UNIVERSITI PUTRA MALAYSIA

OCCURRENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF *Streptococcus canis* ISOLATES AMONG DOGS, CATS AND HUMANS

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OCCURRENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF *Streptococcus canis* ISOLATES AMONG DOGS, CATS AND HUMANS

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Occurrence And Antimicrobial Resistance Profile of *Streptococcus canis* Isolates Among Dogs, Cats and Humans", by Melissa Yam Khai Jie and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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LIST OF ABBREVIATIONS

AMR	antimicrobial resistance
AST	antimicrobial susceptibility test
IgG	Immunoglobulin G
IMR	Institute for Medical Research
MDR	multi-drug resistant
MOH	Ministry of Health
mPLG	mini-plasminogen
MRSA	methicillin-resistant Staphylococcus aureus
PLG	plasminogen
S. canis	*Streptococcus canis*
SCM	*Streptococcus canis* M protein
UPM	Universiti Putra Malaysia
UVH	University Veterinary Hospital

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

KEJADIAN DAN PROFIL KETAHANAN ANTIMIKROBA *Streptococcus canis* PADA ANJING, KUCING, DAN MANUSIA DI MALAYSIA

Oleh

Melissa Yam Khai Jie

2023

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Streptococcus canis (*S. canis*) ialah patogen zoonosis berbilang perumah yang menyebabkan penyakit dalam pelbagai jenis mamalia, termasuk manusia. Anjing dan kucing ialah perumah utama dan mungkin memainkan peranan dalam penularan jangkitan kepada manusia kerana mereka kerap berkongsi persekitaran yang sama. Kajian ini bertujuan untuk menerangkan kejadian kes klinikal *S. canis* dan corak kerentanan antimikrob dalam haiwan kesayangan seperti kucing, anjing dan manusia. Data diperolehi daripada makmal bakteriologi dari Fakulti Perubatan Veterinar Universitii Putra Malaysia dan repositori data kebangsaan Kementerian Kesihatan. Analisis deskriptif retrospektif telah dijalankan untuk data dari Januari 2019 hingga Julai 2023. Sebanyak 30 anjing, 81 kucing dan 42 kes manusia dikenal pasti dengan jangkitan *S. canis*.

Dalam kalangan kucing dan anjing, *S. canis* kebanyakannya diasingkan daripada luka yang bernanah, jangkitan telinga dan kes septikemia dengan penglibatan pelbagai organ. Sebaliknya, *S. canis* kebanyakannya diasingkan daripada sampel darah dari kes bakteremia (45.2%) pada manusia. Sebanyak 24 isolasi daripada anjing, 61 isolasi daripada kucing dan 42 isolasi *S. canis* daripada manusia telah menjalani ujian kerentanan antibiotik. *S. canis* merupakan spesies yang resisten terhadap antibiotic seperti penisilin, sefalosporin, dan amoxicillin-clavulanic di kalangan kucing dan anjing. Tahap resisten yang tinggi diperhatikan terhadap metronidazole, neomycin, dan tetracycline, dengan kadar rintangan dari 67% hingga 100%. Enrofloxacin, antibiotik yang biasa digunakan, menunjukkan kadar kerentanan yang agak rendah iaitu 29% pada kucing dan 23% pada anjing. Isolasi *S. canis* dari kes manusia kekal sangat peka terhadap pilihan antibiotik yang aktif. Walau bagaimanapun, tetrasisiklin dan eritromisin menunjukkan kadar ketahanan masing-masing sebanyak 73% dan 22%. Lebih membimbangkan, sehingga 54% daripada *S. canis* daripada kucing dan anjing menunjukkan rintangan pelbagai ubat. Kesimpulannya, jangkitan Streptococcus canis adalah patogen penting dalam kucing dan anjing dan jangkitan zoonosis yang muncul dalam kesihatan manusia dengan peningkatan corak rintangan antimikrob; diiktiraf terbaik oleh rangka kerja One Health. Kajian lanjut mengenai rintangan dadah dan hasil terapeutik manusia, anjing dan kucing adalah wajar.

Kata kunci: *Streptococcus canis*; ketaahanan antimikroba; ujian kepekaan antimikroba; anjing; kucing; zoonotik

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine as partial requirement on the course VPD 4999 – Final Year Project.

OCCURRENCE AND ANTIMICROBIAL RESISTANCE PROFILE OF *Streptococcus canis* ISOLATES AMONG DOGS, CATS AND HUMANS

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Streptococcus canis (*S. canis*) is a multi-host zoonotic pathogen that causes disease in a wide range of mammals, including humans. Dogs and cats appear to be the primary hosts and may play a role in transmitting infection to humans as they commonly share the same environment. This study aimed to describe the occurrence of clinical cases of *S. canis* and antimicrobial susceptibility patterns in pet cats, dogs, and human. Data was retrieved from the bacteriology laboratory from the Faculty of Veterinary Medicine of Universiti Putra

Malaysia and the national data repository of Ministry of Health. A retrospective descriptive analysis was conducted for data from January 2019 to July 2023. A total of 32 canine, 95 feline and 42 human cases identified with *S. canis* infections. In cats and dogs, *S. canis* was mostly isolated from suppurative wounds, ear infections and septicaemia cases with multiple organs involvement. In contrast, *S. canis* was mainly isolated from blood of bacteraemia cases (45.2%) in human. *S. canis* remains highly susceptible to penicillin, cephalosporins, and amoxicillin-clavulanic acid among cats and dogs. High resistance level was observed against metronidazole, neomycin, and tetracycline, with resistance rates from 67% to 100%. Enrofloxacin, a commonly used antibiotic, exhibited a relatively low susceptibility rate of 29% in cats and 23% in dogs. *S. canis* isolates from human cases remained high susceptible to active antibiotic options. However, tetracycline and erythromycin showed resistant rates of 73% and 22% respectively. Alarmingly, up to 54% of *S. canis* from cats and dogs demonstrated multidrug resistance. In conclusion, *Streptococcus canis* infection is an important pathogen in cats and dogs and emerging zoonotic infection in human health with increasing antimicrobial resistance pattern; best acknowledged by One Health framework. Further studies on drug resistance and therapeutic outcomes of human, dogs and cats are warranted.

Keywords: *Streptococcus canis*; antimicrobial resistance; antimicrobial susceptibility testing; canine; feline; zoonotic

1.0 INTRODUCTION

Streptococcus canis (*S. canis*), originally isolated from dogs, is now recognized to infect a variety of mammalian species including cats (Timoney *et al.*, 2017), dogs (Slaviero *et al.*, 2023), cattle (Eibl *et al.*, 2021), horses (Pinho *et al.*, 2013), rats (Corning *et al.*, 1991), sea lions (Numberger *et al.*, 2021), and even humans (Lam *et al.*, 2007). In cats and dogs, *S. canis* typically colonizes mucosal surfaces as an opportunistic pathogen (Ling & Ruby, 1978; Timoney *et al.*, 2017). These bacteria belong to the β-hemolytic Streptococcus group and can form biofilms during infections. It is worth noting that the actual prevalence of *S. canis* infections might be underestimated, as Lancefield Group G streptococci are not routinely identified at the species level, particularly in human infections (Galpérine T *et al.*, 2007).

Despite its wide host range, there is limited scientific literature exploring *S. canis*' virulence mechanisms, population structures, and antimicrobial resistance (AMR) mechanisms (Pagnossin *et al.*, 2022). A study in Japan has shown an increasing trend in antimicrobial resistance profiles among *S. canis* strains between 2015 and 2017 (Fukushima *et al.*, 2019). Currently, there is a dearth of research on the prevalence and antimicrobial resistance profiles of *S. canis* in small animals and humans, despite its zoonotic potential and emerging antimicrobial resistance issues. Thus, this study aims to highlight the significance of *S. canis* in small animal and human medicine, besides providing clinicians with data for informed clinical decision-making.

In Malaysia, *S. canis* infections are underreported especially in humans, and the data generated from this study will contribute to the epidemiological understanding of *S. canis* infections among dogs, cats, and humans. In fact, this will be the inaugural report on clinical cases of *S. canis* in humans in Malaysia. By investigating antimicrobial profiles, baseline information on antimicrobial resistance characteristics of this bacterium can be established. However, it is important to acknowledge the limitations inherent in retrospective studies, including potential bias in the antibiotics tested due to clinician preferences.

The aims of this study are to (1) determine the occurrence and describe the characteristics of clinical cases of *S. canis* infection in dogs and cats diagnosed at the Bacteriology Laboratory of Faculty of Veterinary Medicine of University Putra Malaysia from 2019 to July 2023; (2) determine the occurrence and describe the characteristics of clinical cases of *S. canis* infection in human patients presented to the Ministry of Health (MOH) Hospitals in Malaysia between 2019 to July 2023; and (3) describe the antimicrobial susceptibility profile and AMR status of *S. canis* isolates from dogs, cats, and humans.

The hypotheses of this study are:

1. *S. canis* is commonly isolated from pyogenic wounds in human, dog, and cat clinical cases.
2. *S. canis* has no multi-resistant drug (MDR) property.

2.0 LITERATURE REVIEW

2.1 Introduction to *Streptococcus canis*

2.1.1 Characteristics of *Streptococcus canis*

In terms of taxonomy, *S. canis* belongs to the phylum Bacillota, the class Bacilli, the order Lactobacillales, the family Streptococcaceae and the genus Streptococcus. This bacterium shows Gram-positive characteristics under microscopic examination and presents as cocci chains after Gram staining. The Lancefield grouping categorizes *S. canis* as a member of the group G streptococci, primarily based on the antigenicity of its cell wall carbohydrates (Yoshida *et al.*, 2021).

