UNIVERSITI PUTRA MALAYSIA

THE DISTRIBUTION AND RISK FACTORS OF BACTERIA ISOLATED FROM FELINE URINARY TRACT INFECTIONS AND THE ANTIMICROBIAL RESISTANCE PROFILE FROM 2017 TO 2022

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THE DISTRIBUTION AND RISK FACTORS OF BACTERIA ISOLATED FROM FELINE URINARY TRACT INFECTIONS AND THE ANTIMICROBIAL RESISTANCE PROFILE FROM 2017 TO 2022

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CERTIFICATION

It is hereby certified that we have read this project paper entitled **"The Distribution and Risk Factors of Bacteria Isolated from Feline Urinary Tract Infections and the Antimicrobial Resistance Profile from 2017 to 2022",** by H'ng Wann Jye and in our opinion it is satisfactory in term of scope, quality and presentation as partial fulfilment of the requirement for the course VPD4999 – Final Year Project.

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Specially dedicated to Dr Khor, Dr Sharina, my family and friends.

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LIST OF ABBREVIATIONS

AAFP	:	Association of Feline Practitioners
AAHA	:	American Animal Hospital Association
AMR	:	Antimicrobial resistance
AST	:	Antimicrobial Susceptibility Test
CLSI	:	Clinical Laboratory Standards Institute
CKD	:	Chronic kidney disease
DSH	:	Domestic Shorthair
E. coli	:	*Escherichia coli*
EUCAST	:	European Committee on Antimicrobial Susceptibility Testing
FIC	:	Feline infectious cystitis
FLUTD	:	Feline Lower Urinary Tract Disease
ISCAID	:	International Society For Companion Animal Infectious Diseases
LUTS	:	Lower urinary tract signs
LUTD	:	Lower urinary tract disease
NSAIDs	:	non-steroidal anti-inflammatory drugs
UA	:	Urinalysis
UTI	:	Urinary tract infection
UVH-UPM	:	University Veterinary Hospital- University Putra Malaysia
SPSS	:	Statistical Package for the Social Sciences®
Staph. spp	:	*Staphylococcus* species

Strep. spp	:	*Streptococcus* species
TMS	:	Trimethoprim- sulphonamides
VLSU	:	Veterinary Laboratory Services Unit

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kursus VPP4999 - Projek Tahun Akhir.

PENGEDARAN DAN FAKTOR-FAKTOR RISIKO BAKTERIA DIISOLASI DARIPADA JANGKITAN SALURAN KENCING DAN PROFIL KERENTANAN ANTIMIKROB DARI TAHUN 2017 HINGGA 2022

oleh

H'ng Wann Jye

Penyelia : Dr. Khor Kuan Hua

Penyelia Bersama : Dr. Sharina Omar

Jangkitan saluran kencing (UTI) merupakan penyebab lazim uropati pada populasi kucing dan sebab utama untuk preskirpsi antibiotik dalam kalangan haiwan kesayangan. Banyak kajian telah menekankan bahawa kemunculan bakteria tahan antimikrob adalah berkaitan dengan penggunaan antibiotik yang tidak berhemah. Walau bagaimanapun, terdapat kekurangan data mengenai tahap rintangan antimikrob dalam haiwan kesayangan. Kajian retrospektif ini bertujuan untuk menilai penyebaran bakteriuria dalam populasi felin, faktor-faktor risiko, dan profil kerintangan antimikrob. Jumlah 312 kucing yang disyaki menjangkiti UTI telah dikenalpastikan dari Januari 2017 hingga Disember 2022, dan rekod perubatan juga diperolehi. Data pesakit seperti umur, jenis baka, jantina, pengurusan dan sejarah serta latar belakang direkodkan. Data dianalisis secara deskriptif, dan *Peason's chi-squared test* telah dijalankan untuk menganalisis faktor-faktor risiko dengan aras signifikan (p = 0.05). Sebanyak 84.0% populasi kucing yang disyaki UTI menghadapi bakteriuria dengan petanda termasuk hematuria (46.4%), stranguria (42.8%), kekurangan selera makan (26.3%), pollakiuria (25.8%), dan anuria (22.2%). Hanya jantina menunjukkan perbezaan signifikan ($p< 0.05$, 95% CI), dan kucing jantan berisiko 2.9 kali lebih tinggi dijangkiti bakteriuria. Sejumlah 227 *isolates* bakteria telah dikenalpasti, dan sebahagian besarnya, *Escherichia coli* (15.4%), *Enterococcus faecalis* (14.1%), *Klebsiella pneumoniae* (13.2%), *Pseudomonas aeruginosa* (10.6%) dan *Proteus mirabillis* (10.1 %) telah diisolat. Bakteria *isolates* telah menunjukkan kerintangan yang tinggi terhadap antibiotik biasa yang digunakan, termasuk *fluoroquinolones, penicillin, cephalosporin,* dan *trimethoprim-sulphonamides*. Antibiotik yang berspektrum lebar biasanya digunakan dalam kes felin UTI telah menyebabkan peningkatan tren keringtangan antimikrob. Dengan petanda klinikal, rawatan antimikrob yang rasional perlulah mengikut garis panduan perlu diberikan berdasarkan keputusan kultur air kencing dan profil kerentanan antimikrob.

Kata kunci: *Jangkitan saluran kencing, faktor-faktor risiko, profil kerentanan antimikrob, bakteriuria, kultur air kencing*

ABSTRACT

An abstract of the project paper presented to Faculty of Veterinary Medicine in partial fulfilment of course VPD 4999- Final Year Project.

THE DISTRIBUTION AND RISK FACTORS OF BACTERIA ISOLATED FROM FELINE URINARY TRACT INFECTIONS AND THE COMMON BACTERIA ANTIMICROBIAL RESISTANCE PROFILE FROM 2017 TO 2022

By

H'ng Wann Jye

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Urinary tract infection (UTI) is a common uropathy affecting the feline population and may be the leading reason for antimicrobial prescription in companion animals. Many studies emphasised that the emergence of antimicrobial-resistant bacteria was related to the imprudent use of antibiotics. Locally, there was a lack of data on antimicrobial resistance in companion animals. This retrospective study assessed the distribution of bacteriuria in cats, the risk factors, and the antimicrobial resistance profile. A total of 312 cats suspected of UTI between January 2017 to December 2022 were identified, and the medical records were retrieved. Signalments such as age, breed, gender, management, and clinical history were recorded. Data was descriptively analysed, and the Pearson's chi-squared test was conducted to analyse risk factors with a significant level (p = 0.05). About 84.0% of suspected UTI cats had bacteriuria with signs of haematuria (46.4%), stranguria (42.8%), inappetence (26.3%), pollakiuria (25.8%) and anuria (22.2%). Only gender showed a significant difference ($p<0.05$, 95% CI), and male cats were at 2.9 times more risk for having bacteriuria. A total of 227 bacterial isolates had been identified, and predominantly, *Escherichia coli* (15.4%), *Enterococcus faecalis* (14.1%), *Klebsiella pneumoniae* (13.2%), *Pseudomonas aeruginosa* (10.6%) and followed by *Proteus mirabillis* (10.1 %) were isolated. These bacteria isolates showed high resistance to common antibiotics, including fluoroquinolones, penicillin, cephalosporin, and trimethoprim-sulphonamides. Broad-spectrum antibiotics were generally used against feline UTIs, causing the increasing trend of antimicrobial resistance. With clinical signs, a rational antimicrobial therapy adhered to guidelines should be given based on the urine culture result and antimicrobial susceptibility profile.

Keywords: *urinary tract infection, risk factors, antimicrobial susceptibility profile, bacteriuria, urine culture*

CHAPTER 1.0

INTRODUCTION

1.1 INTRODUCTION

Due to its short urinary tract anatomy and high production of concentrated urine, cats are highly susceptible to urinary diseases, including cystitis and urolithiasis (Kobayashi *et al*., 2021). Although the submission of feline urine for bacteria culture with antimicrobial resistance profiling has increased gradually in veterinary practice, bacterial urinary tract infections (UTI) are uncommon due to physical and immunological barriers to infection (Litster *et al*., 2011). Feline idiopathic cystitis has been reported as the most common cause of FLUTD, while bacterial cystitis is only reported in a minimal number of cases (2%) (Eggertsdóttir *et al.*, 2007). However, UTI is a compelling cause of feline lower urinary tract disease (FLUTD), and there is a higher risk in geriatric female cats (> 10 years old). A higher prevalence of bacterial cystitis was found in several studies conducted in Thailand (12%) and Europe (8%) (Lekcharoensuk *et al.*, 2001; Gerber *et al.*, 2005). In general, UTI occurs when bacteria travel along the urinary tract, from the urethra up to the bladder, which subsequently causes multiple bladder problems such as idiopathic cystitis, urolithiasis, urethral plugs and neoplasia.

The most common presenting signs of feline bacterial cystitis include stranguria, pollakiuria, dysuria and haematuria, but the non-infectious condition may show similar clincal signs. Moreover, bacteria may be present in the urine without showing any clinical abnormalities, known as subclinical bacteriuria. Interpretation must be made by the clinicians based on the urine's gross and cytological appearance with bacteria culture, resulting in finding the likelihood of a clinically significant UTI (Weese *et al.*, 2011). Therefore, urinalysis can be done as the first-line screening test for clinical bacteriuria in determining bacterial infection (Siu *et al.,* 2022).

