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Epidemiological investigations on microbial infection and crystals causing feline lower urinary tract disease in tomcats in Ismailia, Egypt

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Abstract

Background: Feline lower urinary tract disease (FLUTD) is a common disorder associated with the dysfunction of the urinary bladder or urethra in tomcats.

Aim: A prospective study was carried out on the point prevalence and odds ratio (OR) of the FLUTD in Shirazi and Baladi tomcats at Ismailia Governorate, Egypt, recording the prominent clinical manifestation and identifying the antibiogram, virulence, and antimicrobial resistance genes of the causative microorganisms.

Methods: A total number of 420 tomcats admitted to the veterinary clinics of Ismailia during the period June 2020 to May 2021 were examined for FLUTD. A total of 1,260 urine samples were collected and analyzed.

Results: Hematuria, dysuria, and pollakiuria were the most evident signs recorded in a total of 120 tomcats diagnosed with FLUTD. The diagnosed cases of FLUTD were associated with causes like crystals (35.83%), pyogenic microorganisms (19.16%), and mixed cases (45.00%). The prevalence revealed highly significant ($p < 0.01$) increases in the cases caused by *Escherichia coli*, *E. coli* mixed cases, and calcium oxalate at >4 years; *Staphylococcus aureus* at ≤ 2 years; amorphous urate and phosphate at 2–4 and >4 years in Shirazi and ≤2 years in Baladi; triple phosphate at ≤2 years in Shirazi and >4 years in Baladi; and *S. aureus* mixed cases at ≤2 years. The OR of FLUTD revealed higher odds of associations with *E. coli*, *E. coli* mixed cases, *S. aureus*, amorphous urate, and triple phosphate, as well as lower odds with *S. aureus*, calcium oxalate, amorphous phosphate, and *S. aureus* mixed cases. Isolated *E. coli* revealed higher resistance to amoxicillin (AMX, 83.4%), ceftriaxone (83.4%), ceftazidime (CAZ, 75.0%), and cefoxitin (FOX, 50.0%), and *S. aureus* to oxacillin (100%), FOX (100%), AMX (85.8%), CAZ (76.2%), and FOX (50.0%). *S. aureus*-detected virulence genes were *mecA*, *coa*, *spa*, and *tetK*, and *E. coli* were *fimH*, *iss*, *iutA*, *papC*, *blaTEM*, *blaCTX-M*, and *tetA*. About 100% of *E. coli* and 76.1% of *S. aureus* isolates exhibited multidrug resistance.

Conclusion: FLUTD in tomcats is associated with higher odds in *E. coli*, *E. coli* mixed cases, and triple phosphate at older ages (>4 years) with high antimicrobial resistance in the microbial isolates contributing to the disease.

Keywords: Antibiotic resistance, Crystals, Feline lower urinary tract disease, Prevalence, Virulence.

Introduction

Feline lower urinary tract disease (FLUTD) is not a specific disease but rather is a term used to describe the dysfunction of the urinary bladder or urethra of cats and has been commonly reported with a prevalence of up to 8% (Adam and Dean, 2009; Lund *et al.*, 2012). FLUTD has been revealed in some cases of urethral obstruction associated with oliguria and anuria caused by bacterial cystitis, neoplasia, urolithiasis, anatomical abnormalities, and urethral plugs (Saevik *et al*., 2011; Dorsch *et al.,* 2014; Forrester and Towell, 2015). The prevalence of urinary tract infections (UTI) is associated with the cat's age and to some extent breed as the prevalence increase with older age (Eggertsdottir *et al*., 2011; Martinez-Ruzafa *et al*., 2012).

The prevalence has been defined as the proportion of the population affected with a particular disease at a particular time and is usually expressed as a percentage (Bruce *et al*., 2017). Prevalence (P) can also be expressed as the multiplication of the incidence by the duration of the study (Rothman, 2012). Point prevalence (PP) is a more specific proportion for the number of cases developed at a particular time to the number of the given population at this time (Viera, 2008; Brink, 2021). Odds ratio (OR) is known by the association of the exposure to a certain predisposing cause to an outcome (Carneiro, 2011; Motulsky, 2018). OR usually measures the strength of the association to the presence and absence of the cause (Noordzij et al., 2017). OR values are expressed as OR > 1 when the exposure is associated with higher odds of outcome

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and OR < 1 when the exposure is associated with lower odds of outcome (Szklo and Nieto, 2019).

Escherichia coli (*E. coli*) is the principal etiologic agent of UTI causing up to 90% (Loh and Sivalingam, 2007), and is recognized as uropathogenic *E. coli* (UPEC) (Schwartz *et al*., 2013). UPEC have various virulence factors, such as fimbriae for bacterial adhesion and invasion, iron-acquisition systems for surviving in the iron-deficient environment, flagella, and toxins for bacterial dissemination. Virulence genes are found on transmissible genes (plasmid) and/or the chromosome (Farshad *et al*., 2012). Extraintestinal disease *E. coli* (ExPEC) has numerous virulence factors that cannot be established by traditional diagnostics and is a prominent cause of UTIs. Urinary *E. coli* isolated from cats have shared similarities with ExPEC isolated from humans (Johnson *et al*., 2001). ExPEC isolates have unique virulence factors which enable them to invade host surfaces, damage host tissues, and prevent or reverse host defenses (Johnson and Stell, 2000). *Staphylococci* are nonmotile, nonspore-forming, and opportunistic cocci widely found on mammalian skin, producing various illnesses ranging from mild, soft tissue infections to life-threatening conditions, such as bacteremia and endocarditis (Chang *et al.*, 2003). *Staphylococcus aureus* and *S. schleifer* are the most prevalent pathogenic species (Yamashita *et al*., 2005). *Staphylococcus aureus* is a common cause of UTIs in the general population (Siegman-Igra, 2005). Cats can play a part in *S. aureus* transmission and recurring human methicillin-resistant *S. aureus* (MRSA) infections (Litster *et al.*, 2007).

