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Characterization of attributes beneficial to pathogen fitness of Philippine *Streptococcus suis* strain 8-57C serotype 1/2 from diseased pig lung

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ABSTRACT

Research on the important swine zoonotic pathogen *Streptococcus suis* is disproportionately directed towards serotype 2 since this is the most common and virulent serotype. However, mechanisms of epidemiology and pathogenesis of possibly opportunistic serotypes like serotype 1/2, also common in pigs, are usually understudied. *S. suis* research in swine in the Philippines is severely lacking, with only two publications in the past half-decade. Hence, this study performed a genome-based evaluation of the Philippine *S. suis* strain 8-57C serotype 1/2 isolated from diseased pig lung to determine characteristics beneficial to pathogen fitness, like the presence of antimicrobial resistance (AMR) genes, virulence factors (VFs), and biosynthetic gene clusters (BGCs). PCR-based AMR gene screening and antimicrobial susceptibility testing were also performed to verify the predicted AMR activity. Genome analysis revealed the presence of several VF classes (adherence, enzyme, immune evasion, and protease) which could aid pathogenesis with the ability to adhere to cells and organs, cross the blood-brain barrier, protect against phagocytosis, and exhibit hemolytic activity. Genome-based AMR prediction, PCR-based AMR profiling, and antimicrobial susceptibility testing showed that *S. suis* strain 8-57C was resistant to lincosamides, macrolides, streptogramin B, tetracycline, and amphenicol antibiotic classes. Additionally, BGC prediction identified a lanthipeptide BGC similar to the bacteriocin suicin 90-1330. Further experimentation is recommended to verify if these virulence factors and metabolites are expressed and if they contribute to pathogen fitness. These findings could pave the way for exploring characteristics relevant to the pathogen fitness of other non-serotype 2 *S. suis* serovars in the Philippines.

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Date received: 15 January 2025
Dates revised: 19 April 2025; 28 May 2025
Date accepted: 10 June 2025
DOI: https://doi.org/10.54645/2025181KIA-75

INTRODUCTION

Streptococcus suis is a gram-positive bacterium of global economic and epidemiological importance in the pig industry with associated diseases exemplified by arthritis, endocarditis, meningitis, polyserositis, and septicemia, as well as the zoonotic potential that in extreme cases leads to fatality (Estrada et al. 2019). However, *S. suis* also normally and harmlessly populates the upper respiratory tract of almost all pigs globally as a commensal (Votsch et al. 2018; Neila-Ibañez et al. 2023). This dual characteristic makes *S. suis* a pathobiont, sub-clinically infecting pigs until homeostasis is disrupted to allow pathogenesis (Votsch et al. 2018). The switching of *S. suis* from a porcine commensal to a disease-causing pathogen is determined by a range of factors, inclusive of environmental conditions, host health, as well as a combination of *S. suis*

KEYWORDS

antimicrobial resistance genes, biosynthetic gene clusters, genome analyses, microbiology, pathogenicity, serotype ½, Streptococcus suis, virulence factors

characteristics (e.g., serotype, sequence type, virulence factors, antimicrobial resistance genes, and capacity to produce secondary metabolites) that shape the host microbiota in favor of *S. suis* (Votsch et al. 2018; Estrada et al. 2019; Aradanas et al. 2021; Obradovic et al. 2021; Dresen et al. 2023).

There are already numerous studies on *S. suis*, particularly on pathogenesis, virulence factors and markers, vaccine development, immune response, taxonomy, genetic comparative analysis, epidemiology, and public health responses (Segura et al. 2017; Segura et al. 2020). However, they mostly focus on serotype 2, the most common and virulent serotype, leaving other serotypes understudied (Goyette-Desjardins et al. 2014; Segura 2015; Rieckmann et al. 2020). *S. suis* serotype 1/2 is another highly isolated serotype from diseased pigs worldwide (Goyette-Desjardins et al. 2014) and is usually mischaracterized as serotype 2. Estrada et al. (2019) established that serotype 1/2 is the most predominant serotype in diseased pigs in North America, while a study in Spain revealed that as much as 43% of isolates initially classified as serotype 2 belong instead to serotype 1/2 (Rilo et al. 2024).

Roy et al. (2017) determined that serotypes 2 and 1/2 have high similarity between their capsular polysaccharides, which is conventionally used in serotyping, and only a single nucleotide polymorphism at the glycosyltransferase (*cpsK)* gene could differentiate between them. Like serotype 2, serotype 1/2 can be classified as a pathogenic pathotype that can infect the brain or meninges, heart, joints, or liver of pigs, causing meningitis, epicarditis, arthritis, or septicemia. (Estrada et al. 2019). A study on diseased growing-finishing pigs showed that serotype 1/2 has a high lung isolation frequency (Wu et al. 2023). However, serotype 1/2 isolated from pigs' lungs that do not have other signs of neurological or systemic disease are usually classified as possibly opportunistic (Estrada et al. 2019), making this organ a relatively underexplored *S. suis* infection site.

Global multilocus sequence typing (MLST) studies of *S. suis* identified ST1, ST25, and ST28 as the most prevalent sequence types (STs) in swine (Onishi et al., 2012) with ST28 as associated with the pathogenic pathotype of swine and human disease (Goyette-Desjardins et al. 2014). With *S. suis* serotype 1/2 and ST28 associated as pathogenic, investigating a *S. suis* serotype 1/2 ST28 from diseased pig lungs could determine beneficial characteristics that improve the survivability and eventual pathogenesis of this possibly opportunistic serotype. Identifying the attributes leading to pathogen fitness in *S. suis* is significant in identifying possible pathogenic strains (Estrada et al. 2019), understanding the epidemiological role of *S. suis* in disease progression and transmission (Gottschalk et al. 2010), formulating strategies in preventing its infection and spread in pigs at different production stages (Wu et al. 2023), and constructing diagnostic tools for pathogen surveillance (Segura et al. 2017). Furthermore, expanding the investigation of virulence traits to other *S. suis* serotypes can enhance the inhibition and control of this strain (Estrada et al. 2019).

Locally, *S. suis* research in the Philippines is severely lacking as compared with its Asian neighbors (Huong et al. 2014), with the first isolates from local swine published just in 2020 (Sedano et al. 2020), which included biochemical profiling and phenotypic testing of antimicrobial resistance. Most recently, Sedano et al. (2023) studied 269 *S. suis* isolates of varying serotypes across nine provinces in the Philippines and performed serotyping, sequence typing, and identification of putatively critical virulence factors such as muramidase-released protein (mrp), extracellular protein factor (epf), and suilysin (sly) (Nicholson and Bayles 2022). To further address the *S. suis* information gap in the Philippines, this current study performed the first whole genome sequencing of Philippine *S. suis* strain 8-57C which was identified as serotype 1/2 ST28 isolated from a diseased pig lung and determined attributes beneficial to pathogenicity such as virulence factors (VFs), antimicrobial resistance (AMR) and prediction of biosynthetic gene clusters (BGCs). With the inherent limitations of in silico WGS predictions (Ellington et al. 2017), predicted AMR genotypes were supplemented with amplification-based AMR gene testing and phenotypic antimicrobial susceptibility testing.

MATERIALS AND METHOD

Sample Collection and Bacterial Isolation

The slaughterhouse was inspected and properly sanitized before and after the slaughter operations. Prior to the operations, all the visible debris and organic matter were removed. The floors, walls and equipment were washed with 50 ppm calcium hypochlorite solution and rinsed thoroughly with clean water. The tools used were thoroughly cleaned and disinfected after each use. A separate area was designated for sample collection to prevent cross contamination. After the operations, all the remnants of the carcass and organic waste were properly disposed of. The surfaces were scrubbed with detergent and clean water, followed with disinfection of the floors, walls, and equipment, and sanitation of knives, hooks and machinery. These sanitation procedures were duly enforced by the slaughterhouse supervisor to ensure the prevention of cross-contamination in the slaughterhouse.

Oropharyngeal, nasopharyngeal swabs, and lung samples were collected from 264 asymptomatic finisher pigs from April 2020 to March 2022 in a selected abattoir in Laguna. Swab samples were placed in an AMIES transport medium (OxoidTM, UK), while lung samples were kept on ice before processing in the laboratory. For the lung samples, the workers in the abattoir were informed of the desired diseased lungs as samples. Once the lungs were removed from the slaughtered pigs and identified as diseased, a portion of approximately 250 g was sliced by the butcher. This was immediately placed in resealable bags and kept on ice. The lungs were seared with a hot spatula before processing for isolation.

Lung tissues were acquired aseptically and then suspended in a sterile normal saline solution for at least 2 hours. Aliquots of 0.1 mL lung tissue suspensions were serially diluted and spread on Columbia Blood agar (CBA) plates containing 5% defibrinated sheep blood with and without added Streptococcal supplement (Oxoid^{TM}, UK). The plates were incubated at 37 ^{o}C for 24 hours at 5% CO_2. Meanwhile, serial dilutions of the AMIES transport medium were prepared, and 0.1 mL of the various dilutions was spread plated on CBA and incubated as previously mentioned.

A total of 506 *S. suis* strains were isolated from this collection. All of these isolates were confirmed as *S. suis* using the recN primers, underwent biochemical characterization, serotyped by multiplex PCR and subjected to PCR-based screening of AMR genes. However, in this paper, only the *S. suis* strain 8-57C serotype 1/2 isolated from diseased lung tissues was studied for molecular and genomic characteristics to determine attributes beneficial to its pathogenicity.

Biochemical Characterization

Small, translucent, and α-hemolytic colonies that cause greenish discoloration in the medium were selected and successively purified on CBA. Using a sterile toothpick, a smear of the colony was placed on a glass slide and tested for catalase by adding a drop of 10% hydrogen peroxide. The absence of bubbles indicates the typical reaction of *S. suis.*

Genomic DNA Isolation

The crude DNA extract of *S. suis* strain 8-57C was used in PCR-based identification of *S. suis* using the *recN* gene, multiplex PCR-based serotyping of *Streptococcus suis*, and PCR-based AMR gene screening. Crude DNA extract was obtained by inoculating a sufficient amount of bacterial cells in 0.1 M NaOH solution and boiling it for 5-7 minutes at 100°C. This was used as the template for PCR. On the other hand, genomic DNA was isolated using the Monarch Genomic DNA Purification Kit (New England Biolabs USA) following the manufacturer's protocol for whole genome sequencing. No modifications were made to the procedure.

PCR-Based Identification of *S. suis* using the *recN* Gene

PCR-based identification of *S. suis* strain 8-57C was done following the amplification method of Ishida et al. (2014) with a few modifications. The recombination/repair protein gene (*recN*) was amplified using SSrecN forward (5'-CTACAAACAGCTCTCTTCT-3') and SSrecN reverse primers (5'-ACAACAGCCAATTCATGGCGTGATT-3'), producing a 336 bp-sized amplicon. The PCR reaction was performed in 25 µL total volume using 1x MyTaq HS Red Mix (Meridian Bioscience, UK), a final concentration of 0.2 µM each of *recN*-specific forward and reverse primers, and 1 µl of crude DNA extract as template. Amplification was carried out in a Techne Prime thermal cycler (Cole Parmer, USA) with the following conditions: an initial denaturation step at 94ºC for 2 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 58ºC for 10 s and extension at 68ºC for 30 s, and a final extension step at 68ºC for 5 min. The amplified PCR products were loaded in a 2% agarose gel in 0.5% TBE and electrophoresed for 30 min at a constant voltage of 100V (Mini Gel II, Select Bioproducts, USA). DNA staining solution (Biotium GelRed, Fremont, California, USA) was added to the PCR products and subjected to UV light using a gel documentation system (Syngene GBOX, United Kingdom). The sizes of the PCR products were determined by comparison with a 100 bp molecular size standard (Vivantis, Malaysia). Genomic DNA from *S. suis* serotype 2 (DSM 28762) was used as the *recN* positive control.