The diagnosis of bacterial cystitis should accompanied with urine culture and susceptibility testing, in which the urine is collected by cystocentesis. Urinalysis can be done in all cases to provide supporting evidence, including cytological examination of urine, such as the presence of leukocytes and bacteria microscopically. The common isolated bacteria species include *Proteus* species*, Staphylococcus* species*, Streptococcus* species and *Escherichia coli,* where *E. coli* has the highest prevalence being isolated from cats with UTIs (Dokuzeylül *et al.,* 2015). In contrast, *Enterococcus* species are commonly isolated from cats with subclinical bacteriuria (Teichmann-Knorrn *et al*., 2018). Based on a study conducted in Poland, there is an emergence of multidrug-resistant *E. coli* in companion animals (Rzewuska *et al.,* 2015).

According to Dorsch *et al*. (2019), the decision to use antibiotics should depend on clinical signs and/or concurrent disease and be guided by the result of urine culture and bacteria susceptibility testing. Withholding antimicrobial use while waiting for the urine culture result is reasonable to avoid unwanted use of antimicrobials. Initial therapy should consist of amoxicillin, amoxicillin/clavulanic acid or trimethoprim-sulphonamide as first-line drug options with a recommended duration of three to five days. An extended antibiotic therapy (7-14 days) will be recommended in cases with persistent and recurrent cystitis. These classes of drugs are selected as they are excreted in the urine in their active form.

1.2 OBJECTIVES

The objectives of this study were:

1. To determine the risk factors of cats associated with UTI.
2. To study the distribution of bacteria isolated from cats with UTI.
3. To investigate the AMR profile of common bacteria identified and isolated.

1.3 HYPOTHESIS

1. H_O: There are no risk factors associated with feline UTI due to bacterial infection.
   H_A: There are risk factors associated with feline UTI due to bacterial infection.

1.4 JUSTIFICATION

Vercelli *et al.* (2021) demonstrated the importance of understanding and analysing the antimicrobial resistance of bacteria causing UTIs in veterinary medicine to improve clinical practice in developed individualised antibiotics treatment. Information on the bacteria agent that commonly causes UTI is not often reported locally. This study investigates the risk factors of cats associated with UTI, the bacteria distribution, and the common bacteria's AMR profile isolated and identified.

CHAPTER 2.0

LITERATURE REVIEW

2.1 FELINE URINARY TRACT INFECTION

A urinary tract infection (UTI) is defined as an infection caused by the adhesion, multiplication and persistence of virulent microbes in the urinary tract of a healthy individual, causing a compromise in the host defence mechanism (Dokuzeylül *et al.*, 2015). Feline bacterial cystitis is one of the differentials for feline lower urinary tract disease (FLUTD) that has an increasing incidence rate in older cats. Development of UTI in cats is generally an opportunistic infection that is secondary to or complications of other urinary tract disorders, including neoplasia, renal insufficiency, urolithiasis, urethral obstruction, and idiopathic haemorrhagic cystitis (George *et al.*, 1996). Colonisation of bacteria within the urinary system may occur due to diminished immunological defence contributing to symptomatic or asymptomatic UTIs.

2.2 CLINICAL SIGNS

Cats with FLUTD will typically have altered patterns of urination (e.g. pollakiuria, anuria) and abnormal urine conditions (e.g. haematuria, pyuria). Pyuria indicates high white blood cell content in urine, highly suggestive of bacterial cystitis. On the other hand, the presence of haematuria and proteinuria suggests other differential for FLUTD (e.g., urolithiasis, feline idiopathic cystitis), which is not usually accompanied by pyuria (George *et al.,* 1996). Alarmingly, a substantial number of affected cats may not show prominent clinical signs of FLUTD and on cytological showed evidence of UTI, so-called subclinical bacteriuria, where a diagnosis can only be made with positive urine culture (Dorsch *et al.,* 2017).

2.3 RISK FACTOR OF FELINE URINARY TRACT INFECTION

Several studies have shown that multiple risk factors including age, sex, breed, lifestyle, and diet, contribute to the development of bacterial cystitis. According to Lew-Kojrys *et al.* (2017), gender, neutering status, age, living environment, and diet affect cats' susceptibility to urinary tract disease. However, the risk factors may vary significantly with geographical distribution, contributing to the difference in prevalence and antimicrobial resistance of each bacterial isolate (Piyarungsri *et al.,* 2020; Taylor *et al.*, 2021).

2.3.1 Gender

With regards to gender, male cats were prone to urethral obstruction, which gives a higher risk of urinary tract infection. In Indonesia, there is a higher prevalence of FLUTD in the male cat population, and feline UTI has contributed as the second most significant cause (25.3%) in the research population (Nururrozi *et al.*, 2020). Even though male cats are more predisposed to FLUTD due to the unique narrowing and curving anatomical structure of the penile urethra, but the female cats have a higher risk of UTI at a senior age. According to Lekcharoensuk *et al.* (2001), spayed females are predisposed to urocystolithiasis, UTI and neoplasia compared to intact females. There is also a higher rate of culture-positive urine samples from older female cats in many studies (Litster *et al.*, 2008; White *et al.*, 2016).

2.3.2 Breed

On the other hand, purebred cats are primarily predisposed to general health conditions and are more likely to suffer from illness. Persian, Himalayan and Manx breeds cats have shown high prevalence in experiencing lower urinary tract disease, whereas Abyssinian cats were predisposed to UTI (Lekcharoensuk *et al.*, 2001). Willeberg *et al.* (1976) also suggested that Persian cats appeared predisposed to FLUTD, while Siamese cats have a lower risk. Besides the pedigree breeds, the domestic longhair breed (DLH) has shown a substantial risk of FLUTD development (Jones *et al.,* 1997). In Poland, it was reported that domestic cats had a higher risk of FLUTD (Lew-Kojrys *et al.*, 2017). The preference of the type of breeds in the population of different regions can be one of the factors affecting the findings in each study.

2.3.3 Age

Geriatric cat is more susceptible to UTI due to the high incidence of health conditions (e.g., renal insufficiency, diabetes mellitus and hyperthyroidism) and/or diminished urinary tract defence against infection due to ageing (George *et al.,* 1996). Young and adult cats (< 10 years old) were reported to be more susceptible to feline infectious cystitis (FIC), urolithiasis, and urethral plugs. The average age of infected cats was 8.2 years; male cats get infected at a younger age (6.3 years) compared to female cats (10.6 years) (George *et al.,* 1996). In Indonesian, Polish and Norwegian studies, the mean age in the cat population affected reported were 9.1, 8.9 and 5.6 years, respectively (Sævik *et al.,* 2011; Lew-Kojrys *et al.,* 2017; Nururrozi *et al.,* 2020).

2.3.4 Management

Sævik *et al.* (2011) revealed that cats diagnosed with FLUTD were generally male cats being managed indoors. It was speculated that cats with low activity levels, and managed were at risk of obesity, which could contribute to the risk of FLUTD. According to Lew-Kojrys *et al.* (2017), the majority of the feline FLUTD patients were managed strictly indoors (83.0%), followed by multicat household management at (53.0%). Gun-Moore (2003) found that the prevalence of FLUTD increases with the risk factors of eating dry kibbles diet and indoor and multicat household management. Moreover, the number of litter boxes available in the multicat household is highly associated with feline urinary problems (Piyarungsri *et al.*, 2020).

2.4 PATHOGENESIS

UTI is commonly secondary to other diseases related to FLUTD that may have caused the immunological barrier in the urinary tract to be compromised. Bacterial virulence and poor host defence mechanisms are factors in the development of UTIs (Dorsch *et al.,* 2017). A healthy urinary tract is not conducive to microbial multiplication and colonisation as its natural host defence mechanisms protect the bladder's health. These include physical barriers (e.g. urethral length, urethral peristalsis and the pressure within the urethra) and mucosal defence barriers (e.g. resident immune cells lining, normal resident microflora) within the lower urinary tract (Abraham *et al.,* 2015; Dorsch *et al.,* 2019). Besides that, the virulence of bacteria can contribute to the development of UTI. When the mucosal integrity is breached, the interaction between bacteria and compromised urothelial layers aids in the ascending migration of bacteria within the bladder up to the upper urinary tract (Hickling *et al.,* 2015). For instance, the adhesin protein in uropathogenic *E. coli* interacts with the uroplakins protein in the urothelium, resisting urine flow and causing bacteriuria.

2.5 DIAGNOSTIC METHODS

Besides the observed LUTS, diagnostic methods, including urinalysis and urine culture, were essential as evidence supporting uncomplicated feline UTIs. However, for recurrent feline UTI, underlying concurrent diseases must be identified using other diagnostic methods such as ultrasound imaging, radiography and contrast imaging for long-term success.

2.5.1 Urinalysis

Urine samples obtained via cystocentesis can minimise the risk of contamination, thus giving higher accuracy in the diagnosis of UTI. Catheterisation and free-catch samples or manual expression should be avoided due to the risk of contamination. Litster *et al.* (2009) have revealed a greater value of pH, erythrocyte, and leukocyte counts found in urinalysis results from a positive culture urine sample. UTI cats infected with gram-negative bacteria have lower urine specific gravity and higher leukocyte counts in their urine samples than those infected with gram-positive bacteria. The reagent strip technique shows that the nitrite test is unreliable due to the presence of ascorbic acids in urine (Klausner *et al.*, 1976). Other parameters, including urine glucose, protein count, bilirubin, ketones, and the presence of cast and crystals, have shown insignificant changes in positive culture urine samples in cats with FLUTD (Litster *et al.*, 2009). Bacteria can be easily observed under microscopic examination of urine sediment. Nevertheless, interpretation is operator-dependent as an experienced observer may provide a false positive (able to detect bacteria, but there is no infection) or false negative results (no bacteria observed in urine from the infected cat) (George *et al.*, 1996).

