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RESEARCH ARTICLE

Whole genome sequencing of *Streptococcus suis* revealed potential drug resistance and zoonotic transmission in companion cat

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ABSTRACT

Streptococcus suis is a bacterium of clinical importance in diverse animal hosts including companion animals and humans. Companion animals are closely associated in the living environment of humans and are potential reservoirs for zoonotic pathogens. Given the zoonotic potential of *S. suis*, it is crucial to determine whether this bacterium is present among the companion animal population. This study aimed to detect *Streptococcus suis* in companion animals namely cats and dogs of the central west coast of Peninsular Malaysia and further characterize the positive isolates via molecular and genomic approach. The detection of *S. suis* was done via bacterial isolation and polymerase chain reaction assay of *gdh* and *recN* gene from oral swabs. Characterization was done by multiplex PCR serotyping, as well as muti-locus sequence typing, AMR gene prediction, MGE identification and phylogenomic analysis on whole genome sequence acquired from Illumina and Oxford Nanopore sequencing. Among the 115 samples, PCR assay detected 2/59 of the cats were positive for *S. suis* serotype 8 while all screened dog samples were negative. This study further described the first complete whole genome of *S. suis* strain SS/UPM/MY/F001 isolated from the oral cavity of a companion cat. Genomic analysis revealed a novel strain of *S. suis* having a unique MLST profile and antimicrobial resistance genes of *mef*A, *msr*D, *pat*A, *pat*B and *van*Y. Mobile genetic elements were described, and pathogenic determinants matched to human and swine strains were identified. Phylogenetic tree analysis on the core genome alignment revealed strain SS/UPM/MY/F001 was distinct from other *S. suis* strains. This study provided insight into the detection and genomic features of the *S. suis* isolate of a companion cat and highlighted its potential for antimicrobial resistance and pathogenicity.

Keywords: *Streptococcus suis*; companion animal; feline; genome; sequencing.

INTRODUCTION

Streptococcus suis is one of the major pathogens of swine with almost 100% of the farms worldwide having carrier animals (Goyette-Desjardins *et al*., 2014). *S. suis* infections are usually diagnosed based on clinical signs of meningitis and sudden death (Staats *et al*., 1997). It affects all ages of pigs, particularly in the nursery herd (Arndt *et al*., 2018). It has also been isolated from various host species namely humans, cattle, horses, cats, dogs, birds, wild boar, sheep, rabbits, wild cats and fish (Perch *et al*., 1968; Higgins *et al*., 1990; Devriese & Haesebrouck, 1992; Devriese *et al*., 1992, 1994; Baums *et al*., 2007; Muckle *et al*., 2014; del Rey *et al*., 2013; Tang *et al*., 2016; Dinh-Hung *et al*., 2023).

Detection of *S. suis* in cats and dogs was reported way back 30 years ago in 1992, detecting from tonsil swabs and fecal swabs of healthy animals (Devriese *et al*., 1992). Recently, clinical reports of *S. suis* in cats and dogs were available with different clinical presentations such as meningoencephalitis (Roels *et al*., 2009), urinary tract infection (Muckle *et al*., 2010), spinal meningitis (Ruggeri *et al*., 2019), and endomyocarditis (Wood *et al*., 2021). However, there was scarcity of information regarding the source of infection and the strain of *S. suis* infecting the feline and canine population. Therefore, it is imperative to conduct screenings for *S. suis* in these animal species and conduct further inquiries into the potential sources of infection.

Companion animals such as cats and dogs have a close attachment to humans. Surveys showed more than 75% of households were in contact with pets and had close interactions with pets (Stull *et al*., 2012, 2013). The public have low awareness towards pet-borne diseases and are at high risk for pet-associated disease (Stull *et al*., 2012). Since the severity of human infection with *S. suis* has been reported worldwide and the presence of this

pathogen have been reported in companion animals, early detection of *S. suis* is crucial as a surveillance for the prevention of zoonotic pathogen transmission from canine and feline to human population.

The advancement of sequencing technology has facilitated the extensive study of genomics in various organisms. Comparative genomics has enabled people to draw evolutionary relationships and compare between organisms (Sengupta & Azad, 2023). Besides, various bioinformatic tools provide a platform allowing researchers to predict and identify virulence factors in a genome. Therefore, this study employed molecular methods of detection to screen for *S. suis* in Malaysian cats and dogs; as well as subsequently utilized a genomic approach to fully characterize the isolate of *S. suis* in this country.

MATERIALS AND METHODS

Institutional Review Board Statement

The animal study protocol was approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (IACUC No. UPM/IACUC/AUP-R064/2020).

Written informed consent was obtained from the owner of the companion pets.

Sampling

Sampling was done in the central region of west coast Peninsular Malaysia mainly from states of Perak, Selangor and Negeri Sembilan from August 2020 to May 2022. Sampling was performed from the stray population and pets presented to participating veterinary clinics. Oral swab samples were collected from the entire oral cavity of cats and dogs using a sterile nylon-flocked swab (Copan, USA). The health status, clinical conditions, diet, exposure to swine material (either possible contamination from the environment or consumption as food) and information on exposure to antibiotic treatment at least 48 hours prior to sampling of each animal were recorded. Animals on current antibiotics treatment were excluded from further sampling.

Bacteria isolation

Bacteria isolation was done by streaking the swabs on Columbia sheep agar plates (Thermo Fisher Scientific, USA) and colistin and oxolinic acid supplemented blood agar (COBA) plates. The cultures were incubated at 37°C for 24 to 48 hours under aerobic conditions. Suspected growth appearing as a small, translucent, alpha haemolytic colony was sub-cultured to obtain pure colonies.

Bacteria identification and detection of *S. suis* via PCR assay

A streak of bacteria from the pure colonies were suspended into 100 µL sterile deionized water. At the same time, the swabs were suspended into 100 µL sterile deionized water. DNA was extracted from the bacteria suspension and swab suspension using a commercial extraction kit (DNeasy® Blood and Tissue Kit, Qiagen, Germany) and stored at -20°C until use. Extracted DNA was subjected to the conventional polymerase reaction assay method for detection of *S. suis* using published primers (Kerdsin *et al*., 2012; Ishida *et al*., 2014) detecting both *gdh* and *recN* genes of *S. suis* respectively. PCR reactions were set up at 12.5 µL 2× polymerase taq master mix (Meridian Bioscience®, USA), primer at 10 µM each at final concentration 0.5 µM, template at 3 µL and DNase free water topped up to 20 µL. PCR conditions for the detection of both genes were set up at 95°C for 5 minutes, and 30 cycles of 95°C at 15 seconds, 58°C at 30 seconds and 72°C at 30 seconds, and at 72°C for 5 minutes. PCR products were electrophoresed together with a 100-bp molecular weight marker (GelPilot®, Qiagen, Germany) in 2% agarose gel and Tris-acetate-EDTA buffer at 80 volts for 40 minutes. Results were viewed and documented using a gel viewer system (Syngene, USA). Samples were considered as *S. suis* positive only if PCR assay results were positive for both, showing PCR bands at approximately 695bp (*gdh* gene) and 325bp (*recN* gene).