The current study aims to conduct a prospective epidemiological study on the PP and OR associated with the FLUTD, as well as the associated antibiogram, virulence, and the antimicrobial resistance genes of *E. coli* and *S. aureus* in Ismailia Governorate, Egypt, regarding breed and age during the period from June 2020 to May 2021.

Material and Methods

Study area and design

The study was carried out in small animal clinics in Ismailia Governorate which is located in the northeastern part of Egypt with coordinates of 30°36′15.37″N and 32°16′20.1″E. A prospective epidemiological study was designed to investigate the PP and OR of predisposing risk factors associated with FLUTD of tomcats in Ismailia Governorate, Egypt, considering tomcat's breed and age during the period from June 2020 to May 2021.

Sampling

A total number of 420 tomcats were admitted to the clinics during the study period. Out of them, 120 tomcats (28.57%) were suffering from urine retention. All the cats were recorded for the breed, age, and some other environmental influences, such as feeding habits, housing system, housing pattern, vaccination history, deworming actions, and disease and medication history. The cats were all fed on commercial dry feed and housed individually with the owners in their houses. The clinical examinations had been carried out according to the procedures described by Cote *et al*. (2010).

A total of 1,260 urine samples (three urine samples per case) were collected from all admitted cats via cystocentesis to run a complete urine analysis and detect the predisposing causes for the development of FLUTD (pyogenic organisms, crystals, or both).

Urine analysis

The urine samples (1,260 samples) were examined in a clinicopathological laboratory in Ismailia Governorate, Egypt. The recorded major causes, like crystals and pyogenic causes, were surveyed for calculating the frequencies. The result reports were categorized in a Microsoft Excel sheet (Microsoft Excel 365) for statistical analysis.

Epidemiological measures

The epidemiological measures included PP and OR and they were calculated as recommended by Thrusfield and Christley's (2018) modifications. PP rates of the diseased-specific tomcats admitted to clinics were calculated as follows:

$$\text{PP rate} = (\alpha / \mu) \times 100$$

where α is the number of cats admitted with the FLUTD during the study period and μ is the number of susceptible cats admitted considering breed and age during the same period. OR investigated the risk of contracting FLUTD in the exposed cats' population concerning breed and age as follows:

$$\text{OR} = [(a / b) / (c / d)]$$

where (a / b) is the odds of exposure among the cases (in presence of dependent b) and (c / d) is the odds of exposure among the controls (in absence of dependent b).

Isolation of E. coli and S. aureus

Escherichia coli and *S. aureus* strains were categorized into strains related to pyogenic infection and strains related to mixed infection. Standard bacteriological and biochemical investigations were carried out to isolate and identify *E. coli* and *S. aureus* strains.

Escherichia coli strains were streaked onto MacConkey and eosine methylene blue agar medium (Oxoid) and incubated at 37°C for 24 hours. Blood agar was used to detect the hemolytic activity related to the pathogenicity of *E. coli*. The purified colonies were placed into a nutrient agar slant, incubated at 37°C for 24 hours, and maintained for further identification at −80°C in 10% glycerol (v/v). Isolated colonies have been purified and biochemically tested to validate their identity as *E. coli* according to Quinn *et al*. (2002).

Staphylococcus aureus was streaked onto blood agar, nutrient agar, and mannitol salt agar plates (Oxoid, Hampshire, UK). The streaked plates were then incubated at 37°C for 24–48 hours. *Staphylococcus aureus* circular, convex, and golden yellow colonies were collected

and maintained for further examination at −80°C in 10% glycerol (v/v). Isolated colonies were purified and biochemical tests were conducted to confirm their identity as *S. aureus* according to Quinn *et al*. (2002).

Antibiotic susceptibility

Twenty-four isolates of *E. coli* and 21 isolates of *S. aureus* (one sample-one strain) were analyzed for their antibiotic susceptibility using the Kirby–Bauer disk diffusion method, and zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI). The antibiotics used in the presented study were as follows: amoxicillin (AMX, 30 µg), AMX and clavulanic acid (AMC, 10 µg), oxacillin (OXA, 25 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CTR, 30 µg), cefoxitin (FOX, 30 µg), meropenem (MEM, 10 µg), Nitrofurntin (NIT, 25 µg), gentamicin (GEN, 10 µg), norfloxacin (NOR, 10 µg), amikacin (AK, 30 µg), doxycycline (DOX, 10 µg), trimethoprim-sulfamethoxazole (SXT, 25 µg), and aztreonam (ATM, 30 µg) (Oxoid, Basingstoke, UK). The diameter of the inhibition zone was estimated as described by CLSI 27. The phenotypic resistance patterns are classified into PDR, extensive drug-resistant (XDR), and multidrug resistance (MDR).

Bacterial extraction and PCR amplification

Escherichia coli and *S. aureus* genomic DNAs were extracted following QIAamp DNA Mini Kit instructions from the manufacturer (Qiagen, Germany, GmbH). Specific primers were employed to amplify *fim*H, *pap*C, *iut*A, and *iss* operons by PCR. Primers' details and predicted sizes of the amplified products and specific annealing temperatures are shown in Table 1. Amplification of bacterial DNA was carried out in a total volume of 50 µl containing 5 µl of 10x PCR reaction buffer, 1 µl 200 µM (of each dNTP) of dNTP mix (10 mM), 10 µl of bacterial DNA, 0.4 µl 2

Table 1. Primers and amplicon sizes of the amplified products and specific annealing temperatures.

