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Review article

Genomic epidemiology in *Streptococcus suis*: Moving beyond traditional typing techniques

Rujirat Hatrongjit [a], Nahuel Fittipaldi [b], Marcelo Gottschalk [b], Anusak Kerdsin [c,*]

[a] Faculty of Science and Engineering, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand
[b] GREMIP, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada
[c] Faculty of Public Health, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand

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ABSTRACT

Streptococcus suis is a bacterial gram-positive pathogen that causes invasive infections in swine and is also a zoonotic disease agent. Traditional molecular typing techniques such as ribotyping, multilocus sequence typing, pulse-field gel electrophoresis, or randomly amplified polymorphic DNA have been used to investigate *S. suis* population structure, evolution, and genetic relationships and support epidemiological and virulence investigations. However, these traditional typing techniques do not fully reveal the genetically heterogeneous nature of *S. suis* strains. The high-resolution provided by whole-genome sequencing (WGS), which is now more affordable and more commonly available in research and clinical settings, has unlocked the exploration of *S. suis* genetics at full resolution, permitting the determination of population structure, genetic diversity, identification of virulent clades, genetic markers, and other bacterial features of interest. This approach will likely become the new gold standard for *S. suis* strain typing as WGS instruments become more widely available and traditional typing techniques are gradually replaced.

1. Introduction

Streptococcus suis is a swine and zoonotic pathogen that causes invasive infections in pigs and humans [1]. In 2005, a major human outbreak of *S. suis* in Sichuan Province, China, brought global attention to this pathogen [2]. Since then, numerous studies have investigated various aspects of *S. suis*, such as pathogenesis, virulence factors and markers, vaccine development, immune response, taxonomy, genetic comparative analysis, epidemiology, and public health responses [3,4]. Understanding the population structure, genetic diversity, evolution, and epidemiology of this pathogen is critical for evaluating its virulence, zoonotic potential, clinical manifestations, prevention control and management.

This review aimed to provide a comprehensive overview of traditional and modern molecular typing techniques used for *S. suis* studies from the 1990s to the present. By examining these techniques, this review aimed to highlight the research progress made in understanding *S. suis* diversity and its impact on public health.

2. Serotyping

Serotype identification is considered the typing gold standard for *S. suis* strains. Serotyping has recently been associated with

* Corresponding author.
E-mail address: Anusak.ke@ku.th (A. Kerdsin).

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identifying pathogenic isolates [5]. Traditional serotyping of *S. suis* strains is based on an antigenic reaction directed against the capsular polysaccharide of the pathogen. This technique is used to classify *S. suis* into 35 serotypes (1/2 and 1–34) [6–11]. Because some described serotypes belong to other bacterial species, the number of official *S. suis* serotypes has been reduced to 29 [12–14]. However, after 2010, serotypes 21/29, NCL21-NCL26, and Chz were described in China [15–17].

Since 1999, several polymerase chain reaction (PCR) assays have been developed and widely used to indirectly determine the serotype of *S. suis* strains [15,18–28]. However, one major drawback of these PCR assays is that they cannot distinguish between serotypes 2 and 1/2 or between serotypes 1 and 14. Therefore, antisera are still required to differentiate these serotypes. In 2016, Athey et al. reported that a specific single nucleotide polymorphism (SNP) of the *cpsK* gene at position 483 could differentiate serotype pairs 1 and 14 and pairs 2 and 1/2 [29]. These authors also developed a bioinformatics pipeline to determine the serotype from whole-genome sequencing (WGS) data [29]. Then, PCR-restriction fragment length polymorphism (RFLP), a mismatch amplification mutation PCR assay, and multiplex PCR were developed to differentiate these serotypes based on *cpsK* SNP [30–32]. A loop-mediated isothermal amplification was also reported to precisely identify serotypes 2 and 14 [33].

Other than antisera and PCR-based serotyping, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is useful in determining the serotype of *S. suis* strains [34,35]. However, strain variation would probably preclude this method from being sufficiently accurate.

