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Identification of Marbofloxacin-Susceptible Bacterial Uropathogens in Feline Urinary Tract Infection of Different Age Groups

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Abstract

Urinary tract infections (UTI), an adhesion and multiplication of bacterial uropathogens in the urogenital system, are one of the most important indications for antimicrobial use in veterinary medicine and contribute to the development of antimicrobial resistance. Previously, it was revealed that older cats with an average of 9.1 years old are most likely to develop UTI, however a contradicting investigation stated kittens and young adults are more likely to contract UTI. Along with the uprising of amoxicillin- and ampicillin-resistant uropathogens, the study aims to turn towards marbofloxacin reserve and to analyze their potency. Furthermore, the current study aims to enumerate and compare the number of uropathogens between age groups. Enumeration and identification of uropathgoens from urine samples of feline UTI patients were was conducted through the urine culture on EMB, MSA, and BHI medium followed by an IMViC test and inoculation to the Mueller-Hinton agar to observe the zone of inhibition. The investigation revealed that a higher number of younger cats and kittens were reported to have UTI and catheter-induced UTI with a higher number of uropathogens in the population. The uropathogens were further identified as *Staphylococcus* spp., *Enterococcus* spp., *Enterobacter* spp., *Escherichia* spp., and *Klebsiella* spp. Additionally, marbofloxacin was found to be effective in eliminating and inhibiting the growth of these uropathogens. In conclusion, younger cats were observed to be more prone to UTI of a diverse range of uropathogens, however, marbofloxacin may aid in the treatment of the cats as it has been proven to be most effective.

Keywords: antimicrobial use; marbofloxacin; uropathogens; UTI.

Abbreviations: CAUTI: catheter-associated urinary tract infection, UTI: Urinary tract infections, FIC: Feline Idiopathic Cystitis, FLUTD: Feline lower urinary tract disease, DNA: deoxyribonucleic acid, EMB: mannitol salt agar, MSA: mannitol salt agar, BHI: brain heart infusion, TPC: total plate count.

INTRODUCTION

Urinary tract infections are one of the most important indications for antimicrobial use in veterinary medicine and contribute to the development of antimicrobial resistance. Dorsch *et al.* (2019) proposed that UTIs in cats occur less frequently than in dogs with only 1–2% of cats suffering from UTIs in their lifetime. However, according to a study conducted in Yogyakarta, approximately 25% of 185 cat patients had been diagnosed with UTI, alongside a count of 16.4% of 73 cat patients in the Sleman Regency, Yogyakarta. Overall, these ratings were found to have a higher prevalence compared to the population in Europe or the US (Astuty *et al.*, 2020; Nururrozi *et al.*, 2020). Dorsch *et al.* (2019) stated that UTI cases in Germany were more commonly found in adult cats than in younger cats, however, a study by Piyarungsri *et al.* (2020) conducted in Thailand found that UTIs in young cats were more likely to develop UTIs, especially in those with pre-existing complications, such as kidney disease. These findings may be a result of different demographical clinical practices and healthcare, which indicates that a study of the trend and cause of UTI cases in Yogyakarta must be studied and reviewed (Martinez-Ruzafa, 2016).

UTIs are dominantly caused by bacteria, including *Escherichia coli*, *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. (Dokuzeylül *et al.*, 2019). In both simple and complicated UTIs, *Escherichia coli* is the organism most frequently cultured in both simple and complicated UTIs, thus referred to as the main pathogen causing UTIs (Freitag *et al.*, 2006; Fonseca *et al.*, 2021). To treat feline UTIs, patients are often prescribed amoxicillin, ampicillin, and cephalothin, which are predominantly excreted in the urine in their active form, making them a good first-line option for UTIs. However, a significantly high number of *E. coli* strains were reported by Fonseca *et al.* (2021) to be resistant to amoxicillin (39.4%), ampicillin (19.2%), and cephalothin (58.8%). Meanwhile, several strains of uropathogens

were observed to be the least resistant to marbofloxacin with a percentage of 7.1%.

Marbofloxacin is a synthetic third-generation fluoroquinolone antibiotic reserved for documented resistant UTIs. Similar to ampicillin and amoxicillin, it is excreted in urine predominantly in active form as well (Weese *et al.*, 2011). Although it is currently not widely available, its effectiveness and low MPC values may support the elimination of UTI in felines without the risk of a rise in marbofloxacin-resistant uropathogens (Gebru *et al.*, 2011).