Biochemically, *S. canis* is catalase negative, not resistant to 6.5% NaCl or 40% bile, and can ferment glucose. In terms of carbohydrate fermentation, it can metabolize lactose, but does not ferment sorbitol and trehalose generally. This helps distinguish it from other streptococci, such as *Streptococcus dysgalactiae*. After incubation at 37 ℃ on blood agar for 24 hours, *S. canis* typically forms medium-sized, hemolytic colonies that have circular shape with entire edges, and a diameter of 1–2 mm. *S. canis* is a facultative anaerobic bacterium that grows well at a temperature of 37°C. According to a study, various biochemical tests give specific results: the test is negative for hippurate, fibrinolysin, tyrosine, starch hydrolysis, Voges-Proskauer and pyrrolidonylarylamidase reactions (Devriese *et al.*, 1986). Conversely, it is positive in L-aminopeptidase, alkaline phosphatase, leucine arylamidase and arginine dehydrolase tests. Most strains produce p-galactosidase, although only a few strains are positive for hyaluronidase and beta-glucuronidase. Responses to galactosidase tests vary. However, these tests are not routinely performed in the Veterinary Bacteriology Laboratory at the Faculty of Veterinary Medicine of UPM.

The presence of a novel plasminogen (PLG) receptor or also known as *S. canis* M protein (SCM), is suggested to be one main contributor of the pathogenicity (Fulde *et al.*, 2011; Pagnossin *et al.*, 2022). Detailed biochemical analysis has demonstrated that SCM exhibits strong binding affinity for mini-plasminogen (mPLG), a particular segment of PLG (Fulde *et al.*, 2011). The conversion of PLG, which is attached to the bacterial surface, into plasmin is orchestrated by PLG activators originating from the host (Fulde *et al.*, 2011, 2013). This ability enables *S. canis* to degrade extracellular matrix molecules and dissolve fibrin thrombi, contributing to tissue damage and immune evasion. By having PLG bound to its surface, *S. canis* can effectively avoid entrapment within blood clots in the host's blood vessels and this facilitates the bacterium in spreading to deeper tissues within the host. Moreover, the interaction with PLG has been conclusively demonstrated to provide substantial defense against phagocytosis by polymorphonuclear granulocytes (Fulde *et al.*, 2013).

In 2017, a study demonstrated the ability of SCM to bind with immunoglobulin G (IgG) , which is also suggested to contribute to the anti-phagocytotic properties of *S. canis* (Bergmann *et al.*, 2017). In short, the SCM-PLG and SCM-IgG interaction in *S.*

canis play a crucial role in its pathogenicity by enabling tissue damage, immune evasion, and dissemination within the host's body.

2.1.2 Overview of *S. Canis* as a normal flora and opportunistic pathogen

S. canis is a component of the natural microorganisms inhabiting the mucosal surfaces of dogs and cats, a condition referred to as normal flora *(*Ling & Ruby, 1978; Timoney *et al.*, 2017). This implies that the bacterium can be regularly found in healthy individuals. In the study conducted in 2017, *S. canis* was detected in both the upper respiratory and reproductive tracts of healthy cats (Timoney *et al.*, 2017). In dogs, *S. canis* can be found in oral cavity of healthy dogs (Imanishi *et al.*, 2022), and was notably prevalent in samples obtained from the reproductive tract, particularly in the vagina (Ling & Ruby, 1978; Kustritz, 2006) . Moreover, *S. canis* was reported to be recovered from skin including external ear canal, and mucosal surfaces of conjunctiva, vagina, and oral cavity (Dégi *et al.*, 2012, Cloet *et al.*, 2023).

In addition to companion animals, *S. canis* has been identified as part of the normal flora in the genital tracts of wild cats, including lions and leopards, as documented by Callealta *et al.* (2018). Furthermore, the bacterium has been found as part of the integumentary system in mink, as reported by Chalmers *et al.* (2015). In aquatic environments, *S. canis* has also been detected in the nasal cavities of two healthy Hawaiian monk seals, a type of marine mammal, as reported by Numberger *et al.* (2021). While it typically resides as part of the normal flora, *S. canis* can act as an opportunistic pathogen. It has been documented to cause infections and diseases in various mammalian species, including cats (Timoney *et al.*, 2017), dogs (Slaviero *et al.*, 2023), cattle (Eibl *et al.*, 2021), horses (Pinho *et al.*, 2013), rats (Corning *et al.*, 1991), sea lions (Numberger *et al.*, 2021), and even humans (Lam *et al.*, 2007).

2.2 *Streptococcus canis* in Dogs, Cats and Human

2.2.1 Epidemiology

A study by Kustritz (2006) reported *S. canis* as the second most frequently isolated bacteria from both the vagina of normal bitches and female dogs with vaginitis. A journal published by Pesavento *et al.* (2007) documented three devastating outbreaks of *S. canis* infections in cat shelters in the United States. These outbreaks are estimated to have impacted more than 150 cats, resulting in a distressing mortality rate of 30%. Remarkably, the journal noted that *S. canis* was the predominant and often the sole pathogen isolated in the affected cases. Additionally, Lysková *et al.* (2007) reported that 6.5% of healthy dogs (out of a total of 539 specimens) and 5.9% of healthy cats (out of a total of 169 specimens) tested positive for the presence of *S. canis*. This bacterium was primarily isolated from the rectum in both species, as well as from the preputium of dogs and the oral cavity of cats. Furthermore, within the same study, *S. canis* was identified at various body sites in 22.2% of dogs (out of a total of 176 specimens) and in 4.8% of cats (out of a total of 42 specimens) with active infections.

Between 2015 and 2017, *S. canis* was reported to be the third most common bacterial species isolated in cats and dogs in a public veterinary diagnostic laboratory at a university in Malaysia (Haulisah *et al.*, 2022). The study revealed that among the total

467 isolates, *S. canis* accounted for 9.4%, ranking just behind *Staphylococcus pseudintermedius* (14.3%) and *Escherichia coli* (13.9%). Interestingly, the frequency of *S. canis* isolates in dogs was not as prominent as in cats in the study. In a similar study conducted by Yudhanto *et al.* (2022), an extensive analysis of 803 isolates derived from 2,583 canine urine samples, submitted to the Veterinary Diagnostic Laboratory at the University of Illinois between 2019 and 2020 for suspected urinary tract infections, was undertaken. Intriguingly, the findings revealed that *S. canis* once again held the position of the third most prevalent Gram-positive isolate, constituting 6.10% of the total isolates. It trailed just behind *Staphylococcus pseudintermedius*, which accounted for 17.93%, and *Enterococcus faecalis*, which represented 9.46% of the isolates (Yudhanto *et al.*, 2022).

In another research by Imanishi *et al.* (2022), it was reported that between August 2016 and March 2020, among 546 cases of gram-positive cocci-associated deep pyoderma in dogs from animal hospitals in Japan, 27 cases (approximately 5%) were attributed to *S. canis* infections. Recently, Cloet *et al.* (2023) conducted a study at the Veterinary Medical Centre, University of Saskatchewan, Canada where 118 samples from the normal and abnormal ocular surfaces of 59 dogs were swabbed for culture. The findings revealed a prevalence rate of 6.8% for *S. canis*. In summary, *S. canis* was evidently a well-known and not uncommon name in the context of companion animals.

In humans, however, the situation was different, as *S. canis* infections are typically considered rare events. Nonetheless, sporadic reports have surfaced, encompassing a spectrum of severity, mainly from severe to life-threatening conditions.

Notably, species identification tends to be conducted only when the situation has escalated to a critical level. For example, there were victims of *S. canis* infection reported worldwide since 1997 till now (Amsallem *et al.*, 2014; Bert & Lambert-Zechovsky, 1997; Lacave *et al.*, 2016; Mališová *et al.*, 2019; Taniyama *et al.*, 2017). Although there is a lack of extensive research on the prevalence of *S. canis* infections in humans, a recent retrospective descriptive study conducted at a University Hospital in northern Spain has shed some light on the matter. This study by Fernández Vecilla & Díaz De Tuesta Del Arco (2022), identified a total of 39 human patients with positive cultures of *S. canis*. These cases encompassed instances where *S. canis* was isolated either as the sole culture or in combination with other microorganisms. However, it is worth noting that the actual number of *S. canis* infections in humans may be underestimated as Lancefield G is not routinely identified at the species level (Galpérine *et al.*, 2007; Tsuyuki *et al.*, 2017).

2.2.2 Clinical Manifestations of Infections in Cats and Dogs

In dogs and cats, *S. canis* infections can exhibit as diverse clinical conditions, ranging from localized skin and mucosal infections to life-threatening conditions, such as toxic shock syndrome, necrotizing fasciitis , septicaemia, and meningitis (Ling & Ruby, 1978; Iglauer *et al.*, 1991; DeWinter & Prescott, 1999; Lysková *et al.*, 2007; Sura *et al.*, 2008; Cloet *et al.*, 2023). For example, Pesavento *et al.* (2007) reported on cases of *S. canis* infection in the context of three separate outbreaks in different cat shelters, which manifested in various ways. Some affected cats displayed symptoms such as skin ulceration and chronic respiratory infections that, in certain instances, progressed to more severe conditions like necrotizing sinusitis and meningitis. In one of the shelters, there was a rapid progression of the infection, evolving from necrotizing fasciitis with skin ulceration to toxic shock-like syndrome, sepsis, and ultimately, fatal outcomes. These clinical manifestations underscore the seriousness and diversity of *S. canis* infections in feline populations. Additionally, a noteworthy case was documented in a dog by Slaviero *et al.* (2023), where prostatitis attributed to *S. canis* evolved into endocarditis. This complex infection further led to the development of splenic, renal, and cerebral thromboembolism. Intriguingly, this canine case was associated with a Sertoli cell tumour in a cryptic testis and diffuse prostatic squamous metaplasia, highlighting the multifaceted nature of *S. canis* infections and their potential complications in veterinary medicine.

2.2.3 Clinical Manifestations of Infections in Humans

In humans, *S. canis* have been isolated from infections, and clinical manifestations include pneumonia, endocarditis, cellulitis, bacteraemia, arthritis, urinary and bone infections (Bert & Lambert-Zechovsky, 1997; Galpérine *et al.*, 2007; Lam *et al.*, 2007; Amsallem *et al.*, 2014; Taniyama *et al.*, 2017; Mališová *et al.*, 2019; Fernández Vecilla & Díaz De Tuesta Del Arco, 2022). In the retrospective study conducted by Galpérine *et al.* (2007), spanning from 1997 to 2002 and involving 54 human patients from University Hospital in Bordeaux, *S. canis* accounted for 1% of all the isolated streptococci. The clinical manifestations were comprehensively reviewed, revealing that all but eight of the patients exhibited symptoms. These symptoms included soft tissue infections in 35 cases, bacteraemia in 5 cases, urinary tract infections in 3 cases, bone infections in 2 cases, and pneumonia in 1 case.