Molecular serotyping

Molecular serotyping was performed according to primers, PCR kit and methods described by Kerdsin *et al*. (2014) (20). In brief, samples were subjected to a set of four multiplex PCR reactions. PCR reactions were set at 12.5 µL 1× KAPA2G Fast Multiplex PCR mix (KAPA Biosystem, Roche, Switzerland), 0.2 µM of each primer, 2 µL DNA template and DNase-free water topped up to 25 µL. PCR cycling conditions were. set up at 95°C for 3 minutes, 30 cycles of 95°C at 20 seconds and 62°C at 90 seconds; and at 72°C for 5 minutes. PCR products were subjected to electrophoresis.

Biochemical testing

Biochemical testing was performed using the API 20 Strep system (bioMérieux, France) following the manufacturer's protocol.

Whole genome sequencing, assembly, and annotation

DNA was extracted from a plateful of *S. suis* subculture using a commercial kit (DNeasy® Blood and Tissue Kit, Qiagen, Germany) with a lysis pre-treatment adapted for extracting gram-positive bacteria (innuPREP Bacteria Lysis Booster, IST Innuscreen GmbH, Germany). Genomic DNA was outsourced to a sequencing company and sequenced using a combination of Illumina and Oxford Nanopore whole genome sequencing technology. For Illumina sequencing, approximately 100 ng of gDNA was fragmented using Bioruptor to approximately 350 bp followed by a library preparation using the NEBUltraII Illumina library preparation kit. Sequencing was performed on a NovaSEQ6000 (2 × 150 bp). As for Oxford Nanopore sequencing, approximately 1000 ng of DNA as measured by Qubit was processed using the Nanopore ligation LSK110 kit according to the manufacturer's instructions (Oxford Nanopore Technologies Ltd., UK). The sample was sequenced on a Nanopore Flongle flow cell. Base calling of the fast5 file used Guppy v5.0.7 (super accuracy mode).

Raw nanopore reads were quality- and length-filtered to retain reads longer than 1,000 bp with a q-score of 9 or higher. The filtered nanopore reads were assembled de novo using Flye v2.9-b1768 (Kolmogorov *et al.*, 2019). The raw assembly was polished with Medaka v1.4.1 (https://github.com/nanoporetech/medaka) (Oxford Nanopore Technologies Ltd., UK). Illumina reads were aligned to the polished assembly followed by two rounds of polishing using Pilon v1.2.1 (Walker *et al*., 2014). The assembly statistics was computed using QUAST v5.0.2 (Gurevich *et al*., 2013) and genome completeness was assessed with BUSCO v5.3 using the "–auto-lineage-prok" (Simão *et al*., 2015). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al*., 2016; Haft *et al*., 2018; Li *et al*., 2021). A genome map was plotted using Proksee (https://proksee.ca/) (Grant *et al*., 2023) with annotations from PGAP and mobileOG-db (Data Version: Beatrix 1.6 v1) (Brown *et al*., 2022).

In-silico Multilocus Sequence Typing

An *in-silico* multilocus sequence typing (MLST) was performed using the open-source MLST tool (https://github.com/tseemann/mlst) that conducts search against the PubMLST database (Jolley *et al*., 2018).

Screening of Antimicrobial Resistance Genes

Antimicrobial resistance (AMR) genes were identified using several curated tools such as NCBI AMRFinderPlus v3.11.14 (database 2023-04-17) (Feldgarden *et al*., 2021), CARD's v3.2.7 Resistance Gene Identifier (RGI) v6.0.2 (https://card.mcmaster.ca/analyze/rgi) (Alcock *et al*., 2023) and ResFinder v4.1 (https://cge.food.dtu.dk/services/ResFinder/) (Camacho *et al*., 2009; Bortolaia *et al.*, 2020;

Zankari *et al*., 2020). CARD's RGI was set to "default – perfect and strict hits only" while ResFinder was set to a default parameter (threshold ID: 90%; minimum length: 60%) with "Other" as species selection.

Screening of Mobile Genetic Elements (MGE)

Plasmids and other genetic elements (insertion sequences, integrative and conjugative element (ICE), integrative and mobilizable elements (IME), composite transposons, etc.) were identified using Center for Genomic Epidemiology's (CGE) web-based tool PlasmidFinder v2.0.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) (Gram-positive database at 2023-01-18, min identity 90% and min coverage 60%) (Camacho *et al*., 2009; Carattoli *et al*., 2014) and MobileElementFinder v1.0.3 (https://cge.food.dtu.dk/services/MobileElementFinder/) (Johansson *et al*., 2020). CGE's MobileElementFinder was set to display results for the following parameters: ≥ 95% alignment coverage; ≥ 90% sequence identity and maximum truncation of 30 nucleotide. ICEfinder v1.0 (https://bioinfo-mml.sjtu.edu.cn/ICEfinder/index.php) (Liu *et al*., 2019) was also used to further screen for additional ICE and IME using the genome query for gram-positive organisms and default dataset. PHASTER (Arndt *et al*., 2016) was utilized to identify prophage regions.

Virulence Associated Factors (VAF) Identification

CGE's PathogenFinder v1.1 (https://cge.food.dtu.dk/services/PathogenFinder/) was used to conduct searches against using the database to predict pathogenicity and "Automatic Model Selection" was selected for the search (Cosentino *et al.*, 2013). The genome was subjected to BLASTN v2.14.1 (Zhang *et al*., 2000) search against 80 compiled VAFs (Fittipaldi *et al*., 2012; Estrada *et al*., 2021; Nicholson & Bayles, 2022) as listed in Supplementary Table 1.

