UNIVERSITI PUTRA MALAYSIA

THE OCCURRENCE OF ANTIMICROBIAL RESISTANCE ISOLATED IN URINARY TRACT INFECTIONS (UTIs) IN CATS

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 - Projek.

RINTANGAN ANTIMIKROB DARI JANGKITAN SALURAN KENCING YANG DIASINGKAN DARIPADA KUCING

Oleh

Maria Amalia Binti Mokhlis

2023

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Kemunculan kerintangan antimikrob (AMR) adalah membimbangkan, terumatamanya kepada kesihatan manusia, memandangkan antimikrobial ini digunakan secara meluas dalam perubatan haiwan kesayangan. Interaksi rapat antara manusia dan haiwan peliharaan mereka boleh menjadi salah satu daripada sumber penjalaran organisma AMR yang berkemungkinan meningkatkan kadar keberkesanan rawatan antibiotik pada kedua-dua pihak. Di dalam amalan haiwan kesayangan, penyakit jangkitan saluran kencing (UTIs) merupakan penyakit yang biasa dirawat dengan antibiotik secara empirikal. Oleh kerana itu, tujuan kajian ini dijalankan adalah untuk mengenal pasti status kerentanan antimikrob dalam kucing yang dijangkiti UTIs. Dua puluh dua kes dari arkib sampel telah diambil dari Makmal Bakteriologi, Universiti Putra Malaysia. Sampel tersebut merangkumi penyakit UTIs dari kucing di antara tempoh 1 Januari 2021 hingga 30 September 2022. Dua puluh lapan bakteria telah diasingkan daripada spesis kucing di kedua-dua jantina, pelbagai peringkat umur dengan latar belakang yang berbeza. Setiap arkib sampel disimpan pada suhu -30 °C dan dicairkan sebelum subkultur pada nutrien agar. Subkultur kedua dilakukan untuk mendapatkan kultur tulen. Ujian sensitiviti antimikrob (AST) kemudian dijalankan mengikut kaedah Kirby-Bauer. Secara keseluruhan, pengasingan majoriti bacteria dalam isolate adalah *Enterococcus faecalis* (18%, 5/28), *Escherichia coli* (18%, 5/28), *Proteus mirabilis* (14%, 4/28), dan *Pseudomonas aeruginosa* (11%, 3/28). Antara isolate tersebut (n=17), rintangan antimikrob tertinggi dilihat terhadap amoxicillin, cefotaxime, sefalotin, klindamisin, doksisiklin dan kloramfenikol. Penisilin dan lincosamides telah menunjukkan kadar rintangan tertinggi pada 88% (15/17). Kesimpulannya, antibiotik mesti digunakan secara berhemat dalam bidang veterinar. Rawatan menggunakan antibiotik mestilah berdasarkan kultur dan AST untuk mengurangkan berlakunya rintangan antimikrob.

Kata kunci: rintangan antimikrob, jangkitan saluran kencing, kucing, ujian sensitiviti antimikrob

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901- Project.

THE OCCURRENCE OF ANTIMICROBIAL RESISTANCE ISOLATED IN URINARY TRACT INFECTIONS (UTIs) IN CATS

By

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2023

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The emergence of antimicrobial resistance (AMR) is alarming, particularly to human health, as antimicrobials are widely used in the small animal practices. Close interaction between humans and their pets can become one of the sources of AMR organisms transmission, which increases the likelihood of reducing the efficacy of antibiotics treatment in both of them. In small animal practices, urinary tract infection (UTIs) is a common condition to be treated with antibiotic empirically. Hence, the purpose of this study is to identify the occurrence of antimicrobial susceptibility status in feline species infected with UTIs. Twenty-two cases of archived isolates derived from samples of UTIs in cats from Bacteriology Laboratory, University Putra Malaysia. The isolates were collected between the 1st January 2021 until 30th September 2022. Twenty-eight archived bacteria were isolated from feline species in both genders, various ages with different backgrounds. The archived isolates were kept at -30 °C and thawed before sub-cultured on nutrient agar. Second sub-culture was done to obtain pure culture prior to antimicrobial susceptibility test (AST) using Kirby-Bauer disk diffusion technique. Overall, majority of the isolates were identified as *Enterococcus faecalis* (18%, 5/28), *Escherichia coli* (18%, 5/28), *Proteus mirabilis* (14%, 4/28), and *Pseudomonas aeruginosa* (11%, 3/28). these isolates (n=17) showed resistance towards amoxicillin, cefotaxime, cephalothin, clindamycin, doxycycline and chloramphenicol. In conclusion, among the isolates, both penicillin and lincosamides have shown the highest rate of resistance at 88% (15/17). Therefore, antibiotics must be used prudently in the veterinary field. Treatment using antibiotics must be based on culture and AST to reduce the occurrence of antimicrobial resistance.

Keywords: antibiotic resistance, cats, urinary tract infection, antimicrobial susceptibility test

INTRODUCTION

1.0 Background

In general, antimicrobial resistance refers to the changes of bacteria characteristics transforming to become more resilient to antibiotics that are used for the purpose to combat the infection caused by the microorganism. Resistant against antimicrobial drugs is inevitable in the veterinary field, as these drugs are widely being used as therapeutic purposes to treat diagnosed bacterial infections, to reduce the complications of infection in animals and also to improve animal performance through usage in feed additives (van den Bogaard & Stobberingh, 1999).

The emergence of antimicrobial resistance has sparked global alarm, particularly to human health, as these antimicrobials are vastly being used in small animal medicine. The close interaction between humans and their pets can pose a potential hazard of transferring antibiotic resistance to humans because of the broadly overuse of antimicrobial medicines in animals. When this antimicrobial resistance being transferred between humans and pets occurs, it will increase the likelihood of reducing the efficacy of many antibiotics' treatment in both humans and animals (Guardabassi et al., 2004; Li et al., 2021). In small animal practices, inappropriate antibiotics are being administered due to the lack of bacterial identification and antimicrobial susceptibility testing performed (Guardabassi et al., 2004). The inappropriate usage of antibiotics can cause detrimental effects to not only the animals' welfare in terms of its health, but also can affect the emotional and socio-economic health of pet owners and livestock farmers (Vaarten, 2012).

It was mentioned that urinary tract infections are commonly diagnosed in the small animal practices, which were initiated without microbiological examination, and often inappropriately receive empirical antibiotic treatment (Vercelli et al., 2021). In the veterinary field, urinary tract infections caused by bacteria disease was one of the reasons antimicrobial drugs were widely being used. Hence, improper usage of antimicrobial drugs in this practice leads to increase in antimicrobials resistance concern (Siti Noor Shuhada Iberahim, 2018).