M.Os	Target gene	Primers sequences	Amplicon size (bp)	Amplification (35 cycles)		
				Denaturation	Annealing	Extension
E. coli	*fimH*	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	94°C 30 seconds	50°C 45 seconds	72°C 45 seconds
	papC	TGATATCACGCACTCAGTAGC CCGGCCATATTCACATAA	501	94°C 30 seconds	58°C 40 seconds	72°C 45 seconds
	iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	226	94°C 30 seconds	54°C 30 seconds	72°C 30 seconds
	iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	94°C 30 seconds	63°C 30 seconds	72°C 30 seconds
	$bla_{CTX\text{-}M}$	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	94°C 30 seconds	54°C 40 seconds	72°C 45 seconds
	bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	94°C 30 seconds	54°C 40 seconds	72°C 45 seconds
	tetA	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576	94°C 30 seconds	50°C 40 seconds	72°C 45 seconds
S. aureus	*mecA*	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	94°C 30 seconds	50°C 30 seconds	72°C 40 seconds
	coa	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	630	94°C 30 seconds	55°C 40 seconds	72°C 40 seconds
	spa	TCA ACA AAG AAC AAC AAA ATG C GCT TTC GGT GCT TGA GAT TC	226	94°C 30 seconds	50°C 30 seconds	72°C 40 seconds
	tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	364	94°C 30 seconds	54°C 40 seconds	72°C 40 seconds

U of Taq DNA polymerase (5 U/µl), 30 pmol of each used primer (0.1–0.6 µM), and then sterile ddH2O was added (up to 50 µl).

The amplification was carried out in thermal protocols. Initial denaturation at 94°C for 10 min was required, followed by 35 cycles at a specific temperature (Table 1), and extension at 72°C for 1 min. A 10-µl aliquot of PCR was subjected to a 2% agarose gel electrophoresis, then stained with an ethidium bromide solution. Amplified DNA fragments of specific sizes were detected by UV-induced fluorescence.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS, 2016). The data were analyzed using multifactorial analysis of variance (two-tailed analysis of variance) to determine the influence of breed and age on the urine analysis pictures in the examined tomcats. The statistical model empathized as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \mathcal{E}_{ijk}$$

where Y_{ijk} is the measurement of dependent variables, μ is the overall mean, α_i is the fixed effect of the breed, β_j is the fixed effect of age, $(\alpha\beta)_{ij}$ is the interactions of the breeds by tomcats' age, and $\mathcal{E}_{ijk}$ is the random error.

Ethical approval

The materials, protocol, and study design were approved by the Scientific Research Ethics Committee on Animal and Poultry Researches at the Faculty of Veterinary Medicine, Suez Canal University, Egypt, with approval number (2021050).

The examined tomcats were subjected to the examination humanly and carefully, taking all the necessary precautions to minimize the pain during sample collection. Some of the owners witnessed the examinations and sampling for more clarity and obvious consent for sampling of the animal for research purposes.

Results

Clinical examinations

The clinical examinations of the tomcats admitted to the clinics revealed a prevalence of 28.57% for FLUTD-infected animals (a total of 120 diseased animals out of the 420 examined animals). The prominent recorded clinical signs in the diseased animals were hematuria, dysuria, stranguria, pollakiuria, periuria, vocalization when trying to urinate, urinating in unusual places, excessive licking of the genital area, aggression associated with a large, and distended bladder on abdominal palpation. All of the previous symptoms were concurrent with a history of anuria or oliguria and in some cases passage of a few drops of blood-stained urine for more than 2 days was noted.

Urine analysis

The FLUTD in the infected 120 tomcats out of the examined 420 animals was related to some predisposing factors that were revealed in the urine analysis, such as crystals (n = 43, PP_{Total} = 35.83%), pyogenic microorganisms (n = 23, PP_{Total} = 19.16%), and mixed cases (n = 54, PP_{Total} = 45%). The total recorded crystals were amorphous urate (n = 36, PP_{Total} = 30.00%, $PP_{Specific}$ = 83.72%), calcium oxalate (n = 8, PP_{Total} = 6.66%, $PP_{Specific}$ = 18.60%), amorphous phosphate (n = 15, PP_{Total} = 12.50%, $PP_{Specific}$ = 34.88%), and triple phosphate (n = 38, PP_{Total} = 31.66%, $PP_{Specific}$ = 88.37%). The isolated pyogenic microorganisms were *E. coli* (n = 61, PP_{Total} = 50.83%) and *S. aureus* (n = 16, PP_{Total} = 13.33%).

Epidemiological investigations

The prevalence reveals in Table 2 highly significant ($p < 0.01$) increases of *E. coli*, *E. coli* mixed cases, and calcium oxalate crystals in tomcats older than 4 years in both breeds with no significant differences between the breeds. Highly significant ($p < 0.01$) increases of prevalence were recorded in *S. aureus* in ≤2 and 2–4 years Shirazi with no significant differences between the two ages and in ≤2 years Baladi tomcats. Amorphous urate and phosphate reveal highly significant ($p < 0.01$) increases in 2–4 and >4 years in Shirazi with no significant differences between the two ages and in ≤2 years in Baladi tomcats. Triple phosphate crystals revealed highly significant ($p < 0.01$) increases in ≤2 years in Shirazi and >4 years in Baladi tomcats. *Staphylococcus aureus* mixed cases revealed highly significant ($p < 0.01$) increases in ≤2 years in both breeds with no significant differences between the breeds.

The OR of FLUTD reveals in Table 2 higher odds of associations with *E. coli*, mixed cases of *E. coli*, *S. aureus* (≤2 years in Shirazi), amorphous urate crystals (2–4 and >4 years in Shirazi and ≤2 and 2–4 years Baladi), and triple phosphate crystals (≤2, 2–4, and >4 years in Shirazi and 2–4, and >4 years in Baladi), while OR reveals lower odds of associations with *S. aureus* (2–4 and >4 years in Shirazi, and <2, 2–4, and >4 years in Baladi), amorphous urate (<2 years in Shirazi and >4 years in Baladi), calcium oxalate, amorphous phosphate, triple phosphate (<2 years in Baladi), mixed cases of *S. aureus*.

Antimicrobial susceptibility of the recovered isolates

The antimicrobial susceptibility testing revealed (Table 3) a high resistance pattern of the *E. coli* isolates to AMX (83.4%), CTR (83.4%), CAZ (75.0%), and FOX (50.0%). The examined strains were highly susceptible to MEM (87.5%), nitrofurantoin (83.4%), and gentamycin (75.0%) as described in Table 4 and Figure 1. Statistically, the resistance of retrieved isolates to various tested antimicrobial agents was significantly different ($p < 0.05$).