3. Restriction-fragment length polymorphism (RFLP)

In the early 1990s, RFLP was applied to study *S. suis* strains [36,37]. Restriction enzymes (*Hae*III or *Hin*dIII) generated a convenient digest restriction pattern, with *Hae*III showing higher discriminatory power than *Hin*dIII [36]. RFLP is used successfully to differentiate outbreak from nonoutbreak *S. suis* strains [37]. The main limitation of RFLP is the variable length of cleavage DNA fragments, which ranges from very small to large fragments and can occasionally create challenges in distinguishing between closely related patterns.

4. Ribotyping

To improve the RFLP assay described above, hybridization of rDNA or rRNA probes to digested RFLP DNA fragments is commonly used in ribotyping assay, which was first described and applied to *S. suis* strain typing in 1994 [38]. In 1995, Okwumabua et al. showed that a 1.8 kb *Pst*I fragment from ribotyping is a useful genotypic marker for identifying *S. suis* isolates [39]. Another study revealed that pathogenic serotypes 1 and 2 strains have unique ribotype profiles [40]. Similarly, highly virulent *S. suis* serotype 2 isolates from pigs have unique ribotype profiles [41]. A similar study demonstrated that specific ribotype profiles are related to clinical diseases and antimicrobial resistance [42]. A study from Spain showed very closely related ribotype profiles between human and pig *S. suis* strains, suggesting that the isolates were epidemiologically linked [43].

5. Pulse-field gel electrophoresis (PFGE)

This technique is commonly used with *S. suis* strains from swine and humans since it was first described in 2001 [44]. The most commonly used restriction enzyme for *S. suis* PFGE is *Sma*I [44–49]. PFGE has a relatively high discriminatory power, with a numerical index of discrimination (D) of $\geq$0.87 [50]. PFGE-based analysis of human *S. suis* isolates was carried out for the first time in 2002 [45]. That study and subsequent ones revealed that *S. suis* isolates from humans are genetically more homogeneous than those recovered from pigs [26,45,49,51]. Some *S. suis* isolates from humans and pigs also had identical PFGE patterns, suggesting transmission from pigs to humans [45,47,49]. A study from Spain revealed an important genetic heterogeneity level among clinical pig *S. suis* isolates within the same herd and the predominance of specific clonal isolates in some herds [46]. However, pig *S. suis* isolates causing invasive disease were genetically more homogenous than those recovered from pneumonia cases or carrier animals [44]. Another study showed a close correlation between PFGE patterns and the presence of virulence marker genes among human *S. suis* serotype 2 strains [48].

6. Virulence-associated gene (VAG) profiling

Genes encoding a muramidase-released protein (*mrp*), an extracellular protein factor (*epf*), and the hemolysin known as suilysin (*sly*) are used as markers to predict the virulence or pathogenic potential of *S. suis* serotype 2 strains. These virulence markers are less frequently found in *S. suis* serotypes 1/2, 9, 7, and 3 recovered from diseased or healthy pigs in European countries [52]. In 2002, Wisselink et al. described a multiplex PCR specific to serotypes 1 (or 14), 2 (or 1/2), 7, and 9 and the *epf* [20]. In 2006, a multiplex PCR was used to detect VAGs, including *epf*, *sly*, *mrp*, and *arcA*, among isolates of serotypes 1 (or 14), 2 (or 1/2), 7, and 9 [22]. The virulence marker profile *epf*+/*sly*+/*mrp*+ was significantly associated with human *S. suis* serotype 2 sequence type (ST) 1 strain, whereas the profile *epf*−/*sly*+/*mrp*− was predominantly found in human *S. suis* serotype 2 ST104 strain in Thailand [27]. A genomic meta-analysis has recently proposed that *mrp* and *sly* may be putative zoonotic virulence markers [53].

An enhanced VAG panel comprising 22 genes (*mrp*, *epf*, *sly*, *fbps*, *rgg*, *ofs*, *srtA*, *pgdA*, *gapdh*, *iga*, *endoD*, *ciaRH*, *salKR*, *manN*, *purD*, *rgg*, *DppIV*, *neuB*, *dltA*, SMU_61-like, SpyM3_0908, and *SspA*) was used to characterize *S. suis* serotype 2 strains from China [54]. The distribution of these 22 virulence-related genes enabled assigning the isolates to two genetic clusters, namely, A and B, the latter including the more virulent [54]. A study in 2021 reported a cluster C with *S. suis* serotype 24 strains from humans [55]. However, the virulence of cluster C strains needs to be clarified.