Serotyping of *Streptococcus suis*

The crude DNA extract of *S. suis* strain 8-57C was used for serotyping using a four-reaction set multiplex PCR targeting the capsular polysaccharide synthesis (*cps*) genes by Kerdsin et al. (2014). Set 1 included the primers for serotypes 1/2, 1, 2, 3, 7, 9, 11, 14 and 16; Set 2 for serotypes 4, 5, 8, 12, 18, 19, 24 and 25; Set 3 for serotypes 6, 10, 13, 15, 17, 23 and 31; and Set 4 for serotypes 21, 27, 28, 29 and 30. The PCR mixture contained 1x MyTaq HS Red Mix (Meridian Bioscience, UK) and 0.2 µM of each primer for each set of PCR. Crude DNA extract (1 µl) was used as the template. The reference strains of *S. suis* serotypes 1, 1/2, 2, 3, 7, 11, 14, and 16 used as positive controls in the multiplex PCR reaction were gifted by Dr. Anusak Kerdsin of the Faculty of Public Health, Kasetsart University Chalermphrakiat Sakon Nakhon Province, Thailand (Kerdsin et al., 2014).

The following PCR thermal profile of PCR was used: initial activation of DNA polymerase and initial DNA denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 20 s, primer annealing and extension at 62 °C for 90 s, and a final extension at 72 °C for 5 min (Techne Prime Large-Format Thermal Cycler, Cole Parmer, USA). The amplified PCR products were loaded in a 2% agarose gel in 0.5% TBE and electrophoresed for 30 min at a constant voltage of 100V (Mini Gel II, Select Bioproducts, USA). DNA staining solution (Biotium GelRed, Fremont, California, USA) was added to the PCR products and subjected to UV light using a gel documentation system (Syngene GBOX, United Kingdom). The sizes of the PCR products were determined by comparison with a 100bp molecular size standard (Vivantis, Malaysia). Primer sequences are presented in Table S1. Further in silico verification was performed using serotype-specific *cpsK* sequences by Matiasovic et al. (2020) to verify if the strain belonged to serotype 1/2 and not the closely similar serotypes 1, 2, or 14. The cpsK sequence specific to serotype 1/2 was 5' GGTGGCCTG**T**AATAAAC 3', wherein thymine at position 10 is specific for the serotype 1/2 *cpsK* gene.

Whole Genome Sequencing

The *S. suis* strain 8-57C genome was sequenced by Macrogen, Korea, using the HiSeq Illumina platform. Genomic DNA was isolated using the Monarch Genomic DNA Purification Kit (New England Biolabs USA) following the manufacturer's protocol. The genome library was prepared using the TruSeq Nano DNA Kit (Illumina, USA) by Macrogen, Korea. Bridge amplification was then used to amplify distinct clonal clusters, and the resulting templates were sequenced using the Illumina HiSeq 4000 platform with 100 bp paired-end and 0.5 Gb throughput. The resulting raw fastq files were subjected to trimming of adapters and end-bases coupled with an average quality cutoff of 20 using Trimmomatic v0.36 (Bolger et al. 2014) and FastQC v0.11.5. GC (%), Q20 (%), Q30 (%) were calculated before and after filtering (Table S4). Heterozygosity, k-mer coverage, and estimated genome size were determined using Jellyfish v.2.2.10 (Marcais and Kingsford 2011). *De novo* assembly was done using the SPAdes v3.13.0 assembler (Bankevich et al. 2012), and the assembled genome was validated using a self-mapping strategy and Benchmarking Universal Single-Copy Orthologs (BUSCO, v.3.0) analysis (Simão et al. 2015).

For submission to the GenBank database, the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (v6.5) (Li et al. 2021) was used to annotate the assembled genome of *S. suis* strain 8-57C. The Genome Annotation Service in the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (https://www.bv-brc.org/) (Olson et al. 2023) was utilized to generate the genome map containing coding sequences (CDSs) classified into subsystems of specific biological processes. The resulting genome was deposited in GenBank under the accession number JASFZV000000000.1.

Phylogenetic and phylogenomic analyses

Phylogenetic analysis using the *S. suis* strain 8-57C 16S rRNA and phylogenomic analyses using its genome sequence were performed using the Type Strain Genome Server (TYGS) (Meier-Kolthoff and Göker 2019). TYGS compared the *S. suis* strain 8-57C genome against all the type strains in its database using the MASH algorithm which allows fast approximation of intergenomic relatedness. Ten type strains with the smallest MASH distances were chosen. TYGS also added at least 10 closely related type strains based on the 16S rRNA gene sequences. The *S. suis* strain 8-57C full length 16S rRNA sequence (1.5 kb) was extracted from its genome using the RNAmmer program and BLASTed against the 16S rRNA sequences in the TYGS database. This was used as a proxy to find the best matched type strains based on bitscore. The precise distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula d5. A phylogenetic tree was generated based on pairwise comparisons among sets of genomes using the GBDP approach. Accurate intergenomic distances were inferred under the algorithm "trimming" and distance formula d5. The resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1, including subtree pruning and regrafting (SPR) postprocessing. Branch support was inferred from 100 pseudo-bootstrap replicates each. Default settings were used to generate

the phylogenetic trees.

Sequence Typing Using MLST

Genome sequence-based sequence typing of *S. suis* strain 8-57C was performed using Multilocus Sequence Typing (MLST) 2.0.9 with database version 06/19/2023 by the Center of Genomic Epidemiology (https://cge.food.dtu.dk/services/MLST/) (Larsen et al. 2012). Determination of *S. suis* sequence types (ST) using the MLST scheme was determined using seven housekeeping genes: *aroA* (5-enolpyruvylshikimate-3-phosphate synthase), *cpn60* (60 kDa chaperonin), *dpr* (Dps-like peroxide resistance protein), *recA* (homologous recombination factor A), *gki* (glucose kinase), *mutS* (DNA mismatch repair protein), and *thrA* (aspartokinase A).

Genotypic Assessment of Antimicrobial Resistance, Virulence Factors and Secondary Metabolite Gene Cluster

Identification of antimicrobial resistance, virulence factors, and secondary metabolite gene clusters was determined using the *S. suis* strain 8-57C genome sequence. Identification of acquired genes or chromosomal mutations that mediated antimicrobial resistance was performed using ResFinder 4.1 (http://genepi.food.dtu.dk/resfinder) by the Center for Genomic Epidemiology (Bortolaia et al. 2020). Virulence factors were predicted using VFAnalyzer through the virulence factor database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi) (Liu et al 2022), while the prediction for BGCs encoding compounds which may be beneficial for *S. suis* infection was assessed using antiSMASH 7.0 (https://antismash.secondarymetabolites.org/#) (Blin et al. 2023).

PCR-Based AMR Gene Screening

S. suis strain 8-57C was also subjected to PCR-based detection of AMR genes such as aminoglycoside resistance genes (Vakulenko et al., 2003), macrolide and tetracycline resistance genes (Malhotra-Kumar et al., 2005), florfenicol resistance genes (Kehrenberg and Schwarz, 2006), as well as phenicol and oxazolidinone resistance genes (Shang et al., 2019; Brenciani et al., 2019). The amplification of the corresponding sizes of the target AMR gene being analyzed was evaluated as positive. The summaries of the target genes, their antimicrobial resistance phenotype, primer sequences, amplicon size, and PCR profiles are shown in Tables S2 and S3.

Phenotypic Antimicrobial Susceptibility Profiling

The antibiotic susceptibility profile of *S. suis* strain 8-57C was further evaluated using the disk diffusion assay from the standard protocol of the Clinical and Laboratory Standards Institute (CLSI 2023) and Sedano et al. (2020) with minor modifications. A single colony of *S. suis* strain 8-57C was picked and grown overnight in CBA plates and incubated at 37°C with 5% CO_2. The bacterial lawn was harvested, suspended in 0.85 % normal saline solution, and adjusted to 0.5 McFarland. The resulting bacterial suspension was spread plated (100 µL) on Mueller-Hinton agar supplemented with 5% sheep blood and used as assay plates. Sterile 6 mm circular paper disks were then aseptically placed onto the assay plates. The antibiotics added on the paper disks included ampicillin (AMP) (10 µg), cefalexin (CN) (30 µg), cefotaxime (CTX) (30 µg), ceftriaxone (CTR) (30 µg), penicillin (PEN) (10 units), azithromycin (AZM) (15 µg), erythromycin (E) (15 µg), doxycycline (DO) (30 µg), tetracycline (TE) (30 µg), gentamicin (GEN), chloramphenicol (CLM) (30 µg), levofloxacin (LE) (5 µg) and clindamycin (CD) (2 µg). The antibiotic disk assay was done in triplicate. *Streptococcus pneumoniae* ATCC 49169 was used as a control. The assay plates were incubated at 36ºC for 24 hr using the microaerophilic method, and the zones of inhibition were measured. Zone measurements were assessed as susceptible, intermediate, or resistant according to CLSI (2023), except for GEN, which used those of Sedano et al. (2020).

RESULTS AND DISCUSSION

Sample Collection and Bacterial Isolation

S. suis was detected in 133 out of the 264 samples of asymptomatic finisher pigs obtained from April 2020 to March 2022 in a selected abattoir in Laguna, and a total of 506 *S. suis* strains were isolated. Out of these *S. suis* isolates, 248 strains were isolated from oropharyngeal swabs, 242 from nasopharyngeal samples, and 46 from diseased lung samples. *S. suis* strains isolated from the laryngeal, tonsil, or nasal tissues are classified as commensals that can reside in or on the body without harming human health, while those isolated from lung tissues without signs of neurological or systemic disease are classified as possibly opportunistic. Out of the 46 *S. suis* strains isolated from lung tissues, 7 were identified as serotype 2 or 1/2. Serotypes 2 and 1/2 are the most virulent serotypes and the most frequently isolated from diseased animals in North America (Estrada et al. 2019). *S. suis* strain 8-57C isolated from diseased lung tissues, was identified at serotype 1/2 and ST28, which is a known causative agent of swine and human disease (Goyette-Desjardins et a., 2014). Because of this, this strain was further studied for molecular and genomic characteristics to determine attributes beneficial to its pathogenicity.

Phenotypic, Biochemical, and Molecular Characterization

S. suis strain 8-57C which was isolated from a diseased pig lung, displayed typical *S. suis* characteristics such as the presence of whitish translucent colonies, green-colored, alpha-hemolytic reaction in Columbia Blood Agar plates (Goyette-Desjardins et al. 2014), and negative catalase reaction (Xia et al. 2019). Likewise, the putative *S. suis* isolate amplified the rec*N* gene previously used for *S. suis* identification (Tien et al. 2013; Ishida et al. 2014).

S. suis lung isolates are rarely assessed for virulence. These are usually classified as possibly opportunistic (Estrada et al. 2019; Wileman et al. 2019), unlike those from the upper respiratory tract, which are commensals (Votsch et al. 2018; Neila-Ibañez et al. 2023) and those from systemic (heart, joints) or neurological (brain/meninges) tissues, which are identified as primary pathogens (Estrada et al. 2019). This is due to the common path of *S. suis* pathogenesis involving the colonization of the mucus and epithelial cells of the swine's upper respiratory tract to infiltrate the bloodstream, where it induces a range of diseases and avoids phagocytosis (Segura et al. 2016).

Serotyping

Serotypes 2 or 1/2, 3, 7, 9, 11, and 16 were identified from the *S. suis* strains obtained from the Laguna abattoir collection, wherein serotype 3 was the most prevalent. These serotypes were associated with causing similar infections in pigs and humans. Multiplex PCR results classified *S. suis* strain 8-57C as serotype 1/2. However, most PCR-based techniques are unable to accurately discriminate between serotypes 2 and 1/2 due to the very high similarity between the *cpsK* loci of these serotypes, which differ by only a single nucleotide in position 481 (Okura et al. 2014; Athey et al. 2016). Therefore, a secondary genome sequence-based serotype verification was performed using a serovar 1/2 -specific *cpsK* sequence (Matiasovic et al. 2020). This confirmed the multiplex PCR result showing that *S. suis* strain 8-57C belonged to serotype 1/2.