Unfortunately, Caneschi *et al.* (2023) stated that antibiotic overuse and misuse have contributed to the recent rise in antimicrobial resistance, and the use of antibiotics in animals has selected for and spread resistant microorganisms. According to Lei *et al.* (2017), uropathogens has acquired marbofloxacin-resistant genes as a result of past antibiotic abuse and cross-resistance that has been mitigated by prior fluoroquinolone medications. As a result, this study aimed to observe the UTI trends in Yogyakarta and analyze the difference in bacterial population in feline UTI of various age groups in terms of diversity and abundance, followed by further analysis on the susceptibility of the uropathogens towards marbofloxacin.

MATERIALS AND METHODS

Study area

The samples were collected from 4 veterinary clinics around Yogyakarta in which the cats had already undergone catheterization. Following the sampling, the urine samples were further analyzed in the Laboratory of Microbiology, Faculty of Biology, Universitas Gadjah Mada.

Procedures

Urine Sample Collection

Through collaborative work with local vets and clinics, eight urine samples were acquired from cats that had been diagnosed with UTI. The 6 cats originate from different life stages, namely seniors, prime, young adults, and kittens. The cats originated from different life stages, namely seniors, prime, young adults, and kittens (Quimby, 2021). The samples were then labeled in the following sequence: C(life stage code)(age number), where C stands for “cat”.

Physical and Chemical Analysis of Urine Samples

A urine dipstick was dipped into the urine samples to determine the chemical properties of the samples, including, the pH, protein, and glucose concentration. Additionally, the color of the urine was determined to distinguish whether it was diluted or concentrated (Graziani *et al*., 2008).

Enumeration of the Bacterial Uropathogens

Initially, the urine samples were subjected to a 10-fold serial dilution eight times by placing 1 mL of the urine sample into 9 mL of NaCl, followed by transferring 1000 µL of the diluted solution from the previous tube to the next tube. The following steps were repeated until the remaining 5 tubes had been filled (U.S. Food and Drug Administration, 2023).

Afterward, the 3rd, 4th, 5th, and 6th dilution tubes were each inoculated to two replicates of BHI, MSA, and EMB mediums through the pour plate method by inoculating 1000µL into the petri dishes followed by warm liquified BHI, MSA, and EMB agar solutions. Once it solidified, the samples were incubated for 24-48 hours at 37°C. The colonies were enumerated with the aid of a marker, followed by subculturing the pure colonies into nutrient-slant agar mediums.

Identification of the Bacterial Uropathgoens with IMViC

In terms of the indole test, 5mL of SIM stab agar medium was prepared. Pure colonies isolated from the EMB, MSA, and BHI agars were inoculated into the stab agar. The samples were left to incubate for 24-48 hours at 37°C. Afterward, 5 drops of Kovac reagent were added into the medium to detect the presence of a red ring and a diffusion zone of growth (MacWilliams, 2009).

Following the indole test, the methyl red and Voges-Proskauer (VP) tests were performed in which 5 mL of MRVP broth mediums were prepared. An ose of the pure colonies were inoculated into the MRVP broth then left to incubate for 48 hours at 37°C. Once the samples had been incubated, for the methyl red test, 2.5 mL of the culture was placed into a separate tube, followed by an addition of 6 drops of 0.02% methyl red indicator in order to observe a red coloration which indicated a positive result. In terms of the VP test, 2.5 mL of the remaining culture was placed into a separate tube, followed by addition of 6 drops of 5% a-naphthol and 2 drops of 40% KOH in order to observe a formation of a pink color indicating a positive result (McDevitt, 2009).

Lastly, the citrate test was performed by preparing 7 mL of slant Simmons Citrate agar. Afterward, the pure colonies were streaked onto the slant agar and left to incubate for 24-48 hours at 37°C. The formation of a blue coloration indicates a positive result, whereas the remaining green coloration of the medium indicates a negative result (MacWilliams, 2009).