In recent studies, most of the collected data emphasize more on the emergence of antimicrobial resistance in livestock compared to companion animals as higher risk of transmission of antimicrobial resistance organisms to humans through food chain (Guardabassi et a.l, 2004; (Nurul Asyiqin Haulisah et al., 2021). In Malaysia, studies regarding the occurrence of antimicrobial resistance organisms associated with companion animals is scarce. Thus, the occurrence of resistance against antibiotics in companion animals will be determined to guide veterinarians to choose antimicrobial drugs prudently in small animal practices.

1.1 Objectives

The objectives of this study are:

1. To determine the most common bacteria isolated in archived isolates of urinary tract infection cases submitted to Veterinary Bacteriology Laboratory, VLSU, UPM between 2021 and 2022.
2. To determine the antimicrobial susceptibility status from archived isolates of urinary tract infection collection between 2021 and 2022 from Veterinary Bacteriology Laboratory, VLSU, UPM.

1.2 Hypothesis

In this study, the hypothesis is as below:

Null Hypothesis, H_0 = Less than 50% of the isolates are resistant to more than two of the selected antibiotics.

Alternative Hypothesis, H_a = More than 50% of the isolates are resistant to more than two of the selected antibiotics.

LITERATURE REVIEW

2.1 Usage of antibiotics in small animal practices

Awareness towards animal's health has increased among the public, especially to pet owners in order to save their animals against diseases and give their pets a better life. This shows that the pet owners have high awareness of their animal's welfare, eventually increasing their expenses in veterinary care. With this, usage of antimicrobial agents now often being used in pet animals, particularly the usage of licensed drugs that are equally important in humans' healthcare (Guardabassi et al., 2004). In general, the concern of resistance of antimicrobials is more important in the livestock industry. This is because most antimicrobials are known to be religiously used in the large animal practices to promote growth and prevent diseases, which can inevitably cause the transmission of antimicrobial resistance to humans through consumption (Nurul Asyiqin Haulisah et al., 2021). However, the usage of antimicrobials in small animal practices can also contribute to antimicrobial resistance. This increases the likelihood of resistance transmission as pets can be a reservoir in spreading the resistance genes horizontally to its owners through environment or physical contact (Guardabassi et al., 2004).

One of the most common diseases diagnosed by veterinarians with empirical usage of antibiotics is urinary tract infections (UTIs) with or without waiting for microbiological diagnostic workup (Chan et al., 2022; Vercelli et al., 2021; Weese et al., 2021). According to Weese et al. (2019), the major cause of morbidity in small animals is bacterial urinary tract diseases that eventually lead to the usage of antimicrobials. Improper prescription of antibiotics can lead to many health concerns such as the antimicrobial resistance, owner's economic issue, which is the need for prolonged treatment, and public health relation referring to the transmission of antimicrobial resistance between humans and animals (Weese et al., 2019). Based on a study by Hur et al. (2021), regarding evaluating the dose, indication and agreement with guideline of antimicrobial use in companion animal practice with nature of language processing, it was mentioned that urinary tract disorder is one of the highest diseases that received highest usage of systemic and non-systemic antimicrobials, with the highest usage of amoxicillin-clavulanate and cefovecin. This is because animals with UTIs cases commonly are given antimicrobial therapy while waiting for bacterial culture and antibiotic susceptibility tests in order to relieve the patient's discomfort. Hence, the reason antimicrobial is highly prescribed in urinary tract diseases of dogs and cats.

Table 2.1.1: Guideline of prescribing antibiotics in dogs and cats' infection with urinary tract infections regarding the usage of antimicrobials in urinary tract disease (Guardabassi et al., 2004).

Diagnosis	**First-line of antimicrobials**	**Alternatives**
Lower urinary tract infection; cystitis	Amoxicillin Amoxicillin-clavulanate Cefotaxime Sulfonamides/trimethoprim	Tetracycline Fluoroquinolones Cefalexin Metronidazole
Pyelonephritis	Amoxicillin Amoxicillin-clavulanate	Fluoroquinolones Sulfonamides/trimethoprim
Prostatitis; UTI in dogs	Sulfonamides/trimethoprim Chloramphenicol	Macrolides Lincosamides Fluoroquinolones

In any case of UTIs, it is important to consider complicated cases and non-complicated cases before prescribing antibiotics to small animals. Often, for uncomplicated cases, antibiotics are prescribed according to the clinical signs, although aerobic bacterial culture is preferred, but treatment can be done empirically, except for cats is it recommended to confirm the likelihood of diagnosing bacterial cystitis by doing culture and antimicrobial susceptibility test (Chew et al., 2010).

2.2 Common bacteria isolated in urinary tract infections (UTIs) in cats

Urinary tract infection is considered a rare occurrence in cats, as this disease is most commonly infected in dogs, especially in female dogs. As mentioned, about 0.1% to 1% of urinary tract infection cases are being diagnosed in feline species (Chew et al., 2010). As stated in BSAVA Manual of Canine and Feline Nephrology and Urology, the aetiopathogenesis of bacteria being isolated in the urinary tract in cats could be due to urinary tract infected with ascending pathogens from the distal urogenital tract into sterile condition of proximal urethra, bladder and/or upper urinary tract, hence lead to infection to occur (Bartges and Olin, 2017).

In general, bacteria can be divided into gram-positive and gram-negative bacteria. According to Lister et al. (2009) in her updated study on feline bacterial urinary tract infections, the most common gram-positive bacteria that isolated in the urinary tract infection are *Enterococcus faecalis*, while for Gram-negative is *Escherichia coli* in feline species alongside with *Proteus spp*. It is also known that in the updated study, *Staphylococcus felis* had been identified as another unique strain that can be isolated in feline species infected with urinary tract infection (Lister et al., 2009; Bartges and Olin, 2017). Other bacteria that are commonly isolated in feline species are *Klebsiella* spp, *Pasteurella* spp, *Pseudomonas* spp, and *Corynebacterium* spp (Bartges and Olin, 2017).

Various studies in different demographic areas had shown similar findings in identifying common bacteria isolates in urinary tract infection cases in cats. For example, studies in Europe countries such as Norway and United Kingdom, identified that *Escherichia coli* is the most common Gram-negative isolated, while most common Gram-positive isolates identified were *Staphylococcus spp*, *Streptococcus canis* and *Enterococcus spp* (Eggertsdóttir et al., 2007; Fonseca et al., 2021). Similar study in Italy identified that *Escherichia coli*, *Staphylococcus pseudintermedius,* and *Staphylococcus aureus* were the most common isolates in cats' samples (Vercelli et al., 2021). A study of feline species in Spain mentioned that *Enterococcus* spp. were highly isolated and most prevalent in cats (Darwich et al., 2020).

Although most research was on dogs in different countries, it was mentioned by several studies that bacteria isolated in cats are similar to dogs (Bartges and Olin, 2017). A retrospective study by Hariharan et al. (2016) regarding the bacteria isolates from urinary tract infection dogs in Grenada supported the similar findings of bacteria isolates in cats, such as *Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa*. Other bacteria such as *Staphylococcus intermedius* were also isolated in dogs (Pedersen et al., 2007).