Phylogenomic analysis

Reference complete genomes were downloaded from the NCBI Genbank database with the search criteria "*Streptococcus suis*(organism)" and "complete genome" with circular DNA. All genomes including strain SS/UPM/MY/F001 were re-annotated using Prokka (Seemann, 2014) and subjected to pangenome analysis using Roary (Page *et al*., 2015). A maximum likelihood tree was constructed from the Roary core genome alignment using FastTree2 (Price *et al*., 2010). All the GenBank accession numbers and their respective metadata were listed in Supplementary Table 2. Phandago v1.3.0 web-based visualization tool (www.phandango.net) (Hadfield *et al*., 2018) was used to compile core genome alignment tree, gene clusters and respective metadata. TreeCluster (Balaban *et al*., 2019) was used to define clusters within the phylogenetic tree using the default (maximum pairwise distance) clustering method with the recommended distance threshold value 0.045. Leaves yielding -1 value were considered as singletons.

Statistical analysis

Data were statistically analysed using Minitab® Statistical Package version 21.3 (Minitab, LLC., USA). Quantitative data were reported as mean and median while qualitative data were given as frequencies and percentages.

RESULTS

Samples

A total of 115 oral swabs were collected from 59 cats and 56 dogs sampled in different states of central Peninsular Malaysia (Selangor, *n* = 75; Negeri Sembilan, *n* = 22; Perak, *n* = 13; W.P. Kuala Lumpur, *n* = 3; W.P. Putrajaya, *n* = 1; Malacca, *n* = 1). The demographic information and clinical status of the animals sampled were summarised in Table 1.

Table 1. Summarized demographic information of sampled animals

Animal		Cat (n = 59)	Dog (n = 56)
Age	Min	6 months	4 months
	Max	18 years	15 years
	Mean	4.38 years	5.96 years
	Median	2 years	5 years
Sex	Male	45.8%	53.6%
	Female	54.2%	46.4%
Breed		Domestic Short Hair breed (45), Domestic Long Hair (6), Persian (3), mix-breed (3), Siamese (1), American Curl crossbreed (1)	Local breed (31), Shih Tzu (4), Poodle (3), mix-breed (2), Rottweiler (2), German Shepherd (2).
Pet		69.5%	69.6%
Stray		30.5%	30.4%
Management	Indoor	57.6%	62.5%
	Semi-roamer	11.9%	5.4%
	Outdoor	30.5%	32.1%
Ate pork	Yes	6.8%	33.9%
	Unsure	27.1%	19.7%
	No	66.1%	46.4%
Clinical condition	Healthy	47.5%	51.8%
	Clinically ill	52.5%	48.2%
		neoplasia (n=6), respiratory disease (n=5), urogenital disease (n=5), traumatic injury (n=5), neuromuscular conditions (n=2), systemic condition (n=2), skin disease with poor body condition (n=1), cardiovascular disease (n=1), infectious disease (n=1), hepatobiliary disease (n=1), reproductive condition (n=1), oral ulcer (n=1).	neoplasia (n=19), parasitic infestation (n=2), skin disease (n=2), hepatobiliary disease (n=1), infectious disease (n=1), traumatic injury (n=1), urogenital disease (n=1).

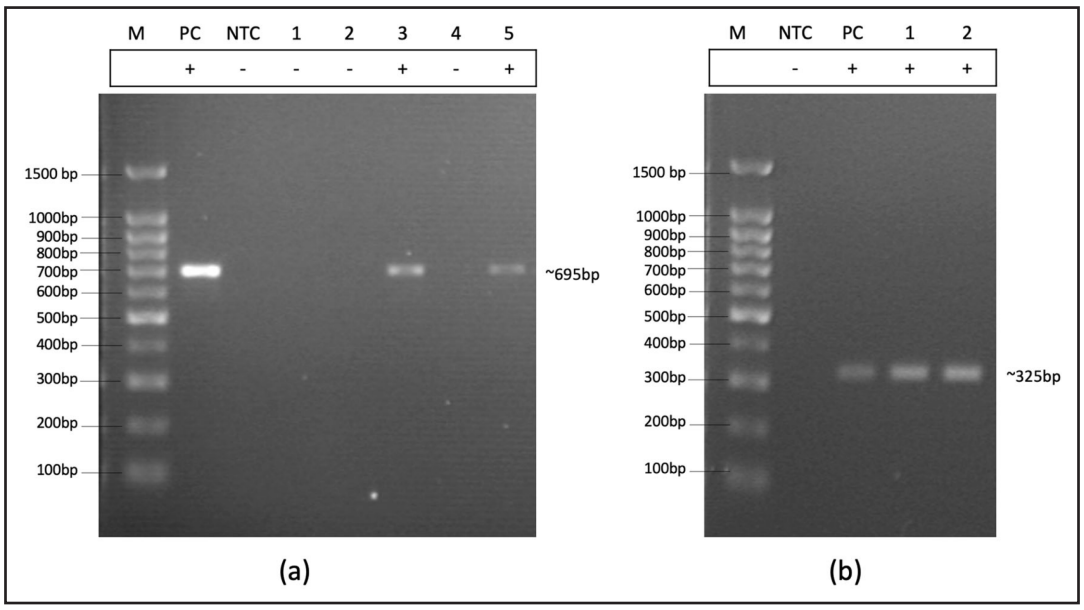

Figure 1. (a) Electrophoresed gel photo of PCR assay amplifying *gdh* gene of *S. suis* with amplified band size of 709bp (approx. 695bp). M, 100bp molecular weight marker; PC, positive control (*S. suis* isolated from swine field sample); NTC, no template control; Lanes 1,2,4, negative samples; Lanes 3,5, positive sample (cat C8 and C11). (b) Electrophoresed gel photo of PCR assay amplifying *recN* gene of *S. suis* with amplified band size of 322bp (approx. 325bp). M, 100bp molecular weight marker; NTC, no template control; PC, positive control (*S. suis* from swine field sample); Lane 1, C8; Lane 2, C11.

Detection, isolation, biochemical reactions, and molecular serotyping of *S. suis*

Among the 59 cats, only 3.4% (n=2) of cats were positive for *S. suis* via PCR assay detection directly from swabs (Figure 1a) and among the two, only one *S. suis* isolate was successfully isolated from the samples collected. Both *S. suis*-positive felines did not display any clinical signs suggestive of *S. suis* infection. Molecular serotyping indicated that the two PCR-positive samples were of serotype 8 supported by the NCBI blast search of the sequenced PCR product fragment (Figure 1b). The *S. suis* was isolated from a semi-roamer companion cat presented to a veterinary clinic for wound treatment. Upon sampling, the cat was not showing any signs of fever or neurological syndrome and there was no wound in the oral cavity. The *S. suis* isolate which was renamed as SS/UPM/MY/F001 appeared as small translucent colonies, alpha-hemolytic, after 24 hours on Columbia sheep blood agar and COBA agar as well as exhibited biochemical properties listed in Table 2. Meanwhile, none of the canine samples were detected positive upon PCR directly from oral swabs or bacterial isolation.