The serotyping result of *S. suis* strain 8-57C from diseased pig lung concurred with previous reports in the United States that a

greater proportion of serotype 1/2 isolates are associated with the pathogenic pathotype (Estrada et al. 2019). Likewise, most *S. suis* serotype 1/2 were found in the lungs of diseased growing-finishing pigs in Taiwan based on a study by Wu et al. (2023). Serotypes 1/2, along with serotypes 2 and 3, are identified globally as crucial serotypes regarding clinical infections (Segura et al. 2020).

In the Philippines, however, serotype 1/2 appears to have low prevalence. In the collection of *S. suis* isolates from Laguna abattoir done in this study, only 18/506 (3.55%) were identified as serotype 2 or 1/2. Out of the 18 serotype 2 or 1/2 detected, 7 strains were isolated from diseased lung tissues. In a separate study done by Sedano et al. (2023), *S. suis* serotype 1/2 was not detected from the 269 *S. suis* tonsil isolates serotyped across 9 provinces in Luzon (Albay, Batangas, Marinduque), Visayas (Bohol, Cebu, Iloilo) and Mindanao (Misamis Occidental, Misamis Oriental, Zamboanga del Norte). However, the absence of serotype 1/2 in this isolation matrix could be attributed to serotype 1/2 to be more likely to exist in the lungs of pigs (Wu et al. 2023), while mostly commensal *S. suis* strains are found on the upper respiratory tract (tonsil, nose) of asymptomatic pigs (Estrada et al. 2019).

Genome Features

Whole genome sequencing of *S. suis* strain 8-57C revealed a total genome size of 2,172,039 bp consisting of 40 contigs with an average depth of 417, an N50 of 95,379, G+C of 41.06%, and a total of 2,011 coding sequences. BUSCO analysis was done using bacteria_odb10 lineage (4085 genomes, 124 BUSCOs), resulting in 97.58% complete and single-copy BUSCO (121 BUSCOs) and 2.42% fragmented BUSCOs (3 BUSCOs). The summary of the contig features is presented in Table 1, while Figure 1 shows the *S. suis* strain 8-57C genome map. The *S. suis* strain 8-57C genome was analyzed to determine phylogenetic relationships, perform MLST, identify genes associated with pathogen fitness, such as AMR and VFs, and predict the presence of BGCs.

Table 1: Annotated genome features of *Streptococcus suis* strain 8-57C isolated from diseased pig lung

Features	Total
Total read bases (bp)	1,035,059,918
Total reads (bp)	10,248,118
Assembly length (bp)	2,107,130
GC content (%)	41.06
Longest contig (bp)	209,331
Number of contigs	40
N50 (bp)	95,379
Average genome coverage (%)	100%
Depth	409.12
Complete BUSCOs	
Complete and single-copy (121 BUSCOs)	97.58 %
Complete and duplicated BUSCOs (0 BUSCOs)	0%
Fragmented BUSCOs (3 BUSCOs)	2.42 %
Missing BUSCOs (0 BUSCOs)	0%
Genes (Total)	2,106
CDS (Total)	2,064
Genes (coding)	2,011
CDSs (with protein)	2,011
Genes (RNA)	42
rRNA	3, 1, 1 (5S, 16S, 23S)
Complete rRNAs	1, 1, 1 (5S, 16S, 23S)
Partial rRNAs	2 (5S)
tRNA	33
ncRNA	4
Pseudo Genes (total)	53
CRISPR Arrays	1

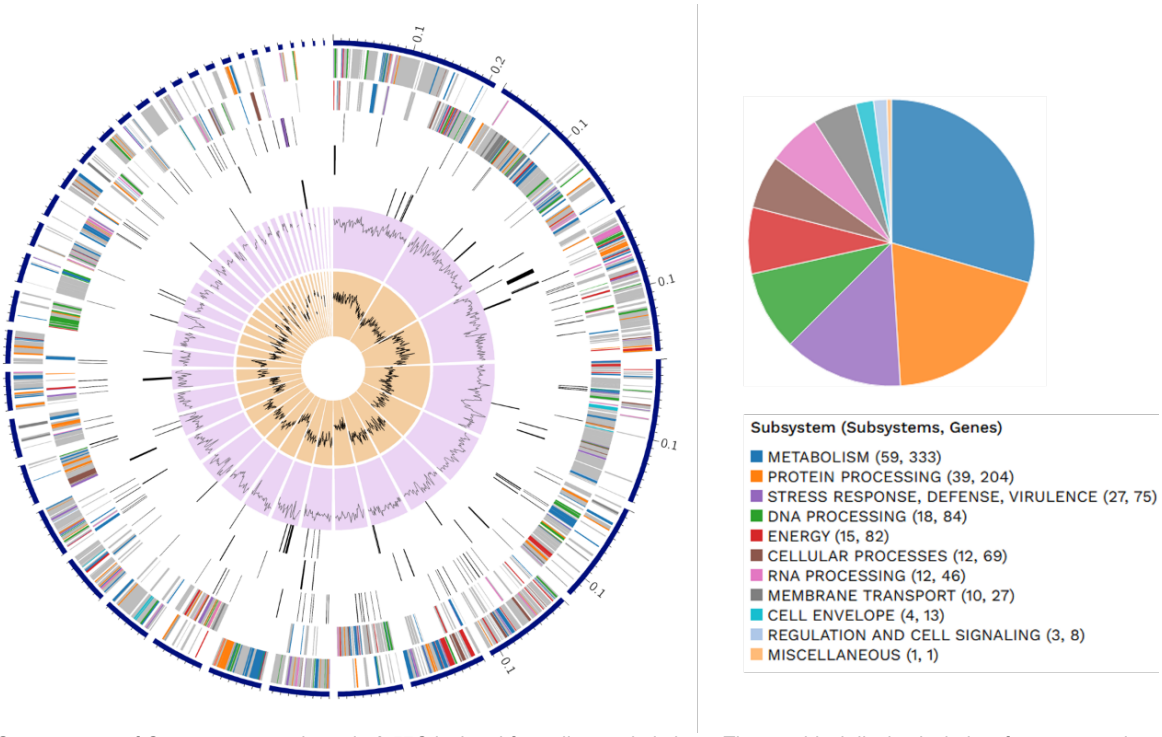

Figure 1: Genome map of *Streptococcus suis* strain 8-57C isolated from diseased pig lung. The graphical display includes, from outer to inner rings, the contigs (dark blue), CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content, and GC skew. An overview of the subsystems for the *Streptococcus suis* strain 8-57C genome is also presented on the right.

Phylogenetic and Phylogenomic Analysis

Phylogenetic relationships of *S. suis* strain 8-57C based on the 16S rRNA gene and pairwise genome sequence alignment using the Type Strain Genome Server showed that it grouped with *Streptococcus suis* S735 with a bootstrap value of 100% (Figure 2). This supports the earlier putative identification of strain 8-57C as *S. suis* using morphological, biochemical, and PCR-based analyses.

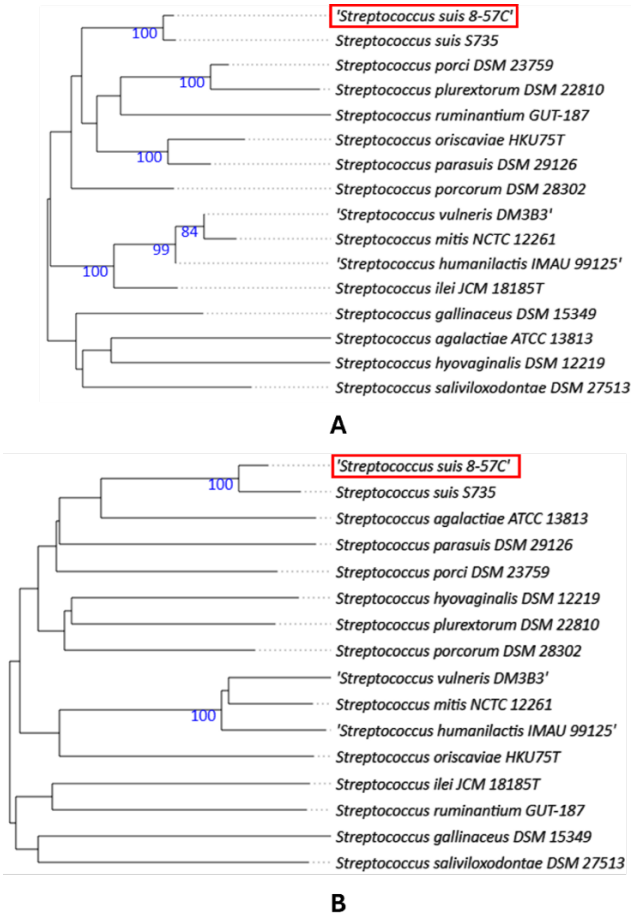

Figure 2: Phylogenetic relationship of *S. suis* strain 8-57C with other *Streptococcus* species based on the full length 16S rRNA (1.5 kb) (A) and pairwise genome sequence alignment (B) using the Type Strain Genome Server. The phylogenetic relationship was inferred with FastME 2.1.6.1 from Genome BLAST Distance Phylogeny (GBDP) distances calculated from 16S rDNA gene sequences. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above the branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 64.0 %. The tree was rooted at the midpoint. On the other hand, the phylogenetic relationship of the genome sequences was also calculated with FastME 2.1.6.1 from 1 GBDP distances. The branch lengths are scaled in terms of the GBDP distance formula d5. The values above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 42.1 %. The tree was also rooted at the midpoint. Default settings were used to generate the phylogenetic trees.

Multilocus Sequence Typing of *S. suis*

Using the MLST scheme, *S. suis* strain 8-57C was identified as ST 28. Globally, *S. suis* STs 1, 25, and 28 represent the highest isolation frequency in swine (Estrada et al. 2019). While ST28 is prevalent in diseased pigs in North America (United States and Canada) (Estrada et al. 2019; Segura et al. 2020), it is also present in significant proportions in diseased pigs in Asia, particularly Japan and China (Tang et al. 2011; Onishi et al. 2012; Chen et al. 2013).

In the United States, an association was proposed between most *S. suis* ST 28 to the pathogenic pathotype (Estrada et al. 2019). Aside from pigs, ST 25 and 28 were verified to be causative agents of human disease (Goyette-Desjardins et al. 2014). However, ST on its own is inconclusive in the prediction of virulence as it leaves out significant and relevant genomic information (e.g., DNA polymorphisms outside the housekeeping genes, gene variability in mobile genetic elements, etc.) (Athey et al. 2015). Indeed, virulence variation exists between ST 28 strains (Athey et al. 2015), with several previous reports implicating it with low virulence (Fittipaldi et al. 2011; Goyette-Desjardins et al. 2014; Yao et al. 2015). However, experimental disease modeling predicts that a portion of ST 28 strains are capable of causing severe disease (Athey et al. 2015).

Evaluation of Characteristics Beneficial to Pathogen Fitness

Antimicrobial Resistance Profiling

Prediction for *S. suis* strain 8-57C AMR genes using ResFinder identified the *erm(B)* gene, which encoded for resistance to lincosamides, macrolides, streptogramin b; and the *tet(O)* gene, which coded for tetracycline resistance (Table 2). With errors associated with inherent algorithm limitations of genome assemblers (Ellington et al. 2017), in silico AMR gene prediction was coupled with PCR-based detection of resistance genes specific to aminoglycosides, tetracyclines, macrolides, and lincosamides, among others (Table 3), which are main antibiotic classes used for clinical treatment of *S. suis* (Haenni et al. 2018). AMR genes (e.g., *fexA*, *poxtA*, *optrA*, and *cfr*) that confer resistance to phenicols and oxazolidinone were also included (Table 3). PCR analysis verified the presence of *tet(O)*, *optrA*, and *cfr* genes but not the *erm(B)* gene as presented in Table 3.

Table 2: Summary of identified antimicrobial resistance genes in *Streptococcus suis* strain 8-57C isolated from diseased pig lung using ResFinder 4.1.