Marbofloxacin Susceptibility Test

Using multi-disc antibiotics, an antimicrobial susceptibility test was conducted on Mueller-Hinton agar (MH). Initially, the isolates were vortexed with aquadest until it was homogenized. Suspension was standardized according to the 0.5 McFarland turbidity standard and inoculated onto the MH agar with the aid of a sterile cotton swab. A concentration of 20µL of marbofloxacin

was impregnated onto the discs and using a set of sterile forceps, the marbofloxacin-impregnated paper disc is placed in the center and gently pressed into the agar. The medium will be inverted and left to incubate for 24 hours at 37°C. Clear zones of inhibition were indicative of sensitivity, whereas no cleared zones were indicative of resistance (Dirisu *et al.*., 2016).

Data analysis

Once the data has been collected, an analysis of the physical and chemical properties of the urine samples was compiled and recorded, followed by a determination of whether the urine is diluted or concentrated and the severity of the UTI.

In terms of the antibiotic susceptibility test, the acquired results were qualitatively analyzed by measuring the size of the zone of inhibition. A zone of inhibition that was larger than ≥ 18 mm was categorized susceiptible; zones that were 15-17 mm in diameter were considered moderate; and no zone of inhibition or ≤ 14 mm was considered resistant (Schneider *et al*., 2004).

Table 1. Zone of Inhibition Interpretations (Fonseca *et al*., 2004).

Zone diameter (mm)	Categories
＞20	Susceptible
15 - 19	Intermediate
≤ 14	Resistant

RESULTS AND DISCUSSION

Urinalysis Results and Clinical Implications

A total of 3 urine samples were collected throughout the duration of the investigation, namely, CY2. CK1, and CP4 with a complete urinalysis and profiling of the patient (Table 2). Patient CK1 was categorized as a male kitten at the age of 1 year old and excreted yellow urine at a pH of 6.5. Patient CK1 had been treated for 5 days as well. Meanwhile, patient CY2 was categorized as a male young adult at the age of 2 years old who had been treated for 6 days. The patient excreted a pink urine color with a pH of 7.5 similar to the patient CP4 who was a prime male cat at the age of 4 years old, however patient CP4 excreted yellow urine and had only been treated for 4 days. Lastly, all 3 patients tested positive for leukocytes and nitrates, but negative for glucose in the urine.

Table 2. Urinarlysis of the Samples of Different Ages

Sample	Life Stage	Age	Gender	N-th Day of Treatment	Urine color	pH	Glucose	Leukocyte	Nitrate
CK1	Kitten	1 y.o	Male	5	Yellow	6.5	-	++	+
CY2	Young Adult	2 y.o.	Male	6	Pink	7.5	-	++	+
CP4	Prime	4 y.o.	Male	4	Yellow	7.5	-	+	+

Bacterial Uropathogen Population

According to Table 2, all three patients had a bacterial count of more than 1000 CFU/mL indicating a UTI case. A trend was observed where younger cats had a higher count of bacteria compared to the older cats. Patient CK1 had a total bacterial count of 247,462,500 CFU/mL, whereas CY2 and CP4 had a bacterial count of 28,080,000 CFU/mL and 100,000 CFU/mL, respectively. Following the bacterial count, the isolates were subjected to a series of tests from the IMVIC test in order to facilitate the identification of the isolates through their phenotypic characteristics. From sample CY2, 3 strains of *Staphylococcus* spp., 3 strains of *Klebsiella* spp., and 1 strain of *Enterobacter* spp. were identified. Meanwhile, patient CK1 was found to contain 3 strains of *Enterobacter* spp., 1 strain of *Klebsiella* spp., and 1 strain *Staphylococcus* spp. Lastly, patient CP4 was found to contain 2 strains of *Enterobacter* spp., 1 strain of *Klebsiella* spp., 1 strain of *Staphylococcus* spp., and 1 strain of *Escherichia coli* (Table 4).

Table 3. Number of Uropathogens Isolates (CFU/mL).