Whole genome sequence

A total of 8.4 million Illumina short reads were generated while 80,371 nanopore raw reads were generated with a read length N50 of 5.17 kb and mean read quality of 10.8. The genome of SS/UPM/MY/F001 was assembled into a single contig marked as "circular" by Flye assembler and BUSCO scans yielded 100% of the 402 conserved lactobacillales genes were complete and of single copy, indicating it was a complete genome. It comprised a circular chromosome of 2470830 bp and GC content of 40.98%. PGAP annotations revealed the genomic features as listed in Table 3 and the genome map was as shown in Figure 2.

Table 2. Biochemical reaction of strain SS/UPM/MY/F001

Biochemical test	Result	Typical *S. suis* reactions*
	Enzymatic reaction	
Catalase	Negative	Negative
Voges Proskaeur	Negative	Negative
Hippuric acid	Negative	Negative
Esculine	Negative	Positive
Pyrolidonyl arylamidase	Negative	Negative
α-galactosidase	Positive	Positive
β-glucoronidase	Negative	Variable
β-galactosidase	Positive	Positive
Alkaline phosphatase	Negative	Negative
Leucine amino peptidase	Positive	Positive
Arginine dihydrolase	Negative	Variable
	Sugar acidification	
Ribose	Negative	Negative
Arabinose	Negative	Negative
Mannitol	Negative	Negative
Sorbitol	Negative	Negative
Lactose	Positive	Positive
Trehalose	Positive	Positive
Inulin	Positive	Variable
Raffinose	Positive	Positive
Amidon	Positive	Positive
Glycogen	Positive	Positive

*Typical reactions based on published articles (Kilpper-Bɲlz & Schleifer, 1987; Higgins & Gottschalk, 1990; Tarradas *et al*., 1994; Facklam, 2002; Stanojkoviт *et al*., 2014).

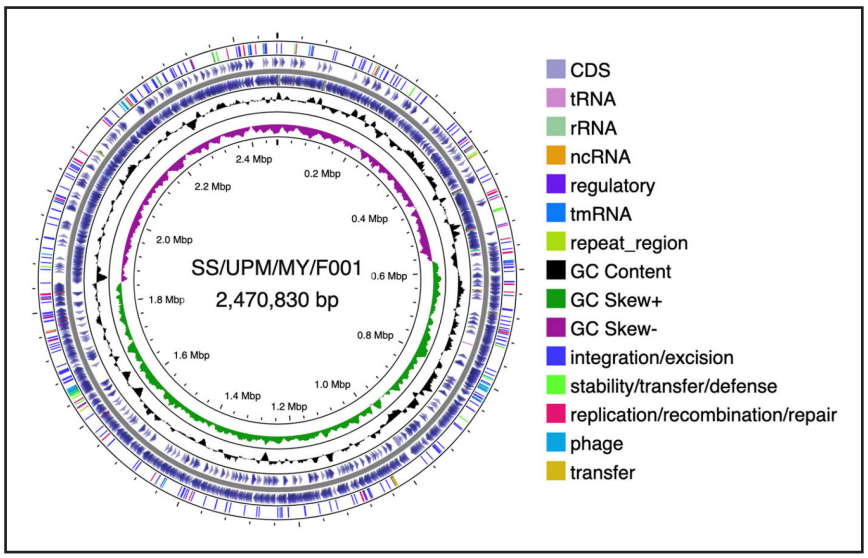

Figure 2. The genome map of *S. suis* strain SS/UPM/MY/F001. From the inner most circle, the strain ID and genome size was presented. This was followed by the GC skew, GC content, reverse strand, forward strand, and mobile orthologous groups representing mobile genetic elements.

In-silico MLST

In-silico MLST search yielded only one exact match for the locus *mut*S (102). Subsequently, allelic sequences of loci *aro*A, *cpn*60, *dpr, gki, rec*A and *thr*A were submitted into the PubMLST *Streptococcus suis* MLST databases and a new allelic number was assigned for each loci. The full MLST profile and their positions in the genome were listed in Table 4. and a new sequence type 2105 (ST2105) was assigned.

AMR genes and MGE identification

There were two AMR genes predicted from AMRFinderPlus and ResFinder as well as additional three genes were predicted using CARD's RGI (Table 5). The isolate was found to carry genes resistant to five classes of drugs namely macrolide, lincosamide, streptogramin B, fluoroquinolone, and glycopeptide. No plasmid was identified for strain SS/UPM/MY/F001. ICEFinder identified two regions of putative ICE with T4SS (Figure 3a). Region 1 (83kb, 43.14%CG) consisted of 85 ORFs, encoded with MOB_F relaxase, Pfam-B_1690, *rve* integrase and TraJ I, T4SS components (Figure 3b). Region 2 (134kb, 39.95% GC) consisted of 146 ORF, encoded with *rve* integrase, AAA_10, FA_orf15, TIGR02224 integrase, phage integrase, MOB_T relaxase, T_virB11, FtsK_SpoIIIE, traK_typeI, and Orf14_Tn, T4SS components (Figure 3c). Eighty-five MGEs were further identified including insertion sequences and composite transposons belonging to the IS110, ISL3 and IS200/IS605 family. Seven prophage regions were determined, of which three are intact and four are incomplete (Figure 4). Among the seven prophage regions, the AMR genes *mef*A-*msr*D were found located within the intact region 5 prophage (Figure 5).

VAFs

There were 56 VAFs identified in the SS/UPM/MY/F001 strain out of the 80 VAFs screened (Supplementary Table 1). Meanwhile, it was predicted that *S. suis* strain had a 0.88 probability of being a human pathogen and revealed matches to 14 pathogenic families of the *S. suis* human pathogenic strains 05ZYH33 (10 families), BM407 (1 family) and 98HAH33 (3 families).