Hence, these findings underscore the clinical significance of *S. canis* infections in both veterinary and human medicine, emphasizing the need for a thorough understanding of their implications.

2.3 AMR Patterns of *Streptococcus canis*

2.3.1 AMR Pattern in Cats and Dogs

AMR in bacteria has been a growing concern for several decades, particularly as the use of antibiotics became widespread in healthcare and agriculture during the mid-20th century. The emergence of resistant bacterial strains and the looming threat of widespread drug ineffectiveness have made this issue a continuously evolving and critically important subject in the fields of medicine, microbiology and public health. As the spotlight increasingly turns toward *S. canis*, its antimicrobial resistance patterns have become a pertinent and essential topic of discussion within this broader context.

In a study conducted by Tsuyuki *et al.* (2017) in a Japan laboratory, which involved 68 *S. canis* isolates obtained from dogs and cats during a two-month period in 2015, patterns of antimicrobial resistance were observed. Specifically, 16 isolates, amounting to 23.5% of the total, exhibited resistance to tetracycline and/or clarithromycin. Additionally, four isolates (5.9%) demonstrated resistance to clindamycin, while two isolates (2.9%) displayed resistance to levofloxacin. Remarkably, none of the strains exhibited resistance to β-lactam antibiotics.

Imanishi *et al.* (2022) investigated antimicrobial resistance patterns in *S. canis* within the context of canine deep pyoderma. They found that a small fraction of strains, constituting 6%, displayed resistance to penicillin, specifically penicillin G and amoxicillin. None of the strains exhibited resistance to amoxicillin with clavulanic acid, which was an encouraging finding. In stark contrast, quinolones, such as enrofloxacin (57%), marbofloxacin (36%), levofloxacin (57%), and gatifloxacin (53%), were met with relatively high resistance rates. Additionally, the strains exhibited resistance to lincosamides, with clindamycin (43%) and lincomycin (32%) showing notable resistance percentages, along with resistance to aminoglycosides like gentamicin (42%) and sulfamethoxazole with trimethoprim sulfadiazine (51%). Tetracyclines encountered resistance as well, with doxycycline (57%), minocycline (25%), and tetracycline (57%) displaying varying resistance rates.

Resistance to cephems (cephalexin (6%) and cefovecin (28%)), macrolides (erythromycin (26%) and kanamycin (28%)), and fosfomycin (25%) was also identified. Strains resistant to anti-Methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, like linezolid and vancomycin, although uncommon in veterinary medicine, were found in the range of 2% to 6%. A striking observation was the presence of MDR strains, with 70% of the strains, specifically 37 out of 53, displaying resistance to three or more antibiotic classes (Imanishi *et al.*, 2022).

In the study by Yudhanto *et al.* (2022), the *Streptococcus canis* isolates from canine urine samples exhibited striking patterns of antimicrobial resistance, with a notably high prevalence of resistance to enrofloxacin (65.3%) and marbofloxacin (55.1%). Additionally, a moderate prevalence of resistance was observed for erythromycin (10.2%) and clindamycin (10.2%). There was no observed resistance to cephalosporins, vancomycin, penicillin, and ampicillin within the studied isolates.

Moreover, Haulisah *et al.* (2022) examined the resistance patterns of 62 *S. canis* isolates obtained from cats between 2015 and 2017, in a bacteriology laboratory in Malaysia. The findings revealed high resistance levels for gentamicin (66.7%) and enrofloxacin (58.8%). An interesting finding was the increase in isolates categorized as 'intermediate' for amoxicillin with clavulanic acid, rising from 13.3% in 2015 to 22.2% in 2017. However, *S. canis* consistently demonstrated high susceptibility (90.7%) to amoxicillin with clavulanic acid over the three-year period. Additionally, the study reported a relatively low rate of multidrug resistance at 9.1% (4 out of 44 isolates).

In a study conducted by Stefańska *et al.* (2022), antimicrobial susceptibility test (AST) was conducted on a selection of clinically relevant antibiotics. The results revealed substantial resistance among *S. canis* isolates, with 66.2% demonstrating resistance to tetracycline, and 55.4% showing resistance to both erythromycin and clindamycin. However, in line with previous research findings, there is a positive note in the fact that 93.9% of the isolates remained susceptible to β-lactam antibiotics.

2.3.2 AMR Pattern in Humans

In humans, a retrospective study of 54 cases of *S. canis* infections, spanning from 1997 to 2002, reported an emergence resistance to macrolides, penicillin G and gentamicin (Galpérine *et al.*, 2007). Nevertheless, the study shows that almost all strains were sensitive to penicillin G apart from four strains (7%) categorized as 'intermediate'. Only one strain that was isolated from skin samples, was highly resistant to gentamicin. Regarding macrolides and related drugs, the study identified 12 strains (22%) that displayed isolated resistance to erythromycin. In contrast, all strains remained sensitive to pristinamycin and glycopeptides. Only one strain was rifampin-resistant and 22 strains (40.7%) exhibited resistance to tetracycline.

In a set of case reports focusing on ulcer infections, *S. canis* strains were found to be generally susceptible to multiple antibiotics, including erythromycin, vancomycin, imipenem, clindamycin, penicillin G, ceftriaxone, gatifloxacin and rifampin (Lam *et al.*, 2007). It is worth noting, that one of the isolates from two patients displayed resistance to gentamicin, while the other remained susceptible. Moreover, a noteworthy study delved into a case of *S. canis* bacteraemia in a human patient and conducted comprehensive antimicrobial susceptibility assays on the isolated strain (Taniyama *et al.*, 2017). The results unveiled that the *S. canis* isolate displayed susceptibility to an array of antibiotics, including penicillin, cephalosporin, tetracyclines, glycopeptides, lincomycins, sulfamethoxazole/trimethoprim, chloramphenicol, and fluoroquinolones. However, the susceptibility to macrolides was somewhat varied, as the isolate exhibited susceptibility to erythromycin but demonstrated intermediate susceptibility to azithromycin. These studies shed light on the diverse antimicrobial susceptibility profiles of *S. canis* in human infections over the years, highlighting the need for continued monitoring and research in this area.

In veterinary and human medicine, limited information is available regarding the antimicrobial resistance patterns of *S. canis*. While these studies provide valuable insights, further research is needed to comprehensively understand the antimicrobial resistance patterns of *S. canis* in veterinary and human infections.

2.4 Transmission dynamics and zoonotic potential

2.4.1 Potential Transmission Routes between Animals and Humans

S. canis is known to affect multiple host species, and one piece of suggested molecular evidence is the presence of species-unspecific SCM-IgG interaction, attributed to the highly conserved binding region of SCM proteins in *S. canis* isolates (Bergmann *et al.*, 2017). Due to the relatively low frequency of *S. canis* infections in humans compared to other pathogens, doubts have arisen regarding its capacity to affect humans. A study in 2013 has confirmed the zoonotic potential of *S. canis* by demonstrating identical genetic lineages among *S. canis* strains that infect both domestic pets and humans, employing multi locus sequence typing and pulsed-field gel electrophoresis (Pinho *et al.*, 2013). This study also highlights several prominent lineages of *S. canis* found in household pets, which have the capability to induce invasive infections in humans.

S. canis infections in humans are not common, there have been several case reports that suggest potential transmission route from pets to humans. In many of these reported cases, a common piece of information gathered from patient histories is previous contact with pets, with or without a history of bites (Bert & Lambert-Zechovsky, 1997; Amsallem *et al.*, 2014; Taniyama *et al.*, 2017; Mališová *et al.*, 2019; Fernández Vecilla & Díaz De Tuesta Del Arco, 2022; Van Tol *et al.*, 2022). As a result, direct contact with infected pets is considered the most straightforward way for *S. canis* to potentially transfer from pets to humans. This direct contact can involve activities like petting, grooming, or experiencing scratches or bites from an infected animal. Any breach in the skin during these interactions can potentially allow the bacteria to enter the human body, leading to infection. However, it is important to note that the exact transmission routes of *S. canis* from pets to humans have yet to be thoroughly studied, and currently, there is limited research or literature that focuses on this specific topic. Therefore, further evidence and research are needed to establish and support this proposed transmission route.

2.4.2 Zoonotic risk and Public Health Implications

There are several publications that suggest a possible zoonotic link. For instance, a 77-year-old man developed septicaemia caused by *S. canis*, and it was presumed that the organism was transmitted from his pet dog. The ulcers on his lower limbs were identified as likely entry points for the infection (Bert & Lambert-Zechovsky, 1997). Additionally, in Singapore, a man with a traumatic wound became infected with *S. canis* after coming into contact with his pet dog, a Siberian Husky (Tan *et al.*, 2016). A more recent case reported in 2020 involved an immunocompromised woman who developed severe cellulitis and bacteraemia due to *S. canis* infection after close contact with her pet dog (Lederman Z. *et al.*, 2020).

In the study conducted by Fernández Vecilla & Díaz De Tuesta Del Arco (2022), they suggested the presence of risk factors for *S. canis* infections, including immunosuppression and underlying diseases such as diabetes mellitus, cancer, HIV, haematological pathology, and the use of corticoid drugs. Besides, their study, which involved thirty-nine patients, found that the mean age was 51.2 years, with a median age of 51 years. Interestingly, when reviewing other case reports, it becomes apparent that all *S. canis* infection victims were over the age of 50. Therefore, the role of age as a potential risk factor remains a topic that warrants further investigation and scrutiny.

Indeed, given the confirmed zoonotic potential and the potentially serious consequences as mentioned, it is imperative for society, particularly individuals who frequently interact with animals, to remain vigilant about the associated risks. To minimize the risk of transmission, maintaining proper hygiene is paramount. This involves consistent handwashing, especially after handling pets, cleaning their living spaces, or if you've experienced scratches or bites from an animal.