The antimicrobial susceptibility testing revealed that the *S. aureus*-recovered isolates exhibited a high resistance pattern to penicillins: OXA (100%), cephalosporins: FOX (100%), AMX (85.8%), CAZ (76.2%), and FOX (50.0%). In addition, the examined strains were highly susceptible to MEM (100%), AK (90.4%),

Table 2. Prevalence and odds ratio of FLUTD's contributing causes (pyogenic, crystals, and mixed cases) in Shirazi and Baladi tomcats.

Causes	*Measures*	*Breeds by age*						*p-value*
		Shirazi Tomcat			Baladi Tomcat			
		≤2	2: 4	>4	≤2	2: 4	>4	
Pyogenic causes								
	n	9	9	12	9	9	13	---
E. coli	PP	12.86^{b}	12.86^{b}	17.14^{a}	12.86^{b}	12.86^{b}	18.57^{a}	0.012
	OR	5.36^{b}	6.52^{b}	27.27^{a}	2.65^{c}	10.71^{b}	21.67^{a}	0.001
	n	4	4	0	5	2	1	---
S. aureus	PP	5.71^{a}	5.71^{a}	0.00^{b}	7.14^{a}	2.86^{b}	1.43^{c}	0.001
	OR	1.06^{a}	0.72^{b}	0.19^{c}	0.82^{a}	0.53^{b}	0.13^{c}	0.000
Urine crystals								
	n	3	5	5	12	7	4	---
Amorphous urate	PP	4.29^{b}	7.14^{a}	7.14^{a}	17.14^{a}	10.00^{b}	5.71^{c}	0.005
	OR	0.68^{c}	1.19^{b}	1.32^{a}	2.68^{a}	2.57^{a}	0.55^{b}	0.001
	n	0	0	2	1	1	4	---
Calcium oxalate	PP	0.00^{b}	0.00^{b}	2.86^{a}	1.43^{b}	1.43^{b}	5.71^{a}	0.002
	OR	0.00^{b}	0.00^{b}	0.40^{a}	0.09^{c}	0.21^{b}	0.55^{a}	0.000
	n	1	3	3	3	2	3	---
Amorphous phosphate	PP	1.43^{b}	4.29^{a}	4.29^{a}	4.29^{a}	2.86^{b}	4.29^{a}	0.007
	OR	0.19^{c}	0.60^{b}	0.66^{a}	0.32^{c}	0.45^{a}	0.38^{b}	0.001
	n	10	7	5	4	5	7	---
Triple phosphate	PP	14.29^{a}	10.00^{b}	7.14^{c}	5.71^{c}	7.14^{b}	10.00^{a}	0.002
	OR	6.25^{a}	2.08^{b}	1.32^{c}	0.45^{c}	1.47^{a}	1.22^{b}	0.000
Mixed causes								
	n	4	6	7	9	6	11	---
E. coli and crystals	PP	5.71^{c}	8.57^{b}	10.00^{a}	12.86^{b}	8.57^{c}	15.71^{a}	0.001
	OR	1.06^{c}	1.34^{b}	1.85^{a}	0.95^{c}	1.92^{a}	1.43^{b}	0.000
	n	3	2	0	5	0	1	---
S. aureus and crystals	PP	4.29^{a}	2.86^{b}	0.00^{b}	7.14^{a}	0.00^{c}	1.43^{b}	0.000
	OR	0.79^{a}	0.45^{b}	0.00^{c}	0.53^{a}	0.00^{c}	0.13^{b}	0.000

Means with different superscripts in the same row are significantly different at $p \leq 0.05$ or highly significantly different at $p < 0.01$. Means with the same superscripts in the same row are nonsignificantly different at $p< 0.05$. Total number of cases caused by crystals = 43; pyogenic microorganisms = 23; and mixed cases = 54. The recorded crystals were amorphous urate = 36; calcium oxalate = 8; amorphous phosphate = 15; and triple phosphate = 38. PP: Point prevalence; OR: Odds ratio; *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*; SE: Standard error.

nitrofurantoin (71.4%), and NOR (66.6%) as described in Table 3 and Figure 1. Statistically, the resistance of retrieved isolates to various tested antimicrobial agents was significantly different ($p < 0.05$). MAR index values, in this study, showed multiple-resistant patterns, indicating recovered isolates originated from high-risk contamination.

The distribution of the virulence genes and antibiotic-resistance genes among the recovered strains

Virulence genes and antibiotic resistance genes (Table 4) were detected successfully in 21 *S. aureus* isolates. The most frequent *S. aureus* virulence genes were *mecA* (100%), *coa* (100%), *spa* (85.7%), and *tetK* (42.9%) (Fig. 2). The distribution of virulence genes and antibiotic-resistant genes among the examined strains was statistically not significantly different.

Virulence genes and antibiotic resistance genes (Table 4) were detected successfully in 24 *E. coli* isolates. The most frequent *E. coli* virulence genes were *fimH* (100%), *iss* (75%), *iutA* (54.2%), *papC* (37.5%), *blaTEM* (83.4%), *blaCTX-M* (75%), and *tetA* (41.6%) (Figs. 3 and 4). The distribution of virulence-determinant genes and antibiotic-resistant genes among the examined strains is statistically not significantly different.

Table 3. Antimicrobial susceptibility of the recovered isolates.