2.5.2 Urine Culture

Urine culture is the gold standard for a definitive diagnosis of bacterial UTI. Care must be taken during urine collection to ensure the validity of the result and to reduce the contamination risk that will deteriorate the quality of specimens. Therefore, obtaining urine specimens via cystocentesis with the least potential for iatrogenic bacterial contamination is preferable.

There is a minority of feline UTI cases that are infected by two or more bacterial microorganisms (12- 22%) (Dorsch *et al*., 2017). *E. coli* has been predominantly isolated from feline patients with concurrent predisposing disorders (e.g., hyperthyroidism, chronic kidney disease (CKD), diabetes mellitus) (Bailiff *et al*., 2006; White *et al*., 2013). In several studies conducted from 1995 to 2014, the most common bacterial isolates in feline UTI were *E. coli, Enterococcus* spp., *Staphylococcus* spp.*, and Streptococcus* spp. (Dorsch *et al.,* 2017). In Norway, the identical common bacterial isolates were found in Norwegian cats with bacterial cystitis (Lund *et al*., 2014). This statement was supported by another study conducted by D'Août *et al.* (2022), stating that *E. coli* (43.7%) was the most common bacteria isolated from urine specimens obtained via cystocentesis, followed by *Enterobacterales* (26.4%), *Enterococcus* spp. (14.9%) *and Staphylococcus* sp. (9.2%). Moreover, the frequency of polymicrobial growth was reported in 22.6% of urine specimens (Martinez-Ruzafa *et al*., 2012), which can also be found in another study conducted with 15.8% of urine specimens showing polymicrobial growth (Litster *et al.,* 2007).

2.6 ANTIMICROBIAL SUSCEPTIBILITY TEST (AST)

AST is a laboratory procedure conducted to identify the effective antimicrobial treatment for patients. It is a routine practice performed in veterinary hospitals and clinics for continuous assessment of treatment regimens and detection of antimicrobial resistance profiles in individual bacterial isolates, which aids in enhancing the effectiveness of empirical therapy (Reller *et al.*, 2009). Each bacterial isolate from the bacteria culture will be tested using the disk diffusion or Kirby-Bauer methods. A standardised inoculum is prepared using isolated bacteria from specific bacteria cultures and then inoculated in a growth medium (e.g. Meuller Hinton Agar). Antibiotic-saturated discs are then placed on the selected agars. Incubation is done under specific conditions, and the zone of inhibition is then measured to determine the susceptibility and further classified into three different classes, including “susceptible”, “intermediate”, or “resistance”. (Tenover *et al.*, 2018). The Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are well-known breakpoint guidelines available which CLSI was the standard methodology used in Southeast Asia (Thailand, Laos and Cambodia) (Cusack *et al.,* 2019).

According to D’Août *et al*. (2022) in their study conducted in the United Kingdom, *E. coli* and *Enterobacterales* isolated from urine specimens collected via cystocentesis showed resistance pattern towards amoxicillin and cephalexin, while *Enterococcus* sp. and *Staphyloccocus* sp. showed resistance pattern towards trimethoprim/sulfamethoxazole (94.3%) and amoxicillin (20.0%), respectively.

2.6.1 Antimicrobial Resistance, Antimicrobial Stewardship and Recommended Treatment Regime

Antimicrobial resistance (AMR) is a phenomenon developed when the microbes are not responsive to antimicrobial treatment given, causing infections difficult to be cured (World Health Organisation, 2021). The imprudent use of antimicrobials, including improper prescription, overuse as a growth promoter, prophylaxis or metaphylaxis, has contributed to selective pressure, forcing microbes to adapt by developing bacteria resistance strategies in which the AMR traits can be amplified and transmitted intensively (Roncada & Tilocca, 2022). AMR has elevated the risk to public health and threatened economic growth. To prolong the efficacy of lifesaving antibiotics, a proper disease management among major sectors (human, animal and environment) is required, in line with the One-Health concept (Murugaiyan *et al.*, 2022). Appropriate antibacterial drug therapy is a crucial step to prevent AMR. An appropriate antibacterial drug can be selected based on its antimicrobial susceptibility profile.

By optimising antimicrobial use, comprehensive antimicrobial stewardship programs can improve cost-effectiveness and ensure the continued efficacy of available antibiotics for a longer time in the clinical field (MacDougall & Polk, 2005). However, implementing antimicrobial stewardship programmes is relatively uncommon in veterinary practices (Hardefeldt *et al.,* 2022).

A short treatment interval of three to five days is sufficient for uncomplicated UTI. However, the treatment interval for complicated UTIs highly depends on the nature of the underlying diseases (e.g., urolithiasis, neoplasia, and urethral plugs). Current guidelines on antimicrobial use for the treatment of FLUTD have suggested the use of amoxicillin (11 – 15 mg/kg PO q8h) or trimethoprim-sulphonamide (TMS) (15- 30 mg/kg PO q8h) for uncomplicated UTIs (Weese *et al.,* 2019). The study noted that the use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) is recommended as initial therapy to minimise the use of antimicrobials. The use of amoxicillin/clavulanic acid (12.5-25mg/kg PO q8h) is reasonable when there is an absence of amoxicillin, suggested by Weese *et al.,* however, there is a lack of evidence on the need for clavulanic acid use, but it was suggested to be used to combat gram-negative bacteria. Alternative antibiotics, including nitrofurantoin, fluoroquinolones, and third-generation cephalosporin, can be reserved to individualise the treatment regime for each patient based on the analysis of urine culture and AST results.

According to Dorsch *et al.* (2017), the inherent resistance of *Enterococcus faecalis* against cephalosporins, clindamycin and TMS must be considered when interpreting its AST result. In the United Kingdom, there is a significant bacteria resistance pattern isolated from feline urine specimens towards cephalexin (20.7%) and amoxicillin (16.7%) (D'Août *et al.*, 2022). Recent studies have reported an alarming condition of multidrug-resistant bacteria emergence in antibiotics agents used in treating bacterial cystitis in Portugal and Germany (Garcês *et al.,* 2022; Aurich *et al.*, 2022).

Treatment of antibiotics should only depend on the result of quantitative urine culture and AST in each case (Weese *et al.,* 2019). Prophylactic treatment of antibiotics should never be considered. In clinic settings, veterinarians should monitor the changes or trends of local resistance patterns, which may contribute to the risk of developing antimicrobial resistance. When the baseline resistance rate of the most common organism increases against a specific drug of choice, changes should be made to avoid therapeutical failure (Weese *et al*., 2011). However, the rate is commonly overestimated due to an elevated number of recurrent cases or infections. The evolution of multidrug-resistant bacteria due to inappropriate usage of antibiotics is a vital exposure that contributes to a global threat to human and animal health.

CHAPTER 3.0

MATERIALS AND METHODS

3.1 SOURCE OF DATA

This retrospective study reviewed the medical records of cat patients presented at the University Veterinary Hospital, Universiti Putra Malaysia (UVH-UPM) from January 2017 to December 2022. Cat patients with clinical signs of LUTD (e.g. dysuria, haematuria, oliguria, stranguria, pollakiuria or periuria) and had their urine specimens submitted to the Veterinary Laboratory Services Unit (VLSU) for urinalysis and urine culture as part of the diagnostic workup were included. Information such as signalment (age, breed, gender), management (housing management and number of cats in the household), and clinical history of the affected cats were recorded. Results of the urinalysis and urine culture were obtained and recorded. If multiple samples were collected from the same patient during the designated period, only the first result was used for analysis.

3.2 INCLUSION CRITERIA

The inclusion criteria of feline UTI recruited in this study were i) retrievable patient file records with complete information of the patient, ii) urine specimens collected via cystocentesis, and iii) available urinalysis result, urine culture and AST profile of each patient.

3.3 PATIENT SIGNALMENT AND MANAGEMENT

The age of cats was categorised into five distinctive groupings based on the 2021 American Animal Hospital Association/Association of Feline Practitioners (AAHA/AAFP) Feline Life Stage Guidelines, with four age-related stages (kitten, young adult, mature adult, senior and end-of-life). In this study, the age groups were divided as follows: i) Kitten (newborn – 1 year old), ii) Young adult (1 - 6 years old), iii) Mature adult (7-10 years old), and iv) Senior (>10 years old) (Refer to Appendix 8.1). Young adult and mature adult were then combined for data analysis.

The breed of each cat patient was recorded accordingly, and the gender was reported as either male or female. The management and lifestyle of the cats were noted and categorised into indoor housing, outdoor housing, or semi-roaming (access to the outdoors and outdoor hunting).

3.3.1 Clinical Signs

Cat patients with FLUTD were commonly found with signs of dysuria, haematuria, stranguria, pollakiuria or periuria. Other systemic signs (e.g., vomiting, inappetence, bladder turgidity) were also recorded with the specific urinary clinical signs.

3.3.2 Urinalysis result

Based on the record in the file, only urine specimens collected via cystocentesis with sterile precaution were analysed. Urinalysis profiles for each feline patient were retrieved, and microscopic examinations of bacteria were recorded as bacteriuria and non-bacteriuria.