3.0 MATERIALS AND METHODS

3.1 Data Collection

3.1.1 Study Design

The data from canine and feline species for this study were originated from routine diagnostic case samples submitted to the accredited Bacteriology Laboratory at the Faculty of Veterinary Medicine, UPM from various veterinary health premises and animal facilities in central Peninsular Malaysia. All the cases with positive *S. canis* isolates, from 1 January 2019 to 31 July 2023, were identified from the log books, reports were compiled, and data were inputted into Microsoft Excel Spreadsheet. Retrospective analysis was performed.

3.1.2 Cats and Dogs Data

From all the identified cases, the collected data include the following: animal ID, year of diagnosis, species, breed, age, sex, neuter status, type of clinical samples (swab, fluid, tissue, urine), clinical manifestation, sample diagnosed with single isolate or in polymicrobial sample, first or second-time culture is performed from the same animal and AST results.

3.1.3 Human Data

All the data were sourced from the Institute for Medical Research (IMR). Nation-wide Ministry of Health (MOH) hospital patient AST data between January 2019 and July 2023 were acquired from the local surveillance program in Malaysia. These data were organized using a downloadable database software developed by the World Health Organization (WHO) known as WHONET. This program homogenizes diverse data from distinct laboratory information systems utilized by individual hospitals, establishing a uniform code and file format. This standardization streamlines the process of centralized data monitoring and analysis. The data were mainly contributed by Malaysian hospitals participating in the WHONET-Malaysia network and consistently providing laboratory data throughout the year. This dataset included information such as the year of diagnosis, types of samples, demographic data, isolation as single or multiple microorganisms and AST results.

3.2 Consent

For the human clinical cases, approval was sought from the Institute for Medical Research (IMR) to have access to the human clinical isolate database from MOH hospitals in Malaysia. Personal information pertaining to the individual human patients were not available. For the animal cases, consent from the Bacteriology Laboratory of the Faculty of Veterinary Medicine was sought to get access to laboratory reports and logbooks while the University Veterinary Hospital (UVH) of Universiti Putra Malaysia

(UPM) was sought for approval to retrieve clinical data on the positive cases involving cats and dogs.

3.3 Data Tabulation and Statistical Analyses

Data collected was tabulated using Microsoft Excel spreadsheet. Data of each categorical variable was subjected to descriptive analysis. Most of the data is presented in frequencies and percentages. AMR status was determined by analysing the AST data where any isolate with resistance to three or more classes of antibiotics is considered as an MDR isolate, following the definition provided in the published journal by Magiorakos *et al.* (2012). Categorical data comparison between cats and dogs, and humans was done using the Chi-squared test where SPSS version 26 was used for the statistical analysis, where p value of less than 0.05 was considered statistically significant.

4.0 RESULTS

4.1 Demographic data for cats and dogs

A total of 95 *S. canis* isolates were identified in 81 cats, and 32 *S. canis* isolates in 30 dogs. The frequency of cases in each year is listed in Figure 1. In the feline cases, the age range spanned from one month to 15 years old, while in canine cases, the age ranged from three months to 14 years old. The distribution of breeds of cats and dogs is presented in Table 1a and 1b. The distribution of sex and neuter status is depicted in the pie charts shown in Figure 2a and 2b.

Table 1a: Cat breed distribution

Breeds	**Frequency**	**Percentage**
DSH	49	60.5%
MIX	10	12.3%
PERSIAN	8	9.9%
MAINE COON	5	6.2%
SPHYNX	2	2.5%
AMERICAN CURL	1	1.2%
AMERICAN SHORTHAIR	1	1.2%
BENGAL	1	1.2%
BRITISH SHORTHAIR	1	1.2%
HIMALAYAN	1	1.2%
LEOPARD CAT	1	1.2%
MUNCHKIN	1	1.2%
Total	**81**	**100.0%**

Table 1b: List with dog breeds

Breed	**Frequency**	**Percentage**
LOCAL & MIX	9	30.0%
GERMAN SHEPHERD	4	13.3%
ROTTWEILER	2	6.7%
BEAGLE	2	6.7%
PUG	2	6.7%
AMERICAN BULLY	1	3.3%
AMERICAN PITBULL	1	3.3%
BULLDOG	1	3.3%
FRENCH BULLDOG	1	3.3%
GOLDEN RETRIEVER	1	3.3%
LABRADOR RETRIEVER	1	3.3%
POMERANIAN	1	3.3%
POODLE	1	3.3%
SHIH TZU	1	3.3%
SIBERIAN HUSKY	1	3.3%
UNIDENTIFIED	1	3.3%
Total	**30**	**100.0%**

4.2 Demographic data for humans

A total of 42 *S. canis* isolates were identified in 42 human patients. The frequency of cases in each year is illustrated in Figure 1, alongside the data for cats and dogs. In the human cases, the age ranged from 35 to 82 years old. Both males and females were equally represented among the human patients, with 12 individuals in each sex, while 18 cases had unidentified sex due to limited data access.

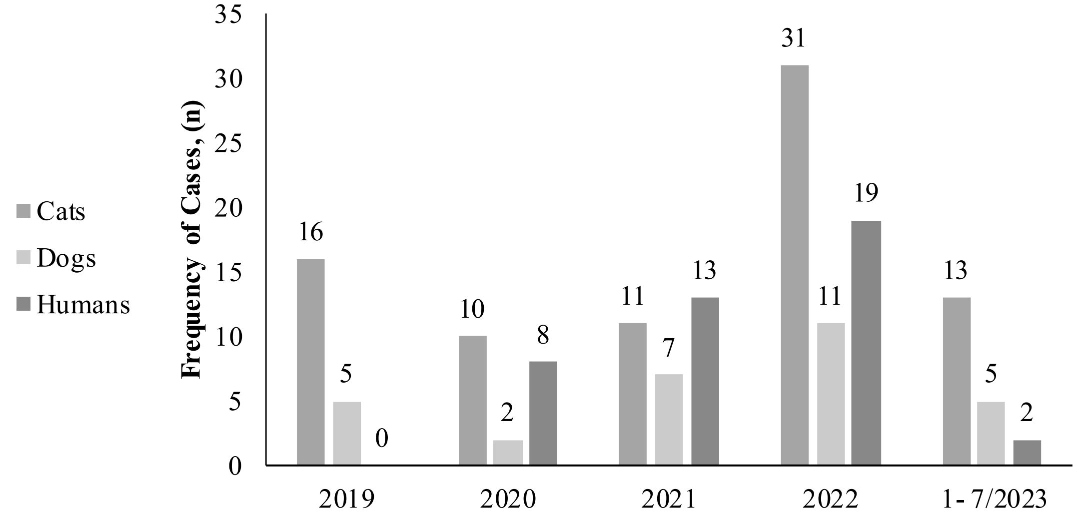
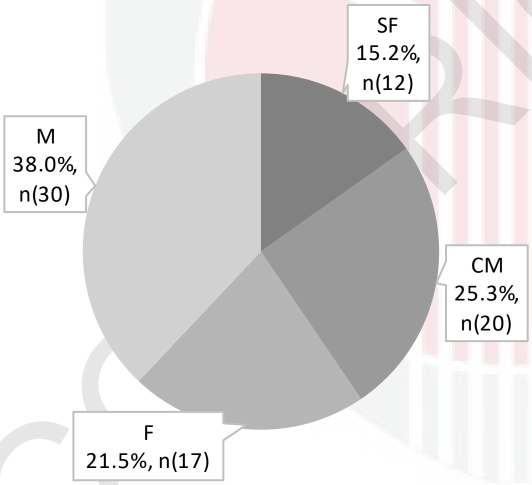


Figure 1: Frequency of isolates from Jan 2019 to July 2023



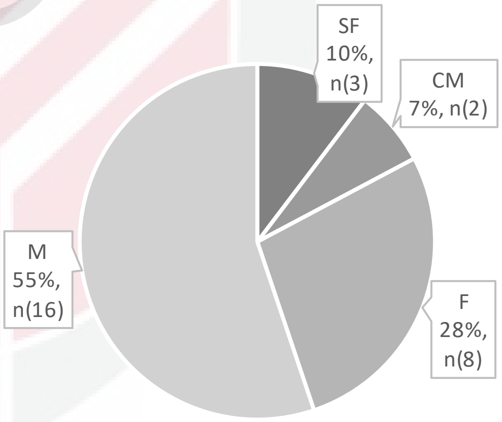


Figure 2a: Sex and neuter status distribution (cats). M, males; CM, Castrated males; F, Females; SF, Spayed females

Figure 2b: Sex and neuter status distribution (dogs). M, males; CM, Castrated males; F, Females; SF, Spayed females

4.3 Types of clinical samples

In cats, the most frequent site where *S. canis* was isolated include wound or abscess (49.4%), followed by ears (18.5%) and the multiple organ samples obtained during post-mortem examinations (11.1%). *S. canis* was identified in a total of 70 clinical cases and 11 post-mortem cases among cats.

Among dogs, samples from the ears were the most common (40.0%), followed by wounds or abscess (33.3%), and the respiratory tract (10.0%). *S. canis* was identified in a total of 27 clinical cases and 3 post-mortem cases among dogs.

Table 2: Summary of anatomical sites with *S. canis* isolations in cats and dogs

Anatomical Sites	**Cats**		**Dogs**	
	Frequency	**%**	**Frequency**	**%**
Wound/ Abscess	41	49.4	10	33.3
Ear	15	18.5	12	40.0
Multiple Organs	9	11.1	1	3.3
Respiratory Tract	8	8.9	3	10.0
Bone/ Joint	4	4.9	0	0
Urinary tract	3	3.7	0	0
Eye	1	1.2	0	0
Oral cavity	1	1.2	0	0
Gastrointestinal Tract	0	0.0	2	6.7
Reproductive Tract	0	0.0	2	6.7
Total patients (n)	81	100	30	100

Table 3: Types of specimens from humans

Type of specimen	**Number of *S. canis* isolates**	**%**
Blood	19	45.2
Tissue	11	26.2
Pus	7	16.7
Aspirate	1	2.4
Pleural fluid	1	2.4
Swab	1	2.4
Tracheal aspirate	1	2.4
Urine	1	2.4
Total	42	100

4.4 Single and Polymicrobial Culture

Figure 3 shows that, among the *S. canis* isolates identified, 64.2% and 75.0% were recovered in a polymicrobial culture in cats and dogs, respectively. In humans, 100.0% of *S. canis* was identified in a pure culture. Table 4, Figures 4a and 4b show the types of bacteria that are isolated along *S. canis* in the polymicrobial samples from cats and dogs.