Antibiotic class	*Antibiotic*	*E. coli* (*n*=24)			S. aureus (*n*=21)		
		***S* (%)**	***I* (%)**	***R* (%)**	***S* (%)**	***I* (%)**	***R* (%)**
Penicillin	AMX	2 (8.3)	2 (8.3)	20 (83.4)	3 (14.2)	0 (0)	18 (85.8)
	AMC	3 (12.5)	6 (25.0)	15 (62.5)	3 (14.2)	3 (14.2)	14 (66.6)
	OXA	6 (25.0)	5 (20.8)	13 (54.1)	2 (9.6)	0 (0)	21 (100)
Cephalosporin	CAZ	0 (0)	6 (25.0)	18 (75.0)	5 (23.8)	0 (0)	16 (76.2)
	CTR	4 (16.6)	0 (0)	20 (83.4)	9 (42.9)	3 (14.2)	9 (42.9)
	FOX	6 (25.0)	6 (25.0)	12 (50.0)	2 (9.6)	0 (0)	21 (100)
Fluroquinolones	NOR	12 (50.0)	7 (29.2)	5 (20.8)	14 (66.6)	0 (0)	7 (33.4)
Monobactam	ATM	14 (58.4)	6 (25.0)	4 (16.6)	13 (62.0)	3 (14.2)	5 (32.8)
Aminoglycoside	AK	16 (41.6)	8 (33.4)	0 (0)	19 (90.4)	1 (4.7)	1 (4.7)
	GEN	18 (75.0)	0 (0)	6 (25.0)	10 (47.6)	6 (28.6)	5 (32.8)
Carbapenem	MEM	21 (87.5)	3 (12.5)	0 (0)	21 (100)	0 (0)	0 (0)
Nitrofuran	NIT	20 (83.4)	3 (12.5)	1 (4.1)	15 (71.4)	3 (14.2)	0 (0)
Tetracycline	DOX	8 (33.4)	6 (25.0)	10 (41.6)	9 (42.9)	3 (14.2)	9 (42.9)
Sulfonamide	SXT	12 (50.0)	0 (0)	12 (50.0)	10 (47.6)	0 (0)	11 (52.4)
Mean ± SE		10.14^{a}±1.860	4.143^{a}±0.7402	9.714^{a}±1.908	9.643^{a}±1.663	1.571^{a}±0.5105	9.786^{a}±1.962

[A]significant difference at $p < 0.0001$; AMX: Amoxicillin; AMC: Amoxicillin and clavulanic acid; OXA: Oxacillin; CAZ: Ceftazidime; CTR: Ceftriaxone; FOX: Cefoxitin; NOR: Norfloxacin; ATM: Aztreonam; AK: Amikacin; GEN: Gentamicin; MEM: Meropenem; NIT: Nitrofurntin; DOX: Doxycycline; SXT: Trimethoprim-sulfamethoxazole.

Table 4. The distribution of the virulence genes and antibiotic resistance genes among the recovered strains of *E. coli* and *S. aureus*.

M.Os	Type of genes	Genes of *E. coli*	No.	%	Chi-square *P*-value
E. coli	Virulence genes	*fim*H	21	87.5	9.1009^{NS}
		iss	18	75.0	
		*iut*A	13	54.2	
		*pap*C	9	37.5	
	Antibiotic resistance genes	bla_{TEM}	20	83.4	
		bla_{CTX-M}	18	75.0	
		*tet*A	10	41.6	
S. aureus	Virulence genes	*coa*	21	100	0.34177^{NS}
		*mec*A	21	100	
	Antibiotic resistance genes	*spa*	18	85.7	
		*tet*K	9	42.9	

A combination between phenotypic multidrug resistance and AMR genes

About 100% of *E. coli* isolates were multidrug-resistant (MDR, Table 5) to various antimicrobial agents and 32.8% of *S. aureus* were XDR to seven antimicrobial agents, and 76.1% of *S. aureus* were multidrug resistance (MDR) to various antimicrobial agents.

Discussion

FLUTD can be considered a term to describe the affections of the urinary bladder and/or urethra of tomcats rather than to be considered a disease. The clinical manifestations are similar to other diseases and affections of the lower urinary tract, which is why further investigations should be carried out to differentiate as reported by Lund *et al*. (2016). The most common and reported clinical manifestations of the FLUTD in tomcats are dysuria (painful urination), pollakiuria (increased urination frequencies), hematuria (blood in urine), periuria (urination outside the litter), self-over-grooming around the perineum, and behavioral vices, like aggression, irritation, and stranguria (blockage of the urethra), following Kim *et al.* (2018).

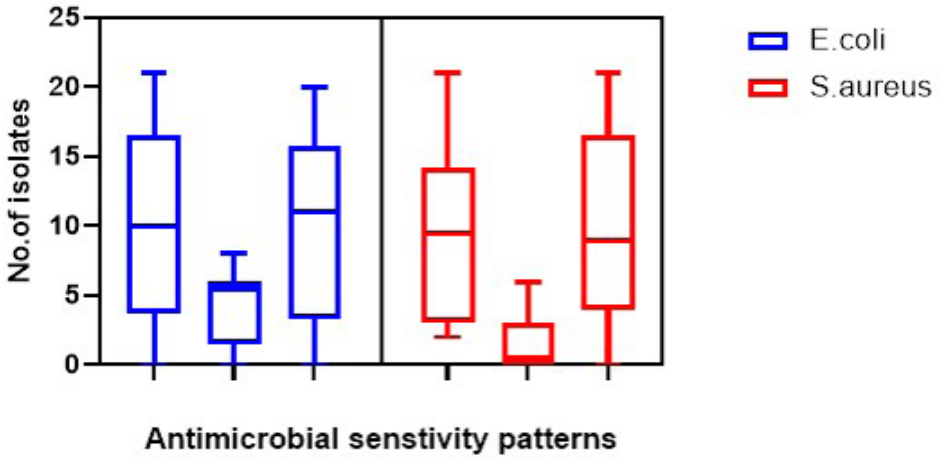

Fig. 1. Antibiotics' sensitivity patterns in the isolated *E. coli* and *S. aureus*.