Antimicrobial	Class	WGS-Predicted phenotype	Genetic background
Clindamycin	Lincosamide	Resistant	*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Lincomycin			*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Erythromycin	Macrolide	Resistant	*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Pristinamycin Ia	Streptogramin b	Resistant	*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Quinupristin		Resistant	*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Virginiamycin S		Resistant	*erm(B) (erm(B)_JN899585), erm(B) (erm(B)_X72021)*
Doxycycline	Tetracycline	Resistant	*tet(O) (tet(O)_Y07780)*
Minocycline		Resistant	*tet(O) (tet(O)_Y07780)*
Tetracycline		Resistant	*tet(O) (tet(O)_Y07780)*

Table 3: Targeted molecular-based antimicrobial resistance gene profiling of *Streptococcus suis* strain 8-57C isolated from diseased pig lung

Gene	Antimicrobial Resistance Phenotype	Presence
aac(6')-Ie, aph(2")-Ia	Aminoglycoside acetyltransferase	(-)
aph(3')-IIIa	Aminoglycoside phosphotransferase	(-)
ant(4')-Ia	Aminoglycoside nucleotidyltransferase	(-)
ant(4')-Ia		(-)
aph(2")-Ib	Aminoglycoside phosphotransferase	(-)
aph(2")-Ic		(-)
aph(2")-Id		(-)
tet(K)	Tetracycline	(-)
tet(L)		(-)
tet(M)		(-)
tet(O)		(+)
mef(A)	Macrolide	(-)
mef(E)		(-)
erm(A)	Streptogramin A and B, Macrolide, Lincosamide	(-)
erm(B)		(-)
fexA	Phenicol	(-)
optrA	Phenicol, Oxazolidinone	(+)
poxtA	Phenicol, Oxazolidinone, Tetracycline	(-)
cfr	Phenicol, Oxazolidinone, Lincosamide, Streptogramin	(+)

The negative amplification of the *erm(B)* gene could be attributed to the crude DNA that was used as the template for PCR. Crude DNA extracts often contain contaminants such as polysaccharides, proteins, lipids, and phenolic compounds that may interfere with the activity of the polymerase enzyme, thus causing false negative results. Furthermore, other than post- and pre- ribosomal modifications by methylation encoded by erythromycin ribosome methylase (erm), the S*treptococcus* genus has other resistance mechanisms for macrolides which includes, macrolide expulsion by the major facilitator superfamily (MFS) efflux pumps (macrolide efflux family, mef) and target protection (Berbel et al. 2022). These mechanisms could have been utilized by *S. suis* strain 8-57C for the exhibited phenotypic macrolide resistance.

Meanwhile, manual checking for the hybridization of the *optrA* and *cfr* primers, which are specific for amphenicol resistance genes, to the *S. suis* strain 8-57C genome sequence yielded negative results. This suggested that the genes responsible for amphenicol resistance in *S. suis* strain 8-57C may be contained in a plasmid that was not resolved properly during whole genome sequencing. Han et al. (2023) isolated *S. parasuis* strains that carry a chromosomal *optrA* gene and a plasmid-borne *cfr(D)* gene showing that the *cfr(D)* gene can be associated with a plasmid. The most utilized genome sequencing platform for bacteria uses short-read sequencing technology such as Illumina-based platforms. While useful, it could also lead to fragmented genome assemblies, wherein plasmid sequences become challenging to reconstruct, especially for large plasmids that are larger than 50 kb or those containing repetitive sequences from transposons or insertion sequences (Harris and Alexander, 2021).

A study of plasmid assemblers (PlasmidSPAdes, Recycler, cBar and PlasmidFinder) by Arredondo-Alonso et al. (2017) showed that while these programs can correctly predict majority of plasmids, predictions still resulted in fragmented, contaminated by chromosomal sequences or incomplete plasmid sequences. Furthermore, without a reference plasmid sequence, the binning of sequences into separate plasmids was unclear and challenging. This could explain the negative detection of the amphenicol resistance genes in the *S. suis* strain 8-57C genome. Furthermore, a study by Lemon et al. (2017) showed a similar occurrence wherein the *erm(B)* gene was not found in the *E. coli* genome assembled using SPAdes. This was attributed to difficulty in assembling highly repetitive plasmid sequences from short reads as well as low representation of the plasmids in the DNA used for whole genome sequencing, highlighting the importance of long-read sequencing for improving genome assembly and resolving repetitive domains. This is one of the reasons why genome sequence-based, PCR-based, and phenotypic characterization should be done in AMR characterization.

Antimicrobial resistance profiling of *S. suis* strain 8-57C revealed that it was resistant to tetracyclines, macrolides, lincosamides, and amphenicol (Table 4). These results showed that *S. suis* strain 8-57C was a multi-drug resistant (MDR) strain

with resistance to more than three antibiotic classes (Schwarz et al. 2010). This has significant implications for the management and prevention of *S. suis* disease since macrolides, lincosamides, and tetracyclines are antibiotic options for the biocontrol and clinical treatment of *S. suis* (Haenni et al. 2018). Except for chloramphenicol, results of this study follow the global trend of *S. suis* AMR with comparatively higher incidences of resistance to tetracyclines, lincosamides, and macrolides as opposed to other antimicrobial classes such as aminoglycosides, beta-lactams, chloramphenicol, fluoroquinolones, and trimethoprim-sulfamethoxazole, (Van Hout et al. 2016; Aradanas et al. 2021).

Table 4: Phenotypic antibiotic susceptibility profile of *Streptococcus suis* strain 8-57C isolated from diseased pig lung.

Class	Mode of Action	Antibiotic	Amount per disk	Susceptibility Category
Aminoglycosides	Breaks cell wall formation and disrupts protein synthesis	Gentamicin	10 µg	Susceptible
Beta-Lactams	Inhibits cell wall synthesis	Ampicillin	10 µg	Susceptible
		Cefotaxime	30 µg	Susceptible
		Cephalexin	30 µg	Susceptible
		Ceftriaxone	30 µg	Susceptible
		Penicillin	10 Units	Susceptible
Amphenicol	Disrupts protein synthesis	Chloramphenicol	30 µg	Resistant
Fluoroquinolone	Inhibits DNA gyrase	Levofloxacin	5 µg	Susceptible
Lincosamide	Disrupts protein synthesis	Clindamycin	2 µg	Resistant
Macrolides	Disrupts protein synthesis	Azithromycin	15 µg	Resistant
		Erythromycin	15 µg	Resistant
Tetracyclines	Disrupts protein synthesis	Doxycycline	30 µg	Intermediate
		Tetracycline	30 µg	Resistant

AMR, particularly in bacterial pathogens, is a worldwide issue with extensive morbidity and mortality implications in clinical settings (Akova 2016). Globally, the occurrence of AMR in *S. suis* is widespread, with an assortment of identified AMR-associated genes conferring resistance to various antibiotic classes, which are corroborated with empirical evidence of the corresponding AMR phenotype (Segura et al. 2020; Tan et al. 2021; Nicholson et al. 2021). Aside from the clinical and epidemiological importance of AMR in *S. suis,* the pathogen is also known to have the capacity to harbor and transfer antimicrobial resistance genes to other *S. suis* strains as well as other relevant *Streptococcal* pathogens like *S. pyogenes*, *S. pneumoniae*, and *S. agalactiae* (Huang et al. 2016b; Chen et al. 2021).

MDR strains like *S. suis* strain 8-57C could have been obtained by pigs in the abattoir environment. Previous research in Brazil revealed that isolates from a butchery environment are usually resistant to multiple drugs. In the Philippines, Sedano et al. (2020) found a different AMR profile for *S. suis* isolated in Laguna backyard farms, with at least a single isolate having resistance to beta-lactams, aminoglycosides, and tetracyclines. However, their testing did not include the macrolide antibiotic class. Overall, the multi-drug resistance of the putatively opportunistic pathogen *S. suis* strain 8-57C presents an epidemiological advantage in swine, which could eventually lead to pathogenesis upon disruption of homeostasis (Votsch et al. 2018; Liang et al. 2022), especially in the presence of antimicrobial pressures in the pig industry with indiscriminate and improper antimicrobial use (Varela et al. 2013; Hadjirin et al. 2021; Uruen et al. 2022).

Genotypic Identification of Putative Virulence Factors

Based on genome sequence prediction for VFs using VFAnalyzer, *S. suis* strain 8-57C has four classes of VFs, which include adherence, enzyme, immune evasion, and protease (Table 5). *S. suis* typically infects the upper respiratory tract of pigs asymptomatically. Virulence only occurs when triggered by a range of biotic and abiotic factors (Votsch et al. 2018).

Table 5: Virulence-associated factors predicted in *Streptococcus suis* strain 8-57C isolated from diseased pig lung using ResFinder 4.1

Virulence Factor Class	Virulence Factors	Related Genes
Adherence	Agglutinin receptor	Undetermined*
	Choline-binding proteins	cbpD
	Fibronectin-binding proteins	pavA
	Muramidase-released protein	mrp
	Streptococcal plasmin receptor/ GAPDH	Plr/gapA
Enzyme	Streptococcal enolase	eno
Immune evasion	Capsule	Undetermined
Protease	Trigger factor	tig/ropA
	Zinc metalloproteinase	zmpC

*Undetermined: The database does not have information on related genes associated with a virulence factor class.

Adhesion, along with invasion, is usually considered among the first steps in host colonization of pathogens like *S. suis* (Fittipaldi et al. 2012). Adhesion VFs allow *S. suis* to cling to both epithelial and endothelial cells as well as organs like tracheal ring cells, joints, and the central nervous system among others (de Greef et al. 2002; Brassard et al. 2004; Wang and Lu 2007). *S. suis* strain 8-57C adhesion VFs included agglutinin receptor, choline-binding proteins, fibronectin-binding proteins (fbp), muramidase-released protein (mrp), and streptococcal plasmin receptor/GAPDH (Table 5). The mrp VF is one of the most important to virulence (Fittipaldi et al. 2012; Aradanas et al. 2021; Nicholson and Bayles 2022) and is putatively associated with zoonotic potential (Roodsant et al. 2021). However, its criticality to virulence is uncertain with mrp-deficient strains still reported to cause disease (Segura et al. 2017).

Streptococcal enolase is the only VF predicted under the enzyme category (Table 5). Its activity takes place after streptococcal infection, with the induction of various autoimmune diseases and pathological processes (Pancholi and Fischetti 1998). Sun et al. (2016) found that enolase, through interaction with astrocytes and porcine brain microvascular endothelial cells, is upregulated, which aids in the penetration of the blood-brain barrier. Aside from its role in direct pathogenesis, *S. suis* enolase is putatively ascribed with immune evasion properties with resistance to phagocytosis in the human blood bactericidal model (Huo et al. 2014).

The capsule is among the most studied and important *S. suis* VFs (Fittipaldi et al. 2012; Nicholson and Bayles 2022) associated with immune evasion and imparting resistance to phagocytosis (Baums and Valentin-Weigand 2009; Okura et al. 2013). Contrary to VFDB results, which classify the function of capsule-related genes as undetermined, numerous studies point out that the *cps* gene cluster, which is frequently used in serotyping, is involved in the biosynthesis and differentiation of capsular polysaccharides in *S. suis* (Okura et al. 2013; Athey et al. 2015; Hatrongjit et al. 2020).

Lastly, zinc metalloprotease (zmp) and trigger factor were identified in the *S. suis* strain 8-57C genome, which represents VFs associated with proteases (Table 5). Zmp's bioactivity is associated with the inactivation of type A immunoglobulins, which prevent epithelial cell adhesion of microorganisms (Zhang et al. 2010; Segura et al. 2016). Despite its prevalence in a portion of 300 *S. suis* isolates based on a study by Weinert et al. (2015), the criticality of this VF is also in question, as *in vitro* porcine bronchial epithelial cells or *in vivo* swine upper respiratory tract assays were unaffected by the absence of a functional *S. suis* zmp protein (Dumesnil et al. 2018).