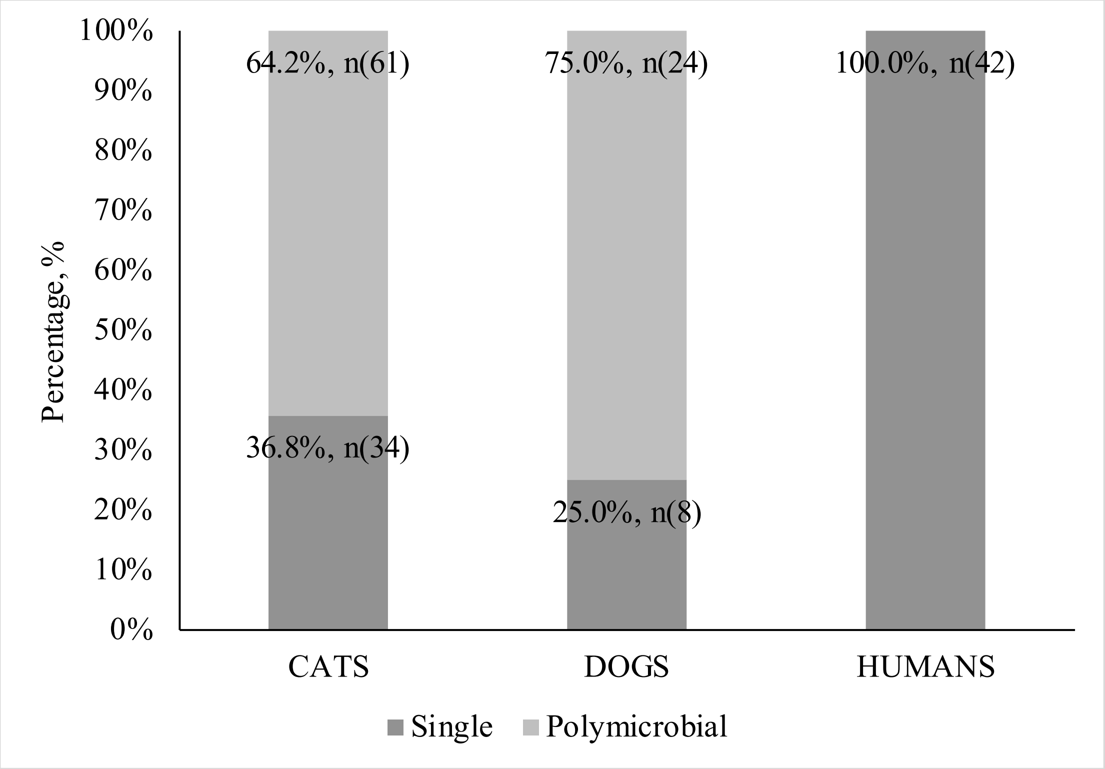


Figure 3: Proportion of single and polymicrobial isolates of *S. canis* isolates

Table 4: Bacteria isolated alongside *S. canis* from polymicrobial culture (cats & dogs)

	Cats		Dogs	
Bacteria isolated	**Number of Isolates**	**Percentage**	**Number of Isolates**	**Percentage**
Gram-positive				
Staphylococcus pseudintermedius	21	23%	2	4%
Enterococcus faecalis	7	8%	2	4%
Staphylococcus intermedius	6	7%	7	14%
Staphylococcus aureus	3	3%	-	-
Corynebacterium sp.	3	3%	1	2%
Rhodococcus equi	1	1%	-	-
Actinomyces sp.	1	1%	-	-
Streptococcus viridans	1	1%	-	-
non faecalis Enterococcus spp.	1	1%	-	-
Subtotal	**44**	**48%**	**12**	**24%**
Gram-negative				
Klebsiella pneumoniae	11	12%	4	8%
Pasteurella multocida	10	11%	-	-
Pseudomonas aeruginosa	9	10%	11	22%
Escherichia coli	9	10%	7	14%
Chromobacterium sp.	3	3%	1	2%
Enterobacter aerogenes	2	2%	1	2%
Acinetobacter baumanii	2	2%	1	2%
Proteus mirabilis	1	1%	8	16%
Citrobacter freundii	1	1%	-	-
Streptococcus dysgalactiae ss equisimilis	-	-	2	4%
Proteus vulgaris	-	-	1	2%
Aeromonas sp.	-	-	1	2%
Subtotal	**48**	**52%**	**37**	**76%**
Total (N)	**92**	**100%**	**49**	**100%**

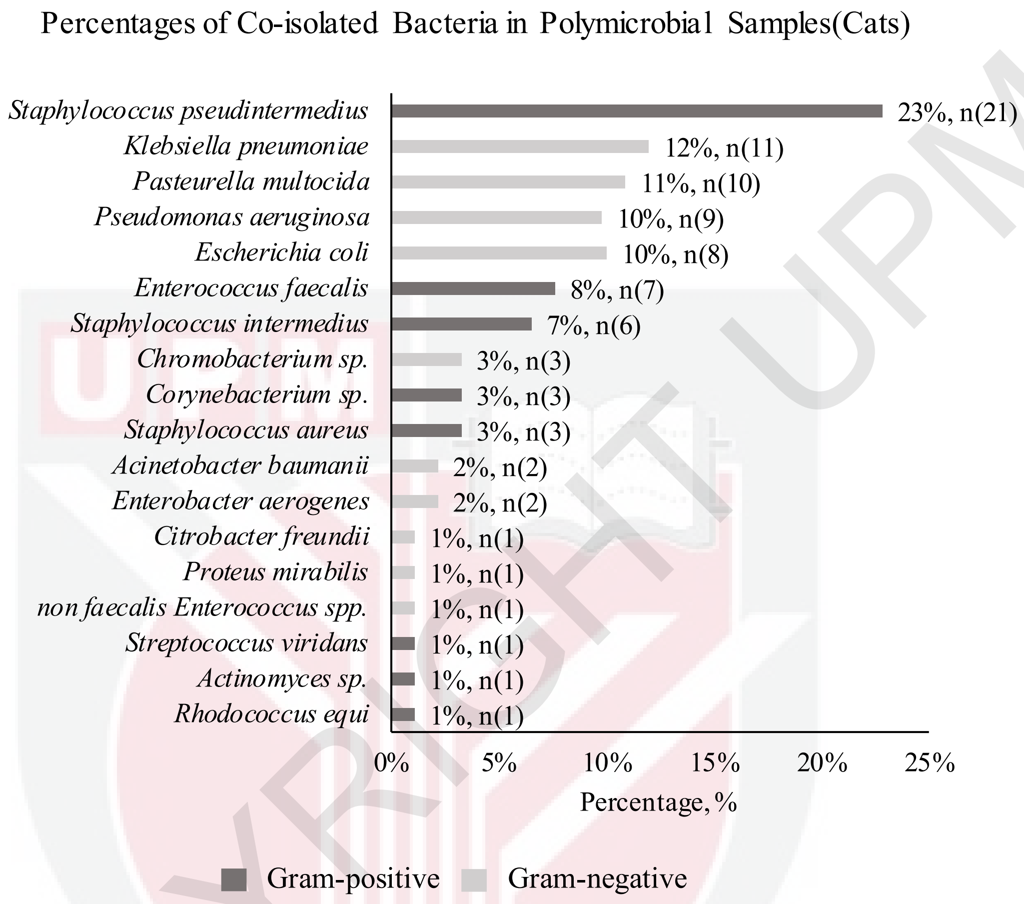


Figure 4a: Percentages of co-isolated bacteria in polymicrobial samples (cats)

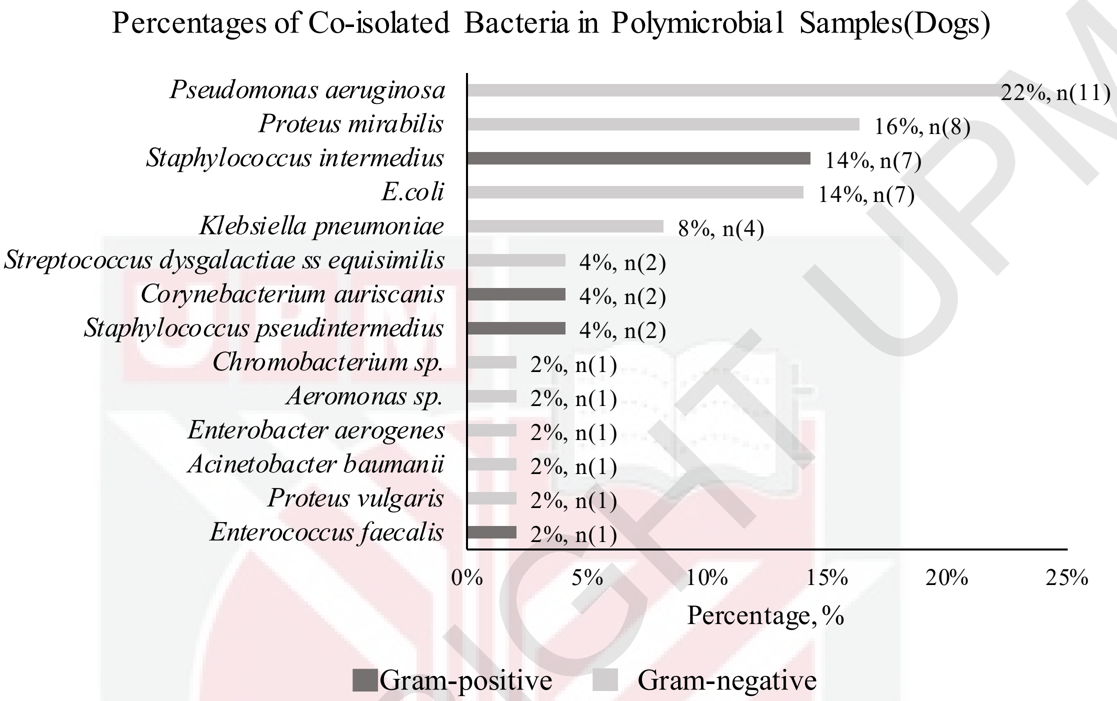


Figure 4b: Percentages of co-isolated bacteria in polymicrobial samples (dogs)

4.5 Antimicrobial testing for isolates from cats

The AST results for *S. canis* isolates from cats are summarized in Figure 5a. The highest levels of resistance were observed against neomycin (100%), ampicillin (100%), metronidazole (92%), and polymyxin B (81%). The isolates displayed a high susceptibility rate to several antibiotics, including amoxicillin (100%), cephalexin (90%), cefixime (100%), amoxicillin with clavulanic acid (92%), chloramphenicol (86%), doxycycline (91%), and sulfamethoxazole with trimethoprim sulfadiazine (75%).