3.3.3 Urine culture

Based on the bacteriology reports of each patient, the bacteria isolates found from culture-positive urine specimens were recorded and tabulated accordingly. In terms of AST, the disk diffusion method is the standard procedure to determine the susceptibility of bacterial isolate towards selected antibiotics based on the size of the inhibitory zone around the disc measured. The zone size can be categorised into 3 groups: susceptible, intermediate, and resistant. Based on Clinical & Laboratory Standards Institute (CLSI) guideline, the susceptibility of each bacterial isolate on selected antibiotics were focused on, which are penicillin (e.g., ampicillin, amoxicillin, amoxicillin/clavulanate acid, penicillin, marbofloxacin, ampicillin/sulbactam), macrolide (e.g. erythromycin, azithromycin), cephalosporin (e.g. cephalexin, ceftriaxone, cefuroxime), tetracycline (e.g. tetracycline, doxycycline), Aminoglycoside (e.g. amikacin, gentamicin and neomycin), carbapenem (e.g. imipenem, meropenem), folate pathway inhibitors (e.g. TMS), fluoroquinolone (e.g. enrofloxacin, ciprofloxacin and norfloxacin) and others (e.g. nitrofurantoin, vancomycin, fosfomycin, lincomycin, metronidazole and clindamycin).

3.4 DATA ANALYSIS

Data from the case file and logbook were tabulated in Microsoft Excel. Data obtained was descriptively analysed using relative frequencies and percentages for categorical variables. By using the Statistical Package for the Social Sciences® (SPSS) software (IBM, USA), Pearson's chi-squared tests with a significant level ($p<0.05$) were used to analyse the association of risk factors with feline UTI, including lower urinary tract sign, age, gender, breed and management.

CHAPTER 4.0

RESULTS

4.1 DISTRIBUTION OF CATS WITH SUSPECTED UTI IN UVH- UPM (2017 TO 2022)

A total of 312 cats that had urine specimens being examined for the suspicion of UTI over 6 years (2017 to 2022) were retrieved. It was observed that most of the urine specimens were collected via cystocentesis (n= 189; 60.9%), followed by urine catheterisation (n= 123; 39.4%).

Of the 312 urinalysis reports, only 289 cats with complete information and evidence of bacteriuria were included in the study. About 23 cats were removed from the analysis due to incomplete signalment (n=12), missing information on clinical signs (n=4), and not retrievable results of bacteriuria (n=7).

4.1.1 Clinical presentation

The comparison of reported clinical signs and physical examination findings of the cat with evidence of bacteriuria and non-bacteriuria as shown in Table 1 and Table 2.

Table 1: Comparison of the frequency of the reported lower urinary tract signs (LUTS) of cats with UTI (n=289) with bacteriuria and non-bacteriuria.

	Bacteriuria n=194, n (%)	**Non-bacteriuria n=95, n (%)**	**Total n=289, n (%)**
With LUTS	163(84.0%)	71 (74.7%)	234 (81.0%)
Without LUTS	31 (16.0%)	24 (25.3%)	55 (19.0%)
Clinical Sign			
Haematuria	90 (46.4%)	45 (47.4%)	135 (46.7%)
Stranguria	83 (42.8%)	36 (37.9%)	119 (41.2%)
Inappetence	51 (26.3%)	24 (25.3%)	75 (26.0%)
Pollakiuria	50 (25.8%)	19 (20.0%)	69 (23.9%)
Anuria	43(22.2%)	15 (15.8%)	58 (20.1%)
Vomiting	29(15.0%)	15 (15.8%)	44 (15.2%)
Polyuria	13 (6.7%)	1 (1.1%)	14 (4.8%)
Dull/Weak	13 (6.7%)	6 (6.3%)	19 (6.6%)
Pyuria	11 (5.7%)	3 (3.2%)	14 (4.8%)
Halitosis	5 (2.6%)	5 (5.3%)	10 (3.5%)
Polydipsia	4 (2.1%)	2 (2.1%)	6 (2.1%)
Diarrhoea	0 (0.0%)	2 (2.1%)	2 (0.7%)

Out of the 234 (81.0%) cats suspected of UTI with LUTS, bacteria were found in 163 (69.7%) urine specimens but absent in 71 (30.3%). The remaining 55 (19.0%) cats were suspected of UTI but no LUTS. However, out of these 55 cases, 31 (56.4 %) were presented with no LUTS showing evidence of bacteriuria, and the remaining 24 (43.6%) cats had no sign of LUTS and bacteriuria.

Out of the 289 cats in the study, haematuria (n=135; 46.7%) was the most common presenting complaint by owners, followed by stranguria (n= 119; 41.2%), pollakiuria (n= 69; 23.9%), anuria (n= 58; 20.1%), polyuria (n= 14; 4.8%), pyuria (n= 14; 4.8%) and polydipsia (n= 6; 2.1%). Systemic signs observed include inappetence (n= 75; 26.0%), vomiting (n=44; 15.2%), weakness (n= 19; 6.6%) and halitosis (n=10; 3.5%) (Refer Table 1).

Table 2: Comparison of the physical examination findings of the 289 cats' urine sample with evidence of bacteriuria and non-bacteriuria.

Physical findings	Bacteriuria n=194, n (%)	Non-bacteriuria n =95, n (%)	Total n =289, n (%)
Dehydration	2 (1.0%)	3(3.2%)	5 (1.7%)
Enlarged Bladder	9 (4.6%)	2(2.1%)	11 (3.8%)
Bladder compression			
Turgid	70 (36.0%)	32 (33.7%)	102 (35.3%)
Semi turgid	8 (4.1%)	2(2.1%)	10 (3.5%)
Non-turgid	11 (5.7%)	7(7.4%)	18 (6.2%)
Penile tip			
Bruised	10 (5.2%)	10(10.5%)	20 (6.9%)
Necrotic	2 (1.0%)	0(0.0%)	2(0.7%)

Based on the information for physical examination findings, five cats were dehydrated (1.7%). About 35.3% of the cat patient was reported with turgid non-compressible bladder (n= 102), while 18 were found with non-turgid bladder (6.2%) and 10 with semi-turgid bladder (3.5%). Numbers of male cats were found with bruises (n=20; 6.9%) and necrotic penile tips (n= 2; 0.7%), respectively.

4.2 THE AGE, SEX, BREEDS, AND MANAGEMENT AS THE ASSOCIATED FACTORS OF FELINE UTI IN UVH-UPM (2017-2022)

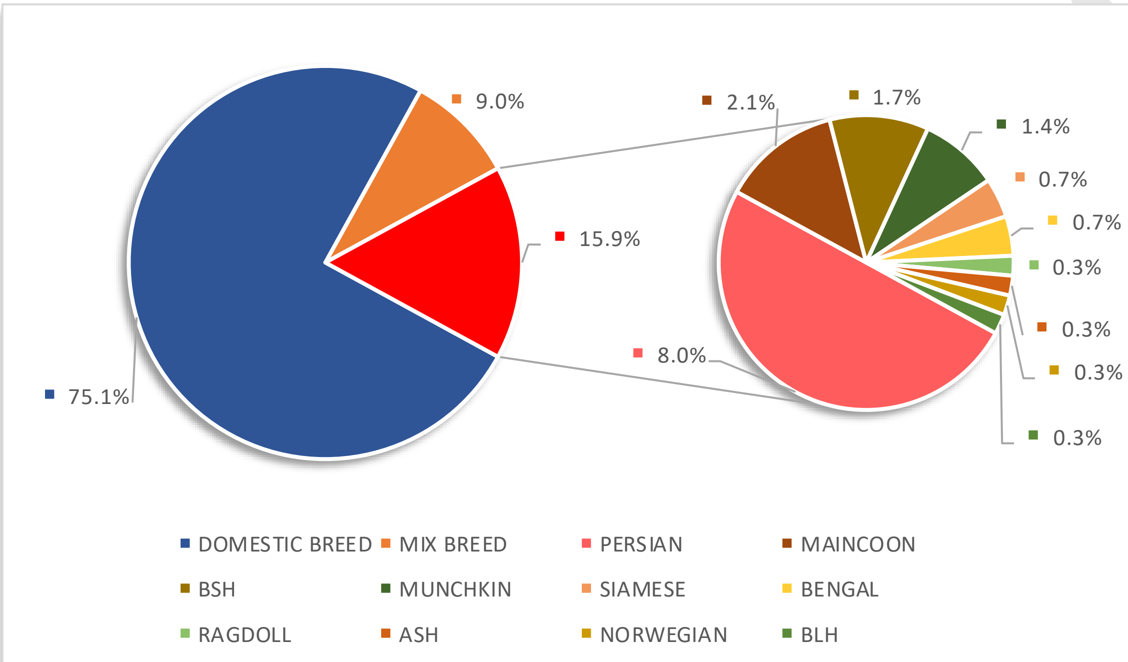
4.2.1 Sex, Age, Management and Breed

The distribution of the cats and the risk factor analysis for bacteriuria as shown in Table 3.

Table 3: The distribution of signalments and lifestyle management of cats (n=289) with evidence of bacteriuria and non-bacteriuria.