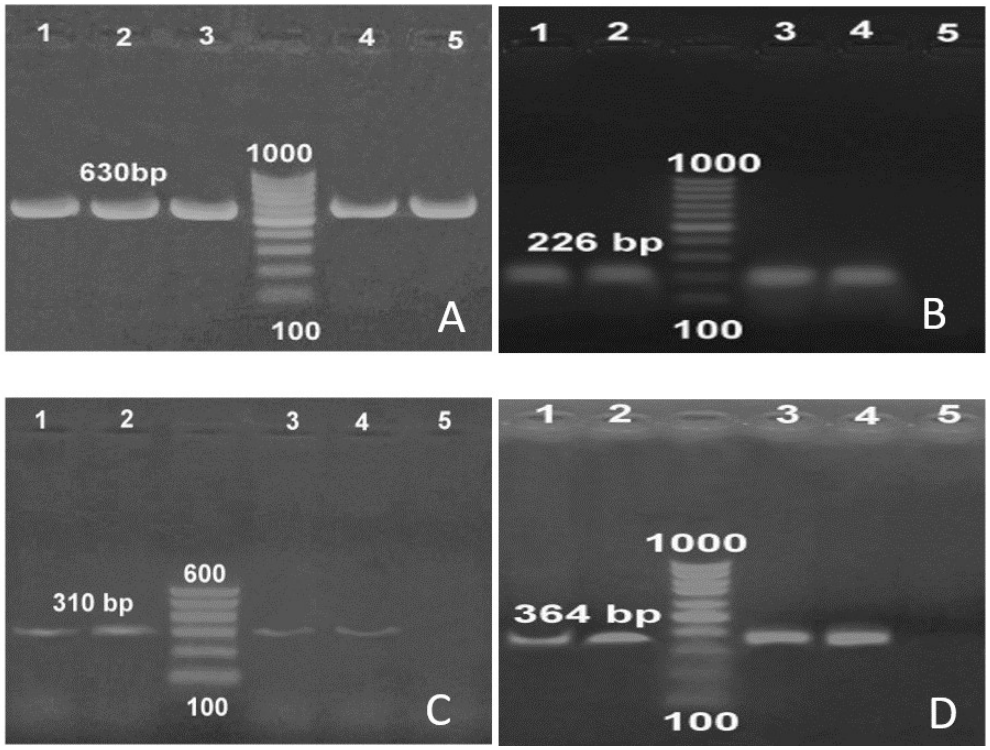

Fig. 2. (A) PCR amplification of the specific gene of *S. aureus* (*coa*) at 630 bp. (B) PCR amplification of the specific gene of *S. aureus* (*spa*) at 226 bp. (C) PCR amplification of the specific gene of *S. aureus* (*mec*A) at 310 bp. (D) PCR amplification of the specific gene of *S. aureus* (tetK) at 364 bp.

The current study reveals that FLUTD prevailed as 28.57% (a total of 120 diseased animals out of the 420 examined animals). These infected cases were correlated to the main causes that were detected through the urine analysis as follow: crystals (n = 43, PP_{Total} = 35.83%), pyogenic microorganisms (n = 23, PP_{Total} = 19.16%), and mixed cases (n = 54, PP_{Total} = 45.00%). The most predominant recorded crystals were amorphous urate (n = 36, PP_{Total} = 30.00%, $PP_{Specific}$ = 83.72%), calcium oxalate (n = 8, PP_{Total} = 6.66%, $PP_{Specific}$ = 18.60%), amorphous phosphate (n = 15, PP_{Total} = 12.50%, $PP_{Specific}$ = 34.88%), and triple phosphate (n = 38, PP_{Total} = 31.66%, $PP_{Specific}$ = 88.37%). The isolated pyogenic microorganisms were *E. coli* (n = 61, PP_{Total} = 50.83%) and *S. aureus* (n = 16, PP_{Total} = 13.33%).

On the other hand, FLUTD was reported by Lew-Kojrys *et al*. (2017) with a prevalence up to 51.9% higher than the recorded prevalence in the current study, and they

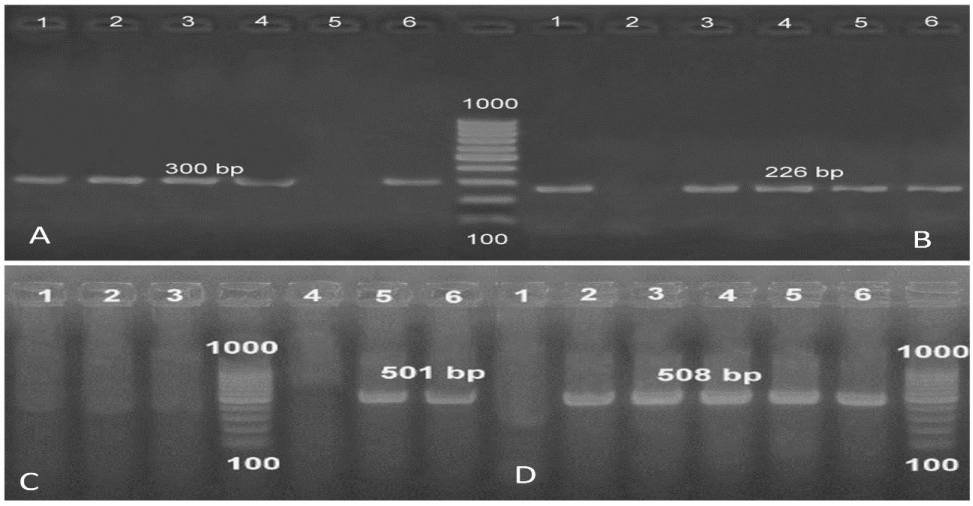

Fig. 3. (A) PCR amplification of the specific gene of *E. coli* (*iut*A) at 300 bp. (B) PCR amplification of the specific gene of *E. coli* (*iss*) at 226 bp. (C) PCR amplification of the specific gene of *E. coli* (*pap*C) at 501 bp. (D) PCR amplification of the specific gene of *E. coli* (*fim*H) at 508 bp.