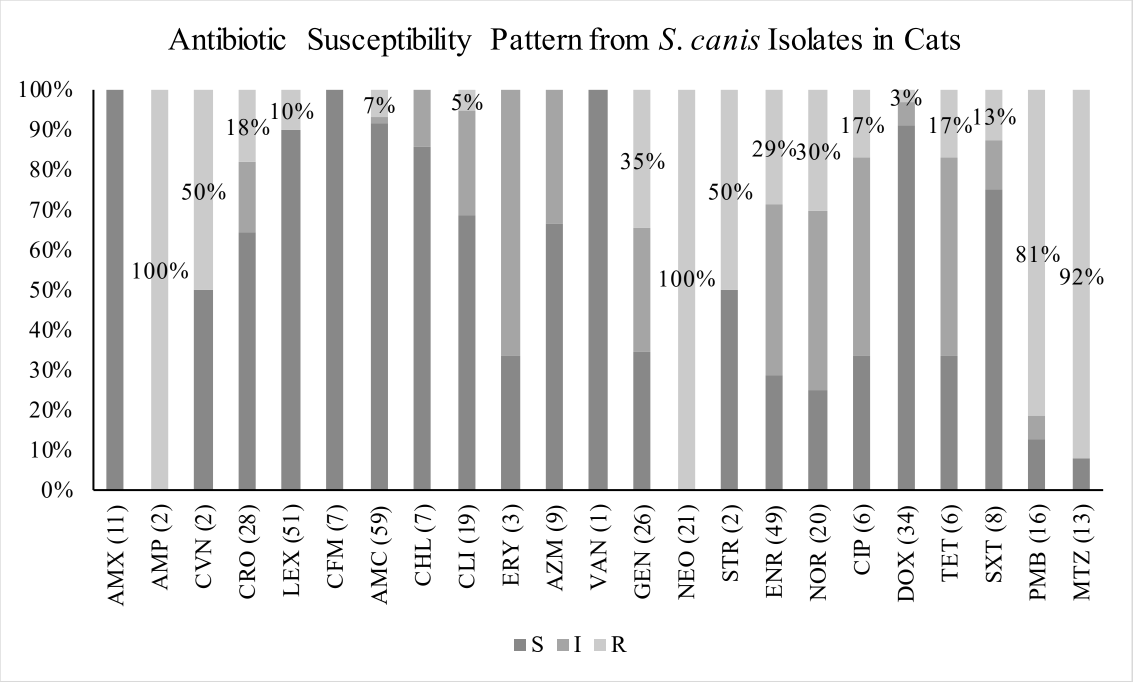


Figure 5a: Antibiotic susceptibility pattern from *S.canis* isolates from cats (2019–July 2023). S–susceptible; I-intermediate; R–resistance; AMX–amoxicillin; AMP–ampicillin; CVN–cefovecin; CRO–ceftriaxone; LEX–cephalexin; CFM–cefixime; AMC–amoxicillin with clavulanic acid; CHL–chloramphenicol; CLI–clindamycin; ERY–erythromycin; AZM–azithromycin; VAN–vancomycin; GEN–gentamicin; NEO–neomycin; STR–streptomycin; ENR–enrofloxacin; NOR–norfloxacin; CIP–ciprofloxacin; DOX–doxycycline; TET–tetracycline; SXT–sulfamethoxazole with trimethoprim sulfadiazine; PMB–polymyxin B; MTZ–metronidazole. Numbers inside brackets '()' indicate the total number of tested isolates for each antibiotic.

4.6 Antimicrobial testing for isolates from dogs

The AST results for *S. canis* isolates from dogs are summarized in Figure 5b. The highest levels of resistance were observed against metronidazole (100%), neomycin (78%) polymyxin B (81%) and tetracycline (67%). The isolates displayed a high susceptibility rate to several antibiotics, including amoxicillin (83%), cephalexin (79%), cefixime (100%), amoxicillin with clavulanic acid (91%) and chloramphenicol (100%),

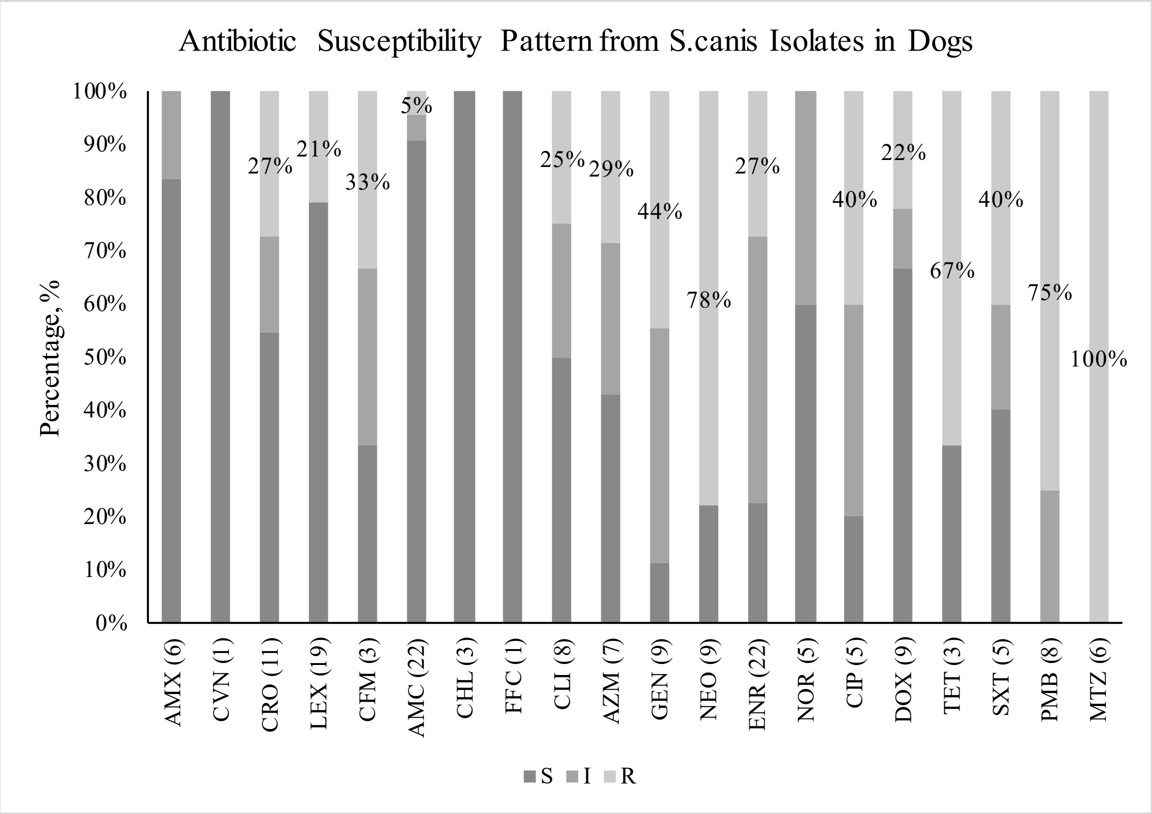


Figure 5b: Antibiotic susceptibility pattern from *S.canis* isolates from dogs (2019–July 2023). S–susceptible; I-intermediate; R–resistance; AMX–amoxicillin; CVN–cefovecin; CRO–ceftriaxone; LEX–cephalexin; CFM–cefixime; AMC–amoxicillin with clavulanic acid; CHL–chloramphenicol; CLI–clindamycin; AZM–azithromycin; GEN–gentamicin; NEO–neomycin; ENR–enrofloxacin; NOR–norfloxacin; CIP–ciprofloxacin; DOX–doxycycline; TET–tetracycline; SXT–sulfamethoxazole with trimethoprim sulfadiazine; PMB–polymyxin B; MTZ–metronidazole. Numbers inside brackets '()' indicate the total number of tested isolates for each antibiotic.

4.7 Antimicrobial testing for isolates from humans

The AST results for *S. canis* isolates from humans are presented in Figure 5c. The isolates exhibited relatively high susceptibility rates to most antibiotics commonly used in human medicine. However, tetracycline and erythromycin displayed non-susceptibility rates of 73% and 22%, respectively.