Pangenome and phylogenetic analysis

A total of 117 whole genome of circular DNA reference genome sequences were retrieved from NCBI Genbank. The Roary core genome alignment analysis of the 118 strains including SS/UPM/MY/F001 revealed 10976 gene clusters comprising of 1268 core genes and 9708 accessory genes. The Roary analysis highlighted a unique region in SS/UPM/MY/F001 spanning from genome coordinate 146060 (locus tag: group_10490) to 150660 (locus tag: group_10720) (Figure 6) which encoded *rsg*A, *ydc*F, *btuD_11, agu*A, *cys*D, *cys*NC, *yve*L, *gly*E, *wfg*D and other hypothetical proteins. The phylogenetic tree constructed based on the core genome alignment revealed that *S. suis* strain SS/UPM/MY/F001 was more closely related to the strains isolated from carrier sites of pigs (strain SRD478, 0061, 1081, and HA1003) and interestingly a recent isolate from the head

Table 3. Genomic features of SS/UPM/MY/F001

Feature	Finding
Length (bp)	2470830
Status	Complete
No. of contigs	1
GC content (%)	40.98%
No. of genes	2515
No. of coding sequences	2442
No. of rRNAs	12
No. of tRNAs	57
CRISPR Array	1

Table 4. The allelic profile of isolate SS/UPM/MY/F001

Locus	Allele	Length	Start position	End position
*aro*A	441	366	1618792	1619157
*cpn*60	578	318	381372	381689
dpr	395	336	1221029	1221364
gki	505	321	1901048	1901368
*mut*S	102	339	623296	623634
*rec*A	358	354	462068	462421
*thr*A	385	336	1110976	1111311

Table 5. AMR genes identified using AMRFinder Plus, ResFinder 4.1 and CARD RGI and its respective predicted antibiotic resistance

AMR gene	AMR Gene family	Drug class	Antibiotic	Start-stop	Strand	Search platform
*mef*A	macrolide efflux MFS transporter	macrolide	erythromycin, azithromycin	1624781–1625998	+	AMRFinderPlus, ResFinder
*msr*D	ABC-F type ribosomal protection protein	Macrolide, lincosamide and streptogramin B	erythromycin, azithromycin, telithromycin, quinupristin, pristinamycin IA, virginiamycin S	1626119–1627582	+	AMRFinderPlus, ResFinder
*pat*A	ATP-binding cassette (ABC) antibiotic efflux pump	fluoroquinolone	ciprofloxacin, norfloxacin	694,310–696,016	+	CARD RGI
*pat*B	ATP-binding cassette (ABC) antibiotic efflux pump	fluoroquinolone	ciprofloxacin, norfloxacin	696,017–697,801	+	CARD RGI
*van*Y *gene in* *van*B *cluster*	vanY, glycopeptide resistance gene cluster	glycopeptide	vancomycin	1745366–1746121	+	CARD RGI

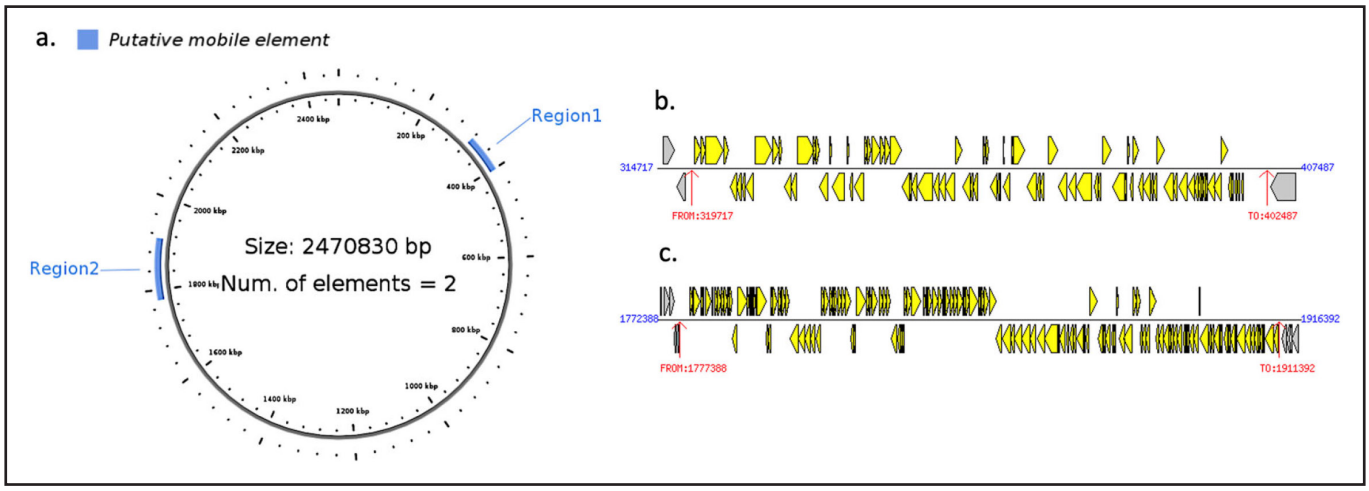

Figure 3. (**a**) Two regions of putative ICE with T4SS mapped on the genome map. (**b**) Region 1 spans from nucleotide position 319717 to 402487. **(c)** Region 2 spans from nucleotide position 1777388 to 1911392.

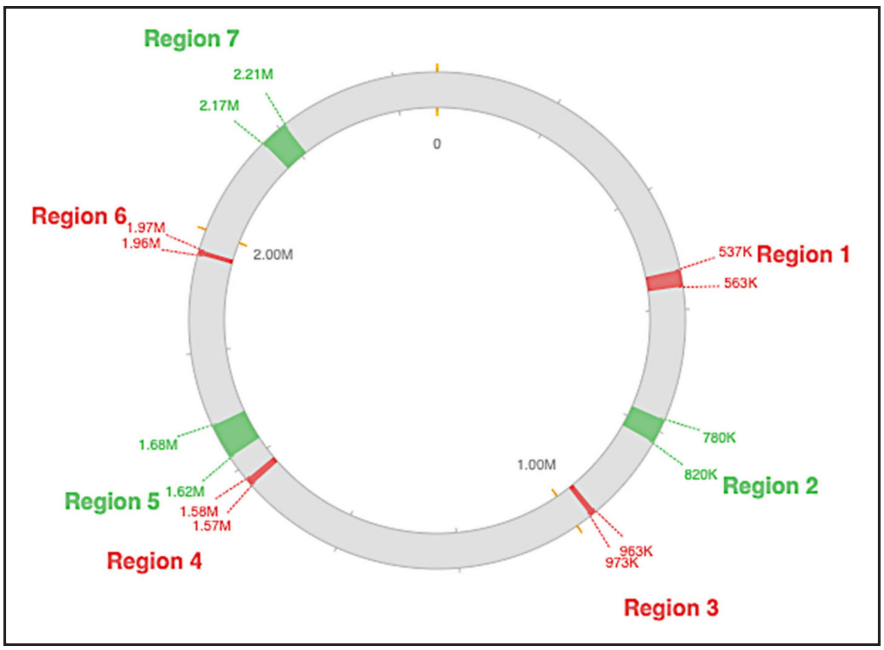

Figure 4. Prophage regions identified by PHASTER. Regions labelled in red are considered incomplete (scores <70). Region labelled in green are considered intact prophage (score > 90).