CY2		CK1		CP4	
Isolates	**CFU/mL**	**Isolates**	**CFU/mL**	**Isolates**	**CFU/mL**
CY2A	143,500	CK1A	12,550,000	CY4A	37,500
CY2B	9,650,000	CK1B	110,000,000	CY4B	57,500
CY2C	615,000	CK1C	5,400,000	CY4C	4,000*
CY2D	131,500	CK1D	119,500,000	CY4D	500*
CY2E	2,545,000	CK1E	12,500*	CY4E	500*
CY2F	345,000				
CY2G	14,650,000				
Total	28,080,000		247,462,500		100,000

(*) Although it was present, CFU was not within the applicable range of 30-300 CFU/mL

Bacterial Uropathogen Identification

A total of 17 isolates were collected from the three urine samples and were subjected to a series of tests from the IMVIC test in order to facilitate the identification of the isolates through their phenotypic characteristics. From sample CY2, 3 strains of *Staphylococcus* spp., 3 strains of *Klebsiella* spp., and 1 strain of *Enterobacter* spp. were identified. Meanwhile, patient CK1 was found to contain 3 strains of *Enterobacter* spp., 1 strain of *Klebsiella* spp., and 1 strain *Staphylococcus* spp. Lastly, patient CP4 were found to contain 2 strains of *Enterobacter* spp., 1 strain of *Klebsiella* spp., 1 strain of *Staphylococcus* spp., and 1 strain of *Escherichia* spp.

Table 4. Phenotypic and Biochemical Characteristics of Bacterial Uropathogens.

Isolates	Colony Morphology	Media	IMViC Test				
			Indole	MR	VP	Citrate	Identification
CY2A	White circular mucoid aerobic colonies	BHI	-	+	+	+	*Klebsiella* spp. *
CY2B	Yellow circular anaerobic colonies	BHI	-	+	+	+	*Staphylococcus* spp. *
CY2C	Translucent circular aerobic colonies with a darker middle	BHI	-	-	+	+	*Enterobacter* spp.
CY2D	Large light purple aerobic circular mucoid colonies	EMB	-	+	+	+	*Klebsiella* spp. *
CY2E	Small circular dark purple anaerobic colonies	EMB	-	+	+	+	*Klebsiella* spp. *
CY2F	White circular aerobic colonies with yellow hues	MSA	-	+	-	-	*Staphylococcus* spp. *
CY2G	Yellow oval anaerobic colonies	MSA	-	+	+	+	*Staphylococcus* spp. *
CK1A	Large white circular mucoid aerobic colonies	BHI	-	-	+	+	*Enterobacter* spp. *
CK1B	Small yellow oval anaerobic colonies	BHI	-	-	+	+	*Enterobacter* spp.
CK1C	Large light purple circular aerobic colonies	EMB	-	-	+	+	*Enterobacter* spp.
CK1D	Small dark purple circular anaerobic colonies	EMB	-	+	+	+	*Klebsiella* spp. *
CK1E	Yellow aerobic colonies with yellow hues	MSA	-	+	+	+	*Staphylococcus* spp. *
CP4A	Large white circular mucoid aerobic colonies	BHI	-	-	+	-	*Enterococcus* spp.
CP4B	Small white ocal anaerobic colonies	BHI	-	-	+	+	*Enterobacter* spp.
CP4C	Small dark purple circular anaerobic colonies	EMB	+	-	-	+	*Klebsiella* spp. *
CP4D	Green circular aerobic colonies	EMB	+	+	-	-	*Escherichia coli*
CP4E	Yellow circular aerobic colonies	MSA	+	-	+	+	*Staphylococcus* spp. *

IMViC test, indole, methyl red, Voges–Proskauer, and citrate tests; EMB, eosin methylene blue;
BHI, brain heart infusion; MSA, mannitol salt agar; (-), negative; (+), positive; (*) signifies that the strains are from within the same genus but remains a different species

Marbofloxacin Susceptibility Test

The marbofloxacin susceptibility test was conducted on each of the isolates that were detected from the patients. According to the table below, *E. coli* (n=1), *Klebsiella* spp. (n=5), *Enterococcus* spp. (n=1), and *Staphylococcus* spp. (n=4) were all susceptible towards marbofloxacin at

a percentage of 100%. Meanwhile, 75% of the *Enterobacter* spp. strains (n=4) were found to be susceptible whereas the remaining 25% were found to be in the intermediate category.

Table 6. Marbofloxacin Susceptibility Test (%).