Trigger factor, on the other hand, is an important contributor to pathogenesis, with deficient strains losing hemolytic activity coupled with a significant reduction of adherence (Wu et al. 2011). The trigger factor is also suggested to influence the expression of several virulence-related genes such as *mrp*, *cps*, *fbps*, *sly*, *epf*, *hrcA*, *hyl*, and *rpob*. This has important implications in *S. suis* strains, which are positive for *sly* and *epf* since the presence of *sly*, *epf*, *mrp*, and *cps* VFs is a crucial factor for virulence (Fittipaldi et al. 2012; Aradanas et al. 2021; Nicholson and Bayles 2022).

Despite *S. suis* strain 8-57C being predicted to contain one or more VFs using a genome-based approach, previous studies on serotype 1/2 by Martinez et al. (2002) revealed that clinical diseases in swine were less likely caused by specific isolate virulence but more of the result of inherent herd factors. Similar to mrp, the presence of other virulence factors deemed important was not guaranteed for the development of disease (Segura et al. 2017). Hence, further assessment is needed to assess whether *S. suis* disease in Philippine swine is related more to farming practices than VFs of *S. suis* strains.

In silico Prediction of Secondary Metabolite Gene Clusters

Gene cluster prediction of *S. suis* strain 8-57C using antiSMASH 7.0 identified an unknown polyketide synthase type III BGC and a BGC with 100% similarity to suicin 90-1330. Suicin 90-1330 is a lanthipeptide class I bacteriocin from a nonvirulent *S. suis* strain 90-1330, which has high homology with the bacteriocin, nisin U (LeBel et al., 2014) (Table 6). The genus *Streptococcus* are known producer of antimicrobial bacteriocins exemplified by *BlpC* from *S. pneumoniae* (Wholey et al. 2016), Smb from *S. mutans* (Yonezawa et al. 2008), Lcn 351 from *S. suis* strain WUSS351 (Liang et al. 2022) as well as suicins (e.g. suicin3908 from *S. suis* strains 3908 and WUSS351; and suicin 90-1330 from *S. suis* 90-1330) (LeBel et al. 2014; Vaillancourt et al. 2015; Liang et al. 2022).

Table 6: Biosynthetic gene clusters identified in *Streptococcus suis* strain 8-57C isolated from diseased pig lung using antiSMASH 7.0.

Region	Type	From	To	Most similar known cluster		Similarity
Region 4.1	Lanthipeptide Class I	95,464	121,494	Suicin 90-1330	RiPP	100%
Region 4.1	Polyketide synthase Type III	1	23,459	ND	ND	ND

Bacteriocins are antimicrobial peptides that could allow a pathogen to shape complex host microbiota in favor of itself by targeting neighboring bacteria, either from different genera, species, or strains for lysis (Li et al. 2014; Wholey et al. 2016; Heilbronner et al. 2021). Previous research in *Streptococcus* species indicated that the production of bacteriocins, along with integral processes for pathogenesis such as host bacterial adhesion, biofilm formation, and virulence, were activated by quorum sensing (Wang and Kuramitsu 2005; Xue et al. 2022). The importance of quorum sensing in streptococcal antibiotic production is compounded by its usual coexistence with other bacterial species, including those of the same genera in host organs (Niazy et al. 2022).

Studies have shown that suicin3908-producing *S. suis* in healthy pigs exhibit self-resistance to suicin3908 and also promote intraspecies competition by exerting biocontrol activities against non-suicin3908-producing pathogenic isolates belonging to sequence types 1, 25, and 28 and serotype 2 (Vaillancourt et al. 2015; Liang et al. 2022). On the contrary, based on experiments by Liang et al. (2022), bacteriocin Lcn351 from *S. suis* strain WUSS351 only exerted antimicrobial activity against one strain (*S. suis* P 1/7) out of 11 tested isolates. This suggests that there is still a lack of information on whether these intraspecies antagonisms caused by bacteriocins are serovar or sequence-type-specific or whether they only occur between non-pathogenic and pathogenic strain combinations.

Implication

Identifying and understanding attributes that promote pathogen fitness in *S. suis* could primarily guide the formulation of strategies to mitigate the infection and spread of *S. suis*. This is economically beneficial for the pig industry to avoid losses due to death from the pathogen as well as the culling of other infected animals (Gottschalk and Segura, 2000). Epidemiologically, this is important in preventing the zoonotic transmission of the pathogen to other animals, including humans, with close contact to infected pigs or pork products (Gottschalk et al. 2010) as well as avoiding the development and further proliferation of antimicrobial resistance in both humans and animals (Huang et al. 2016a). Finally, the increased understanding of pathogen fitness factors could ultimately lead to better diagnostic tools for the surveillance of the pathogen within and outside of farm settings as well as effective therapeutics, both innovative and classical (e.g. vaccines) targeting these attributes or their associated pathways (Fittipaldi et al., 2012; Feng et al. 2014; Segura et al. 2017).

CONCLUSION

Streptococcus suis strain 8-57C serotype 1/2 was isolated from a diseased pig lung tissue in the Philippines. This strain phenotypically showed multidrug resistance to tetracyclines, macrolides, lincosamides, and chloramphenicol. Inconsistencies were observed in validating the presence of AMR genes using PCR, in silico genome predictions, and phenotypic expression. It is strongly suggested that all three methods be used to verify the presence of AMR. *S. suis* strain 8-57C was the first Philippine *S. suis* strain to undergo whole genome sequencing. Genome analysis of this strain showed several genotypic factors that were predicted as beneficial to pathogen fitness, host survivability, and pathogenicity. These included: (1) categorizing *S. suis* strain 8-57C as ST28 which is commonly associated as a pathogenic pathotype of swine and human disease, (2) the verification of antimicrobial resistance genes for lincosamide, macrolide, streptogramin, and tetracycline drug classes, except for chloramphenicol, wherein the presence of these genes is advantageous in antibiotic pressure scenarios against non-AMR susceptible commensals and pathogens, (3) genome-based prediction of a range of virulence factors which could enable *S. suis* strain 8-57C adhesion to host cells and organs, protection against phagocytosis, ability to cross the blood-brain barrier and hemolytic activity and (4) the genome-based identification of the presence of secondary metabolite gene clusters encoding for the production of suicin 90-1330 which promotes intra- and interspecies competitive advantage in favor of *S. suis*. The analysis of *S. suis* strain 8-57C highlights the importance of significantly expanding Philippine *S. suis* research to other serovars beyond the common serovar 2 to establish their pathogenic potential in various aspects (virulence, AMR profiles, secondary metabolites beneficial to pathogen survival). Current Philippine *S. suis* studies primarily delve into the presence of *S. suis* serotypes in swine farms but lack comprehensive data on the isolates' pathogen fitness attributes that could pose huge economic, epidemiological, and public health consequences in the long run if left unchecked.

ACKNOWLEDGMENT

This study was funded by the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños through the General Appropriations Act Project (Fund code: 9151014) entitled Exploring Philippine Bioresources as Source of Bioactive Compounds against Potential Emerging and Re-Emerging Animal Diseases (PHASE I).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

ABC* - Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Reviewing and Editing
JEIZ – Conceptualization, Formal Analysis, Writing – Original Draft Preparation, Reviewing and Editing
SAS* – Formal Analysis, Investigation, Methodology, Writing – Review & Editing

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REFERENCES

Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence 2016; 7(3):252-266. https://doi.org/10.1080/21505594.2016.1159366

Aradanas M, Poljak Z, Fittipaldi N, Ricker N, Farzan A. Serotypes, virulence-associated factors and antimicrobial resistance of *Streptococcus suis* isolates recovered from sick and healthy pigs determined by whole-genome sequencing. Front Vet Sci 2021; 8:742345. https://doi.org/10.3389/fvets.2021.742345

Arredondo-Alonso S, Willems RJ, van Schaik W, Schürch AC. On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. Microb Genom. 2017; 3(10):e000128. http://doi.org/10.1099/mgen.0.000128.

Athey TBT, Auger JP, Teatero S, Dumesnil A, Takamatsu D, Wasserscheid J, Dewar K, Gottschalk M, Fittipaldi N. Complex population structure and virulence among serotype 2 *Streptococcus suis* strains belonging to sequence type 28. PLoS One 2015; 10(9):e0137760. https://doi.org/10.1371/journal.pone.0137760

Athey TBT, Teatero S, Lacouture S, Takamatsu D, Gottschalk M, Fittipaldi N. Determining *Streptococcus suis* serotype from short-read whole-genome sequencing data. BMC Microbiol 2016; 16:162. https://doi.org/10.1186/s12866-016-0782-8

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012; 19(5):455-477. https://doi.org/10.1089/cmb.2012.0021

Baums CG, Valentin-Weigand P. Surface-associated and secreted factors of *Streptococcus suis* in epidemiology, pathogenesis and vaccine development. Animal Health Res Rev 2009; 10(1):65-83. https://doi.org/10.1017/s146625230999003X

Berbel D, González-Díaz A, López de Egea G, Càmara J, Ardanuy C. An Overview of Macrolide Resistance in Streptococci: Prevalence, Mobile Elements and Dynamics. Microorganisms. 2022; 10(12):2316. http://doi.org/10.3390/microorganisms10122316.

Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJN, Van Wezel, GP, Medema MH, Weber T. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualization. Nucleic Acids Res 2023; 51(W1):W46-W50. https://doi.org/10.1093/nar/gkad344

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014; 30(15):2114-2120. https://doi.org/10.1093/bioinformatics/btu170

Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykasenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes

from genotypes. J Antimicrob Chemother 2020; 75(12): 3491-3500. https://doi.org/10.1093/jac/dkaa345

Brassard J, Gottschalk M, Quessy S. Cloning and purification of the *Streptococcus suis* serotype 2 glyceraldehyde-3-phosphate dehydrogenase and its involvement as an adhesin. Vet Microbiol 2004; 102(1-2):87-94. https://doi.org/10.1016/j.vetmic.2004.05.008

Brenciani A, Fioriti S, Morroni G, Cucco L, Morelli A, Pezzotti G, Paniccià M, Antonelli A, Magistrali CF, Rossolini GM, Giovanetti E. Detection in Italy of a porcine *Enterococcus faecium* isolate carrying the novel phenicol-oxazolidinone-tetracycline resistance gene *poxtA*. J Antimicrob Chemother. 2019; 74(3):817-818. http://doi.org/10.1093/jac/dky505

Chen L, Huang J, Huang X, He Y, Sun J, Dai X, Wang X, Shafiq M, Wang L. Horizontal transfer of different *erm*(B)-carrying mobile elements among *Streptococcus suis* strains with different serotypes. Front Microbiol 2021; 12:628740. https://doi.org/10.3389/fmicb.2021.628740

Chen L, Song Y, Wei Z, He H, Zhang A, Jin M. Antimicrobial susceptibility, tetracycline and erythromycin resistance genes, and multilocus sequence typing of *Streptococcus suis* isolates from diseased pigs in China. J Vet Med Sci 2013; 75(5): 583-587. https://doi.org/10.1292/jvms.12-0279

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100. 33rd ed. Wayne PA: Clinical and Laboratory Standards Institute; 2023:130-135.