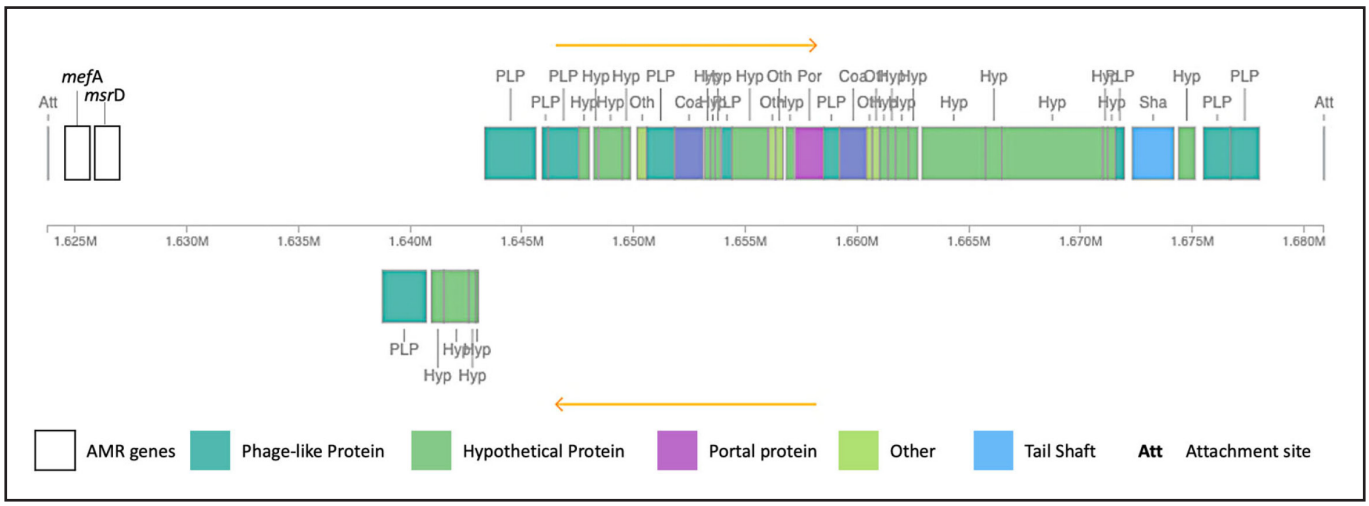

Figure 5. Intact prophage region 5 (57.1kb, 38.70% GC) starting from nucleotide position 1623786 and ending at 1680911. AMR genes *mef*A and *msr*D were located within this region.

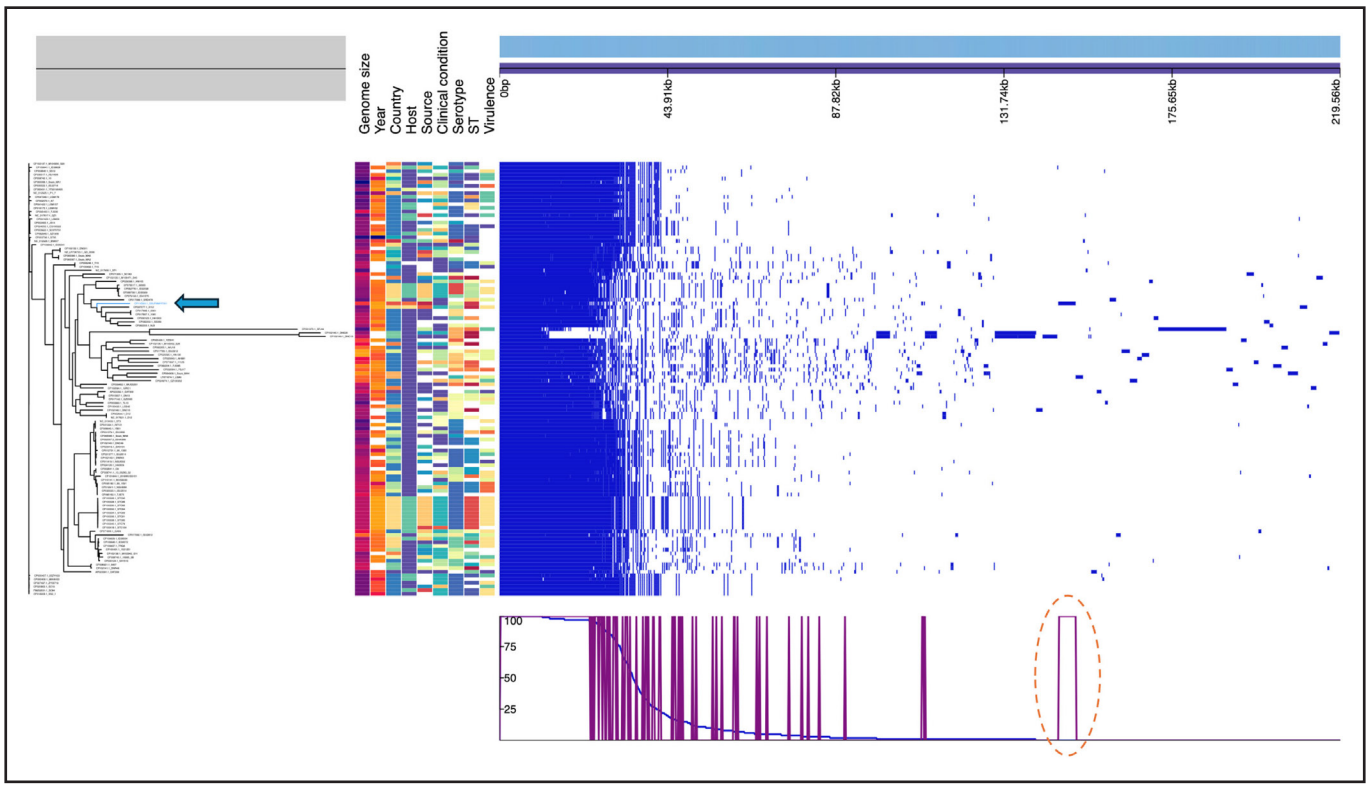

Figure 6. Integrated visualization of phylogenetic tree (left), metadata (middle), and matrix of presence and absence of core genes (right). The strain SS/UPM/MY/F001 was labeled in blue and pin-pointed by a blue arrow in the maximum likelihood phylogenetic tree (Please refer to Supplementary Figure 1) constructed to scale. Metadata was colored according to information available in Supplementary Table 2 while white blanks indicate no data available. The blues shades at the right indicated gene presence whereas white blanks indicated the absence of gene as in reference to Supplementary Table 3. The graph at the lower right indicates the percentage of strains with the gene present in reference to strain SS/UPM/MY/F001 and the unique gene cluster region was indicated by an orange oval.