As mentioned by Teo Chin Xian (2013), different temperate countries may have different bacteria isolates which causes this infection to occur, hence the environment in Malaysia tropical country might differ. Due to the limited study in Malaysia regarding bacteria isolates and antimicrobial resistant patterns in urinary tract infections, it is beneficial for this study to allow clinicians to choose antibiotics appropriately while waiting for diagnostic work up testing results.

2.3 Antibiotics resistant patterns in urinary tract infections (UTIs) in cats

Urinary tract infection is the most common disease that often use empirical treatment while waiting for bacterial culture and antimicrobial sensitivity test results to reduce the pain in the animals infected. Due to this, it has caused the improper usage of antibiotics in the small animal practices, besides owner cost constraint to do bacteria culture and antibiotic susceptibility test, but the overuse of antibiotics causes these animals to eventually be resistant to important first-line drugs. The most common antibiotics that can be used as first-line treatment to treat urinary tract infection are amoxicillin, amoxicillin-clavulanate and sulfonamides/trimethoprim (Guardabassi et al., 2004). When there is no improvement of infected animals to these drugs, alternative drugs will be taken into consideration. The most common alternatives are fluoroquinolones, such as norfloxacin, enrofloxacin, marbofloxacin, ciprofloxacin, orbifloxacin and difloxacin (Chew et al., 2010).

Many retrospective studies have shown a drastic trend of resistance of similar antimicrobial drugs in treating urinary tract infection in cats. Most of the drugs mentioned were the antibiotics listed as first-line antimicrobials treatment for UTIs cases. It was mentioned by Vercelli et al. (2021), feline urine samples showed more than 30% of resistance results towards antibiotics in the antimicrobial classes of penicillin (amoxicillin, ampicillin), cephalosporins (cephalexin, cephalothin, cefovecin), fluoroquinolones (enrofloxacin) and sulphonamides (trimethoprim/sulfamethoxazole). Although Litster et al. (2011), mentioned that most feline urinary tract infections can be treated with oral amoxicillin and amoxicillin/clavulanate acid, however many studies showed that these two drugs were recorded the most resistance towards *Escherichia coli* isolates in feline urine samples (Vercelli et al., 2021; Normand et al., 2000). It was then supported by KuKanich et al. (2019) regarding usage of amoxicillin and amoxicillin/clavulanate acid in treating UTIs in cats and dogs, no shown susceptibility against these two drugs.

A retrospective study done in Hong Kong, China, regarding over usage of first-line antimicrobials in urinary infections cases in cats and dogs, showed results of antimicrobials resistance on the most common isolates, which are *Escherichia coli*, *Enterococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., and *Staphylococcus* sp. About 40%

of *Escherichia coli* samples were resistant towards amoxicillin and ampicillin, while the rest resisted to most cephalosporins and fluoroquinolones antibiotic class. Followed by *Enterococcus*, about 90% were resistant to gentamicin and trimethoprim/sulfamethoxazole, while the rest (35%) were resisted to fluoroquinolones group, doxycycline and meropenem. For *Klebsiella* sp and *Pseudomonas* sp, both were also resisted towards amoxicillin at about 90%, whereas about 45% of penicillin groups were resisted towards *Staphylococcus* sp (Chan et al., 2022).

2.4 One Health concerns of antimicrobial resistance

The risk of antimicrobial resistance not only concerns the veterinary medicine field in dealing with choosing appropriate antimicrobials in treating companion animals, but the risk of transmitting resistance also concerns the public health, especially pet owners and workers directly working with companion animals. Antimicrobial resistance can be transmitted Antimicrobials resistant can be transmitted either from direct or indirect contact between humans and animals, such as through physical touch of touching animals, getting licked or bitten by animal, or through the environment contaminated by resistant bacteria (Guardabassi et al., 2004).

Antimicrobials used in veterinary medicine are almost similar in human medicine. The antibiotic classes that are commonly used in both fields are tetracyclines, macrolides, lincosamides, chloramphenicol, aminoglycosides, penicillins and cephalosporins. Due to this, the possibilities of transmitting antimicrobial resistance organisms through interaction between pets and their owners could affect the same classes of antimicrobial agents used in both practices (Guardabassi et al., 2004). Hence, this might also increase the likelihood of multi-drug resistant concerns that will reduce the treatment efficacy (Pomba et al., 2016).

The main reasons antimicrobial resistance occurs in companion animals is because these animals are frequently being treated empirically rather than being given antimicrobials after or through antimicrobial susceptibility tests. The lack of need for acquired tests eventually led to the inappropriate use of antimicrobial. It was mentioned antimicrobials are often being administered in cases with unknown efficacy, such as feline lower urinary tract disease, and treatment that does not require antibiotics (Pomba et al., 2016).

Due to these concerns towards public health, this led to the need of veterinarians to use antimicrobials prudently. Reducing the usage of antimicrobials is the only way to prevent antimicrobials resistant to occur (van den Bogaards & Stobberingh, 1999). In Malaysia, Malaysian Veterinary Antimicrobial Guideline produced by Department of Veterinary Services is a guideline made for veterinarians and those involved in the animal industry, such as food producing animals, to use antimicrobials prudently in order to maintain the animal's welfare and health. According to one of its principles, antimicrobials should be used responsibly using the 5R's motto: Right drug, Right time, Right dose, Right duration and Right route. Similar to other countries' guidelines of prudence in the use of antimicrobials mentioned in Singapore and the United States of America, the prescription of antibiotics should be chosen appropriately according to its right dosage and period of treatment. Microbiological diagnostic work up should be done, especially in cases of urinary tract infections, and its antimicrobial prescriptions should be changed immediately according to results of antimicrobial susceptibility test (Edwards et al., 2004). Hence, it is important for veterinarians to maintain their stewardships towards antimicrobial usage to prevent antimicrobial resistance occurring.

METHODOLOGY

3.1 Sample collection

The samples used were from archived isolates retrieved from Bacteriology Laboratory, Veterinary Laboratory Services Unit (VLSU), University Putra Malaysia (UPM). The samples taken consist of urine samples of feline species of different gender and age that were sent to the laboratory for bacterial isolation and identification between the period of 1st January 2021 and 30th September 2022. Out of 77 urinary related cases in the span of that period, only 10 and 12 urinary tract cases positive for bacteria reported in 2021 and2022, respectively. from the 22 cases that were positive for bacterial infection, a total of 28 bacterial isolates have been identified and stored in a microcentrifuge tube containing Brain Heart Infusion (BHI) broth added with 10% glycerol which were kept at -30°C. Most of these cases consist of feline species with different tentative diagnosis such as bacterial cystitis, suspected bacterial cystitis, Feline Lower Urinary Tract Disease (FLUTD), Feline Idiopathic Cystitis (FIC) and other correlation with urinary tract infection diseases. The samples submitted to the laboratory were either urine collected through cystocentesis or catheterisation method or swab samples. The archived samples that have been identified consist of mostly Enterobacteriaceae group of bacteria such as *Escherichia coli, Enterobacter cloacae, Klebsiella* spp*, Proteus mirabilis, Salmonella enterica* ssp *arizonae*, and other type of bacteria such *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus* spp.