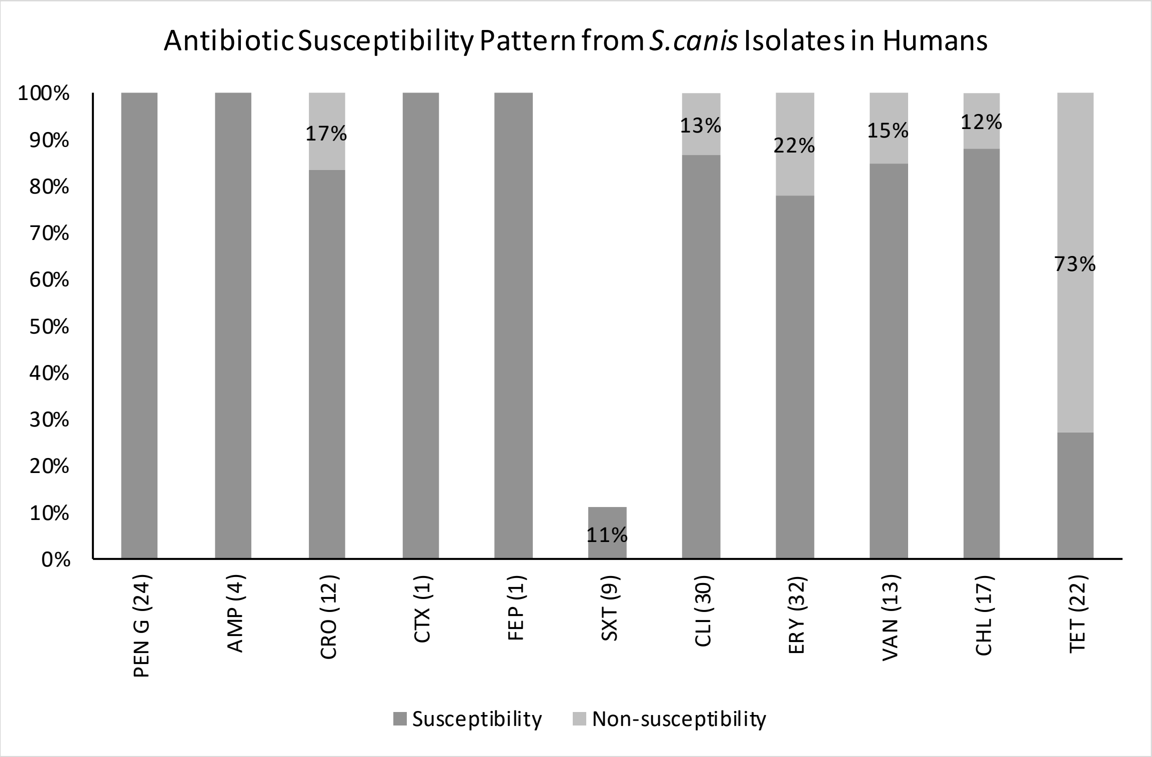


Figure 5c: Antibiotic susceptibility pattern from *S.canis* isolates from humans (2019–July 2023). Non-susceptibility includes both intermediate and resistant strains; AMX–amoxicillin; CVN–cefovecin; CRO–ceftriaxone; LEX–cephalexin; CFM–cefixime; AMC–amoxicillin with clavulanic acid; CHL–chloramphenicol; CLI–clindamycin; AZM–azithromycin; GEN–gentamicin; NEO–neomycin; ENR–enrofloxacin; NOR–norfloxacin; CIP–ciprofloxacin; DOX–doxycycline; TET–tetracycline; SXT–sulfamethoxazole with trimethoprim sulfadiazine; PMB–polymyxin B; MTZ–metronidazole. Numbers inside brackets '()' indicate the total number of tested isolates for each antibiotic.

4.8 Multidrug resistance status across species

Figure 6 shows that, during the 5-year study period, 44% (27/61) of *S. canis* strains isolated from cats and 54% (13/24) from dogs exhibited resistance to three or more antimicrobial classes. Notably, the highest occurrence of MDR strains occurred in the year 2022, with a total of 15 cases observed in both feline and canine populations.

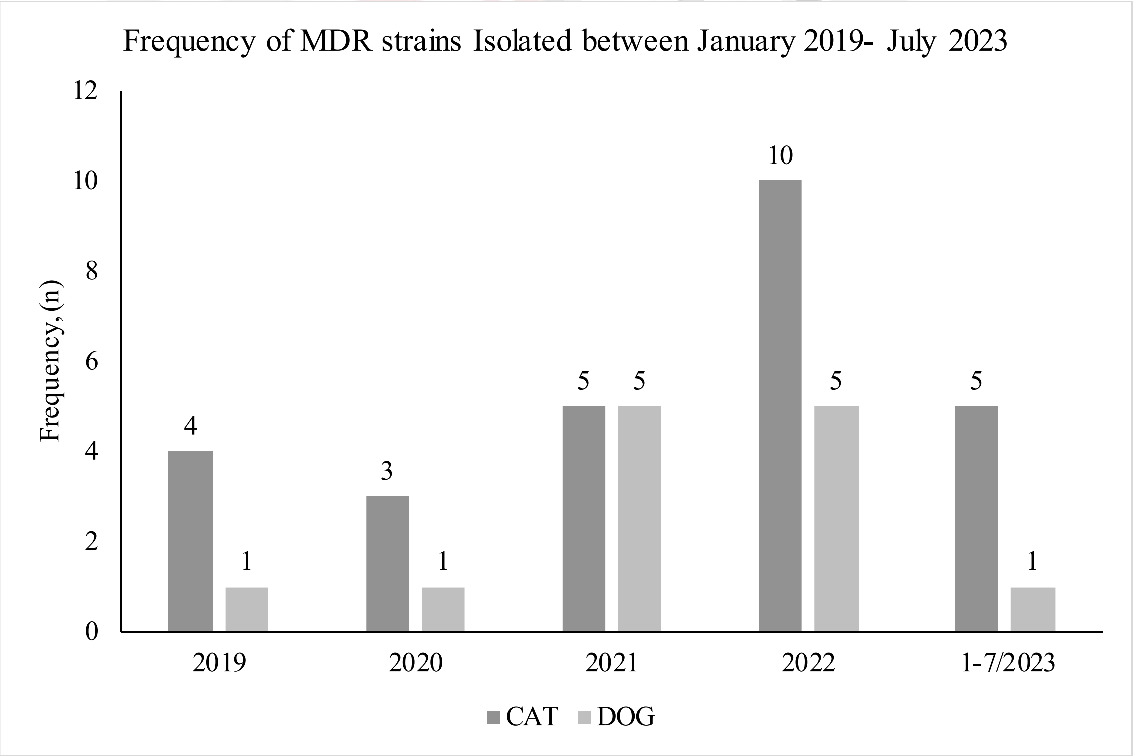


Figure 6: Frequency of MDR *S. canis* strains isolated between 2019 to July 2023 in cats and dogs.

4.9 Analysis of relation between species and the isolation sites

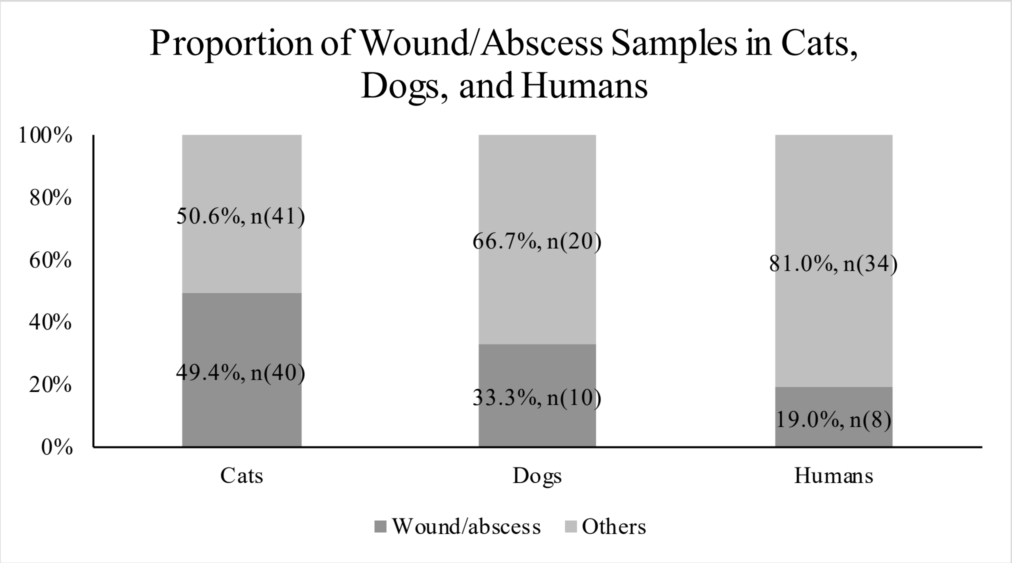


Figure 7: Proportion of Wound/Abscess Samples in Dogs, Cats and Humans. Numbers inside brackets '()' indicate the total number of *S. canis* isolates found in the category of samples.

As shown in Figure 7 and Table 2, wound/ abscess is the common site for the isolation of *S. canis* in both cats (49.4%, 41/81) and dogs (33.3%, 10/30). In contrast, in humans, the common source of *S. canis* isolation is blood (45.2%). When comparing cats and dogs, as well as, dogs and humans, no statistically significant associations were found (p=0.235 and p=0.225, respectively). However, when comparing cats and humans, a statistically significant association was observed (p=0.001), with the odds of finding *S. canis* in 'wound/abscess' samples in cats 4.3 times higher than in humans. Furthermore, when comparing humans to the combined group of cats and dogs, a statistically significant association was also observed (p=0.002), with 3.6 times higher odds of finding S. canis in 'wound/abscess' samples in cats and dogs compared to humans.

5.0 DISCUSSION

This study aimed to determine the occurrence and the characteristics of clinical cases of *S. canis* infection in dogs and cats diagnosed at the Bacteriology Laboratory of the Faculty of Veterinary Medicine, UPM, spanning from 2019 to July 2023. It also sought to achieve the same objectives for human patients who presented to MOH Hospitals in Malaysia with *S. canis* infections during the same period. Furthermore, this study aimed to assess the antimicrobial susceptibility profile and AMR status of *S. canis* isolates obtained from dogs, cats, and humans. The increasing number of clinical cases involving *S. canis*, both in veterinary patients and human infections, should raise significant public health concerns. However, despite the growing relevance of this issue, there remains a dearth of comprehensive studies addressing it (Stefańska *et al.*, 2022). Remarkably, in the Malaysian context, no prior investigations have been specifically conducted on *S. canis* infections, spanning across cats, dogs, and humans. This study, therefore, assumes a pivotal role in addressing this knowledge gap and holds the potential to contribute essential baseline information that can inform clinical decision-making in both veterinary and human medicine, with particular significance for the local population.