De Greef A, Buys H, Verhaar R, Dijkstra J, Van Alphen L, Smith HE. Contribution of fibronectin binding protein to pathogenesis of *Streptococcus suis* serotype 2. Infect Immun 2002; 70(3):1319-1325. https://doi.org/10.1128/IAI.70.3.1319-1325.2002

Dresen M, Valentin-Weigand P, Weldearegay YB. Role of metabolic adaptation of *Streptococcus suis* to host niches in bacterial fitness and virulence. Pathogens 2023; 12(4):541. https://doi.org/10.3390/pathogens12040541

Dumesnil A, Auger JP, Roy D, Votsch D, Willenborg M, Valentin-Weigand P, Park PW, Grenier D, Fittipaldi N, Harel J, Gottschalk M. Characterization of the zinc metalloprotease of *Streptococcus suis* serotype 2. Vet Res 2018; 49:109. https://doi.org/10.1186/s13567-018-0606-y

Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Koser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen O, Woodford N. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 2017; 23(1):2-22. https://doi.org/10.1016/j.cmi.2016.11.012

Estrada AA, Gottschalk M, Rossow S, Rendahl A, Gebhart C, Marthaler DG. Serotype and genotype (multilocus sequence type) of *Streptococcus suis* isolates from the United States serve as predictors of pathotype. J Clin Microbiol 2019; 57(9): e00377-19. https://doi.org/10.1128/JCM.00377-19

Feng Y, Zhang H, Wu Z, Wang S, Cao M, Hu D, Wang C. Streptococcus suis infection an emerging/reemerging challenge of bacterial infectious diseases? Virulence 2014; 5(4): 477-497. https://dx.doi.org/10.4161/viru.28595

Fittipaldi N, Segura M, Grenier D, Gottschalk M. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol 2012; 7(2):259-279. https://doi.org/10.2217/fmb.11.149

Fittipaldi N, Xu J, Lacouture S, Tharavichitkul P, Osaki M, Sekizaki T, Daisuke T, Gottschalk M. Lineage and virulence of *Streptococcus suis* serotype 2 isolates from North America. Emerg Infect Dis 2011; 17(12):2239-2244. https://doi.org/10.3201/eid1712.110609

Gottschalk M, Segura M. The pathogenesis of the meningitis caused by Streptococcus suis: the unresolved questions. Vet Microbiol 2000; 76(3): 259-272. https://doi.org/10.1016/S0378-1135(00)00250-9

Gottschalk M, Xu J, Calzas C, Segura M. Streptococcus suis: a new emerging or an old neglected zoonotic pathogen. Future Microbiol 2010; 5(3): 371-391. https://doi.org/10.2217/fmb.10.2

Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. Emerg Microb Infect 2014; 3(1): e45. https://doi.org/10.1038/emi.2014.45

Hadjirin NF, Miller EL, Murray GGR, Yen PLK, Phuc HD, Willeman TM, Hernandez-Garcia J, Williamson SM, Parkhill J, Maskell DJ, Zhou R, Fittipaldi N, Gottschalk M, Dan Tucker AW, Hoa NT, Welch JJ, Weinert LA. Large-scale genomic analysis of antimicrobial resistance in the zoonotic pathogen *Streptococcus suis*. BMC Microbiol 2021; 19:191. https://doi.org/10.1186/s12915-021-01094-1

Haenni M, Lupo A, Madec JY. Antimicrobial resistance in *Streptococcus* spp. Microbiol Spectr 2018; 6(2): 10.1128/microbiolspec.ARBA-0008-2017. https://doi.org/10.1128/ microbiolspec.ARBA-0008-2017

Han N, Li J, Wan P, Pan Y, Xu T, Xiong W, Zeng Z. Co-Existence of Oxazolidinone Resistance Genes cfr(D) and optrA on Two *Streptococcus parasuis* Isolates from Swine. Antibiotics (Basel). 2023;12(5):825. http://doi.org/10.3390/antibiotics12050825.

Harris PNA, Alexander M W. Beyond the Core Genome: Tracking Plasmids in Outbreaks of Multidrug-resistant Bacteria. Clin Infect Dis. 2021;72(3):421-422. http://doi.org/10.1093/cid/ciaa052.

Hatrongjit R, Fittipaldi N, Gottschalk M, Kerdsin A. Tools for molecular epidemiology of *Streptococcus suis*. Pathogens 2020; 9(2):81. https://doi.org/10.3390/pathogens9020081

Heilbronner S, Krismer B, Brotz-Oesterhelt H, Peschel A. The microbiome-shaping roles of bacteriocins. Nat Rev Microbiol 2021; 19:726-739. https://doi.org/10.1038/s41579-021-00569-w

Huang J, Ma J, Shang K, Hu X, Liang Y, Li D, Wu, Z, Dai L, Chen L, Wang L. Evolution and diversity of the antimicrobial resistance associated mobilome in *Streptococcus suis*: a probable mobile genetic elements reservoir for other Streptococci. Front Cell Infect Microbiol 2016; 6: 118. https://doi.org/10.3389/fcimb.2016.00118

Huang K, Song Y, Zhang Q, Zhang A, Jin M. Characterisation of a novel integrative and conjugative element ICES*sD9*

carrying *erm*(B) and *tet*(O) resistance determinants in *Streptococcus suis*, and the distribution of ICE*sD9*-like elements in clinical isolates. J Glob Antimicrob Resist 2016; 7:13-18. https://doi.org/10.1016/j.jgar.2016.05.008

Huo C, Ji Y, Ren L, Zhang J, Kong J. Enolase of *Streptococcus suis* serotype 2 is involved in the antiphagocytosis of *Streptococcus suis*. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2014; 30:1146-1149.

Huong VTL, Ha N, Huy NT, Horby P, Nghia HDT, Thiem VD, Zhu X, Hoa NT, Hien TT, Zamora J, Schultz C, Wertheim HFL, Hirayama K. Epidemiology, clinical manifestations, and outcomes of *Streptococcus suis* infection in humans. Emerg Infect Dis 2014; 20(7):1105-1114. https://doi.org/10.3201/eid2007.131594

Ishida S, Thien LHT, Osawa R, Tohya M, Nomoto R, Kawamura Y, Takahashi T, Kikuchi N, Kikuchi K, Sekizaki T. Development of an appropriate PCR system for the reclassification of *Streptococcus suis.* J Microbiol Methods 2014*;* 107:66-70. https://doi.org/10.1016/j.mimet.2014.09.003

Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. Antimicrob Agents Chemother. 2006; 50(4):1156-63. http://doi.org/10.1128/AAC.50.4.1156-1163.2006

Kerdsin A, Akeda Y, Hatrongjit R, Detchawna U, Sekizaki T, Hamada S, Gottschalk M, Oishi K. *Streptococcus suis* serotyping by a new multiplex PCR. J Med Microbiol 2014; 63(6):824-830. https://doi.org/10.1099/jmm.0.069757-0

Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012; 50(4): 1335-1361. https://doi.org/10.1128/JCM.06094-11

LeBel G, Vaillancourt K, Frenette M, Gottschalk M, Grenier D. 2014. Suicin 90-1330 from a nonvirulent strain of *Streptococcus suis*: a nisin-related lantibiotic active on gram-positive swine pathogens. Appl Environ Microbiol 2014; 80(17):5484-5492. https://doi.org/10.1128/AEM.01055-14

Lemon JK, Khil PP, Frank KM, Dekker JP. Rapid Nanopore Sequencing of Plasmids and Resistance Gene Detection in Clinical Isolates. J Clin Microbiol. 2017; 55(12):3530-3543. http://doi.org/10.1128/JCM.01069-17.

Li MF, Zhang BC, Li J, Sun L. Sil: a *Streptococcus iniae* bacteriocin with dual role as an antimicrobial and an immunomodulator that inhibits innate immune response and promotes *S. iniae* infection. PLoS One 2014; 9(4):e96222. https://doi.org/10.1371/journal.pone.0096222

Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. RefSeq: expanding the prokaryotic genome annotation pipeline reach with protein family model curation. Nucleic Acids Res 2021; 49(D1):D1020-D1028. https://doi.org/10.1093/nar/gkaa1105

Liang Z, Wu H, Bian C, Chen H, Shen Y, Gao X, Ma J, Yao H, Wang L, Wu Z. The antimicrobial systems of *Streptococcus suis* promote niche competition in pig tonsils. Virulence 2022; 13(1):781-793. https://doi.org/10.1080/21505594.2022.2069390

Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res 2022; 50(D1): D912-D917. https://doi.org/10.1093/nar/gkab1107

Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrob Agents Chemother. 2005; 49(11):4798-800. http://doi.org/10.1128/AAC.49.11.4798-4800.2005

Marcais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics 2011; 27(6):764-770. https://doi.org/10.1093/bioinformatics/btr011

Martinez G, Harel J, Lacouture S, Gottschalk M. Genetic diversity of *Streptococcus suis* serotypes 2 and 1/2 isolates recovered from carrier pigs in closed herds. Can J Vet Res 2002; 66(4): 240-248.

Matiasovic J, Zouharova M, Nedbalcova K, Kralova N, Matiaskova K, Simek B, Kucharovicova I, Gottschalk M. Resolution of *Streptococcus suis* serotypes 1/2 versus 2 and 1 versus 14 by PCR restriction fragment length polymorphism method. J Clin Microbiol 2020; 58(7):e00480-20. https://doi.org/10.1128/JCM.00480-20

Meier-Kolthoff JP, Goker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 2019; 10:2182. https://doi.org/10.1038/s41467-019-10210-3

Neila-Ibañez C, Napp S, Pailler-Garcia L, Franco-Martinez L, Ceron JJ, Aragon V, Casal J. Risk factors associated with *Streptococcus suis* cases on pig farms in Spain. Vet Record 2023; 193(5): e3056. https://doi.org/10.1002/vetr.3056

Niazy M, Hill S, Nadeem K, Ricker N, Farzan A. Compositional analysis of the tonsil microbiota in relationship to *Streptococcus suis* disease in nursery pigs in Ontario. Anim Microbiome 2022; 4:10. https://doi.org/10.1186/s42523-022-00162-3

Nicholson TL, Bayles DO. Comparative virulence and antimicrobial resistance distribution of *Streptococcus suis* isolates obtained from the United States. Front Microbiol 2022; 13:1043529. https://doi.org/10.3389/fmicb.2022.1043529

Nicholson TL, Waack U, Anderson TK, Bayles DO, Zaia SR, Goertz I, Eppinger M, Hau SJ, Brockmeier SL, Shore SM. Comparative virulence and genomic analysis of *Streptococcus suis* isolates. Front Microbiol 2021; 11:620843. https://doi.org/10.3389/fmicb.2020.620843

Obradovic MR, Segura M, Segales J, Gottschalk M. Review of the speculative role of co-infections in *Streptococcus suis*-associated disease in pigs. Vet Res 2021; 52: 49. https://doi.org/10.1186/s13567-021-00918-w

Okura M, Lachance C, Osaki M, Sekizaki T, Maruyama F, Nozawa T, Nakagawa I, Hamada S, Rossignol C, Gottschalk M, Takamatsu D. Development of a two-step multiplex PCR assay for typing of capsular polysaccharide synthesis gene

clusters of *Streptococcus suis*. J Clin Microbiol 2014; 52(5):1714-1719. https://doi.org/10.1128/JCM.03411-13

Okura M, Takamatsu D, Maruyama F, Nozawa T, Nakagawa I, Osaki M, Sekizaki T, Gottschalk M, Kumagai Y, Hamada S. Genetic analysis of capsular polysaccharide synthesis gene clusters from all serotypes of *Streptococcus suis*: Potential mechanisms for generation of capsular variation. Appl Environ Microbiol 2013; 79(8):2796-2806. https://doi.org/10.1128/AEM.03742-12

Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, Dempsey DM, Dickerman A, Dietrich EM, Kenyon RW, Kuscuoglu M, Lefkowitz EJ, Lu J, Machi D, Macken C, Mao C, Niewiadomska A, Nguyen M, Olsen GJ, Overbeek JC, Parrello B, Parrello V, Porter JS, Pusch GD, Shukla M, Singh I, Stewart L, Tan G, Thomas C, VanOeffelen M, Vonstein V, Wallace ZS, Warren AS, Wattam AR, Xia F, Yoo H, Zhang Y, Zmasek CM, Scheuermann RH, Stevens RL. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. Nucleic Acids Res 2023; 51(D1):D678-D689. https://doi.org/10.1093/nar/gkac1003

Onishi H, Sugawara M, Okura M, Osaki M, Takamatsu D. Prevalence of *Streptococcus suis* genotypes in isolates from porcine endocarditis in East Japan. J Vet Med Sci 2012; 74(12): 1681-1684. https://doi.org/10.1292/jvms.12-0301

Pancholi V, Fischetti VA. Alpha enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. J Biol Chem 1998; 273(23):14503-14515. https://doi.org/10.1074/jbc.273.23.14503

Rieckmann K, Pendzialek SM, Vahlenkamp T, Baums CG. A critical review speculating on the protective efficacies of autogenous *Streptococcus suis* bacterins as used in Europe. Porc Health Manag 2020; 6:12. https://doi.org/10.1186/s40813-020-00150-6