3.2 Bacterial subculture from archived samples

The archived bacterial isolates which were kept in a Brain Heart Infusion (BHI) broth with 10% glycerol at -30°C were firstly thawed in chiller about 4°C for an hour and another hour at room temperature. Nutrient agar media (Oxoid) were prepared prior to bacteria culture which was kept in a chiller at 4°C. Once is the isolates were fully thawed; the isolates were then cultured onto a nutrient agar in order to reactivate the bacteria. For *Streptococcus* sp*,* it was cultured onto 4% blood agar. The samples were incubated in an incubator at 37°C for 24-48 hours. The isolates were subculture again to obtain a higher number of pure colonies from a single culture plate. Screening of the identified bacteria was done by Gram staining method to confirm the type of bacteria based on the labelling on the stock samples. Through Gram staining method, the bacteria stained with purple or blue colour is determined as Gram-positive bacteria, while pink colour will be Gram-negative bacteria.

3.3 Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) procedure in this study was done according to the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol by American Society of Microbiology standard procedures (Hudzicki, 2009). AST were performed using disc diffusion method using 8 types of antibiotics tested in this study, which included meropenem, cefotaxime, amoxicillin, chloramphenicol, doxycycline, cephalothin, clindamycin and enrofloxacin. The quality control organisms used in this study were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as recommended by the Clinical and Laboratory Standards Institute (CLSI), VET01, 5th Edition (2019).

Once pure culture obtained from each isolate, AST was performed. About 2ml of autoclaved saline water was added into a sterile test tube, which then it was added with four to five colonies of respective bacteria using a sterile loop. The turbidity of the suspension was compared to 0.5 MacFarland standard that was equivalent to 1-2 x 10^8 cfu/ml. A sterile swab was dipped into the inoculum and then lawn onto Mueller-Hinton (MH) agar using three overlapping directions at 45-degree angles. Antibiotics selected for this study were meropenem (MEM 10µg), cefotaxime (CTX 30µg), amoxicillin (AML 10µg), chloramphenicol (C 30µg), doxycycline (DO 30µg), cephalothin (KF 30µg), clindamycin (DA 2µg) and enrofloxacin (ENR 5µg). All antibiotic discs were from ThermoFisher, Oxoid. These selected antibiotics were pressed onto the agar surface using a multi-disc antimicrobial dispenser (ThermoFisher, Oxoid). A sterile forceps was used to press the disc further to allow complete contact. The inoculated plates were then incubated in an incubator at 37°C for 24 hours. The inhibition zones were measured and recorded using callipers and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) antibiotic susceptibility table (2019) to determine the susceptibility of the isolates to each selected antibiotic. For each selected antibiotic, the zones were recorded as susceptible (S), intermediate (I) and resistance (R) accordingly while absence of inhibition zones were recorded as resistant.

3.4 Data analysis

Data from the samples were recorded using Excel 2019 (Microsoft Office 2019). The value of each zone of inhibition in each sample was measured and recorded. Each result was also confirmed at its breakpoints using AST standards stated in Clinical and Laboratory Standards Institute (CLSI) in M100 - Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition (2020).

RESULTS

4.1 Bacteria isolated from archived samples.

Twenty-two (22) cases with a total of 28 bacteria were obtained from identified bacteria diagnostic cases that were sent to Bacteriology Laboratory, VLSU, UPM archived between the period of 2021 and 2022. The samples consist of feline species with different gender and tentative diagnosis of urinary tract infection (UTIs), with most common methods of sampling being catheterisation and cystocentesis.

Based on the results obtained, the most common Gram-positive bacteria that have been isolated were *Enterococcus faecalis* (18%), whereas the most common Gram-negative bacteria that have been isolated were *Escherichia coli* (18%), *Proteus mirabilis* (14%), and *Pseudomonas aeruginosa* (11%).

Table 4.1.1: Bacteria isolated from archived samples of urinary tract infections in feline species submitted to Bacteriology Laboratory, VLSU, UPM among the period of 2021 and 2022.

Bacteria isolated	No. of archived samples	Percentage (%)
Gram- positive		
Enterococcus faecalis	5	18
Staphylococcus intermedius	1	4
Staphylococcus pseudintermedius	2	7
Gram-negative		
Proteus mirabilis	4	14
Pseudomonas aeruginosa	3	11
Escherichia coli	5	18
Klebsiella sp.	1	3
Klebsiella pneumoniae	2	7
Enterobacter cloacae	1	3
Klebsiella aerogenes	2	7
Salmonella enterica ssp arizonae	1	4
Pasteurella multocida sub subsp multocida	1	4
Total bacteria isolated	**28**	**100**

Based on the laboratory file records retrieved for each archived sample, only four cases submitted did not request for antimicrobial susceptibility test (AST). The bacteria isolated from the cases that were not requested for AST includes *Enterococcus faecalis* (n=2), *Enterobacter aerogenes* (n=1) and *Pasteurella multocida subsp multocida* (n=1).