In this study, the predominant site for the isolation of *S. canis* in cats was pyogenic wounds, classified under the 'wound and abscess' category, accounting for a major proportion (49.4%) of cases. This finding aligns with a prior study conducted by Haulisah *et al.* (2022), where they also reported wound and abscess as the most frequent site for *S. canis* isolation, albeit at a higher proportion (70.5%). In contrast, among dogs, although wound/ abscess was also among the common isolation site (33.3%), the ear emerged as the most common site of *S. canis* isolation, representing 40.0% of isolates. This observation resonates with the research conducted by Lysková *et al.* (2007), where they analysed 39 *S. canis* isolates from dogs, and found otitis externa to be the most common condition associated with *S. canis* isolation, accounting for 48.7% of cases. While specific research on the predominant sites for *S. canis* colonization and infection in companion animals is limited, existing literature consistently recognizes its involvement in skin infections, otitis externa, and upper respiratory tract infections (Lysková *et al.*, 2007; Timoney *et al.*, 2017). This study reinforces these observations by identifying wound and abscess (40.2%, 51/127), ear (23.6%, 30/127), and respiratory tract (9.4%, 12/127) lesions as the primary locations for *S. canis* isolation in companion animals (cats and dogs). Although a number of literatures have emphasized *S. canis* involvement in various clinical manifestations, such as urinary tract infections (Yudhanto *et al.*, 2022), reproductive tract infections (Kustritz, 2006), and ophthalmic infections (Hewitt *et al.*, 2020; Cloet *et al.*, 2023), this study found rather low occurrence rates in these sites - 3.7% in feline urinary tract, 6.7% in the canine reproductive tract, and 1.2% in the feline eye. It is essential to consider the potential sampling bias in this study, as a majority of submitted canine and feline samples likely originated from wounds and ear lesions, which could have influenced the observed occurrence rates at these sites. Therefore, further investigations involving a more diverse sample pool are warranted to provide a comprehensive understanding of *S. canis* distribution and its associated clinical manifestations. Furthermore, most feline and canine isolates were identified in polymicrobial cultures, which is also similarly mentioned in the study by Lysková *et al.* (2007). Regarding this, Lysková *et al.* suggests the role of *S. canis* likely to be more deviated to be a secondary invading species rather than the primary disease-causing pathogen.

In the case of human infections, the understanding of the clinical manifestations of *S. canis* infections is somewhat limited due to data access constraints. However, it is evident that blood samples accounted for the highest rate of *S. canis* isolation at 45.2%. To address data categorization challenges, we consolidated both 'pus' and 'swab' samples into the category of pyogenic wounds, which represented 19.1% of the occurrence rate. However, the findings under this categorization do not align with the findings of Galpérine *et al.* (2007), where cutaneous or soft tissue infections comprised a major portion (64.8%) of *S. canis* infections, while bacteraemia was a less frequent occurrence at 9.2% compared to the bacteraemia cases or represented as 'blood' in our study. Furthermore, our study found that 100% of human *S. canis* isolates were obtained as pure cultures. This signifies the importance of *S. canis* as a virulent pathogen in human infections. However, this finding contrasts sharply with another study where the majority (77.8%) of *S. canis* isolates were identified within polymicrobial cultures (Galpérine *et al.*, 2007). This disparity can be attributed to the fact that a large proportion of our *S. canis* isolates originated from sterile sites, including blood, tissue, and pleural fluid.

Antimicrobial resistance in *S. canis* deserves significant attention, as the zoonotic transmission of this bacterium could potentially contribute to AMR issues in humans as well. In accordance to other studies, *S. canis* isolates in this study typically demonstrates high susceptibility to penicillin, first-generation cephalosporins and amoxicillin-clavulanic acid (Tsuyuki *et*, 2017; Haulisah *et al.*, 2022; Imanishi *et al.*, 2022; Stefańska *et al.*, 2022; Yudhanto *et al.*, 2022). Several pieces of literature have reported a high resistance rate to tetracycline (Gebru *et al.*, 2012; Pinho *et al.*, 2013; Tsuyuki *et al.*, 2017; Stefańska *et al.*, 2022) . However, it is worth noting that these studies appear to have a sampling bias towards dogs. Considering this bias, it helps explain the findings in this study, where *S. canis* isolates from dogs exhibited a high resistance rate of 67%, whereas in cats, the resistance rate was not as high, at 17%. Stefańska *et al.* (2022) attributed the high tetracycline resistance levels to the presence of various tet genes, including tet(O), tet(M), and tet(T), which encode proteins that protect bacterial ribosomes from the effects of tetracyclines. However, it is essential to acknowledge the potential influence of bias in our study's findings regarding tetracycline resistance, given the relatively small number of isolates subjected to antimicrobial susceptibility testing for tetracycline.

In companion animals, numerous studies have previously addressed AMR towards fluoroquinolones, with reported resistance rates typically falling within the range of 36% to 59% (Haulisah *et al.*, 2022; Imanishi *et al.*, 2022; Yudhanto *et al.*, 2022). In contrast, our study revealed a slightly lower rate of resistance, ranging from 17% to 40%. Besides, *S. canis* displayed exceptionally high resistance to neomycin, a finding consistent with the report by Hewitt *et al.* (2020). Hewitt *et al.* also documented a high resistance rate to neomycin, albeit within the category of *Streptococcus* spp., further emphasizing the prevalence of neomycin resistance among streptococcal species.

Metronidazole and polymyxin B exhibited an extremely high resistance rate in this study. Interestingly, no prior studies have specifically investigated the AMR of *S. canis* against metronidazole and polymyxin B. This lack of investigation may be attributed to these antibiotics not being routine choices for treating Streptococcal infections and their infrequent use as single drugs. As for now, there is currently no research to support the effectiveness of metronidazole and polymyxin B against Group G Streptococci.

In the case of human infections, although there have been limited studies conducted on the AMR of *S. canis*, our findings regarding the concerning resistance levels of tetracycline and erythromycin align with the study conducted by Galpérine *et al.* (2007). However, it is worth noting that the resistance rate towards tetracycline was approximately 30% higher in our study compared to their findings.

The proportion of MDR strains among feline and canine isolates in this study closely resembled the findings of Stefańska *et al.* (2022). Notably, the occurence of MDR cases appears to have increased when compared to the study by Haulisah *et al.* (2022), which had a similar study background to ours. While our MDR issue is still not as severe as observed in the study of Imanishi *et al.* (2022), it's worth noting that non-MDR cases in our study might include hidden MDR patterns due to incomplete testing across all necessary antibiotic classes. Nevertheless, the evolving pattern of MDR emphasizes the need for sustained attention to this matter.

This study has limitations inherent to its retrospective design, covering the period from 2019 to July 2023. Retrospective studies may not always offer generalizability to wider populations or different timeframes and entail limited control over collected variables and reliance on existing data. The COVID-19 pandemic during the study period may have affected healthcare-seeking behaviour and diagnostic practices, potentially influencing the determination of *S. canis* occurrence rates. Furthermore, the observed antimicrobial resistance pattern may not fully represent real scenarios, given variations in the strains subjected to antimicrobial susceptibility testing. The study's findings may not fully represent the entire feline and canine populations in Malaysia, primarily originating from a specific region served by the UVH. Similarly, data on human infections represent selected hospitals under the MOH, potentially underrepresenting the true prevalence. Additionally, the underreporting of *S. canis* cases, often grouped under '*Streptococcus* spp.,' is a recognized issue in veterinary and human medicine (Bert & Lambert-Zechovsky, 1997; Lysková *et al.*, 2007; Lacave *et al.*, 2016; Tsuyuki *et al.*, 2017; Stefańska *et al.*, 2022). These limitations, coupled with potential recall bias and historical data quality control, underscore the need for cautious interpretation and call for further investigations to comprehensively understand *S. canis* epidemiology and antimicrobial resistance patterns in Malaysia.

For future research recommendations, a prospective study design is essential to provide more in-depth insights into the epidemiology and clinical manifestations of *S. canis* infections. This approach will allow for the collection of real-time data and more accurate assessment of trends in *S. canis* infections in both veterinary and human medicine. Standardizing the antibiotic panel for antimicrobial susceptibility testing is crucial for facilitating accurate comparisons and assessing the true extent of antimicrobial resistance. Additionally, there is a need to explore human data further to elucidate the zoonotic potential of S. canis and better understand the dynamics of transmission between animals and humans. To achieve this, conducting investigations at the molecular level, including genotyping and whole-genome sequencing, would be beneficial. Investigating treatment outcomes, including the effectiveness of different antibiotics and therapeutic approaches, is paramount to improving patient care in both veterinary and human medical settings. These research avenues will not only enhance our understanding of S. canis infections but also contribute to the development of evidence-based guidelines for diagnosis, treatment, and prevention.

6.0 CONCLUSIONS

In conclusion, *S. canis* is commonly found in pyogenic wounds in cats and dogs, and notably, it is also frequently associated with otitis. In contrast, *S. canis* in human infections mainly occurs as pure isolates in cases of bacteraemia. Antibiotic susceptibility testing revealed that amoxicillin-clavulanic acid, penicillin, and 1st generation cephalosporin remain effective choices for treating *S. canis* infections across all species. However, the complexity arises from the presence of co-existing bacteria with distinct antimicrobial resistance profiles, which could pose challenges in treatment strategies. Most notably, the alarming discovery of multidrug resistance in up to 54% of *S. canis* isolates from cats and dogs underscores the urgent need to address antimicrobial resistance in *S. canis* infections. These findings emphasize the significance of comprehensive treatment guidelines and heightened awareness of the clinical impact of *S. canis* infections in both veterinary and human medicine. As we move forward, it is imperative to consider these results as a basis for further research to improve our understanding of this pathogen and enhance clinical management strategies.

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APPENDIX

APPENDIX A: Quantitative Distribution of *S. canis* (Cats)

	Few colonies	**1+**	**2+**	**3+**	**4+**	**Unidentified**
Bone/ Joint				1	3	
Ear		4	2	8	2	1
Eye		1				
Gastrointestinal Tract						
Multiple Organs	5	3	4	7	1	
Oral cavity				1		
Reproductive Tract						
Respiratory Tract		2	1	2	3	
Urinary tract	1	1			1	
Wound/ Abscess	1	9	9	14	8	
Total	**7**	**20**	**16**	**33**	**18**	**1**

APPENDIX B: Quantitative Distribution of *S. canis* (Dogs)

	Few colonies	**1+**	**2+**	**3+**	**4+**	**Unidentified**
Bone/ Joint						
Ear		1	3	4	4	1
Eye						
Gastrointestinal Tract		1			1	
Multiple Organs				1		
Oral cavity						
Reproductive Tract		1	1			
Respiratory Tract			1	1		2
Urinary tract						
Wound/ Abscess		3	3	1	1	2
Total	**0**	**6**	**8**	**7**	**6**	**5**