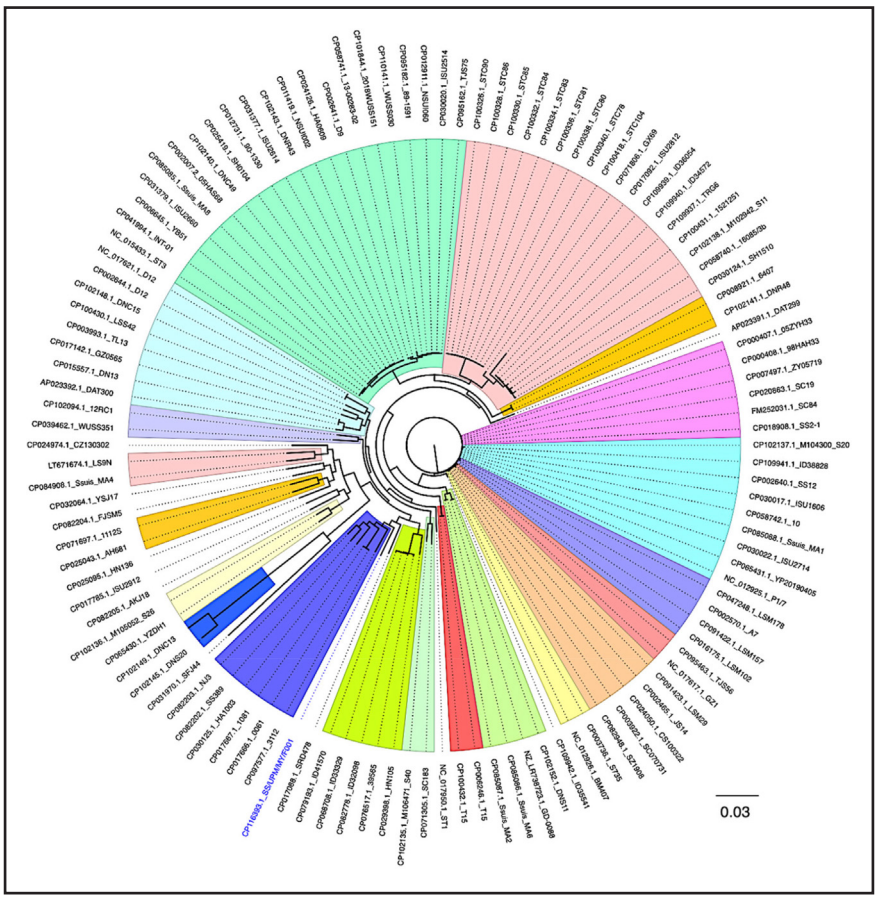

Figure 7. A circular visualization of the maximum likelihood phylogenetic tree constructed from the Roary core genome alignment of 118 strains of *S. suis*. Clusters were coloured according to the cluster number defined by the TreeCluster. Strains that were not highlighted were singletons. Strain SS/UPM/MY/F001 was labelled in blue colour.

kidney of a fish (strain 3112) (Figure 7). However, further clustering of the constructed phylogenetic tree by TreeCluster revealed that the 118 strains were divided into 20 clusters, while strain SS/UPM/MY/F001 yield cluster of value -1, indicated that it was a singleton and distinct among the other 117 strains.

Data availability

The complete genome sequence of *S. suis* strain SS/UPM/MY/F001 has been deposited into the NCBI Genbank database and assigned GenBank accession number CP116393. The genome and MLST profile of SS/UPM/MY/F001 was also deposited into the PubMLST database with the ID 3477.

DISCUSSION

There was only one *S. suis* isolate recoverable from the two PCR-positive samples. This might be due to the overgrown of other dominant normal flora, especially other oral Streptococcus species (Frymus *et al*., 2015) and a low prevalence of *S. suis* in the Malaysian cat population. In this study, the animal detected positive for *S. suis* was not directly linked or showed clinical signs pointing towards *S. suis* infection. It was also unclear whether the semi-roamer cat had direct contact or access to raw pork; however, it was not fed any pork material due to the owner's religious prohibition. *S. suis* is a multi-host organism; the clinical presentation and outcome are only evident in pigs and humans but still obscured in other species of animals. As there are more reports of *S. suis* in numerous hosts without a history of contacting raw pork materials, it is reasonable that cats and many more species of animals might be *S. suis* carriers and potentially develop a clinical infection under unfavorable circumstances e.g., stress, immunosuppression, and existing comorbidities.

Generally, most biochemical reactions matched those typical of *Streptococcus suis* except a few. This is not an uncommon finding as atypical biochemical reactions were frequently documented (Hommez *et al*., 1986; Devriese *et al*., 1992). As *S. suis* is not a common pathogen infecting hosts aside from pigs and humans, an atypical biochemical reaction will be easily misinterpreted as other species. Therefore, interpretation of biochemical reactions needs to be done carefully and, in this study, conventional PCR assays were employed as methods to identify possible *S. suis* isolate from cultures instead of biochemical reaction testing.

Serotyping is a widely used characterization method for bacteria species. After assignment of six serotypes to other species of Streptococcus, there are 29 *S. suis* true serotypes at present (Estrada *et al*., 2019, Segura *et al*., 2020). Previously, a small number of feline isolates were serotyped; they were of serotype 4, 9, ½, 20, 22, 26 and non-typable (Salasia *et al*., 1994; Wood *et al*., 2021) [in which serotypes 20, 22, and 26 were reclassified as *Streptococcus parasuis* (Okura *et al*., 2016)]. There was also a past report of *S. suis* serotype 9 isolated from a wild cat (Tang *et al*., 2016). Instead of these serotypes, serotype 8 was detected in both PCR positive samples. Recently, there has been a recent report of an ill dog with *S. suis* serotype 8 infection (Muckle *et al*., 2010). This could be suggesting that companion animals may be more susceptible to this serotype and further study focusing on serotype 8 can be

done to further determine this. Nonetheless, studies regarding *S. suis* isolates in cats and dogs is limited so far, there could be more serotypes that the feline and canine population can harbour.