4.2 Antimicrobial susceptibility test (AST) of archived isolates.

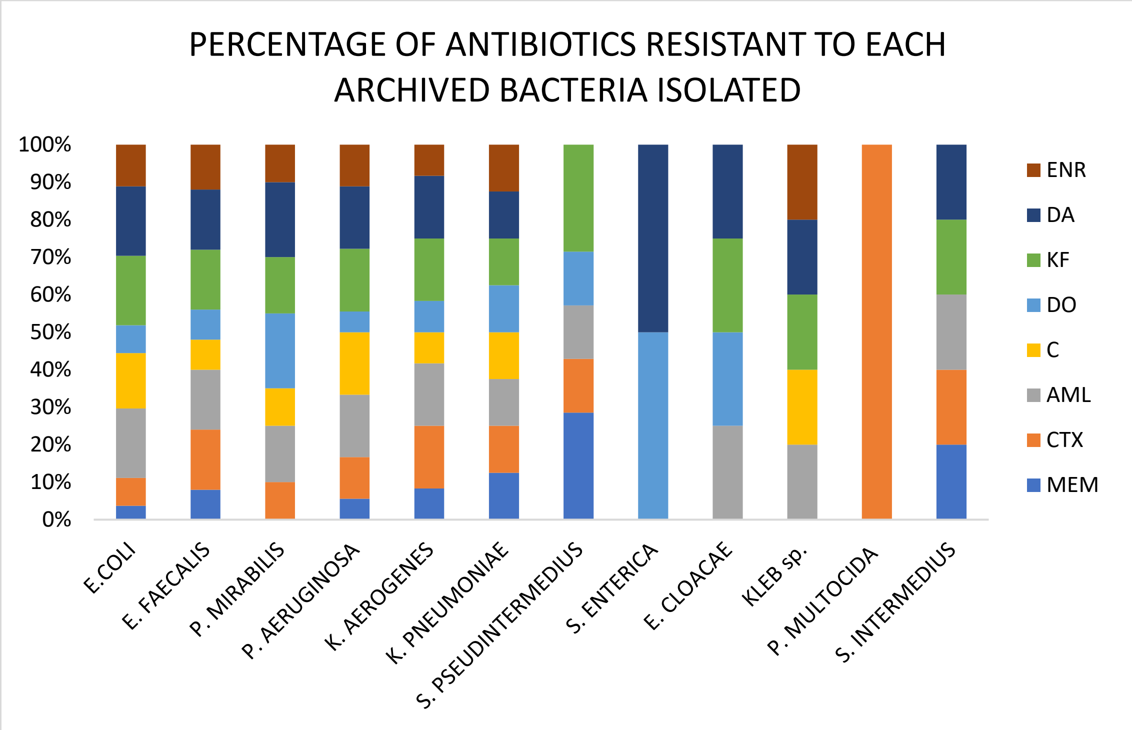
A total of 28 archived bacteria were successfully isolated into pure culture from its stock samples. Antimicrobial susceptibility test in this study used selected antibiotics as listed in Table 4.2.1 with different antibiotic classes. Each bacterium isolated was tabulated based on susceptibility, intermediate and resistance according to its measurement of zone of inhibition. The zone of inhibition measured was manually checked at its breakpoints using AST standards produced by Clinical and Laboratory Standards Institute (CLSI), M100, 30th Edition.

Table 4.2.1: The percentage of susceptibility and resistance of bacteria isolates from the archived samples.

		PERCENTAGE OF RESISTANT ISOLATES (%)							
[a]CLASSES OF ANTIBIOTICS		CARB	CEPH	PENI	CHLOR	TETRA	CEPH	LINC	FLURO
ISOLATES	N	[b]MEM	CTX	AML	C	DO	KF	DA	ENR
Escherichia coli	5	20	40	100	80	40	100	100	60
Enterococcus faecalis	5	40	80	80	40	40	80	80	60
Proteus mirabilis	4	*-	50	75	50	100	75	100	50
Pseudomonas aeruginosa	3	33.3	66.7	100	100	33.3	100	100	66.7
Klebsiella aerogenes	2	50	100	100	50	50	100	100	50
Klebsiella pneumoniae ss. pneumoniae	2	100	100	100	100	100	100	100	100
Staphylococcus pseudintermedius	2	100	50	50	-	50	100	-	-
Salmonella enterica ss. arizonae (Subgroup IIIa)	1	-	-	-	-	100	-	100	-
Enterobacter cloacae	1	-	-	100	-	100	100	100	-
Klebsiella sp.	1	-	-	100	100	-	100	100	100
Pasteurella multocida ss. multocida	1	-	100	-	-	-	-	-	-
Staphylococcus intermedius	1	100	100	100	-	-	100	100	-

[a]CARB: Carbapenem, CEPH: Cephalosporin, PENI: Penicillin, CHLOR: Chloramphenicol, TETRA: Tetracycline, LINC: Lincosamides, FLURO: Fluoroquinolones
[b]MEM: Meropenem, CTX: Cefotaxime, AML: Amoxicillin, C: Chloramphenicol, DO: Doxycycline, KF: Cephalothin, DA: Clindamycin, ENR: Enrofloxacin
**Dash (-) indicates 0%.*
N = Total

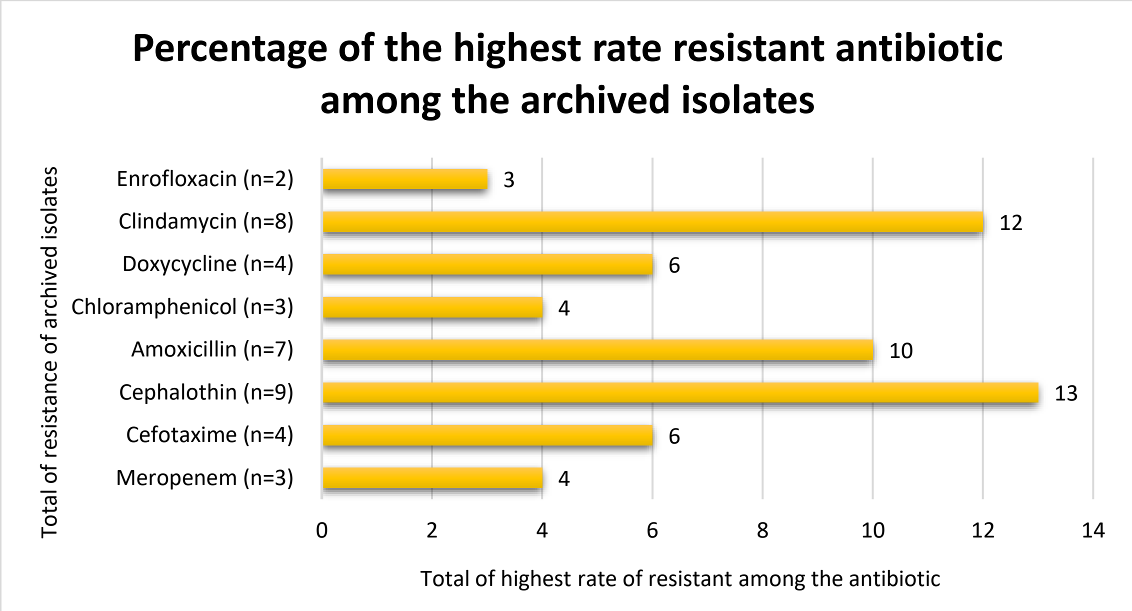
Figure 4.2.1: Figure shows bar chart of the percentage of respective antibiotics resistant to the archived bacteria isolated in this study.



MEM: Meropenem, CTX: Cefotaxime, AML: Amoxicillin, C: Chloramphenicol, DO: Doxycycline, KF: Cephalothin, DA: Clindamycin, ENR: Enrofloxacin

Based on the results, antibiotics that showed 100% refers to most of the isolates in the respective bacteria that were resistant to the antibiotic, while 0% refers to most isolates that were susceptible to the antibiotic. A total of 69 isolates showed resistance to archived isolates. The antibiotics that showed the highest resistance at 100% resistance were cephalothin (13%, n=9/69), clindamycin (12%, n=8/69) and amoxicillin (10%, n=7/69). The least antibiotic that showed significant resistance at 100% among bacteria isolates were meropenem (3%, n=2/69) and enrofloxacin (3%, n=2/69). For isolates that showed no percentage is considered susceptible.