7. Multilocus sequence typing (MLST)

An MLST scheme for *S. suis* was developed by King et al., in 2002 [56]. It uses seven housekeeping genes: *aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA*, and *thrA*. Up to this year, STs have been recorded in the MLST database (PubMLST; accessed on March 8th, 2024). MLST has revealed the presence of many clonal complexes (CCs) within the *S. suis* population. The most important CCs causing infections in pigs and humans are CC1, CC13/149, CC16, CC17, CC20, CC25, CC28, CC94, CC104, CC233, CC221/234, CC1109, CC1112, and CC1237 [57,58]. In North America, CC25 (Canada) and CC28 (United States and Canada) are more commonly reported [59]. These latter CCs are also present in Australia and some parts of Asia [4], whereas CC1 strains are more prevalent in Europe, Asia, and South America [1, 60,61]. CC20 is important in The Netherlands [62], whereas CC104 and CC233 (ST233, ST379, and ST1656) have caused outbreaks and are endemic to Thailand [63–65]. CC16 and CC94 predominate among swine isolates in Europe; however, human cases caused by isolates of the latter CCs have also been reported in Thailand [1,63,66].

Based on clinical information and isolation sites, one study revealed that CC1, CC28, CC94, and CC104 are associated with a pathogenic pathotype, whereas CC750 is associated with a commensal pathotype, as supported by odds ratio analysis [5]. Although MLST has been applied widely by multiple laboratories throughout the world to study the *S. suis* population structure, there are limitations to this technique. For example, because MLST only considers a small fraction of genome information (seven genes), it may not reveal important genetic differences among strains of the same CCs or even STs, as shown for *S. suis* CCs 25 and 28 [60,61]. However, even if MLST is sometimes a poor or misleading predictor of *S. suis* strain virulence [60], it remains a powerful tool that may help identify pathogenic isolates, particularly when used in combination with serotyping, as exemplified by Estrada et al. (2019) [5].

8. Random amplification of polymorphic DNA (RAPD)

S. suis was first typed using this technique in 1999 based on primers OPB07, OPB10, and OPB17 [67]. That study demonstrated that RAPD clusters, in conjunction with the MRP/EF/suilysin phenotype, can provide a reliable assessment of clonal relationships between *S. suis* isolates responsible for infections in pigs or humans and that the technique is useful to characterize isolates displaying the classic virulent phenotype suilysin $^{+}$ MRP^{+} EF^{+} [67]. A subsequent study combined three RAPD primers (OPB07, OPB10, and CLAU), which detected genotypic differences between isolates of *S. suis* serotype 1/2 [68]. Two studies reported the discovery of identical RAPD patterns and specific virulence gene profiles in *S. suis* serotype 2 isolates from Brazil and Thailand, respectively [69,70]. Independently, both studies found matching RAPD profiles aligned with virulence gene profiles [69,70]. In 2003, RAPD analysis with primers OPB06, OPB10, and OPB11 showed different reproducible patterns on *S. suis* serotype 5 isolates from pigs in herds with and without clinical disease [71]. In 2019, another study revealed that RAPD has greater discriminatory power and is a better predictor of MLST CCs associated with swine and human infections than PCR-RFLP [72].

9. 16S–23S rDNA intergenic spacer PCR-RFLP (ISR PCR-RFLP)

Marois et al. (2006) first used this technique to investigate 138 independent *S. suis* strains belonging to various serotypes isolated from swine and human cases [73]. They reported a discriminatory power of 0.954 with *Rsa*I and 0.984 with *Rsa*I plus *Mbo*II [73]. They also showed that *S. suis* serotype 2 strains are significantly associated with clusters of the *Rsa*I subgroup A and group C and *Mbo*II subgroup c [73]. ISR PCR-RFLP permitted better discrimination than PFGE, as eight and five patterns were detected with the use of *Rsa*I and *Mbo*II, respectively, in 34 strains of serotypes 2 and 1/2, relative to only three PFGE patterns among the same strains [74]. Another report revealed that ISR PCR-RFLP could not differentiate among most human CCs [72]. However, DNA sequencing using 16S–23S rDNA ISR distinguished between four clusters: No. 1 consisting of CC25, CC28, CC104, and CC233; No. 2 consisting of CC221/234; No. 3 consisting of CC16 (ST16); and No. 4 consisting of CC1 [72].

