalent at 20.3% (23/113), Staphylococcus spp. *at* 15%, *Klebsiella spp. at 14*%, *Proteus spp. at* 13.2%, and *Pseudomonas spp. at* 0.8% in cats. On the same lines, the bacterial prevalence in dogs was 26% for *E. coli and* 40% for *Staphylococcus. Spp.*

Antibiotic sensitivity test and multidrug resistance (MDR)

The resistance of the UTI bacterial isolates is depicted in Table 4 and Fig.1. The highest resistance in the case of *E. coli* was against amoxicillin/clavulanic acid (100%), followed by tetracycline (55.5%), vancomycin (44.4%), ceftazidime (33.3%), cefepime (22.2%), then ciprofloxacin (11%), and the lowest resistance was recorded against enrofloxacin (0%). MDR strains of *E. coli* were represented by 22.2% of *E. coli* isolates.

Table 4: Showing resistance prevalence of antibiotics toward each bacterial UTI and MDR prevalence

Bacteria	No of isolates	Antibiotics sensitivity %							
		Ciprofloxacin	Cefepime	Vancomycin	Ceftazidime	Amoxicillin / clavulanic acid	Enrofloxacin	Tetracycline	Multi drug resistance
E. coli	9	11	22.2	44.4	33.3	100	0	55.5	22.2
Staph. spp	8	12.5	25	50	62.5	62.5	25	0	37.5
Klebsiella spp	2	50	50	100	50	100	50	0	50
Proteus spp	8	37.5	25	100	50	100	37.5	12.5	12.5
Pseudomonas spp	1	0	100	100	100	100	0	100	100

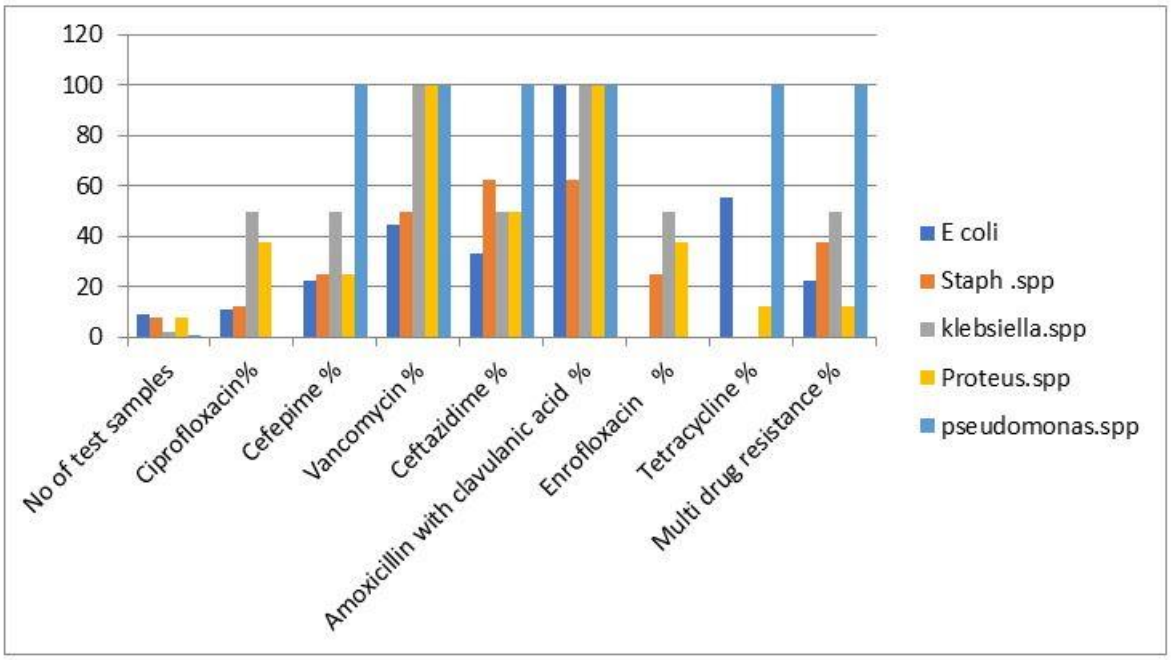

Fig. 1: Showing resistance prevalence of antibiotics toward each bacterial UTI

Klebsiella spp. had the highest resistance to amoxicillin/clavulanic acid and vancomycin (100%), followed by ceftazidime, cefepime, ciprofloxacin, and enrofloxacin (50%) and no resistance to tetracycline (0%). MDR Klebsiella strains were conferred by 50% of the isolates.

PCR Findings

As shown in table 5, *S. aureus* harboured the virulence genes *NorA, blaZ and clfA*) in 100% of the tested isolates, while the *Seb* gene was only detected in 66.6% of the tested isolates. In *K. pneumonia* the virulence genes *magA and mrkA* were detected in 100% of the isolates, while *rmpA* was not detected in any of the tested isolates. Concerning *Pseudomonas aeruginosa*, the virulence genes 16S rRNA, *arr, mexR, qnrS and blaTEM* were detected in all tested isolates. For *P. mirabilis*, the virulence genes *rsbA, blaTEM, and qnrS* were detected while the *ureC* gene was not detected. In *E. coli*, the virulence genes *iss, ompA, luxS, qnrS and blaTEM were* detected in 100% of the isolates (Photos 1–7).