Identification	n	S (%)	I (%)	R (%)
Escherichia coli	1	100%	0%	0%
Enterobacter spp.	4	75%	25%	0%
Klebsiella spp.	5	100%	0%	0%
Enterococcus spp.	1	100%	0%	0%
Staphylococcus spp.	4	100%	0%	0%

S, Susceptibility; I, Intermediate; R, Resistant

Further analysis revealed that *Escherichia coli* (average of 31.1 mm), *Enterococcus* spp. (average of 29.1 mm), *Staphylococcus* spp. (average of 28.6 mm and 30.3 mm), and *Klebsiella* spp. (average of 25.8 mm, 30.1 mm, and 31.2 mm) were all found to be susceptible towards marbofloxacin according to the standards shown on Table 2. On the other hand, a strain of *Enterobacter* spp. was found to be an intermediate (average of 16.9 mm) whereas the remainder of the strains were found to be susceptible (average of 26.2 mm). In conclusion, marbofloxacin was effective against most of the uropathogens isolated from the patients, however the *Enterobacter* spp. isolates may require an adjustion in the dosage due to its prominent biofilm (Kahlmeter, 2017). These findings were consistent with Ferrans *et al.*, (2016) where marbofloxacin exhibited a greater activity in the elimination of biofilm compared to amoxicillin as they have the ability to kill non-dividing bacteria.

Discussion

Feline urinary tract infection is a condition, categorized under FLUTDs, where there is an accumulation and persistent uropathogen within the urogenital system thus inducing an associated inflammatory response and clinical symptoms. UTIs can result simply from the migration of uropathogens that had already resided in the gut into the periurethral area, this is known as uncomplicated UTI. Once the uropathogens have migrated to the urethra, it will continue migration into the bladder where they undergo multiplication and the formation of biofilm. These uropathogens continue to produce toxins and proteases causing the host cell to deteriorate releasing nutrients to the uropathogens. As the uropathogens continue to multiply, migration to the kidney continues and if left untreated it may reach the bloodstream causing bacteraemia. A similar pathway is found in the case of complicated UTI, but the source of the infection is due to a compromised bladder (Flores-Mireles *et al.*, 2015).

Nururrozi *et al.* (2020) reported that Yogyakarta a trend where older cats with an average age of 9.1 years old were mostly diagnosed with complicated UTI due to their association with CKD or compromised host defenses. However, a contradicting investigation by Piyarungsri *et al.* (2020) reported that a high number of younger cats have been diagnosed with UTI compared to elder cats due to the preexisting complications, such as kidney diseases. Both cases mentioned the presence of preexisting diseases associated with cats of all ages, therefore, a continuous update on the feline UTI trends in Yogyakarta must be conducted.

Urinalysis and Clinical Implications

Feline urinary tract infection is a condition, categorized under FLUTDs, where there is an accumulation and persistent uropathogen within the urogenital system thus induces an associated inflammatory response and clinical symptoms. UTIs can result simply from the migration of uropathogens that had already resided in the gut into the periurethral area, this is known as uncomplicated UTI. Once the uropathogens had migrated to the urethra, they will continue migration into the bladder where they undergo multiplication and the formation of biofilm. These uropathogens continue to produce toxins and proteases causing the host cell to deteriorate releasing nutrients to the uropathogens. As the uropathogens continue to multiply, migration to the kidney continues and if left untreated it may reach the bloodstream causing bacteraemia. A similar pathway is found in the case of complicated UTI, but the source of the infection is due to a compromised bladder (Flores-Mireles *et al.*, 2015).

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Throughout the course of the investigation, three patients have been confirmatively diagnosed with UTI due to the presence of more than 10-3 CFU/mL of bacteria in a growth media. The patients had an age of 1 year old, 2 years old, and 4 years old, which were categorized in the kitten, young adult, and prime life stages, respectively. Although the cats had no prior record of preexisting diseases, the anatomical structure of the male cats may be cause of the UTI. Compared to female cats, the male cat's urethra is narrower and more curved making them more susceptible to urethral blockage/plug. Urethral plugs are described as lodges in the urethra due to the accumulation of proteins, cells, and other debris from the bladder, preventing urination. As a result, urine is buildup in the bladder creating a favorable

environment for bacteria to grow leading to the case of UTI (Heyns, 2011).

The case of UTI is further supported by the presence of a higher pH than the normal range as well as the presence of nitrate and leukocytes detected in the patient's urine. The normal pH range of male cats is 5.73-7.39, however, patient CP4 and CY2 had a pH of 7.5 which was consistent with the number of bacteria present in their urine. The presence of ammonia is a result of urease produced by uropathogens such as, P. mirabilis, K. pneumoniae, and S. saprophyticus, which catalyses the hydrolysis of urea to CO2 and ammonia, thus elevating urine pH (Flores-Mireles *et al..*, 2015; Cottam *et al..*, 2002). Elevating the urine pH may also result in the production of calcium crystals and with the additional presence of ammonia the tissue is irritated and damaged causing blood to enter the urine, this condition is knows as crytalluria.