	Bacteriuria, n=194, n (%)	**Non-bacteriuria n=95, n (%)**	**Total n=289, n (%)**	**p-value**
Gender				
Male	174 (89.7%)	71 (74.7%)	245 (84.8%)	
Female	20 (10.3%)	24 (25.3%)	44 (15.2%)	**0.002**
Age				
Kitten	11 (5.7%)	4 (4.2%)	15 (5.2%)	
Adult	169 (87.1%)	81 (85.3%)	250 (86.5%)	
Senior	14 (7.2%)	10(10.5%)	24 (8.3%)	**0.569**
Breed				
Domestic breed	148 (76.3%)	69 (72.6%)	217 (75.1%)	
Pure Breed	28 (14.4%)	18 (19.0%)	46(15.9%)	
Mixed Breed	18 (9.3%)	8 (8.4%)	26 (9.0%)	**0.619**
Management				
Indoor housing	96 (49.5%)	45 (47.4%)	141 (48.8%)	
Outdoor housing	10 (5.1%)	5 (5.2%)	15 (5.2%)	
Semi roaming	88 (45.4%)	45 (47.4%)	133 (46.0%)	**0.116**
Household				
Multicat	92 (47.4%)	54 (56.8%)	146 (50.5%)	
Single-cat	30 (15.5%)	11 (11.6%)	41 (14.2%)	
Unknown	72 (37.1%)	30 (31.6%)	102 (35.3%)	**0.307**

Figure 1: Breed categories of cats with suspected UTI.



In this study, there were 245 (84.8%) male and 44 (15.2%) female cats. Of the 194 cat patients with bacteriuria, 89.7% (n=174) were males and 10.3% (n=20) were females (Refer Table 3). The neutering status of the cats was not reported, as there were cats with unknown status. The risk factor analysis showed a significant association between gender and bacteriuria results, $\chi2$ (1, n= 289) =9.921, p = 0.002, *phi* = 0.196. The male cat was at a 2.9 times greater risk of having bacteriuria.

The majority of the cats reported with bacteriuria were adults (n=250; 86.5%), followed by seniors (n= 24; 8.3%) and kittens (n= 15; 5.2%). Of the total of 194 cases with bacteriuria, 11 (5.7%) cases were from the kitten category, 169 (87.1%) cases were from the adult category, and 14 (7.2%) cases were from the senior category (Refer Table 3). There was no significant association between age groups and bacteriuria results ($\chi2$ (2, n= 289) = 1.128, p = 0.569, phi = 0.062).

The domesticated cats were presented with the highest (n=217; 75.1%) incidence of bacteriuria, followed by purebred cats (n=46; 15.9%) and mixed-bred (n= 26; 9.0%) cats (Refer Table 3). The pedigree population was represented by Persian (n= 23; 8.0%), Maine Coon (n= 6; 2.1%), British Shorthair (n=5; 1.7%), Munchkin (n= 4; 1.4%), Siamese (n= 2; 0.7%), Bengal (n= 2; 0.7%) and only one cat for each breed as follows; Ragdoll, American Shorthair, Norwegian and British Longhair (Refer Figure 1). However, there was no significant association between breed and bacteriuria ($\chi2$ (1, n=289) =0.960, p = 0.619, *phi* = 0.058).

A total of 141 (48.8%) cats were managed in indoor housing, followed by 133 (46.0%) managed as semi-roaming and 15 (5.2%) cats as outdoor. Approximately 49.5% (n= 96) of the indoor-kept cats had bacteriuria, followed by semi-roamer (n= 88; 45.4%) and outdoor (n=10, 5.2%) cats (Refer Table 3). There was no significant association between management and bacteriuria ($\chi2$ (2, n= 289) = 0.116, p = 0.944, *phi* = 0.020).

Besides, of the total of 289 cats, 146 (50.5%) cats were from multicat households, 41 (14.2%) cats were single-cat, and the remaining 102 (35.3%) cats had unknown household information (Refer Table 3). Cats with unknown household management were excluded from the analysis, resulting in risk factor analysis from 187 cats. Cats from a multicat household had the highest percentage of bacteriuria (n=92; 47.4%) compared to cats in a single-cat household (n=30; 15.5%). There was no significant association between the number of cats in a household and bacteriuria ($\chi2$ (1, n= 187) =1.456, p = 0.307, *phi* = -0.88).

4.3 LABORATORY FINDINGS OF FELINE UTI IN UVH-UPM (2017 to 2022)

4.3.1 Urinalysis

Bacterial culture results were compared between cats with and without bacteriuria, as shown in Table 4.

Table 4: Comparison of bacteria culture results of the study population (n= 289) with the evidence of bacteriuria and non-bacteriuria.

	Bacteriuria n= 194, n (%)	**No bacteriuria n=95, n (%)**	**Total n= 289, n (%)**	**P-value**
Culture positive, n	111 (57.2%)	29 (30.5%)	140 (48.4%)	**<0.001**
Culture negative, n	83 (42.8%)	66 (69.5%)	149 (51.6%)	

About 67.1% (n= 194) of the urine samples were found with microscopical evidence of bacteria. Of the 194 urine samples, 111 (57.2%) had positive microbial growth and 83 (42.8%) had negative culture. There were 95 urine samples found to be non-

bacteriuria (32.9%). However, 30.5% (n=29) of the urine samples non-bacteriuria showed positive culture results (Refer Table 4). There was a significant association between gender and bacteriuria results, $\chi 2$ (1, n= 289) = 17.136, p = <0.001, *phi* = 0.251. Cats with bacteriuria are at 3.0 times more significant risk of having culture-positive results.

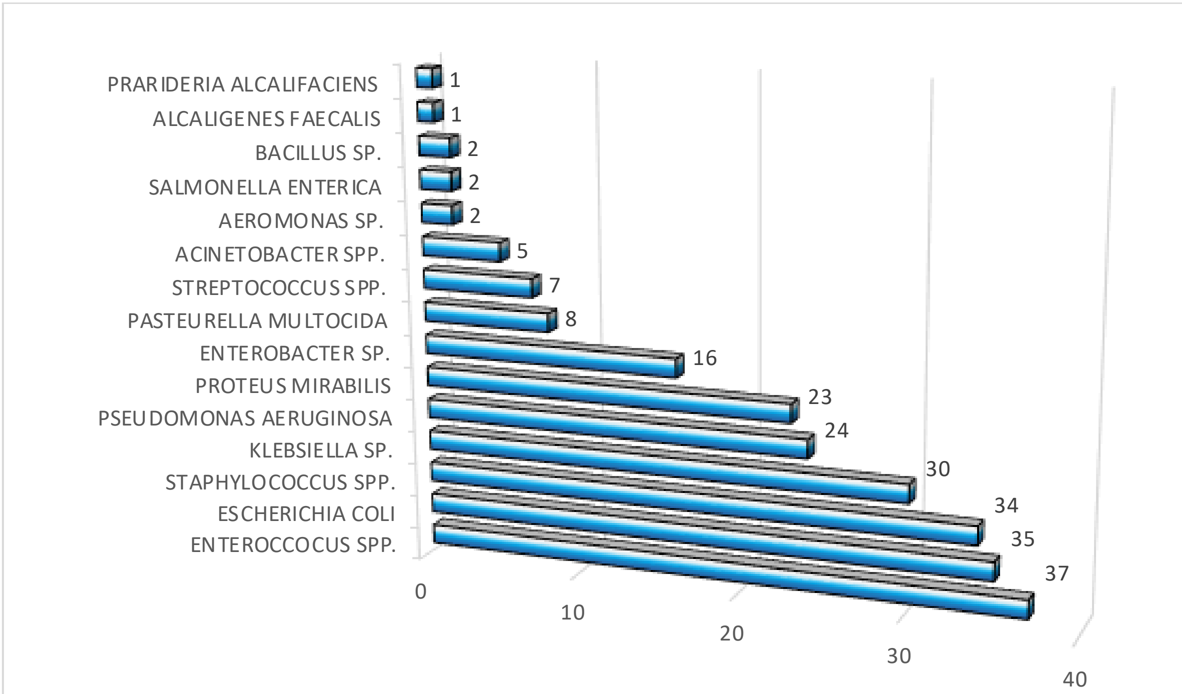
4.3.2 Urine Culture and Bacteria Isolate

Only a total of 161 urine sample reports were available retrievable. A total of 107 out of 161 urine specimens had pure culture (66.5%); about 33.5% (n= 54) were mixed growth of bacteria, which included 42 (26.1%) cultures with two bacterial isolates and 12 (7.5%) cultures with more than two bacterial isolates.

Table 5: Comparison of number of bacterial isolates from 161 urine specimens via different methods of urine specimens collection.

Method of Collection	**No of urine specimens containing one or more bacterial isolates, n**		
	1	**2**	**>2**
Cystocentesis, n= 81, n (%)	54 (66.7%)	20 (24.7%)	7 (8.6%)
Catheterization, n=80, n (%)	53 (32.9%)	22 (27.5%)	5 (6.3%)
Total (n =161)	107 (66.5%)	42 (26.1%)	12 (7.5%)

Figure 2: Type and Number of bacterial species isolated from culture-positive specimens in cats.



The most common isolated bacteria from culture-positive urine samples were *Enterococcus* species (n=37, 16.3%), which included *Enterococcus faecalis* (n=32; 14.1%); *Enterococcus faecium* (n=3; 1.3%); *Enterococcus* sp. (n=2; 0.9%). These were followed by *Escherichia coli* (n=35, 15.4%) and *Staphylococcus* species (n=34; 15.0%). (*Staphylococcus pseudintermedius,* n=21, 9.3%; *Staphylococcus intermedius,* n=12; 5.3%; Staphylococcus *schleiferi,* n=1; 0.4%) About 30 bacterial isolates were identified as *Klebsiella pneumoniae* (13.2%), and *Pseudomonas aeruginosa* and *Proteus mirabilis* had accumulated about 24 (10.6%) and 23 (10.1%) among positive bacterial growth respectively. 16 bacterial isolates were accounted for *Enterobacter* species (7.1%). Lastly, the remaining 28 bacteria isolates was identified as *Pasteurella multocida* (n=8; 3.5%), *Streptococcus* species (n= 7; 3.1%), *Acinetobacter* species (n= 5; 2.2%), *Aeromonas* species (n=2; 0.9%), *Salmonella enteritica* (n=2; 0.9%), *Alcaligenes faecalis* (n=1; 0.4%) and *Prarideria alcalifaciens* (n= 1; 0.4%).