Table 5: PCR of MDR and virulence genes in different bacterial isolates from cat and dog UTIs

Bacteria	MDR genes				
Staphylococcus	*norA*	*blaZ*	*clfA*	*seb*	
1	+	+	+	+	
2	+	+	+	-	
3	ND	ND	+	+	
Klebsiella	*magA*	*rmpA*	*mrkA*		
1	+	-	+		
2	+	-	+		
Pseudomonas	*16S rRNA*	*arr*	*mexR*	*qnrS*	*blaTEM*
1	+	+	+	+	+
Proteus	*rsbA*	*ureC*	*qnrS*	*blaTEM*	
1	+	-	ND	ND	
2	+	-	+	+	
E. coli	*iss*	*ompA*	*luxS*	*qnrS*	*blaTEM*
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	ND	ND
5	+	+	+	ND	ND

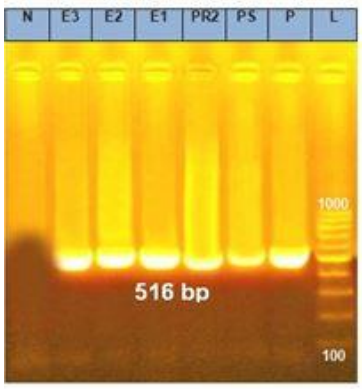

Photo 1: PCR *(blaTEM*) detailing

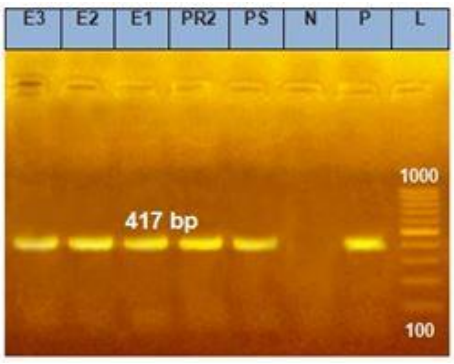

Photo 2: PCR *(qnrS*) detailing

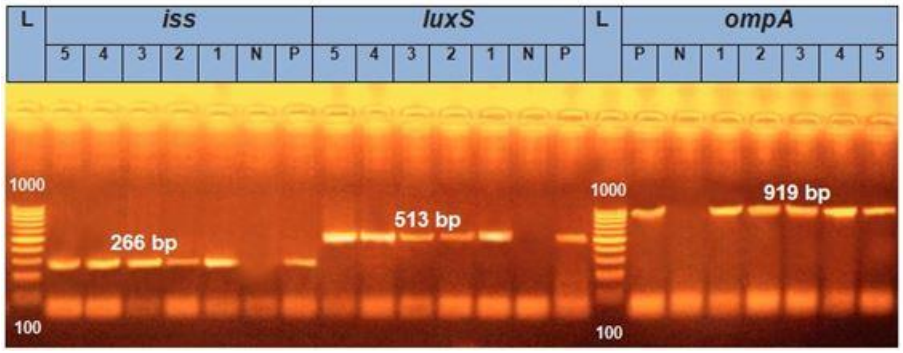

Photo 3: PCR (*qnrS*) detailing

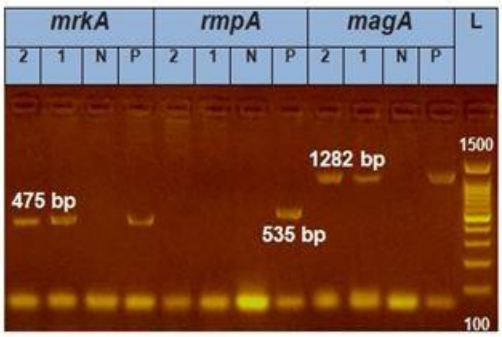

Photo 4: PCR (Klebsiella) detailing

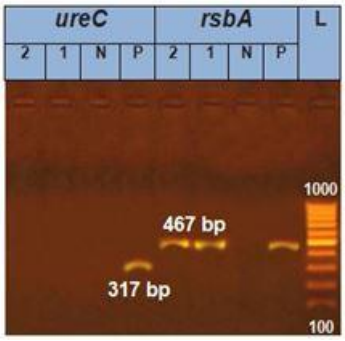

Photo 5: PCR (Proteus) detailing

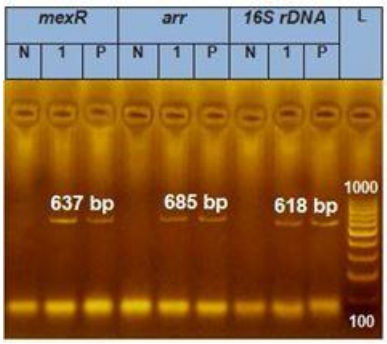

Photo 6: PCR (Pseudomonas) detailing

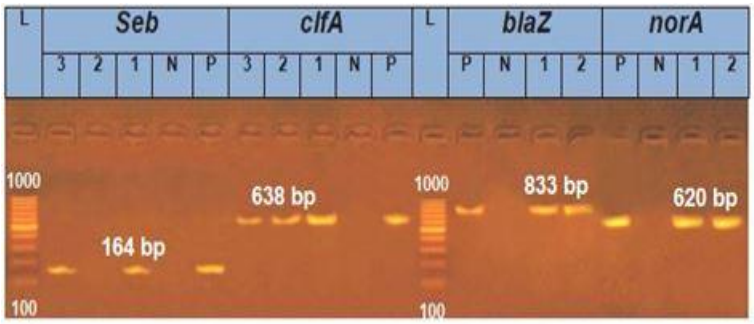

Photo 7: PCR (Staph.) detailing

DISCUSSION

From the current study, the overall bacterial incidence in feline UTIs was 64% (82/128).When comparing animal species, the incidence in cats was 63.7% (72/113) and in dogs was 66.6% (10/15). Therefore, our results indicate higher incidences in both animal species and these results are not aligned with those mentioned by **Litster *et al.*, (2007),** who recorded the feline incidence as rare. Also, **Litster *et al.*, (2011)** mentioned that the bacterial feline urinary tract disease was less than 3% with a higher incidence rate (15–43%) in cats with compromised immune systems. Both of **Banu *et al.*, (2015)** and **Litster *et al.*, (2011)** reported a high incidence of feline bacterial urinary infection (26.2 %). Also, **Hernando *et al.*, (2021)** mentioned that dogs were found to have a higher rate of positive urinary bacterial cultures than cats (39.3% and 24.7%, respectively).

Also, the bacterial incidences of UTIs were nearly equal in both the cat and dog 'groups, despite being a little bit higher in dogs than in cats and these results agreed with those of **Hernando *et al.* (2021),** who mentioned that UTI is a major clinical problem in dogs and that the incidence was much higher in dogs (39.3%) than in cats (24.7%). Regarding to the animal sex, the incidences differed, where in male cats was 62.3% (63/101), while female cats was 75% (9/12). On the other side, male dogs' incidence was 62.5% (5/8), while in female dogs it was 71.4% (5/7). Our recorded incidences are somewhat consistent with the findings of **Amphaiphan *et al.* (2021),** who mentioned a higher