10. Amplified fragment length polymorphism (AFLP)

AFLP for *S. suis* typing was first described in 2007, which allowed the identification of a cluster (cluster A1) associated with invasive clinical strains of serotype 2 of porcine and human origin, which possessed markers *sly*, *mrp,* and *epf* (or *epf* variants) [75]. AFLP was also used to type *S. suis* strains recovered from wild boars. Notably, 80% of *cps2*$^{+}$ (a proxy for serotype 2) AFLP-typed wild boar isolates were grouped within cluster A1 [76]. It was also suggested that AFLP cluster A probably belongs to CC1 [76]. A study from Brazil reported no apparent correlation between the isolation site of the strains and their *mrp/epf/sly* genotypes. However, *S. suis* isolates possessing an *mrp*$^{+}$*/epf*^{variant}*/sly*$^{+}$ genotype had higher genetic similarity and appeared to cluster closely when analyzed using AFLP [77].

11. Multiple-locus variable tandem repeat number analysis (MLVA)

In 2010, MLVA permitted typing *S. suis* isolates using nine selected tandem repeat loci (TR1–TR9). Loci TR1 to TR8 were described as markers of lower or moderate diversity, whereas locus TR9 was proposed as a marker of higher diversity (Simpson's index value 0.96) [78]. Locus TR9 was useful in discriminating between Chinese ST7 outbreak strains that could not be resolved solely using PFGE [78]. A subsequent study showed 17 variants of TR9 locus sequencing among 21 *S. suis* ST1 serotype 2 isolates recovered from humans [51]. The advantages of MLVA are as follows: (i) more discriminatory power than PFGE; (ii) ability to perform high-throughput screenings with large numbers of isolates; (iii) increased feasibility, as MLVA is relatively inexpensive, easy to perform, rapid, and

reliable; and (iv) easier interlaboratory comparisons because a reference strain is not required [78].

12. PCR pathotyping

This technique was developed to target three genetic marker genes associated with observed clinical phenotypes: genes for copper-exporting ATPase 1, a type I restriction-modification system S protein, and a putative sugar ABC transporter [79]. The selected genetic markers were to differentiate *S. suis* into a disease-associated group (pathogenic pathotype) and a non-disease-associated group (nonpathogenic pathotype). PCR pathotyping worked well for pig *S. suis* strains from England and Wales, but contradictory results were observed with pig *S. suis* strains from Switzerland [79,80]. A recent study showed that this assay does not differentiate non-disease-associated from disease-associated groups in clinically healthy pig *S. suis* isolates in Thailand, although it works well for human isolates [81]. The disease-associated group could also be classified into four types, with human *S. suis* CC1 isolates significantly associated with disease-associated type I and CC104 and CC25 significantly associated with disease-associated type IV [81].

A study in 2021 proposed that PCR identified a human-associated clade (HAC) of *S. suis* [82]. Two genes consisting of sigma-70 (G15) and relaxase mobilization nuclease domain protein (G20) were chosen for PCR to be representative of HAC as promising markers for pathogen detection and surveillance [82]. The study showed that both primer pairs of these two HAC marker genes efficiently amplified all 12 training HAC isolates and the 21 uncharacterized patient isolates [82].

13. WGS-based typing

WGS has revolutionized *S. suis* epidemiological studies by offering a high-resolution discriminatory power that can reveal genetic diversity details in *S. suis* populations that are impossible to obtain with other typing approaches. Combining epidemiological and WGS data can effectively describe the genetic diversity of *S. suis* populations, provide critical information on the origin of isolates, facilitate the tracking of outbreaks or strains over time, and aid in identifying novel candidate VAGs. The benefits of using genomic epidemiological approaches have been demonstrated in various *S. suis* studies, as described below.