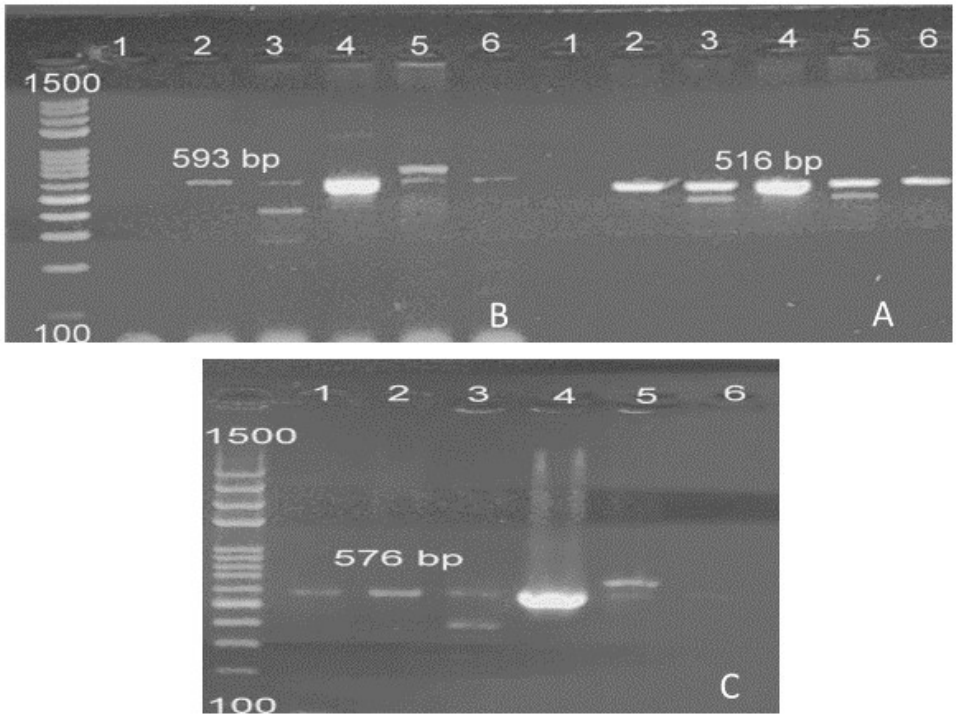

Fig. 4. (A) PCR amplification of the specific gene of *E. coli* (bla_{CTX-M}) at 593 bp. (B) PCR amplification of the specific gene of *E. coli* (bla_{TEM}) at 516 bp. (C) PCR amplification of the specific gene of *E. coli* (*tet*A) at 576 bp.

attributed the high prevalence to idiopathic cystitis in Polish feline populations. They also recorded that FLUTD is associated with numerous factors such as animal factors including breed, sex, age, and neutering conditions, as well as environmental factors including housing and living standards, and diet formulation. Kovarikova *et al*. (2020) reported a higher prevalence compared to the current study of up to 51.9% of 214 examined males and female cats for FLUTD. They attributed the disease to a urethral obstruction in 23 cases and 100 cases were nonobstructive. Lund and Eggertsdottir (2019) reported studied the occurrence

Table 5. The frequency of the phenotypic multidrug resistance and the antibiotic resistance genes among the retrieved strains of *E. coli* and *S. aureus*.

Type of bacteria	No. of strains	%	Type of resistance	Phenotypic multidrug resistance	MAR	Antimicrobial resistance genes
E. coli	12	50.0	MDR	Penicillins: AMX, AMC, OXA Cephalosporins: CTR, CAZ, FOX Sulfonamides: SXT	0.29	bla_{TEM} and bla_{CTX-M}
	6	25.0	MDR	Penicillins: AMX Cephalosporins: CTR, CAZ Tetracycline: DOX Aminoglycoside: CN	0.20	bla_{TEM} bla_{CTX-M} and tetA
	3	12.5	MDR	Penicillins: AMC Tetracycline: DOX Fluroquinolones: NOR Monobactam: ATM	0.16	*tetA*
	2	8.3	MDR	Penicillins: AMX Cephalosporins: CTR Fluoroquinolones: NOR	0.12	bla_{TEM}
	1	4.1	MDR	Penicillins: OXA Tetracycline: DOX Monobactam: ATM Aminoglycoside: CN Nitrofuran	0.20	*tetA*
S. aureus	11	52.4	MDR	Penicillins: AMX, AMC, OXA Cephalosporins: CAZ, FOX Sulfonamides: SXT	0.28	bla_{TEM}
	5	32.8	XDR	Penicillins: OXA Cephalosporins: CAZ, CTR, FOX Aminoglycoside: CN Tetracycline: DOX Monobactam: ATM Fluoroquinolones: NOR	0.38	*tetA*
	2	9.6	MDR	Penicillins: AMX, OXA Cephalosporins: CTR, FOX Tetracycline: DOX Fluoroquinolones: NOR	0.28	*tetA*
	2	9.6	MDR	Penicillins: AMC, OXA Cephalosporins: CRO, FOX Tetracycline: DOX	0.23	*tetA*
	1	4.7	MDR	Penicillins: AMC, OXA Cephalosporins: FOX Aminoglycoside: AK	0.19	

MDR: multidrug resistance; XDR: extensively drug-resistance; AMX: Amoxicillin; AMC: Amoxicillin and clavulanic acid; OXA: Oxacillin; CAZ: Ceftazidime; CTR: Ceftriaxone; FOX: Cefoxitin; NOR: Norfloxacin; ATM: Aztreonam; AK: Amikacin; GEN: Gentamicin; MEM: Meropenem; NIT: Nitrofurntin; DOX: Doxycycline; SXT: Trimethoprim-sulfamethoxazole.

of FLUTD in Norwegian cats during the period 2003–2009. They attributed these cases to nonobstructive feline idiopathic cystitis and urolithiasis. They suggested the application of multimodal environmental modifications and enrichment of cats. Kaul *et al*. (2020) conducted a questionnaire survey on the incidence of FLUTD in 101 cats from 2010 to 2013. They recorded nearly close numbers to the current study and associated them with pathogenic causes such as 52 cats diagnosed with idiopathic cystitis, 21 with urolithiasis, and 13 with bacterial urinary tract infection.