Rilo MP, Martin CBG, Fernandez VA, Turrientes AA, Fernandez AG, Perez RM, Martinez SM. *Streptococcus suis* research update: serotype prevalence and antimicrobial resistance distribution in swine isolates recovered in Spain from 2020 to 2022. Vet Sci 2024; 11(1):40. https://doi.org/10.3390/vetsci11010040

Roodsant TJ, Van Der Putten BCL, Tamminga SM Schultz C, Van Der Ark KCH. Identification of *Streptococcus suis* putative zoonotic virulence factors: A systematic review and genomic meta-analysis. Virulence 2021; 12(1): 2787-2797. https://doi.org/10.1080/21505594.2021.1985760

Roy D, Athey TBT, Auger J-P, Goyette-Desjardins G, Van Calsteren M-R, Takamatsu D, Okura M, Teatero S, Alcorlo M, Hermoso JA, Segura M, Gottschalk M, Fittipaldi N. A single amino acid polymorphism in the glycosyltransferase CpsK defines four *Streptococcus suis* serotypes. Sci Rep 2017; 7:4066. https://doi.org/10.1038/s41598-017-04403-3

Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, Gaastra W. Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 2010; 65(4):601-604. https://doi.org/10.1093/jac/dkq037

Sedano SA, Cantalejo MGCT, Lapitan CGAR, de Guzman AMES, Consignado JT, Tandang NA, Estacio MAC, Kerdsin A, Silva BBI. Epidemiology and genetic diversity of *Streptococcus suis* in smallhold swine farms in the Philippines. Sci Rep 2023; 13:21178. https://doi.org/10.1038/s41598-023-48406-9

Sedano, S A, Silva, BBI, Sangalang, AGM and Mendioro, M S. *Streptococcus suis* and *S. parasuis* in the Philippines: Biochemical, molecular, and antimicrobial resistance characterization of the first isolates from local swine. Philipp Sci Lett 2020; 13(Supplement):107-119.

Segura M, Aragon V, Brockmeier SL, Gebhart C, de Greef A, Kerdsin A, O'Dea MA, Okura M, Salery M, Schultz C, Valentin-Weigand P, Weinert LA, Wells JM, Gottschalk M. Update on *Streptococcus suis* research and prevention in the era of antimicrobial restriction: 4th international workshop on *S. suis*. Pathogens 2020; 9(5):374. https://doi.org/10.3390/pathogens9050374

Segura M, Fittipaldi N, Calzas C, Gottschalk M. Critical *Streptococcus suis* virulence factors: are they all really critical? Trends Microbiol 2017; 25(7): 585-599. https://doi.org/10.1016/j.tim.2017.02.005

Segura M, Calzas C, Grenier D, Gottschalk M. Initial steps of the pathogenesis of the infection caused by *Streptococcus suis*: fighting against nonspecific defenses. FEBS Lett 2016; 590(21):3772-3799. https://doi.org/10.1002/1873-3468.12364

Segura M. *Streptococcus suis* vaccines: candidate antigens and progress. Expert Rev Vaccines 2015; 14(12):1587-1608 https://doi.org/10.1586/14760584.2015.1101349

Shang Y, Li D, Shan X, Schwarz S, Zhang SM, Chen YX, Ouyang W, Du XD. Analysis of two pheromone-responsive conjugative multiresistance plasmids carrying the novel mobile optrA locus from *Enterococcus faecalis*. Infect Drug Resist. 2019; 12:2355-2362. http://doi.org/10.2147/IDR.S206295

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 2015; 31(19):3210-3212. https://doi.org/10.1093/bioinformatics/btv351

Sun Y, Li N, Zhang J, Liu H, Liu J, Xia X, Sun C, Feng X, Gu J, Du C, Han W, Lei L. Enolase of *Streptococcus suis* serotype 2 enhances blood-brain barrier permeability by inducing IL-8 release. Inflammation 2016; 39:718-726. https://doi.org/10.1007/s10753-015-0298-7

Tan MF, Tan J, Zeng YB, Li HQ, Yang Q, Zhou R. Antimicrobial resistance phenotypes and genotypes of *Streptococcus suis* isolated from clinically healthy pigs from 2017 to 2019 in Jiangxi Province, China. J Appl Microbiol 2021; 130(3): 797-806. https://doi.org/10.1111/jam.14831

Tang Y, Zhao H, Wu W, Wu D, Li X, Fang W. Genetic and virulence characterization of *S. suis* type 2 isolates from swine in the province of Zhejiang and Henan, China. Folia Microbiol (Praha) 2011; 56:541-548. https://doi.org/10.1007/s12223-011-0077-2

Tien LHT, Nishibori T, Nishitani Y, Nomoto R, Osawa R. Reappraisal of the taxonomy of the *Streptococcus* serotypes 20, 22, 26, and 33 based on DNA-DNA homology and *sodA* and *recN* phylogenies. Vet Microbiol 2013; 162(2-4):842-849. https://doi.org/10.1016/j.vetmic.2012.11.001

Uruen C, Garcia C, Fraile L, Tommassen J, Arenas J. How *Streptococcus suis* escapes antibiotic treatments. Vet Res 2022; 53:91. https://doi.org/10.1186/s13567-022-01111-3

Vaillancourt K, LeBel G, Frenette M, Gottschalk M, Grenier D. Suicin 3908, a new lantibiotic produced by a strain of *Streptococcus suis* serotype 2 isolated from a healthy carrier pig. PLoS One 2015; 10(2):e0117245. https://doi.org/10.1371/journal.pone.0117245

Vakulenko SB, Donabedian SM, Voskresenskiy AM, Zervos MJ, Lerner SA, Chow JW. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob Agents Chemother. 2003; 47(4):1423-6. http://doi.org/10.1128/AAC.47.4.1423-1426.200

Van Hout J, Heuvelink A, Gonggrijp M. Monitoring of antimicrobial susceptibility of *Streptococcus suis* in the Netherlands, 2013-2015. Vet Microbiol 2016; 194:5-10. https://doi.org/10.1016/j.vetmic.2016.03.014

Varela NP, Gadbois P, Thibault C, Gottschalk M, Dick P, Wilson J. Antimicrobial resistance and prudent drug use for *Streptococcus suis.* Anim Health Res Rev 2013; 14(1): 68-77. https://doi.org/10.1017/S1466252313000029

Votsch D, Willenborg M, Weldearegay YB, Valentin-Weigand P. *Streptococcus suis* – The "two faces" of a pathobiont in the porcine respiratory tract. Front Microbiol 2018; 9:480. https://doi.org/10.3389/fmicb.2018.00480

Wang BY, Kuramitsu HK. Interactions between oral bacteria: Inhibition of *Streptococcus mutans* bacteriocin production by *Streptococcus gordonii.* Appl Environ Microbiol 2005; 71(1):354-362. https://doi.org/10.1128/AEM.71.1.354-362.2005

Wang K, Lu C. Adhesion activity of glyceraldehyde-3-phosphate dehydrogenase in a Chinese *Streptococcus suis* type 2 strain. Berl Munch Tierarztl Wochenschr 2007; 120(5-6):207-209. https://doi.org/10.2376/0005-9366-120-207

Weinert LA, Chaudhuri RR, Wang J, Peters SE, Corander J, Jombart T, Baig A, Howell KJ, Vehkala M, Valimaki N, Harris D, Chieu TTB, Chau NVV, Campbell J, Schultz C, Parhill J, Bentley SD, Langford PR, Rycroft AN, Wren BW, Farrar J, Baker S, Hoa NT, Holden MTG, Tucker AW, Maskell DJ. Genomic signatures of human and animal disease in the zoonotic pathogen *Streptococcus suis*, Nat Commun 2015; 6:6740. https://doi.org/10.1038/ncomms7740

Wholey WY, Kochan TJ Storck DN, Dawid S. Coordinated bacteriocin expression and competence in *Streptococcus pneumoniae* contributes to genetic adaptation through neighbor predation. PLoS Pathog 2016; 12(2):e1005413. https://doi.org/10.1371/journal.ppat.1005413

Wileman TM, Weinert LA, Howell KJ, Wang J Peters SE, Williamson SM, Wells JM, Langford PR, Rycroft AN, Wren BW, Maskell DJ, Tucker AW. Pathotyping the zoonotic pathogen *Streptococcus suis*: novel genetic markers to differentiate invasive disease-associated isolates from non-disease-associated isolates from England and Wales. J Clin Microbiol 2019; 57(7):e01712-e01718. https://doi.org/10.1128/JCM.01712-18

Wu CF, Chen SH, Chou CC, Wang CM, Huang SW, Kuo HC. Serotype and multilocus sequence typing of *Streptococcus suis* from diseased pigs in Taiwan. Sci Rep 2023; 13:8263. https://doi.org/10.1038/s41598-023-33778-9

Wu T, Zhao Z, Zhang L, Ma H, Lu K, Ren W, Liu Z, Chang H, Bei W, Qiu Y, Chen H. Trigger factor of *S. suis* is involved in stress tolerance and virulence. Micro Pathog 2011; 51(1-2):69-76. https://doi.org/10.1016/j.micpath.2010.10.001

Xia X, Qin W, Zhu H, Wang X, Jiang J, Hu J. How *Streptococcus suis* serotype 2 attempts to avoid attack by host immune defenses. J Microbiol Immunol Infect 2019; 52(4):516-525. https://doi.org/10.1016/j.jmii.2019.03.003

Xue B, Shen Y, Zuo J, Song D, Fan Q, Zhang X, Yi L, Wang Y. Bringing antimicrobial strategies to a new level: The quorum sensing system as a target to control *Streptococcus suis*. Life 2022; 12(12):2006. https://doi.org/10.3390/life12122006

Yao X, Li M, Wang J, Wang C, Hu D, Zheng F, Pan X, Tan Y, Zhao Y, Hu L, Tang J, Hu F. Isolation and characterization of a native avirulent strain of *Streptococcus suis* serotype 2: a perspective for vaccine development. Sci Rep 2015; 5:9835. https://doi.org/10.1038/srep09835

Yonezawa H, Kuramitsu HK, Nakayama S, Mitobe J, Motegi M, Nakao R, Watanabe H, Senpuku H. 2008. Differential expression of the Smb bacteriocin in *Streptococcus mutans* isolates. Antimicrob Agents Chemother 2008; 52(8): 2742-2749. https://doi.org/10.1128/AAC.00235-08

Zhang A, Mu X, Chen B, Liu C, Han L, Chen H, Jin M. Identification and characterization of IgA1 protease from *Streptococcus suis*. Vet Microbiol 2010; 140(1-2):171-175. https://doi.org/10.1016/j.vetmic.2009.06.034

SUPPLEMENTARY DATA

Table S1: Oligonucleotide primers and target genes used in the multiplex PCR for *S. suis* serotyping.