4.3.3 AST

Table 6: Disc diffusion antimicrobial susceptibility results (% of susceptible) for 227 Bacterial isolates obtained from 161 culture-positive urine specimens.

Species (*n*)	Antimicrobial(b)																												
	Fluoroquinolone			Penicillin						Macrolide		Cephalosporin				Tetracycline		Aminoglycoside			Carbapenem		Others						
	ENR	CIP	NOR	MAR	AMX	AMC	PEN	AMP	AMP/SUL	ERY	AZI	CEP	CXM	CEX	CEF	TET	DOX	AMI	GEN	NEO	IMI	MEM	NIT	VAN	FOS/TRO	LIN?	MET	CLI	SUL/TRI
Gram- positive bacteria (80)																													
Enterococcus faecalis (32)	13.6		10		75							13.3		14.3			33.3	**100**	**100**		**100**	**100**		**100**		**100**			33.3
Staph. pseudintermedius (21)	66.7		42.9	**100**	50	**100**	**100**				50	58.3		29.5			75		75									**100**	**100**
Staph. intermedius (12)	40				50							50		33.3			50											50	
Strep. canis (4)	75		**100**		**100**							66.7	66.7	50			50	**100**	50		**100**		**100**						**100**
Enterococcus faecium (3)	33.3											33.3		50		100													
Strep dsygalactiae (2)	50		50		**100**							**100**	50	50			66.7	**100**	100		**100**								**100**
Bacillus sp. (2)	50											**100**																	
Enterococcus sp..(2)	50		**100**									50																	
Staph. schiferi(1)																													
Strep. viridian (1)																													
Gram-negative bacteria (147)																													
E. coli (35)	46.2		75		33.3	75	**100**	33.3	99.9		33.3	29.2	57.1	41.7			40	**100**	25	**100**	**100**	**100**	99.9	**100**	**100**	10			37.5
Klebsiella pneumoniae (30)	7.7		16.7								**100**	27.3		14.4		50	20	**100**	33.3		50		**100**						33.3

Pseudomonas aeruginosa (24)	27.4	50	45.5	**100**	33.3							12.5		6.7			22.2	**100**	**100**		**100**								50
Proteus mirabillis (23)	35.4	33.3	57.1		20	33.3		33.3				41.7	33.3	54.5	**100**			**100**	99.9	**100**			50						28.6
Enterobacter aerogenes (13)																	**100**												2
Pasteurella multocida (8)	100		100		33.3				**100**			75	100	66.7	**100**		**100**										**100**	**100**	**100**
Acinetobacter baumanii (4)	50																**100**												
Enterobacter cloacae (3)	71.4												50					**100**	**100**		**100**								**100**
Salmonella enteritica (2)			100								100																		
Aeromonas sp (2)																	**100**												
Acinetobacter iwofii (1)	**100**											**100**																	
Alcaligenes faecalis (1)	**100**											**100**																	
Providencia alcalifaciens (1)	**100**													**100**			**100**										**100**		

a: Isolates considered sensitive or intermediate according to CLSI (Clinical Laboratory Standards Institute) (2018) breakpoint.

b: ENR, enrofloxacin 5µg; CP ciprofloxacin 5µg; NOR, norfloxacin 10µg; MAR, marbofloxacin µg; AMX, amoxicillin µg; AMC, amoxycillin/clavulanic acid 20/10µg; PEN, penicillin 10 units; AMP, ampicillin 10µg; AMS, ampicillin/sulbactam 10/10µg; ERY, erythromycin 15µg; AZI, azithromycin 15 µg; CEP, cephalexin 30µg; CXM, cefuroxime 30 µg; CEX, ceftriaxone 30µg; CEF, cefovecin 30 µg; AMI, amikacin 30 µg; GEN, gentamicin 10 µg; IMI, imipenem 10 µg; MEM, meropenem 10 µg; NIT, nitrofurantoin 300 µg; FOS/TRO, Fosfomycin trometamol 200 µg; LZD, linezolid 30 µg; MET, metronidazole 50 µg; CLI, clindamycin 2 µg; TMS, trimethoprim- sulphonamide 25µg.

Table 7: Disc diffusion antimicrobial susceptibility result for 227 bacterial isolates obtained from 161 culture-positive specimens.

Species (*n*)	Susceptibility (a)	Antimicrobial(b)																												
		Fluoroquinolone			Penicillin						Macrolide			Cephalosporin			Tetracycline		Aminoglycoside			Carbapenem		Others						
		ENR	CIP	NOR	MAR	AMX	AMC	PEN	AMP	AMP/SUL	ERY	AZI	CEP	CXM	CEX	CEF	TET	DOX	AMI	GEN	NEO	IMI	MEM	NIT	VAN	FOS/TRO	LIN?	MET	CLI	SUL/TRI
Gram- positive bacteria (80)																														
Enterococcus faecalis (32)	R	20	2	9	1	1	3	1	2	2	1	1	13	6	18		4	8	0	2	1			2				8	7	4
	I	1		1		2									1			1	1			1								
	S	2				1							2		2			3		1			1		1		1			2
Staph. pseudintermedius (21)	R	6	1	4		3			1			2	5	3	12		1	2		1								10		
	I	2										1			3			4											1	
	S	10		3	1	3	1	1				1	7		2			2		3									1	2
Staphylococcus intermedius (12)	R	3		2		1							1	2	2			1										2	1	
	I					1																							1	
	S	2											1		1			1												
Strep. canis (4)	R	1	1				1		1	1			1	1	1			2		1								1		
	I	3		1																		1								
	S					1							2	2	1			2	1	1				1						2
Strep dsygalactiae (3)	R	1	1	1										1	2			1										2		
	I			1																		1								
	S	1				1							1	1	2			2	1	1										1
Enterococcus faecium (3)	R	2											2		1			2												
	I												1				1													
	S	1													1															
Enterococcus sp (2)	R	1											1		2													2		
	I																													
	S	1		1									1																	
Bacillus sp.(2)	R	1													2													1		
	I																													
	S	1											1																	
Strep. viridian (1)	R	1											1		1			1												
	I																													
	S																													
Staph. schiferi (1)	R	1		1											1													1		
	I																													
	S																													

Gram-negative bacteria (147)																														
E. coli (35)	R	14	3	1	2	4	1		2			2	17	3	14	1	3	9		3								8	4	5
	I	4		1		2	1			2			4	2	1			3	1	1	1			1						1
	S	8		2			2	1	1	1		1	3	2	9			3				1	1	2	1	3	2			2
Klebsiella pneumoniae (30)	R	12	2	5		3	2		2	2			8	3	12		1	4		2								5	3	2
	I			1								1	3		2							1								
	S	1															1	1	2	1		1		2						1
Pseudomonas aeruginosa (24)	R	10	2	6		4	1		1	1		2	14	3	14			7						1				7	2	1
	I	4																2												
	S	5	2	5	2	2							2		1				2	2		1								1
Proteus mirabillis (23)	R	7	2	3		4	2	1	2	1	1		7	2	5		3	4						1				2	3	5
	I	1		1									2		3	1				1	1									1
	S	3	1	3		1	1		1				3	1	3				1	2				1						1
Enterobacter aerogenes (13)	R	3		1									2		2															2
	I																	1												
	S																													
Pasteurella multocida (8)	R					2							1		2													5	1	
	I	1																												1
	S	4		1		1				1			3	1	4	1		1												
Acinetobacter baumanii (4)	R	1		1		1							1		2													2		
	I																	1												
	S	1																												
Aeromonas sp (2)	R	1											1		1															
	I																	1												
	S																													
Enterobacter cloacae (3)	R	2	1	1		1	1		1	1		1	3	1	1			1						1				1		
	I	1																				1								
	S													1					1	1										1
Salmonella enteritica (2)	R	1				1							1	1																
	I											1																		
	S			1																										
Alcaligenes faecalis (1)	R													1																
	I																													
	S	1											1																	
Providencia alcalifaciens (1)	R																													
	I	1																1										1		
	S														1															
Acinetobacter iwofii (1)	R														1													1		
	I																													
	S	1											1																	

a: Isolates considered R, resistant; I, intermediate susceptible; S, susceptible according to CLSI (2018) breakpoint.

b: ENR, enrofloxacin 5µg; CP ciprofloxacin 5µg; NOR, norfloxacin 10µg; MAR, marbofloxacin 5µg; AMX, amoxicillin 10µg; AMC, amoxycillin/clavulanic acid 20/10µg; PEN, penicillin 10 units; AMP, ampicillin 10µg; AMS, ampicillin/sulbactam 10/10µg; ERY, erythromycin 15µg; AZI, azithromycin 15 µg; CEP, cephalexin 30µg; CXM, cefuroxime 30 µg; CEX, ceftriaxone 30µg; CEF, cefovecin 30 µg; AMI, amikacin 30 µg; GEN, gentamicin 10 µg; IMI, imipenem 10 µg; MEM, meropenem 10 µg; NIT, nitrofurantoin 300 µg; FOS/TRO, Fosfomycin trometamol 200 µg; LZD, linezolid 30 µg; MET, metronidazole 50 µg; CLI, clindamycin 2 µg; TMS, trimethoprim- sulfa 25µg.