Cases such as haematuria was detected only in patient CY2 where a pink coloration was observed in the urine sample (Bono *et al..*, 2023). However, presence of blood in the urine was not a definitive diagnosis of UTI as blood may also be a result of FIC and crystalluria. UTIs are defined as an infection with more than 1000 CFU/mL of bacteria in the urine, whereas FIC is a spontaneous non-infectious bladder disease in cats (Nururrozi *et al..*, 2020). Similar to the inflammation due to a bacterial infection, inflammation in FIC may be triggered by histamine and cytokines causing tissue damage, as a result 70% of cats diagnosed with FIC had haematuria. Similarly, crytalluria may also haematuria due to the irritation of the bladder walls by the crystals (Ofakor *et al..*, 2018; Dorsch *et al..*, 2019)

Although leukocytes and nitrites are deemed unreliable in confirming UTI cases, they can still be considered as a supporting factor. A significant number of leukocytes were detected in the urine samples due to the response to bacteria in the urine. According to Hayes and Abraham (2016), a bacterial infection induces the release of leukocytes, such as neutrophils, into the bladder which in turn produces pro-inflammatory cytokines (Hedges and Svanborg, 1994). Meanwhile, nitrite is widely-used indicator in urine dipsticks to determine UTI as uropathogens reduce nitrate to nitrite in order to prevent the conversion of nitric oxide in the urine which limits their growth and metabolism (Domili *et al..*, 2020).

Bacterial Uropathogen Population

According to Table 4, the bacterial isolates that were found in all three patients were *Klebsiella* spp. and *Enterobacter* spp. However, a distinctively high number of *Staphylococcus* spp. and *Enterobacter* spp. were detected in patients CY2 and CK1, respectively. The distinction between the presence of bacterial species may be a result of catheter-associated urinary tract infection (CAUTI) as all three patients underwent catheterization as a form of treatment for FLUTS. Dorsch *et al.* (2019) mentioned that clinical UTIs are difficult to diagnose as the patients would already have been catheterized for prior symptoms of FLUTD, as a result, FLUTD patients that may not have UTI may come to develop CAUTI due to catheterization. Aside from catheterization, UTIs could have occurred without a compromised bladder rather than a pre existing disease. However, as there were no prior records of the patients having prior diseases or reinfection, it was speculated that the patients may have had a persistent infection from a catheter-induced UTI. In the case that This is supported by Dorsch *et al.* (2019), where a persistent infection takes 7-14 days of treatment consistent with the 5th and 6th-day treatment mark for patients CK1 and CY2, respectively.

Additionally, a research conducted by Bubenik *et al.* (2007) discovered that catheterization allows opportunistic *Enterobacter* spp. and *Staphylococcus* spp. to enter the patients. These findings were consistent to the data in which a high number of *Staphylococcus* spp. (87.77%) and *Enterobacter* spp. (2.19%) in CY2, as well as *Enterobacter* spp. (57.71%) in CK1 (Scarpellini *et al*, 2023; Dorsch *et al.*, 2016). In addition *Klebsiella pneumoniae* being an opportunistic bacteria could have compromised the catheters (Dorsch *et al.*, 2019). Meanwhile, patient CP4, may have had a UTI prior to the catheterization as common uropathogens associated with UTI, such as, *Enterococcus* spp., *E. coli*, *Staphylococcus* spp., and *Klebsiella* spp. were detected at a lower number on the 4th day of treatment (100,000 CFU/mL) which was less than the bacterial count of patient CK1 and CY2 (Table 3 and Table 5). Thus, it was speculated that the UTI was not induced by catheterization but rather simply by the migration of uropathogens to the bladder thus resulting a non-persistent infection or a reinfection (Dorsch *et al.*, 2019). A similar case was found in a study conducted by Thompson *et al.* (2011) where a case recurrent canine UTI resulted in similar uropathogen diversity consisting of *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Enterococcus* spp.