The most often isolated bacterium in dogs and cats with UTI was *E. coli*. In general, *Enterobacteriaceae* were responsible for more than half of UTIs in dogs and cats; Simultaneously, *Staphylococcus* spp. were the second most often detected bacteria; however, the *Staphylococcus* species found varied greatly across dogs and cats (Hall *et al*., 2013**)**. *Enterobacteriaceae* along with *Staphylococcus* spp. causes approximately 79% of UTIs in dogs and cats (Marques *et al*., 2018).

UTI are the most commonly diagnosed diseases of cats that are inappropriately treated with empirical antibiotics without microbiological testing (De Briyne *et al*., 2014). The cats are assumed to be potential reservoirs for antimicrobial resistance (AMR) transfer to humans due to the large-scale use of antimicrobial agents and close interaction with humans being extensively treated by broad-spectrum antimicrobial agents (Guardabassi *et al*., 2004; Lloyd, 2007). The risk of antimicrobial treatment failure could be increased in both animals and humans (Algammal *et al*., 2020a; Li *et al*., 2021).

The current findings regarding the bacteriological identification of characteristic colonies on eosine methylene blue agar and the biochemical reaction are similar to those of Edwads and Ewing (1972). The *in vitro* antimicrobial resistance pattern of the recovered *E. coli* strains exhibited a high resistance pattern to AMX (83.4%), CTR (83.4%), CAZ (75.0%), and FOX (50.0%). In addition, the examined strains were highly susceptible to MEM (87.5%) was consistent with those detected by Prescott *et al*. (2000) and Patel *et al*. (1999).

In the present study, the PCR proved that the tested strains harbored the virulence-determinant genes (*fim*H). Adhesion gene was the most common and present in 87.5% UPEC isolates, which is in agreement with studies carried out in Romania (86%) (Vargová *et al*., 2017) and Mongolia (89.9%) (Munkhdelger, 2017) on UTI. The present study shows the presence of virulence genes characteristic of ExPEC strains in bacterial isolates from nondiarrheic cats, as well as cats with UTI (*iss*, *iutA*, and *papC*), with an incidence of 75.0, 54.2, and 37.5%, respectively. Gregova and Kmet (2020) revealed that the most commonly discovered virulence genes in *E. coli* strains isolated from animals were *iutA*, *iss*, and *papC*. The presence of these genes has also been confirmed in ExPEC strains derived from UPEC. Pets are natural reservoirs for various pathogens, including ExPEC strains, that are capable of infecting humans, which is a reason for concern (Johnson *et al*., 2003).

The large increase in the discovery of multidrug-resistant and methicillin-resistant *S. aureus* (MDR MRSA) over time has been a finding because it poses serious therapeutic constraints, which is in agreement with Jay-Russell *et al*. (2014) who isolated MDR MRSA in cats. The rapid emergence and spread of resistant *Staphylococci* in cats is a worrying development. Resistant *Staphylococci* in cats are similar to human *Staphylococci* that are highly resistant to penicillin, ampicillin, and tetracycline. Notably, the *mec*A gene was mostly found in *Staphylococcus* spp. isolated from cats. Nonetheless, MRSA identification in cats with UTIs is a public health hazard because companion animals will be responsible for spreading these bacteria to the household and public surroundings. Tetracyclines were extensively employed for therapy and prevention of bacterial infections in humans, animals, and plants (Malik *et al*., 2005). The genes *tetK* and *tetL* are most often plasmid-borne (Schwarz and Noble, 1999). Staphylococcal virulence factors are generally divided into four categories: exoenzymes, exotoxins, adhesins, and others (Kuroda *et al*., 2001; Zhang *et al*., 2003). The *coa* gene was 100% abundant in the MRSA strains tested. These findings are comparable to those of Akineden *et al*. (2001). This gene showed no polymorphisms of any size (Algammal *et al*., 2021). However, in other investigations, amplification of the coagulase gene led to various amplicons, demonstrating the variability of coagulase gene size. The *spa* gene showed notable gene polymorphisms with various amplicons (140, 270, and 290 bp). The X region of the *spa* gene is usually replaced variable (up to 24 times) in various strains (Algammal *et al*., 2020b).

Conclusion

FLUTD is a common disease affecting tomcats and was detected with high prevalence in older ages >4 and 2–4 years. The OR epidemiological investigations reveal higher odds of associations with *E. coli*, mixed cases of *E. coli* and *S. aureus*, amorphous urate crystals, and triple phosphate crystals, while OR reveals lower odds of associations with *S. aureus*, amorphous urate, calcium oxalate, amorphous phosphate, triple phosphate, and mixed cases of *S. aureus*.

The antimicrobial susceptibility reveals a high resistance of *E. coli* and *S. aureus* to a large number of the tested antibiotic disks. The most frequently detected *S. aureus* virulence genes were *mecA*, *coa*, *spa*, and *tetK*, and *E. coli* were *fimH*, *iss*, *iutA*, *papC*, *blaTEM*, *blaCTX-M*, and *tetA*. About 100% of *E. coli* and 76.1% of *S. aureus* isolates exhibited multidrug resistance and

32.8% of *S. aureus* were extensively drug-resistant to 7 antimicrobial agents.

Authors' contributions

AEM collected the raw data and urine samples from tomcats admitted to the clinics, carried out the clinical examination, conducted the urine analysis, and took part in writing the manuscript. MME participated in the clinical examination, urine analysis, and took part in writing the manuscript. RME conducted the bacteriological examination, antibiotic sensitivity testing, virulence examination, and took part in writing the manuscript. ESS designed the study design, conducted the epidemiological analysis, took part in writing the manuscript, and reviewed the final edit of the manuscript.

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Conflict of interests

The authors declare that they have no financial or personal conflicts which may have inappropriately influenced them in writing this manuscript.

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