The *in-silico* screening of antimicrobial resistance genes indicated that the isolate SS/UPM/MY/F001 possesses resistance genes towards macrolide, gene *mef*A and *msr*D. Both resistances gene are common resistance genes found among *S. suis* isolate of pigs (Chen *et al*., 2013; Varela *et al*., 2013; Huang *et al*., 2019; Dong *et al*., 2023). The gene *mef*A and *msr*D were also found in tandem just as described by a previous report (Iannelli *et al.*, 2018). This study also reports the *in-silico* detection of the *pat*A-*pat*B efflux protein complex genes in *S. suis*, known for the resistance to ciprofloxacin and norfloxacin which was commonly reported in *S. pneumoniae* (El Garch *et al*., 2010; Garvey *et al*., 2010). Previously, only the homologous *sat*A and *sat*B fluoroquinolone efflux pumps were demonstrated in *S. suis* (Escudero *et al*., 2011). Besides, resistance to vancomycin was also predicted with the *in-silico* detection of gene encoding the *van*Y operon of the *van*B gene cluster. So far, only the *van*Z operon and *van*G was fully demonstrated in *S. suis*, conferring resistance to teicoplanin, dalbavancin, and vancomycin. (Lai *et al*., 2017; Huang *et al*., 2018). The main objective of the study was to characterize the feline strain at a genomic level; hence, the phenotypic AMR expression was not further explored at this point.

Discrepancies in the prediction of antimicrobial resistant genes were observed when using three commonly used platforms: AMRFinder PLUS, ResFinder, and CARD's RGI. AMRFinder PLUS and ResFinder provided conflicting predictions, identifying macrolide resistant genes, whereas CARD's RGI predicted fluoroquinolone and glycopeptide resistant genes. These disparities in predictions can be attributed to the variations in the prediction model, curated database, and the specific threshold or cut-off value established by each platform (Papp & Solymosi, 2022). For instances, during a manual search of the databases utilized by AMRFinder PLUS and ResFinder, the *pat*A-B genes were absent from both databases which could explain why these genes were not predicted. On the other hand, CARD's RGI was only able to predict *mef*A-*msr*D when the prediction was conducted at a "loose hit" algorithm, a cut-off beyond the detection of clinical and known AMR genes. Furthermore, the detection of efflux markers *pat*A and *pat*B may not necessarily indicate a resistance because the expression is reliant to gene regulations (Mahfouz *et al*., 2020). Therefore, to support the AMR predictions and assess whether the predicted genes are indeed expressed, the gold standard of antibiotic sensitivity testing should be conducted.

Identification of mobile genetic elements in diverse species provides clues to how certain virulence or antimicrobial resistance were acquired (Vale *et al*., 2022; Morales *et al*., 2023). Based on the gene analysis, we have identified presence of *mef*A and *msr*D in an intact prophage which likely provide evidence where strain SS/UPM/MY/F001 had acquired this resistance through prophage transduction. At the same time, there was a possibility that this strain was capable to act as a reservoir of AMR and exchange genetic material via horizontal gene transfer through the prophage (Kondo *et al*., 2021). There were a few resemblances in MGE between the SS/UPM/MY/F001 strain and other strains described so far. Based on the results, there were two putative type IV secretion systems (T4SSs) regions identified in strain SS/UPM/MY/F001 which were known to be responsible for the horizontal gene transfer of the 89K pathogenic island (PAI) in strains that inflicted human outbreak infections (Li *et al*., 2011). However, it lacks the *salK*/*salR* transduction systems located in the T4SSs crucial for highly invasive *S. suis*. Aside from that, the insertion sequence families ISL3, IS110, and IS200/IS605 present in SS/UPM/MY/F001 were common insertion families reported in the *S. suis* strains of swine and human (Kerdsin *et al*., 2021; Nicholson & Bayles, 2022).

As the companion animal population increases and shifts towards a more common lifestyle of having close contact between human and their pets, the risk of zoonosis spread to the human population also increases. Although *S. suis* serotype 8 has not been reported in human cases so far, *S. suis* strain SS/UPM/MY/F001 poses a threat of disease transmission to human as it possessed pathogenic families of the human strains and predicted to be a potential human pathogen. Furthermore, the presence of many virulence factors indicated that this strain should not be taken lightly. Despite the fact that this study was unable to associate positive detection to the exposure of swine material due to the limited positive samples, the acquirement of *S. suis* by the feline and canine population from feed particularly raw pork needs to be further investigated. This is because *S. suis* serotype 8 remains a common serotype in swine (Goyette-Desjardins *et al*., 2019; Lunha *et al*., 2022) and that raw meat diet feeding to companion animals was practiced by some pet owners.

Study limitation

This study exhibits a notable constraint stemming from the relatively limited quantity of positive samples at our disposal despite optimizing our method of sample collection and detection. Consequently, we encounter impediments in establishing correlations with respect to risk factors. Furthermore, the process of sample collection presented specific challenges where in numerous instances, pet owners were uncertain regarding the potential exposure of their pets to raw pork when the pets were allowed to roam outdoors. To mitigate these constraints, it is crucial to expand the scope of our research by conducting a more comprehensive sample collection, specifically targeting animals with confirmed contact (e.g., consuming raw pork, living proximity with pigs, etc.), and utilizing serology to determine the extent of exposure.

Another noteworthy limitation pertains to the absence of an in-depth exploration of the phenotypic antimicrobial resistance, which could have provided a more substantive foundation for our AMR predictions. It is evident when the different platforms yielded dissimilar results, we are not able to conclude whether predictions were true or expressed. Even so, the results provided an idea to where we can improve and further narrow down our future studies. Given the individual strengths and weaknesses posed by each AMR prediction platform, researcher ought to understand the basis behind each platform to make a meaningful interpretation of result that matches each study objective. For example, AMRFinder PLUS provides better prediction in acquired resistance genes while CARD's RGI aces in predicting mutation conferring resistance genes (Papp & Solymosi, 2022). Nevertheless, numerous databases are constantly undergoing refinement and there is need for different databases to converge as well as synchronize their data to provide a comprehensive and standardize AMR prediction.

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Conflict of Interest

The author declares that they have no conflict of interests.

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