In order to further support the case of CAUTI in patients CY2 and CK1, the concept of biofilm may be applied. Biofilms are described as polymicrobial colonies that attach to surfaces and form an encasement of the extracellular polymeric matrix, allowing them to bypass host defenses and increase antimicrobial resistance through slow penetration, resistant phenotype, and altered microenvironment. Biofilms are one of the contributors of persistent infection and antibiotic resistance in the case of UTI and CAUTI. The uropathogens that contaminate the urinary catheters and form biofilms are *E. coli*, *Proteus* spp., *Enterococcus* spp., *Staphylococcus* spp., and *Klebsiella* spp. which were consistent to the findings of the investigation (Lila *et al.*, 2022). According to Sabir *et al.* (2017), the uropathogen that produces the highest amount of biofilm was *Enterobacter clocae* (87.5%) followed by *Klebsiella*

pneumoniae (87.1%) and *Enterococcus faecium* (79.2%). According to several studies, the abundance of *Enterobacter* spp. may be attributable to the formation of the most potent biofilm at the highest percentage compared to the remainder of the uropathogens as they have the ability to grow and survive in a nutrient-deficient environment such as urine. These findings may uropathogens found in patient CK1 where *Enterobacter* spp. was abundant in the uropathogen population (Table 4) (Liu *et al.* 2022).

Meanwhile, *Klebsiella* spp., being one of the most common uropathogen found in the patients and the second highest biofilm producing uropathogen, has the ability to produce a capsular polysaccharide thicker than the capsule of *E. coli*. These findings may support the abundance of *Klebsiella* spp. in all of the three patients as the biofilm provides adhesion to the uroepithelium tissues of the bladder and the catheter (Lila *et al.*, 2022). Following *Klebsiella* spp., an abundance on *Staphylococcus* spp. was detected in all three patients as well with a distinctively high number in patient CY2 (Table 4) that produces a biofilm that expresses clumping factors that had a high affinity for fibrinogen allowing it to attach to the fibrinogen-coated catheter and damaged tissues. Additionally, their biofilm allows for the production of toxins that promotes tissue damage, and from the findings collected from patient CY2 these characteristics supported the speculation that the UTI was catheter-induced and that the haematuria may had been resulted from the toxins damaging the tissue of patient CY2 (Lila *et al.*, 2022).

Lastly, the presence of a high number of *Entercoccus* spp. was detected in patient CP4 which is unique for their ability to suppress the innate and adaptive immune pathways attributing to the further infections by other uropathogens which may explain the presence of a diverse range of uropathogens in CP4 including, *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Staphylococcus* spp., compared to the other patients (Table 4).

Marbofloxacin Susceptibility Patterns

The isolates obtained from each patient were subjected to the antibiotic susceptibility test for marbofloxacin as ampicillin and amoxicillin had been reported to have been ineffective towards uropathogens that had developed resistance. The standardized measurements for susceptible strains had an inhibition zone diameter of ≥ 18 mm, intermediate strains had an inhibition zone diameter of 15–17 mm, and resistant strains had an inhibition zone inhib1ition diameter of ≤ 14 mm (Schneider *et al..*, 2004). According to Table 5, *E. coli* (average of 31.1 mm), *Enterococcus* spp. (average of 29.1 mm), *Staphylococcus* spp. (average of 30.5 mm), and *Klebsiella* spp. (average of 27.7 mm) were all found to be susceptible to marbofloxacin. On the other hand, a strain of *Enterobacter* spp. was found to be an intermediate (average of 16.9 mm) whereas the remainder of the strains were found to be susceptible (average of 26.2 mm). In conclusion, marbofloxacin was effective against most of the uropathogens isolated from the patients, however the *Enterobacter* spp. isolates may require an adjustment in the dosage (Kahlmeter, 2017). These findings were consistent with Ferrans *et al..*, (2016) where marbofloxacin exhibited a greater activity in the elimination of biofilm compared to amoxicillin as they have the ability to kill non-dividing bacteria.

CONCLUSIONS

From the following findings it was concluded that the population of uropathogens found from different age groups or life stages were diverse, however the factor of age did not seem to influence the diversity of uropathogens, rather environmental factors such as the insertion of the catheter. Therefore, it must highlight those cases of UTI must be distinguished between CAUTI as the species of uropathogens may differ. In addition, the uropathogens isolated from the patients were highly susceptible to marbofloxacin, thus making the antibiotic one of the most effective antibiotics in treating UTI.

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