Figure 4.2.2: Bar graph above shows the amount of antibiotics obtain 100% of resistance over the total of antibiotics that were resistant among the archived bacteria isolates.

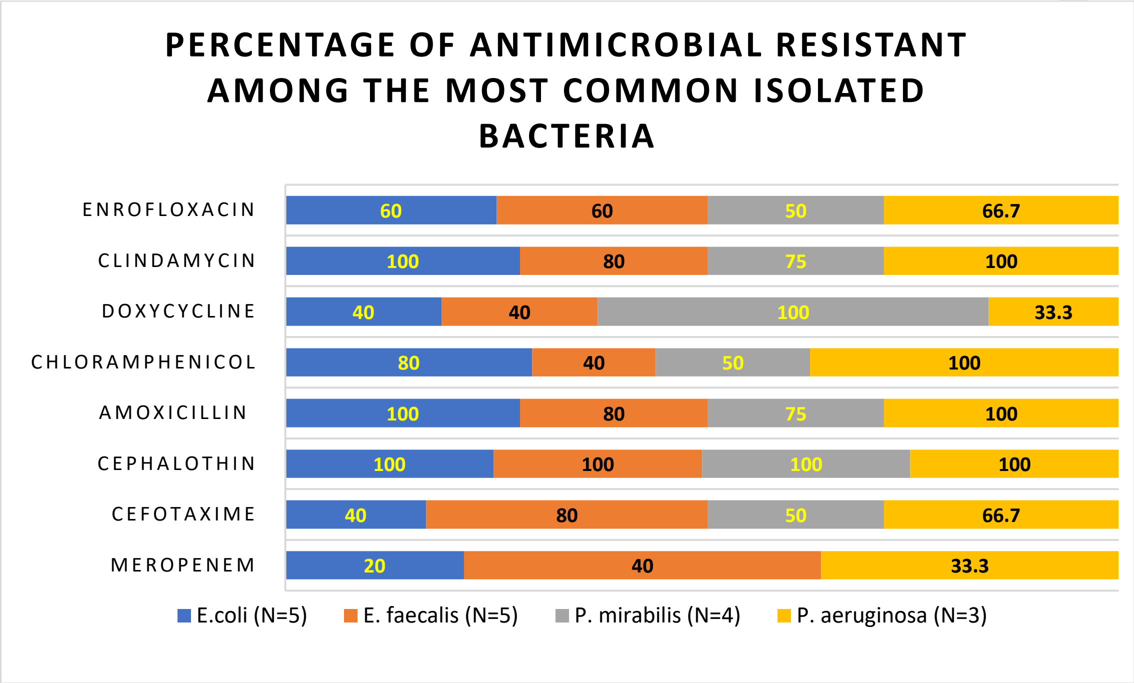


Out of all 28 samples from the archived isolates, 17 isolates showed resistance to all classes of antibiotics in the selected drugs in this study. The isolates were *Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Klebsiella pneumonia*. Four isolates of *Proteus mirabilis* were resistant to 6 classes of antibiotic in this study. Only *Pasteurella multocida* subsp *multocida* one isolate was resistant to only one class of antibiotic that was the cephalosporins (cefotaxime).

4.3 Antimicrobial susceptibility test (AST) of most isolated bacteria.

In this study, the highly isolated bacteria among the archived isolates were *Enterococcus faecalis* (18%), *Escherichia coli* (18%), *Proteus mirabilis* (14%), and *Pseudomonas aeruginosa* (11%). The following bar graph shows the resistance of antimicrobials among those isolated samples.

Figure 4.3.1: Figure above shows the percentage of antimicrobial resistant to selected antibiotics among the most common bacteria isolates in feline species urinary tract infection collected between 2021 and 2022.



E.coli = *Escherichia coli, E. faecalis* = *Enterococcus faecalis, P. mirabilis* = *Proteus mirabilis, P. aeruginosa* = *Pseudomonas aeruginosa*

Among the *Enterococcus faecalis,* 80% were resistant to cephalosporins (cefotaxime, cephalothin), penicillin (amoxicillin) and lincosamides (clindamycin) classes of antibiotics, while 40% were resistance to carbapenem (meropenem), chloramphenicol, and tetracycline (doxycycline). One class of antibiotic which is fluoroquinolones (enrofloxacin) were resistant to *Enterococcus faecalis* at 60%.

For *Escherichia coli,* the most common class of antibiotic with significant 100% (n=5/5) of isolates showed resistance were penicillin (amoxicillin), cephalosporin (cephalothin) and lincosamides (clindamycin), similarly with *Enterococcus faecalis*. Besides that, 40% of the isolates were resistant to cephalosporin (cefotaxime) and tetracycline (doxycycline), including 60% of isolates to fluoroquinolones (enrofloxacin).

Another 80% of isolates (n=4/5) were resistant to chloramphenicol and only 20% of isolates (n=1/5) were resistant towards meropenem in this isolate.

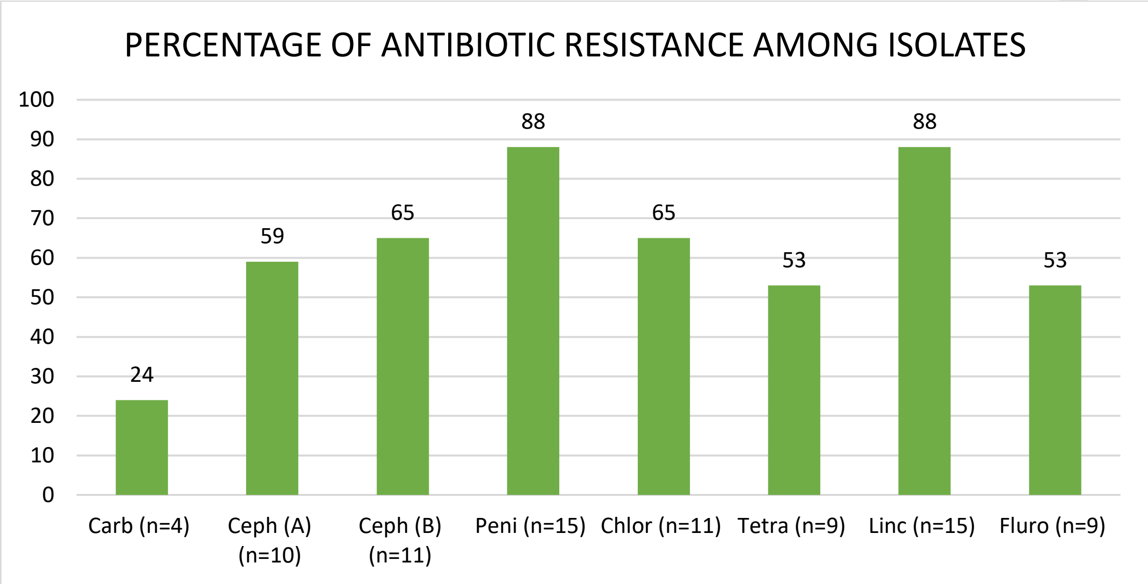
Among *Proteus mirabilis,* no samples were resistant towards meropenem. However, a significant 100% resistance was towards tetracycline (doxycycline) and lincosamides (clindamycin). Others, 50% of isolates were resistant to fluoroquinolones (enrofloxacin), chloramphenicol, and penicillin (amoxicillin). For cephalosporins antibiotics, 50% (n=2/4) were resistant to cefotaxime, while 75% (n=3/4) were resistant to cephalothin. No isolates showed resistance over meropenem in all isolates.