As shown in Figure 3, the top five bacterial isolates from a total of 161 culture-positive urine specimens were *E. coli* (n= 35; 15.4%); closely followed by *Enterococcus faecalis* (n=32; 14.1%); *Klebsiella pneumoniae* (n=30; 13.2%), *Pseudomonas aeruginosa* (n= 24; 10.6%) and *Proteus mirabilis* (n= 23; 10.1%).

The most common bacteria isolated was *Escherichia coli* (n=35), with the detection of remarkable resistance to cephalosporins selected and significant susceptibility to carbapenem. 100% susceptibility was shown to penicillin, amikacin, neomycin, imipenem, meropenem, vancomycin, fosfomycin, and nitrofurantoin, followed by amoxicillin/clavulanic acid (75.0%) and enrofloxacin (46.2%), trimethoprim-sulfa (37.5%) and cephalexin (29.2%),

Enterococcus faecalis (n=32) was detected with notable resistance towards ceftriaxone (14.3%), enrofloxacin (13.6%), cephalexin (13.3%) and norfloxacin (10.0%). However, 75.0% of the isolates were susceptible to amoxicillin and 33.3% to trimethoprim-sulfa and doxycycline. 100% Susceptibility was found towards amikacin, gentamicin, imipenem, meropenem, vancomycin and lincomycin.

Klebsiella pneumoniae (n=30) were not resistant to azithromycin, amikacin, and nitrofurantoin, with 100% susceptibility detected but showed 50.0% susceptibility towards imipenem and tetracycline. However, low susceptibility was found towards enrofloxacin (7.7%), ceftriaxone (14.4%), norfloxacin (16.7%), doxycycline (20.0%), cephalexin (27.3%), gentamicin (33.3%) and trimethoprim-sulfa (33.3%).

Pseudomonas aeruginosa (n=24) isolated in this study showed low susceptibility towards selected antibiotics. 50% of the isolate was susceptible to ciprofloxacin and trimethoprim-sulfa, followed by norfloxacin (45.5%), amoxicillin (33.3%), enrofloxacin (27.4%), doxycycline (22.2%), cephalexin (12.5%) and ceftriaxone (6.7%). 100% susceptibility was found towards marbofloxacin, amikacin, gentamicin and imipenem.

Proteus mirabilis (n=23) were 100% susceptible to neomycin, ceftriaxone, amikacin and gentamicin. Moderate susceptibility was found towards norfloxacin (57.1%), closely followed by cefovecin (54.5%), nitrofurantoin (50.0%), cephalexin (41.7%) and enrofloxacin (35.4%). One-third of isolates were found susceptible to ciprofloxacin, amoxicillin/clavulanic acid, ampicillin, and cefuroxime. The species were found with low susceptibility towards amoxicillin (20.0%), and trimethoprim-sulfa (28.6%)

CHAPTER 5.0

DISCUSSION

Clinical signs such as haematuria, stranguria, pollakiuria, and pyuria, were often described in cats with FLUTD. In our study, about 50.0% of feline patients with UTI were presented with signs of haematuria and stranguria, followed by pollakiuria (25.8%) and anuria (22.2%). Similarly, multiple studies showed relevant findings, indicating LUTS, including stranguria, haematuria and pollakiuria, as the most frequently presented clinical manifestations (Gerber *et al.,* 2005; Dorsch *et al.*, 2014; Lew-Kojrys *et al.*, 2017). Kovarikova *et al.* (2020) reported that haematuria was the most common clinical symptom in feline UTIs. However, there was a lack of association between bacteria culture results and the presence of LUTS in a study conducted by Djoneva *et al.* (2023). Like human medicine, asymptomatic bacteriuria was common and antimicrobial treatments were unnecessary except for immunocompromised individuals and pregnant women (Bono *et al.,* 2022). Thus, the presence of LUTS highly indicated feline UTI, but it was not the gold standard for determining the definitive diagnosis of FLUTD. Bacterial culture plays a vital role in diagnosing UTI, and it will provide the causative bacteria for each case.

In this study, 67.1% (n=194/289) of the study population were diagnosed with feline UTI. This finding was compatible with a study conducted in Germany, stating that male cats had a significant overrepresentation compared to female cats in diagnosing UTI (Aurich *et al.,* 2022). Similarly, in Indonesia, Nururrozi *et al.* (2020) reported a higher prevalence in male cats and UTI (25.0%) was commonly diagnosed. Furthermore, the male cats (n= 174) had a significantly higher risk (OR= 2.942) compared to females (n= 20). In contrast, Lekcharoensuk *et al.* (2001) concluded that old cats and spayed females had an elevated risk for UTI, and Sævik *et al.* (2011) described that there were more female cats diagnosed with UTI than other FLUTD due to the shorter and broader urethral opening. The distance between the anal opening and urethral opening is closer compared to male cats, further increasing the risk of ascending bacterial infection.

Furthermore, uncomplicated UTIs were found frequently in the human female population, which often resolved spontaneously (Bono *et al.,* 2022). Besides, Martinez-Ruzafa *et al.* (2012) stated that sex or breed differences were not the risk factor causing cats to be predisposed to UTI. Nonetheless, the current results showed that the notable prevalence of male cases with UTI is suggestive of the overrepresentation of male felines in the research area from 2017 to 2022. Male cats were more prone to urethral obstruction due to their longer and progressively constricting urinary tract.

In the study conducted by Dorsch *et al.* (2019), UTIs were found to have a significantly higher incidence in geriatric cats (10 years or older). Moreover, subclinical bacteriuria was presented predominantly in older cats (White *et al.,* 2016; Puchot *et al.,* 2017). However, in this current study, adults were a remarkably high percentage (87.1%) of adult cats (ranging from 1 year to 10 years) diagnosed with UTI, compared to kittens

(5.7%) and senior cats (7.2%). Similar findings were observed in studies conducted in the United States and Spain, stating that the median age of cats with UTI was 8 and 9 years old, respectively (Martinez-Ruzafa *et al.*, 2012; Hernando *et al.*, 2021). Moreover, UTIs were found to be more common in cats and dogs between 6 and 10 years old (Garcês *et al.,* 2002). It had been believed that concurrent morbidities, including hyperthyroidism, diabetes mellitus or chronic kidney disease (CKD), were often seen in geriatric cats, which elevated the susceptibility to UTI (White *et al.*, 2016). In human medicine, UTIs have ranked second most prevalent disease in older adults in Malaysia due to compromised immune response and sedentary lifestyle (Akhtar *et al.,* 2021). Multiple factors influence the likelihood of UTI occurrence in geriatrics, underscoring the need for prompt medical attention and preventive measures to mitigate UTIs-related complications in old age.

Our study showed that around three-fourths of the study population were domestic-bred cats (n=216). Among feline patients diagnosed with UTI (n=194), domestic-bred cats had accumulated the highest percentage (75.8%) compared to purebred (n=29, 15.0%) and mixed-bred cats (n=18, 9.3%). Domestic cats were overly represented in this study population as they were the common breed favoured by cat owners in Malaysia. This result was similar to the findings of Nururrozi *et al.* (2020), stating that domestic bred cats represented 58.0% of the study population diagnosed with feline UTI. In Sævik *et al.* (2011) study, domestic cats represented 71.4% of patients with bacterial cystitis, but purebred cats showed a significantly higher risk of contracting bacterial cystitis than urethral plugs. Among the purebred cats' population, the Persian breed was found as the predisposing breed to FLUTD, including urocystolithiasis and UTI (Lekcharoensuk *et al*., 2001; Kaul *et al.,* 2020). It was speculated that the Persian breed is a long hair breed with decreased grooming behaviour in cases of systemic illness, allowing bacteria colonisation around anogenital area predisposed to bacterial UTI.

Management was shown to play a role as a risk factor in feline UTIs. Among 194 cats with feline UTI found in this study, 96 (49.5%) were managed by indoor housing, followed by 88 (45.4%) as semi-roamer. According to Kovariova *et al.* (2020), indoor-managed cats showed a higher percentage (43.5%) of feline UTIs compared to outdoor-managed cats (13.0%). Similar findings were found in a study conducted in Thailand (indoor housing, 61.5%; outdoor housing, 25.6%; and semi-roamer, 12.8%) (Piyarungsri *et al.,* 2020). However, some owners preferred to provide outdoor access to their cats primarily to promote the mental well-being of their cats (Finka *et al.*, 2021), which may expose them to various hazards, including infectious diseases, predators, extreme weather fluctuations, ultimately elevate the occurrence of UTI development. According to Nururrozi *et al.* (2020), the occurrence of UTIs was elevated due to access to contaminated water sources such as flower bowls and drains with bacterial infestations and increased interaction with other stray cats. In this study, the overrepresentation of indoor cats with feline UTI can be explained by the fact that with closer and easier monitoring of cats indoors, their owners were able to bring their cats to the veterinarian for immediate, spontaneous action when noticing any subtle change in behaviours or LUTS. Moreover, indoor cats relatively have rather low activity compared to outdoor cats, and they were highly dependent on the number of litter boxes due to restricted access to the outdoor environment (Gunn-Moore, 2003).