incidence in female cats than in male cats as 41.7% and 37.5%, respectively. In males and females, the incidences in dogs were 70.5 percent and 77.9 percent, respectively. The incidence referred to the catheterization and associated bacterial growth in cats was 43.3% (49/113), and this incidence was higher than that reported by **Dorsh *et al.*, (2016)** (17%) in collected urine for 24 hours, however, it was 33% in collected urine for 48 hours.

Concerning the incidence of bacterial isolates from UTIs in different animal species; the common bacterial isolates and their incidence in dogs were *E. coli* and *Staphylococcus spp.* (26% and 40%, respectively). These findings are nearly in line with two studies accomplished in Thailand, where they reported that *Staphylococcus* was the most predominant bacteria isolated from UTIs in dogs (26 % and 33.60%, respectively). These findings are nearly in line with two studies accomplished in Thailand, where they reported that Staphylococcus was the most predominant bacteria isolated from UTIs in dogs (26% and 33.6%), with a lesser prevalence for *Proteus spp.* and *E. coli* **(Adsanychan *et al.*, 2019).**

One of our aims was to detect the resistance of different bacterial UTI isolates against antibiotics. Multi-drug resistance (MDR) prevalence was high in *Pseudomonas* (100%), *Klebsiella* (50%), *Staph*ylococcus (37.5%), *E. coli* (22.2%), and *Proteus* (12.5%). These results agree with those reported by **Hilde *et al.* (2017)**. Also, amoxicillin/clavulanic acid resistance was 100% in all bacterial samples except for *Staphylococcus spp.*, where it was 62.5%. In general, the differences in prevalence results between this study and others may be due to geographic distribution and antibacterial drug use in UTIs in pet animals **(Amphaiphan, *et al.*, 2021).**

CONCLUSION

In many studies, incidence and prevalence differences may be due to the study type and the condition. However, more studies are necessary about the link between the geographic distribution of antibiotics and the development of bacterial virulence and resistance in those areas. This study's results raised many red flags toward increasing bacterial UTI resistance to antibiotics and the high prevalence of MDR genes in those bacteria which have a high risk of spreading to other flora. Antibiotic misuse is prohibited in the absence of isolation and culture sensitivity tests.

Conflicts of interest

The authors acknowledge that there is no conflict of interest regarding the research data and tools used with this study.

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