- **Comparative genomic analysis of reference strains.** In 2007, an 89 kbp pathogenicity island (named 89K PAI) was found using WGS of Chinese *S. suis* serotype 2 ST7 strains (98HAH12 and 05ZYH33), which were responsible for large outbreaks of human *S. suis* disease that occurred in that country in 1998 and 2005 [83]. In 2009, three strains from Vietnam (BM407), China (SC84), and Europe (P1/7) were also studied using WGS [84]. The Chinese outbreak strain SC84 contained an island almost identical to the previously described 89K PAI of strains 98HAH12 and 05ZYH33 [83,84]. However, the 89K island of strain SC84 had a composite structure and contained several regions that appeared to be integrative conjugative elements (ICE) carrying elements conferring resistance to antimicrobial drugs [84]. The 89K PAI was also used to track epidemic ST7 strains in China and some other countries [27,85–88].
  Another study showed a highly pathogenic ST1 strain that harbored 132 acquired islands, including 5 pathogenicity islands. This strain appears to have evolved from an intermediate pathogenic ST25 strain, whereas epidemic ST7 strains evolved from ST1 and acquired 5 additional genomic islands [89]. Willemse et al. showed that *S. suis* CC20 isolates include a unique 18.5 kb prophage region [90]. In many cases, clinical and zoonotic isolates possess smaller genomes but more VAGs than nonclinical (and/or nonzoonotic) isolates [90,91].
  Comparative genomic analysis of *S. suis* serotypes 2, 3, and 7 demonstrated instances of capsule switching in strains of serotypes 2, 3, and 7 of CCs 28 and 29, and WGS-based phylogenetic analysis showed that serotype 2 isolates belong to two major clades (1 and 2) [92]. Clade 2 *S. suis* serotype 2 strains were genetically similar to *S. suis* serotypes 3 and 7 strains and differed significantly from clade 1 serotype 2 strains [92].

- **Comparative genome hybridization (CGH).** This technique was used to analyze 55 *S. suis* isolates from different serotypes [93]. Two clusters (A and B) were divided based on CGH data. Cluster A exclusively contained CC1 isolates, whereas cluster B contained a more divergent and heterogeneous group of isolates [93]. Another study classified *S. suis* strains tested into three groups of differing virulence: (i) epidemic and highly virulent (E/HV group), including CC1 isolates; (ii) virulent (V group) containing several STs, such as ST13, ST54, ST56, ST81, and ST87 isolates; and (iii) intermediate or weakly virulent (I/WV group) composed of several STs isolates recovered from nonhuman sources [94].
- **Minimum core genome sequence typing (MCG).** Seven MCG groups and an ungroupable MCG were defined among a collection of *S. suis* isolates examined using WGS [95]. MCG Group 1 comprised all ST1 and ST7 isolates, including those from human infections and harboring a high number of VAGs, whereas MCG group 4 contained STs of intermediate virulence, such as ST25 and ST28. Isolates of MCG group 6 possessed fewer virulence genes [95]. In a subsequent study, a set of 10 SNPs present in 6 genes were used to identify the 7 MCG groups based on PCR and sequencing [96].
- **SNP-based phylogenetic studies.** Athey et al. analyzed core genome-SNP phylogenies of *S. suis* serotype 2 ST28 isolates recovered from humans and pigs in Canada, the United States, Japan, and Thailand [60]. The study revealed two larger clades: clade I comprising most Canadian strains and the second major clade further divided into four subclades (clades II–V). Clades II and III had a strong signal of geographical structure [60]. Strains from clade V were also significantly more virulent than clade I strains in a murine model of infection [60]. Another study in 2016 revealed the population structure of serotype 2 ST25 *S. suis* strains from Canada, the United States, and Thailand [61]. Genome-wide SNP-based phylogenetic analysis identified two distinct main clades

consisting of Thai and North American strains, respectively. The North American clade was further divided into two main subclades, which differed mainly in ICE content and had defined patterns of antimicrobial resistance genes [61].
A recent study showed that ST1656 clonal strains, which diverged from other CC233 isolates, including ST233 and ST379, were responsible for disease outbreaks [65]. This study also revealed a Thai-specific zoonotic clade (CC104 and CC233) distantly related to other zoonotic lineages such as CC1, CC20, and CC25 [65].