Serotype	Sequence (5′→3′)*	Gene	Multiplex PCR reaction set	Amplicon size (bp)	Reference
1 and 14	F: AATCATGGAATAAAGCGGAGTACAG	*cps1J*, *cps14J*	1	550	Kerdsin et al. 2012
	R: ACAATTGATACGTCAAAATCCTCACC				
2 and 1/2	F: GATTTGTCGGGAGGGTTACTTG	*cps2J*, *cps1/2J*	1	450	Kerdsin et al. 2012
	R: TAAATAATATGCCACTGTAGCGTCTC				
3	F: TGGGAGAAGGCAGAAAGTACGAGA	*cps3J–cps3K*	1	1273	Kerdsin et al. 2012
	R: ACCCCCAGAAGAGCCGAAGGA				
4	F: ACTTGGAGTTGTCGGAGTAGTGCT	*cps4M–cps4N*	2	783	Kerdsin et al. 2012
	R: ACCGCGATGGATAGGCCGAC				
5	F: TGATGGCGGAGTTTGGGTCGC	*cps5N*	2	166	Kerdsin et al. 2012
	R: CGTAACAACCGCCCCAGCCG				
6	F: TACGGTCTCCCTTGCCTGTA	*cps6I*	3	325	Kerdsin et al. 2014
	R: AACTCAGCTAGTGCTCCACG				
7	F: GATGATTTATGGCACCCGAGTAAGC	*cps7H*	1	150	Kerdsin et al. 2014
	R: AGTCACAATTGCTGGTCCTGACACC				
8	F: ATGGGCGTTGGCGGGAGTTT	*cps8H*	2	320	Kerdsin et al. 2014
	R: TTACGGCCCCCATCACGCTG				
9	F: GGGATGATTGCTCGACAGAT	*cps9H*	1	300	Kerdsin et al. 2014
	R: CCGAAGTATCTGGGCTACTG				
10	F: TTACGAGGGGATTCTGGGGT	*cps10M*	3	153	Kerdsin et al. 2014
	R: CGGGACAACAGATGGAACCT				
11	F: TACAGTGCTTGCAGCCCTAC	*cps11N*	1	896	Kerdsin et al. 2014
	R: CGACTTGTCGTGCCCTGAT				
12	F: TGTGGCGATAGGACAACAGG	*cps12J*	2	209	Kerdsin et al. 2014
	R: ACCAAGAAGTTTCCGCCTGA				
13	F: CTGGTGCTGCAATTTCGCTT	*cps13L*	3	1135	Kerdsin et al. 2014
	R: GCAGACTAGCTGCAGTTCCA				
15	F: GCAAGAAAGCTTCCGGATGGA	*cps15K*	3	274	Kerdsin et al. 2014
	R: CAAGAGAGTGTGCAACCCCA				
16	F: TGGAGGAGCATCTACAGCTCGGAAT	*cps16K*	1	202	Kerdsin et al. 2014
	R: TTTGTTTGCTGGAATCTCAGGCACC				
17	F: ACTTGGGTTGGAATGGCGAA	*cps17O*	3	906	Kerdsin et al. 2014
	R: ACCACCGAAAGTCAGGTCAC				
18	F: CGGGGCAGTCTTACTCATGG	*cps18N*	2	432	Kerdsin et al. 2014
	R: ATGACAGCGAAACGGACAGA				
19	F: AGCAGGGTTGCGTATGGCGG	*cps19L*	2	1024	Kerdsin et al. 2014
	R: ACAAGCACCAGCAAAGACCGCA				

Serotype	Sequence (5′→3′)*	Gene	Multiplex PCR reaction set	Amplicon size (bp)	Reference
21	F: GGTGGCAAGGAGAGCAAAGT	*cps21N*	4	325	Kerdsin et al. 2014
	R: ACATGGTAAGCCATTGCTGGA				
23	F: TGCTCAACAAACGCAGCAAA	*cps23I*	3	454	Kerdsin et al. 2014
	R: TGACTGGTACATCTGCAGCC				
24	F: ACCCGGAAAAACCAGGAGTT	*cps24L*	2	500	Kerdsin et al. 2014
	R: ACCAATCAATGCCAAGCGAC				
25	F: GGAGGAGCTGCGGGCTCATA	*cps25M–cps25N*	2	1211	Kerdsin et al. 2012
	R: TGGCCACAACCTGGATGCGTT				
27	F: CTACGCCAATCGAAGCCAGA	*cps27K*	4	506	Kerdsin et al. 2014
	R: CCAGTAAGAAGCCTGTCGCA				
28	F: GGACTTCGGTACCTTAGCGT	*cps28L*	4	865	Kerdsin et al. 2014
	R: CTCCAGCACATTCCCGTACC				
29	F: GTGCGGGCGTTATTTTTGGT	*cps29L*	4	435	Kerdsin et al. 2014
	R: AGCCTTGCAACCCATTTCCT				
30	F: CTTTAATTGCTTGCGCCCGT	*cps30I*	4	170	Kerdsin et al. 2014
	R: ATTCGGGCTACCCATTGCAG				
31	F: GGAGTGCTCTATGCCACCTT	*cps31L*	3	550	Kerdsin et al. 2014
All	F: TTCTGCAGCGTATTCTGTCAAACG	*gdh*	All reactions	695	Kerdsin et al. 2012
	R: TGTTCCATGGACAGATAAAGATGG				

Table S2: Oligonucleotide primers used in PCR-based antimicrobial resistance genes gene detection.

Primer Name	Sequence (5′→3′)*	Gene	Amplicon Size (bp)	Reference
aac(6')-Ie-	F: CAGGAATTTATCGAAAATGGTAGAAAAG	*aac(6')-Ie-*	369	Vakulenko et al. 2003
	R: CACAATCGACTAAAGAGTACCAATC			
aph(2")-Ia	F: CAGAGCCTTGGGAAGATGAAG	*aph(2")-Ia*	348	Vakulenko et al. 2003
	R: CCTCGTGTAATTCATGTTCTGGC			
aph(3')-IIIa	F: CCTCGTGTAATTCATGTTCTGGC	*aph(3')-IIIa*	523	Vakulenko et al. 2003
	R: CTTTAAAAAATCATACAGCTCGCG			
ant(4')-Ia	F: CAAACTGCTAAATCGGTAGAAGCC	*ant(4')-Ia*	294	Vakulenko et al. 2003
	R: GGAAAGTTGACCAGACATTACGAACT			
aph(2")-Ib	F: CTTGGACGCTGAGATATATGAGCAC	*aph(2")-Ib*	867	Vakulenko et al. 2003
	R: GTTTGTAGCAATTCAGAAACACCCTT			
aph(2")-Ic	F: CCACAATGATAATGACTCAGTTCCC	*aph(2")-Ic*	444	Vakulenko et al. 2003
	R: CCACAGCTTCCGATAGCAAGAG			
aph(2")-Id	F: GTGGTTTTTACAGGAATGCCATC	*aph(2")-Id*	641	Vakulenko et al. 2003
	R: CCCTCTTCATACCAATCCATATAACC			
tet(K)	F: GATCAATTGTAGCTTTAGGTGAAGG	*tet(K)*	155	Malhotra-Kumar et al. 2005
	R: TTTTGTTGATTTACCAGGTACCATT			
tet(L)	F: TGGTGGAATGATAGCCCATT	*tet(L)*	229	Malhotra-Kumar et al. 2005
	R: CAGGAATGACAGCACGCTAA			
tet(M)	F: GTGGACAAAGGTACAACGAG	*tet(M)*	406	Malhotra-Kumar et al. 2005
	R: CGGTAAAGTTCGTCACACAC			
tet(O)	F: AACTTAGGCATTCTGGCTCAC	*tet(O)*	515	Malhotra-Kumar et al. 2005
	R: TCCCACTGTTCCATATCGTCA			
mef(A/E)	F: CAATATGGGCAGGGCAAG	*mef(A)*	317	Malhotra-Kumar et al. 2005
	R: AAGCTGTTCCAATGCTACGG			
erm(A)	F: CCCGAAAAATACGCAAAATTTCAT	*erm(A)*	590	Malhotra-Kumar et al. 2005
	R: CCCTGTTTACCCATTTATAAACG			
erm(B)	F: TGGTATTCCAAATGCGTAATG	*erm(B)*	745	Malhotra-Kumar et al. 2005
	R: CTGTGGTATGGCGGGTAAGT			
fex(A)	F: GTACTTGTAGGTGCAATTACGGCTGA	*fex(A)*	1272	Kehrenberg and Schwarz 2006
	R: CGCATCTGAGTAGGACATAGCGTC			
cfr	F: TGAAGTATAAAGCAGGTTGGGAGTCA	*cfr*	746	
	R: ACCATATAATTGACCACAAGCAGC			
optrA	F: GCACCAGACCAATACGATACAA	*optrA*	794	Shang et al. 2019
	R: TCCTTCTTAACCTTCTCCTTCTCA			
poxtA	F: GAACGCTTGGAGTATTTCGACTTC	*poxtA*	778	Brenciani et al. 2019
	R: CTGGACTGAGAATACCCATC			

Table S3: PCR cycling profile used in detecting antimicrobial resistance genes.

Gene/s detected	PCR conditions				Reference
	PCR steps	Temperature	Time	Cycle	
erm(A) *erm(B)* *mef(A)*	Initial Denaturation	93	3 min	1	Malhotra-Kumar et al. 2005
	Denaturation	93	1 min		
	Annealing	62	1 min	30	
	Extension	65	4 min		
	Final Extension	65	3 min	1	
tet(M) *tet(O)* *tet(L)*	Initial Denaturation	93	3 min	1	
	Denaturation	93	1 min		
	Annealing	62	1 min	30	
	Extension	65	4 min		
	Final Extension	65	3 min	1	
aac(6')-Ie-aph(2")-Ia *aph(3')-IIIa* *ant(4')-Ia* *aph(2")-Ib* *aph(2")-Ic* *aph(2")-Id*	Initial Denaturation	94	3 min	1	Vakulenko et al. 2003
	Denaturation	94	40 sec		
	Annealing	55	40 sec	35	
	Extension	72	40 sec		
	Final Extension	72	2 min	1	
optrA	Initial Denaturation	94	1 min	1	Shang et al. 2019
	Denaturation	94	1 min		
	Annealing	57	30 sec	34	
	Extension	72	3 min		
	Final Extension	72	7 min	1	
poxtA	Initial Denaturation	94	1 min	1	Brenciani et al. 2019
	Denaturation	94	1 min		
	Annealing	57	30 sec	34	
	Extension	72	3 min		
cfr	Initial Denaturation	94	1 min	1	Kehrenberg and Schwarz 2006
	Denaturation	94	1 min	34	
	Annealing	48	30 sec		
	Extension	72	3 min		
	Final Extension	72	7 min	1	
fexA	Initial Denaturation	94	1 min	1	
	Denaturation	94	1 min	34	
	Annealing	57	30 sec		
	Extension	72	3 min		
	Final Extension	72	7 min	1	

Table S4: Pre- and post-trimming FASTQC quality reports of the raw sequence files.

	Total Read Bases	Total Reads	GC (%)	Q20 (%)	Q30 (%)
Raw data stats	1,035,059,918	10,248,118	40.83	96.83	94.64
Filtered data stats	880,607,216	8,719,342	40.75	99.52	99.08

REFERENCES

Brenciani A, Fioriti S, Morroni G, Cucco L, Morelli A, Pezzotti G, Paniccià M, Antonelli A, Magistrali CF, Rossolini GM, Giovanetti E. Detection in Italy of a porcine Enterococcus faecium isolate carrying the novel phenicol-oxazolidinone-tetracycline resistance gene poxtA. J Antimicrob Chemother. 2019; 74(3):817-818. http://doi.org/10.1093/jac/dky505

Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant *Staphylococcus* isolates. Antimicrob Agents Chemother. 2006; 50(4):1156-63. http://doi.org/10.1128/AAC.50.4.1156-1163.2006

Kerdsin A, Dejsirilert S, Akeda Y, Sekizaki T, Hamada S, Gottschalk M, Oishi K. Fifteen *Streptococcus suis* serotypes identified by multiplex PCR. J Med Microbiol 2012; 61:1669–1672. https://doi.org/10.1099/jmm.0.048587-0

Kerdsin A, Akeda Y, Hatrogjit R, Detchawna U, Sekizaki T, Hamada S, Gottschalk M, Oishi K. *Streptococcus suis* serotyping by a new multiplex PCR. J Med Microbiol 2014; 63(6):824-830. https://doi.org/10.1099/jmm.0.069757-0

Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrob Agents Chemother. 2005; 49(11):4798-800. http://doi.org/10.1128/AAC.49.11.4798-4800.2005

Shang Y, Li D, Shan X, Schwarz S, Zhang SM, Chen YX, Ouyang W, Du XD. Analysis of two pheromone-responsive conjugative multiresistance plasmids carrying the novel mobile optrA locus from Enterococcus faecalis. Infect Drug Resist. 2019; 12:2355-2362. http://doi.org/10.2147/IDR.S206295

Vakulenko SB, Donabedian SM, Voskresenskiy AM, Zervos MJ, Lerner SA, Chow JW. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob Agents Chemother. 2003; 47(4):1423-6. http://doi.org/10.1128/AAC.47.4.1423-1426.200