For *Pseudomonas aeruginosa*, most isolates were significant at 100% (n=3/3) resistant to penicillin (amoxicillin), chloramphenicol and lincosamides (clindamycin). For the cephalosporin group, only 2 isolates out of 3 were resistant to cefotaxime (66.7%), while all isolates (n=3/3) were resistant to cephalothin (100%). At 33.3% (n=1/3), this isolate was resistant to carbapenem and tetracycline.

4.4 Pattern of antimicrobial resistance among the isolates

The pattern of resistance among the classes of antibiotics in this study were tabulated to show the resistance among the archived isolated samples that were highly isolated in this study. A total of 17 isolates among the mostly isolated bacteria in this study, which were *Enterococcus faecalis* (n=5), *Escherichia coli* (n=5), *Proteus mirabilis* (n=4), and *Pseudomonas aeruginosa* (n=3). Based on the figure below, the highest class of antibiotics that showed the highest percentage are penicillin and lincosamides, both at 88% (n=15/17), followed by 11 isolates were resistant to cephalothin and chloramphenicol at 65%. The least class of antibiotics that were resistant in most of the isolates is carbapenem at 23% (n = 4/17).

Figure 4.4.1: Figure shows a bar chart of the percentage of resistance over the classes of antibiotics among the most common archived isolates isolated in the feline samples infected with urinary tract infection.



CARB: Carbapenem, CEPH: Cephalosporin, PENI: Penicillin, CHLOR: Chloramphenicol, TETRA: Tetracycline, LINC: Lincosamides, FLURO: Fluoroquinolones A: Cefotaxime, B: Cephalothin

DISCUSSION

The most common Gram-positive bacteria that was isolated among all the archived urinary tract infection samples in feline species was *Enterococcus faecalis*, while the most isolated Gram-negative were *Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa* in this study. These isolates are commonly isolated in cases of urinary tract infection (UTIs) in cats. There were several studies that supported these findings, especially indicating *Escherichia coli* and *Enterococcus* spp. as the most commonly isolated bacteria in urinary tract infection in cats (Chew et al., 2010; Litster et al., 2009; Martinez-Ruzafa et al., 2012; Marques et al., 2016). One report in Thailand revealed similar results of common bacteria isolates in UTIs, which are *Escherichia* coli, *Pseudomonas* spp and *Proteus* spp (Amphaiphan et al., 2019).

However, it was known that studies in different regions have found different bacteria isolated in urinary tract infection in cats. For example, a study by Vercelli et al. (2021) and Hernando et al. (2021), identified that *Staphylococcus* spp was also highly isolated in feline species, next to Escherichia coli and *Enterococcus* spp in European region. Similar to another study in Istanbul which identified *Staphylococcus* as one of the most common isolates in UTIs in cats (Dokuzeylül et al., 2015). In this study, only one to two isolates of *Staphylococcus* spp. were archived and isolated. This could conclude that regions with a similar environment and climate as the hot, humid and tropical climate of Malaysia might obtain similar findings compared to regions outside of Asia.

The antimicrobial susceptibility status in this study was only focused on the bacteria that were highly isolated among the archived samples to show better prevalence of antimicrobial resistance results were considered in this study. Among the isolates, it was shown that the highest percentage of antibiotics that was highly resistance were amoxicillin, cephalosporins group (cefotaxime and cephalothin), clindamycin, doxycycline and chloramphenicol. Based on Guardabassi et al. (2004) and Weese et al. (2019), in cases of urinary tract infections in feline species, the first line of antimicrobials for treatment are amoxicillin and cefotaxime were known to be effective to treat against UTIs cases. In this study, there was a significant result of resistance at almost 100% in most of the common isolates towards amoxicillin, cefotaxime, and chloramphenicol, followed by about 80% of alternative drugs such as clindamycin, doxycycline and enrofloxacin were also showed resistance in most of the isolates. It was revealed that in a study by Hernandez et al. (2013), showed that high resistance of urinary tract infection isolates in felines were also resistant to penicillin, cephalosporins, quinolones and tetracycline. Less resistance was shown in most of the isolates over the carbapenem group, which is meropenem.

For *Escherichia coli* isolates (n=5), all isolates were resistant to amoxicillin, cephalothin and clindamycin. According to KuKanich et al. (2019), a study of *Escherichia coli* against amoxicillin and amoxicillin/clavulanate antibiotics, showed all isolates retrieved from samples of feline infected with urinary tract infections were resistant to both drugs. In this study, resistance of *Escherichia coli* was also supported by Fonseca et al. (2021), mentioned that most of the isolates of cats infected with UTIs were highly resistant to cephalothin. The trend pattern of *Escherichia coli* resistant to most of the first-line antibiotics and non-first line antibiotics has increased from a retrospective study from the year 2018 to 2020 among cats and dogs in China (Chan et al., 2022).

Among *Enterococcus faecalis* isolates (n=5), at almost 80% of isolates showed resistance towards amoxicillin, cefotaxime, cephalothin and clindamycin. Intrinsic resistance is commonly known in *Enterococcus faecalis* towards some penicillins, cephalosporins and lincosamides, however susceptibility may be shown in these drugs (Dorsch et al., 2019; Weese, 2008). If they show susceptibility, the results should not be considered as effective if the isolate is *Enterococcus* sp. (Teichmann-Knorrn et al., 2018). Even though these antibiotics will not show any activity towards the bacteria, this has made the chances of becoming acquired antimicrobial resistance is higher (Weese, 2008). Hence, treatment with antibiotics against *Enterococcus* sp. is limited (Kukanich et al., 2015).

According to Marques et al. (2020), *Proteus mirabilis* is considered the most common UTIs bacteria in dogs compared to cats. However, Litster et al. (2009), mentioned that *Proteus mirabilis* is the second-most common urinary tract infection isolated in cats, next to *Enterococcus faecalis* and *Escherichia coli.* In this study, this bacterium showed high resistance to doxycycline and clindamycin. *Proteus mirabilis* is known for its intrinsic resistance towards tetracycline (Lister et al., 2007). For *Pseudomonas aeruginosa* isolates, amoxicillin, clindamycin and chloramphenicol showed the highest resistance against this bacterium in this study. Based on a study by Weese (2008) and Pomba et al. (2016), mentioned that *Pseudomonas* spp is a common bacterium that is known for multidrug-resistance.