In this study, cats that lived in multicat households were found to be a greater percentage among the study population (n= 92, 47.4%), affecting the occurrence of UTIs from 2017 to 2022. This finding was consistent with the previous studies, which stated that indoor management and multicat households were crucial risk factors associated with FLUTD (Jones *et al*., 1997; Buffington *et al.,* 2006). According to Piyarungsri *et al.* (2020), litter box management is an essential factor that affect urination behaviour, especially in a multicat household. The number of litter boxes with restriction indoors was strongly associated with FLUTD, affecting the cat's urinary tract health. Moreover, Lund and Eggertsdottir (2018) suggested that stress can alter cat's drinking and eating behaviour, potentially affecting water intake and increasing the risk of FLUTD. It had been proposed that stress may be one of the risk factors for cats living in multicat households, contributing to the likelihood of UTI occurrence.

Van Duijkeren *et al*. (2004) concluded that cystocentesis was the best method for collecting urine specimens to diagnose UTI compared to spontaneous micturition or catheterisation. This mirrored the importance of maintaining sterility to avoid iatrogenic contamination of urine samples to improve the accuracy of results. In the current study, only urine samples obtained via cystocentesis, and catheterisation were included for further diagnosis based on findings of urinalysis and bacteria culture. About 194 (67.1%) cats showed bacteriuria, while almost one-half (n= 140; 48.4%) of the samples showed culture-positive results. According to Djoneva *et al.* (2023), bacteria culture results were significantly associated with the microscopical presence of bacteriuria and leukocytes. In contrast, in human medicine, microscopic detection of bacteriuria was found sensitive when ⩾10^5 cfu/mL bacteria count was identified (Wilson *et al.,* 2004) compared to ⩾1000 CFU/mL in feline and canine urine (Osborne and Stevens, 1999; Sørensen *et al.,* 2016). In this current study, there is a strong association between bacterial culture and bacteriuria results in urinalysis, indicating the detection of bacteria in the urine was strongly related to the UTI occurrence in feline cases.

Bacterial culture is not typically warranted as a necessary component of the assessment for outpatients with uncomplicated symptomatic UTIs for antimicrobial prescription, but it is of great significance for cases with recurrent UTI or impatient UTI cases in veterinary and human medicine (Wilson *et al.,* 2004; Olin *et al.,* 2015). Of 161 urine specimens with positive culture, 107 (66.5%) showed pure culture, while the remaining 54 (33.5%) were mixed. According to Dorsch *et al.* (2019), uncomplicated UTIs are typically caused by a single pathogen, while multiple pathogens can be isolated from complicated UTIs or cats with a urinary catheter intact.

Of the 227 bacterial isolates in this study, 80 were gram-positive bacteria, and 127 were gram-negative. Bacterial isolates were identified in this current study, and *E. coli* (n=34; 15.0%) was the most isolated bacteria reported, followed by *Enterococcus faecalis* (n=32; 14.1%), *Klebsiella pneumoniae* (n=30; 13.2%), *Pseudomonas aeruginosa* (n= 24; 10.6%) and *Proteus mirabillis* (n=23; 10.1%). These findings were consistent in multiple studies conducted, suggesting that these bacterial isolates were the common aetiology of feline UTI while *E. coli* had the greatest percentages (Šeol *et al.,* 2011; Martinez-Ruzafa *et al.,* 2012; Teichmann-Knorrn *et al.,* 2018; Smoglica *et al.*, 2022). *E.* coli had been the most frequently isolated bacteria from urine samples in cats, dogs and humans. (Bailiff *et al.,* 2006). This echoed the finding stating *E. coli* was the most common causative agent of UTI, while the same strain was found in the faeces (Johnson *et al.,* 2008). These bacterial isolates were identified as gastrointestinal flora except *Pseudomonas aeruginosa,* which is commonly found in the environment. Ascending bacterial infection further causes bacterial colonisation in the urinary tract of patients with poor immune mechanisms.

The emergence of multidrug antimicrobial resistance in the veterinary field, especially production animals and companion animals, was worrying. Attention was drawn towards the risk of cross-species transfer of AMR genes due to the close relationship between humans and animals in food production and companion animals. As UTI is one of the frequent diagnoses in companion animal practice, empirical antimicrobial uses were commonly indicated for bacterial UTIs in pets with clinical signs (Smoglica *et al.*, 2022). This current study focused on top 5 bacteria isolates (*E. coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis)* and the antimicrobial resistance profile. These isolates were highly resistant to multiple antimicrobials, especially towards the first-line antibiotics (amoxicillin, amoxicillin/clavulanic acid, and TMS). In the United Kingdom, *E. coli, Proteus* species, and *Pseudomonas* species have shown an increasing trend of acquiring multidrug resistance, which *E. coli is* significantly resistant towards amoxicillin-clavulanic acid and streptomycin (Normand *et al.,* 2000). Moreover, *Enterococcus* species resisted broad-spectrum antibiotics, including beta-lactamase and aminoglycoside (Shepard *et al*., 2002). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were included as the top five isolates, also known as the ESKAPE organism, which was majorly clinically relevant bacteria for AMR traits (Roncada & Tilocca, 2022).

Based on the antimicrobial susceptibility profile analysis in this study, the first-line antibiotics were mainly shown with poor susceptibility rate (<50%), indicating an increasing trend of antimicrobial resistance of common uropathogen towards the suggested empirical treatment. From the AST analysis, there was also a high resistance rate (>50%) found towards first (cephalexin) and second-generation cephalosporins group (cefuroxime), which are frequently used as second-line antibiotics of UTIs. Amikacin, an alternative antibiotic from the aminoglycoside group being tested, had shown a 100% susceptibility rate towards the top five bacterial isolates. Still, consideration must be given to feline patients with concurrent kidney impairment due to its nephrotoxicity and ototoxicity properties. Besides, nitrofurantoin and carbapenem were found as suitable alternative antibiotics with a greater susceptibility rate (approximately 100%). Nitrofurantoin, fluoroquinolones and third-generation cephalosporin were used for cases resistant to the first-line antibiotics (Weese *et al.,* 2019). According to Steen (2011), it is recommended to avoid the usage of antibiotics with a 10 to 20% resistance rate towards common uropathogens as the empirical treatment. A high resistance rate can significantly elevate the risk of unfavourable patient outcomes. This present study provided information on the susceptibility pattern of selected antibiotics, focusing on common antibiotics used to treat UTIs, suggesting that AST is advisable and recommended before giving any antimicrobial treatment to avoid antibiotic misuse with no clinical improvement due to high resistance rate, which, in turn, encouraging the development of AMR traits in the prevalent uropathogen.

In Malaysia, there was an improving knowledge of antimicrobial uses and proper treatment in small animal practices among veterinary students and clinicians, but with poor awareness of the importance of antimicrobial stewardship despite having guidelines available (Chee and Chen, 2023). Clinicians in Malaysia tend to prioritise using broad-spectrum antibiotics amoxicillin-clavulanic acid in FLUTD treatment even without bacterial culture and AST results as the empirical treatment for symptomatic uncomplicated UTI. This is supported by International Society for Companion Animal Infectious Diseases (ISCAID) guidelines, which had suggested the usage of NSAIDs for the first 3 to 4 days before administering antimicrobial treatment when AST result was on pending (Weese *et al.,* 2017). Antimicrobial treatment can be given if the condition worsens or clinical signs persist. The optimal use of antimicrobial treatment as an empirical choice should depend on the AST pattern in the region. Nonetheless, there is a potential risk of transmission of AMR traits to humans due to the extensive use of broad-spectrum antibiotics due to the close contact with humans as companion animals share the typical everyday living environment with their caregivers.

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CHAPTER 6.0

CONCLUSION

6.1 CONCLUSION

In conclusion, LUTS was a crucial indication in patients suspected of feline UTI, in which haematuria ranked the first with the highest percentages among other signs. Compromised defence mechanism contributed to the occurrence of feline UTI when the patients had an underlying FLUTD problem such as urolithiasis, neoplasia or urethral plugs. Among all the risk factors, only the gender of cats showed a significant association with the occurrence of feline UTI. Male cats had a greater risk of than female cats, with a notable risk of 2.9. Besides, domestically bred cats were found to be predisposed to UTI development. Patients detected with bacteriuria werehighly associated with culture positive result. *E. coli, Enterococcus* species, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* were the most isolated bacteria in this study. Regarding AST, *E. coli* was found less susceptible to cephalosporin and TMS. Low resistance to fluoroquinolones and trimethoprim-sulfa were detected for the other four bacteria species. Amikacin was found to be as antibiotic with 100% susceptibility towards all bacteria isolated in cases investigated, but consideration must be given to feline patient with renal impairment. Nitrofurantoin and imipenem have shown a higher susceptibility rate and suitable for multi-drug resistant bacteria infection, which is suitable for alternative antibiotics. Optimal antimicrobial use as an empirical choice is essential to control and monitor Malaysia's antimicrobial resistance rate trend. Public awareness is an essential part of a multi-pronged strategy in combating AMR development. It complements medical and scientific endeavours to develop new treatments and monitor the changes in antimicrobial patterns. Prudent use of antibiotics in healthcare and agriculture is required to ensure current antibiotics remain effective against infection.

CHAPTER 7.0

LIMITATION AND RECOMMENDATIONS

The primary constraints of this study were the incomplete data available in medical records. Insufficient information caused several cases to be removed from the study population. Moreover, poor documentation as there are no digital records, causes missing health information required for this study and data collection time to be more consuming. For recommendation, similar study can be done in different geographical regions and demographic backgrounds to understand the general situation in Malaysia in terms of the AST profile of feline patient and the prevalence of feline UTI.

CHAPTER 8.0

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CHAPTER 9.0

APPENDICES

9.1 AAHA/AAFP Feline Life Stage Guidelines