- **Bayesian analysis of population structure (BAPS).** Weinert et al. identified five distinct populations among *S. suis* 459 isolates using WGS and BAPS [91]. All five BAPS populations contained nonclinical and disease-causing isolates. However, BAPS population 1 had the largest number of disease-causing isolates, and serotype 2 was also predominant [91], whereas BAPS population 5 had more nonclinical isolates than clinical isolates and no serotype 2 strains [91]. A pyruvate synthase gene was present in BAPS populations 2 to 4 but absent from all systemic *S. suis* isolates [91]. Disease-causing isolates also had significantly smaller genomes than nonclinical isolates [91]. That study suggested that nonclinical isolates with larger genomes could be sources of new adaptive phenotypes that might be transferred to pathogenic *S. suis* isolates [91].
  Another WGS and BAPS investigation used 116 *S. suis* isolates from human patients and pigs [90]. Seven BAPS groups were identified based on the nucleotide alignment of the core genome. BAPS groups correlated with CCs. BAPS group 1 consisted of all CC13 isolates, BAPS group 2 contained most CC16 isolates, BAPS group 3 comprised a single isolate, BAPS group 4 consisted of all CC1 isolates, BAPS group 5 included CC27 and CC29, and BAPS group 6 contained CC20, and BAPS group 7 comprised mostly diverse unrelated isolates [90]. Human *S. suis* isolates were predominant in BAPS groups 4 and 6, and these two BAPS groups were considered to have zoonotic potential [90].
  Dong et al. (2021) recently analyzed the genome sequences of 1634 *S. suis* isolates from 14 countries and classified them into nine BAPS groups [82]. Among them, BAPS group 7 represented a dominant group of virulent *S. suis* associated with human infections, most being ST1 and ST7 (included in CC1) [82]. Those authors proposed that this cluster constituted a novel HAC that had diversified from swine *S. suis* isolates. The phylogeographical analysis identified Europe as the origin of HAC, coinciding with the exportation of European swine breeds between the 1960s and 1970s [82].

- **Genome-wide association study (GWAS).** GWAS and principal component analysis were used to examine a collection of clinical and nonclinical *S. suis* isolates from the United Kingdom [79]. The investigation proposed that two marker genes, a copper-exporting ATPase 1 and a type I restriction-modification system S protein, were associated with pathogenic isolates, whereas a putative sugar ATP-binding cassette transporter could serve as a marker of strains with a nonpathogenic pathotype [79]. However, a subsequent study found no correlation between these markers and identifying pathogenic isolates from swine [80,81]. GWAS and chi-square analysis were instrumental in identifying 25 HAC-specific genes [82]. These genes might contribute to the increased risk of human infection and be used as markers for HAC identification.
- **Pan-genome analysis.** Pan-genome analysis based on a chi-square test and the least absolute shrinkage and selection operator regression model identified five marker genes significantly associated with the pathogenic pathotype of North American strains [97,98]. The marker genes consisted of *ofs*, *srtF*, *SSU_RS09525*, *SSU_RS09155*, and *SSU_RS03100*. GWAS and pan-genome analysis were performed to identify marker genes on *S. suis* strains of restricted geographical areas (United Kingdom and North America, respectively). Larger *S. suis* isolate collections from different regions or countries, isolation sources, serotypes, and STs should be further evaluated to fully understand the usefulness of these pathogenic pathotype marker genes.
  Guo et al. (2021) conducted a pan-genome analysis of *S. suis* serotype 2 and found that the *srtBCD* pilus gene cluster had a significant discrepancy between virulent and avirulent strains [99]. They proposed that the *sbp2′* gene in the *srtBCD* cluster could be a fitness virulence-associated marker of virulent isolates [99]. They also showed that 53 and 58 genes could be specific markers of high and low virulence groups, respectively [99].