Overall, the highest percentage of antibiotics that were highly resistant to most of the isolates are amoxicillin, clindamycin, cephalothin and chloramphenicol. Amoxicillin is considered as the first-line of antibiotics to treat urinary tract infections (Weese et al., 2011). However, in this study, almost all isolates were resistant to amoxicillin. A study by KuKanich et al. (2019) on amoxicillin resistance in *Escherichia coli* isolates showed no isolates in the urinary tracts infection in cats were susceptible to the antibiotics chosen. This finding is similar to studies that showed amoxicillin is the most highly resistant antibiotics against isolates in urinary tract infection, alongside resistance to cephalothin (Darwich et al., 2021). In this study, the least antibiotic that was resistant to most of the isolates were meropenem and enrofloxacin. Meropenem and enrofloxacin are considered as a reserved drug that should only be used when there are multidrug-resistance infections and should only be used as a last resort option (Teichmann-Knorrn et al., 2018; Weese et al., 2011). Although it is considered as an option to treat urinary tract infection, it should not be used as a routine and only can be given for recurrent cases of UTIs after culture and susceptibility testing only to avoid further resistance (Darwich et al., 2021).

CONCLUSION

Enterococcus faecalis, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were the most common isolated bacteria from the archived samples of feline with urinary tract infections. Among those isolates, it was shown that high resistance of bacteria was to amoxicillin, cephalothin, cefotaxime, clindamycin, doxycycline and chloramphenicol. Multi-drug resistance was detected in almost all of the isolates, with resistance towards more than 2 antibiotics. Due to empirical treatment being frequently used to manage urinary tract infection cases in small animal practices, lack of bacterial culture and susceptibility test was done beforehand increases the chances of antimicrobial resistance occurrence. The likelihood of transferring the potential of multi-drug resistance between humans and pet owners which might reduce the efficacy of most antibiotics in the future. Hence, to reduce the chances of resistance, prudent use of antimicrobials should be done in treating disease in the small animal practices.

LIMITATION

In this study, due to the small number of sample sizes, the potential of identifying the antimicrobial susceptibility status may just be defined as a baseline data that can be used as an updated guide for clinicians to choose drugs prudently. Due to the sample collected being archived, some of the identified bacteria that have been inoculated into the Brain Heart Infusion (BHI) with 10% glycerol might end up having mixed infections from the environment during the procedure. Besides that, there were few samples and cases came during the 5-weeks allocated for sampling, hence most of these isolates relied solely on the archived samples from a year ago.

RECOMMENDATION

Antimicrobial susceptibility and resistance status in urinary tract infections in cats may be successfully studied if there is an extended number of samples that could be collected to be archived. Furthermore, a retrospective study of urinary tract infection would be best in addition to this study to further investigate the trend of antimicrobial resistance in urinary tract infections cases in feline species.

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APPENDIX

Table 9.1: Table shows the bacteria isolated from the archived samples showed susceptibility to the antibiotics tested in this study.

BACTERIA ISOLATES SUSCEPTIBLE	Total	MEM	CTX	AML	C	DO	KF	DA	ENR
Escherichia coli	5	80	75		20	20			20
Enterococcus faecalis	5	60	20	20	60	60			20
Proteus mirabilis	4	100	50	25	25		25		
Pseudomonas aeruginosa	3	66.7							
Klebsiella aerogenes	2	50			50				
Klebsiella pneumoniae ss. pneumoniae	2								
Staphylococcus pseudintermedius	2		50		100	50		50	100
Salmonella enterica ss. arizonae (Subgroup IIIa)	1	100	100	100			100		
Enterobacter cloacae	1	100	100		100				100
Klebsiella sp.	1	100	100			100			
Pasteurella multocida ss. multocida	1	100		100	100	100	100		100
Staphylococcus intermedius	1				100	100			100

MEM: Meropenem, CTX: Cefotaxime, AML: Amoxicillin, C: Chloramphenicol, DO: Doxycycline, KF: Cephalothin, DA: Clindamycin, ENR: Enrofloxacin
Empty box: Indicate no results.

Table 9.2: Table shows the bacteria isolated from the archived samples showed intermediate to the antibiotics tested in this study.

BACTERIA ISOLATES INTERMEDIATE	Total	MEM	CTX	AML	C	DO	KF	DA	ENR
Escherichia coli	5					40			20
Enterococcus faecalis	5						20	20	20
Proteus mirabilis	4				25				25
Pseudomonas aeruginosa	3		33.3			66.7			33.3
Klebsiella aerogenes	2					50			50
Klebsiella pneumoniae ss. pneumoniae	2			50				50	
Staphylococcus pseudintermedius	2	0	0	0	0	0	0	0	0
Salmonella enterica ss. arizonae (Subgroup IIIa)	1	0	0	0	0	0	0	0	0
Enterobacter cloacae	1	0	0	0	0	0	0	0	0
Klebsiella sp.	1	0	0	0	0	0	0	0	0
Pasteurella multocida ss. multocida	1	0	0	0	0	0	0	0	0
Staphylococcus intermedius	1	0	0	0	0	0	0	0	0

MEM: Meropenem, CTX: Cefotaxime, AML: Amoxicillin, C: Chloramphenicol, DO: Doxycycline, KF: Cephalothin, DA: Clindamycin, ENR: Enrofloxacin
Empty box: Indicate no results.

Table 9.3: Table shows the bacteria isolated from the archived samples showed resistance to the antibiotics tested in this study.

BACTERIA ISOLATES RESISTANT	Total	MEM	CTX	AML	C	DO	KF	DA	ENR
Escherichia coli	5	20	40	100	80	40	100	100	60
Enterococcus faecalis	5	40	80	80	40	40	80	80	60
Proteus mirabilis	4		50	75	50	100	75	100	50
Pseudomonas aeruginosa	3	33.3	66.7	100	100	33.3	100	100	66.7
Klebsiella aerogenes	2	50	100	100	50	50	100	100	50
Klebsiella pneumoniae ss. pneumoniae	2	100	100	100	100	100	100	100	100
Staphylococcus pseudintermedius	2	100	50	50		50	100		
Salmonella enterica ss. arizonae (Subgroup IIIa)	1					100		100	
Enterobacter cloacae	1			100		100	100	100	
Klebsiella sp.	1			100	100		100	100	100
Pasteurella multocida ss. multocida	1		100						
Staphylococcus intermedius	1	100	100	100			100	100	

MEM: Meropenem, CTX: Cefotaxime, AML: Amoxicillin, C: Chloramphenicol, DO: Doxycycline, KF: Cephalothin, DA: Clindamycin, ENR: Enrofloxacin
Empty box: Indicate no results.

Figure 9.1: Archived samples kept in Brain Heart Infusion (BHI) media in a microcentrifuge tube