- **Genomic meta-analysis.** Roodsant et al. (2021) analyzed all publicly available *S. suis* genomes with available metadata on the host, disease status, and country of origin [53]. The authors identified 124 *S. suis* putative virulence factors, where 26 were considered putative zoonotic virulent factors (PZVFs) [53]. Genes encoding NisK/NisR, Hhly3, and Fhb-1 were more prevalent among human and CC1 isolates than pig isolates, suggesting that these PZVFs may contribute to the zoonotic potential. Furthermore, they proposed that PZVF could be useful for classifying the zoonotic potential of *S. suis* and for the early detection of emerging zoonotic lineages.
- **Population genomic analysis.** A recent study conducted 3071 *S. suis* genome analyses [100] and identified 10 pathogenic lineages with broad geographical spread and origin dates. These lineages consist of disease-associated serotypes 1 to 9 and 14 with STs belonging to STs 1, 14 to 17, 20, 23, 25, 27 to 29, 54, 87, 89, 94, 108, 123, 136, and 198 [100]. Among 10 pathogenic lineages, lineage 1 is associated with zoonotic disease in humans and almost corresponds to ST1 of serotypes 2 or 14 strains [100].
  Three genomic islands that are mostly specific to these 10 pathogenic lineages were identified [100]. Island 1 (SSU_RS05400-SSU_RS05325 in the published genome of *S. suis* strain P1/7) was present in >95% of isolates from 9 of 10 pathogenic lineages, and only 17% of isolates were from the nonpathogenic clade. Island 1 contained heparan sulfatase and hyaluronate lyase. Island 2 (SSU_RS02325-SSU_RS02355 in P1/7) contained pilin subunit protein-encoding genes and sortase and was distributed in 84% of isolates from 10 pathogenic lineages, and 11% was from nonpathogenic lineages. Island 3 (SSU_RS1130-SSU_RS01185 in P1/7) showed the strongest association with 10 pathogenic lineages and was present in >95% of isolates in these pathogenic lineages, and only 1% was from nonpathogenic lineages. This island contained a gene encoding an ABC transporter, ROK family proteins, and a surface protein similar to a PTSII subunit.

14. Conclusions and perspectives

S. suis is a major pathogen that negatively affects the pig industry and human health. Molecular typing techniques, including traditional and modern methods such as WGS, have been used to characterize and determine strain-specific genetic relationships for epidemiological purposes. With the increasing feasibility and decreasing costs of WGS, high-resolution strain typing has become a useful tool for outbreak investigation, infectious disease surveillance, genomic characterization, and comparative analyses. *S. suis* is a prime example of the effectiveness and usefulness of WGS in replacing traditional typing methods as the new gold standard in the near future. However, although highly virulent serotype 2 strains for humans and pigs have been identified, there is no universal consensus on the definition and role(s) of virulence factors. Information is extremely heterogeneous, with many factors not validated in different geographical regions. Therefore, caution should be exercised when interpreting results from published studies.

Although generating WGS data for *S. suis* isolates has become routine, its full adoption in many laboratories is hindered by a persistent barrier—the lack of user-friendly bioinformatics platforms and standardization of sequencing and analysis workflows. There is also a substantial lack of genomics expertise outside the research environment. To improve *S. suis* clinical and epidemiological investigations, user-friendly bioinformatics pipelines for analysis are necessary, from raw read quality to assembly and SNP calling. Furthermore, it is crucial to build a global *S. suis* genomic database with appropriate tools for analysis. Addressing these barriers will enable more laboratories to adopt WGS for improved strain typing and comparative analyses of *S. suis*, ultimately advancing the understanding and management of this pathogen.

Data availability statement

Data sharing is not applicable to this review as no new data were created or analyzed in this study.

CRediT authorship contribution statement

Rujirat Hatrongjit: Writing – review & editing, Writing – original draft, Conceptualization. **Nahuel Fittipaldi:** Writing – review & editing, Writing – original draft. **Marcelo Gottschalk:** Writing – review & editing, Writing – original draft. **Anusak Kerdsin:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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