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REVIEW

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Potential mechanisms of *Streptococcus suis* virulence-related factors in blood–brain barrier disruption

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Abstract

Streptococcus suis (*S. suis*) has emerged as a prevalent bacterial pathogen within the swine industry, posing a substantial zoonotic threat to global public health. As an inhabitant of the upper respiratory tracts of animals, *S. suis* possesses a sophisticated array of virulence-related factors that enable it to breach cellular barriers and induce multisystem inflammation, notably causing meningitis. This review synthesizes current research findings to provide insights into the complicated virulence-related factors employed by *S. suis*. Special emphasis is given to factors crucial for penetrating the host blood–brain barrier (BBB). By summarizing existing knowledge, this review lays the groundwork for future advanced investigations, paving the way for a deeper understanding of *S. suis* pathogenesis and potential therapeutic interventions. Specifically, comprehensive explorations to unravel the expression dynamics of these virulence-related factors and elucidate the unique pathogenic mechanisms that operate during host attacks could contribute to clinical treatment strategies and advance innovations in vaccine development.

Introduction

Streptococcus suis (*S. suis*), a facultatively anaerobic, Gram-positive bacterium, was initially documented by Dutch and British scholars in 1951 and 1954, respectively [1]. Based on variations in cell wall capsular polysaccharides (CPS), *S. suis* is currently classified into 29 serotypes (original serotypes 20, 22, 26, and 32–34 have been reclassified), most of which were originally isolated from pigs. Increasing numbers of researchers are identifying novel cps loci (NCLs) from non-typeable isolates through bioinformatics analysis leveraging next-generation sequencing technologies. Despite the absence of systematic nomenclature rules, serotype Chz and 27 NCLs have been documented [2]. As of August 21, 2024, the *S. suis* multilocus sequence typing (MLST) database (https://pubmlst.org/organisms/streptococcus-suis/) included a total of 4,165 distinct sequence types.

S. suis is a globally significant bacterial pathogen of swine, acting as both a commensal organism in pigs and a major zoonotic pathogen. With a carriage rate nearing 100% on pig farms, human transmission can occur via close contact with infected or carrier pigs [3]. Although the upper respiratory, digestive, and reproductive tracts of pigs are natural colonization sites for *S. suis*, the mechanisms through which specific strains or serotypes penetrate the host's mucosal barrier to enter the bloodstream and systemic circulation are not yet fully understood [4, 5]. *S. suis* has a broad host range, encompassing humans, domestic pigs, wild boars, flies, rats, birds, and other animals, which may serve as important carriers [6, 7]. Although all serotypes and clonal complexes

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(CCs, clustered using MLST) can be found among *S. suis* isolates from healthy pigs, the invasive isolates from diseased pigs are most commonly serotypes 1/2, 2, 3, 7 and 9, and belong to CC1, CC16, and CC28. While most zoonotic infections are caused by serotype 2 of CC1, infections with other serotypes (e.g., serotypes 9 and 14) and genotypes (e.g., CC20) have also been documented [8]. As the most widely studied serotype, serotype 2 is recognized for its high pathogenicity and virulence, and is commonly associated with human infections and pig farm outbreaks globally. The dissemination of *S. suis* in pig populations often entails asymptomatic nasopharyngeal carriage, including cross-infection among various serotypes [9, 10]. In young pigs, typically aged 5–10 weeks, the infection progresses rapidly, with symptoms evolving from persistent high fever and bacteremia to meningitis, arthritis, endocarditis, and pneumonia. Infected piglets often exhibit more severe symptoms than adults; pregnant sows may experience miscarriages, and acute cases can lead to sudden death without preceding symptoms [11, 12].

In 1968, Denmark reported the world's first human infection case of *S. suis* infection [3], initiating a rising trend in both human and pig cases globally, especially in countries with intensive pig farming. Current studies suggest that human *S. suis* infections are primarily linked to consumption of contaminated food, close contact with carrier pigs, overlap between living environments and farming or slaughterhouse operations, and handling or consumption of sick animals. Foodborne transmission plays a pivotal role in the spread of the disease, impacting swine farm workers, slaughterhouse staff, meat processing and retail employees, and rural veterinarians [13]. *S. suis* infection of the human body typically manifests with symptoms including high fever, coma, elevated white blood cell count, multiorgan failure, and acute respiratory distress syndrome, mirroring symptoms of Streptococcal toxic shock syndrome caused by Group A *Streptococcus* [13–16].

The precise mechanism facilitating the translocation of *S. suis* from the bloodstream to the brain remains elusive. Although numerous virulence-related factors with potential involvement in penetrating the blood–brain barrier (BBB) have been identified, none appear to be indispensable. The redundancy of virulence-related factors in *S. suis* complicates research efforts. BBB destruction and penetration are likely orchestrated by a variety of factors and mechanisms, with remarkable immune evasion playing a key role in the successful traversal capability of *S. suis*. This review synthesizes information on virulence-related factors that may facilitate BBB penetration by *S. suis*, offering insights into potential targets and strategies for preventing and treating bacterial meningitis (Fig. 1).

Adhesins

$AtlA_{SS}$

Indirect immunofluorescence assays have revealed that $AtlA_{SS}$, an autolysin recognized for regulating bacterial chain length in *S. suis*, is localized at the bacterial surface, specifically at the division septum. With a predicted molecular mass of 76 kDa, $AtlA_{SS}$ possesses a signal peptide sequence at its N-terminus and an N-acetylmuramidase (GH25 muramidase) domain at the C-terminus. Additionally, it contains a cell wall anchoring domain (GW module) and a Group B *Streptococcus* Bsp-like peptidoglycan-binding domain. The remarkable and specific fibrinogen (FG)/fibronectin (FN)-binding activity of $AtlA_{SS}$ significantly contributes to *S. suis* adherence to brain microvascular endothelial cells (BMECs). Furthermore, $AtlA_{SS}$-mediated bacterial cell autolysis plays a role in *S. suis* biofilm formation, with the deletion of $atlA_{SS}$ resulting in a significant decrease in virulence in mice [17].

SadP

Streptococcal adhesin P (SadP), an 80-kDa cell wall protein anchored via SrtA-mediated catalysis, possesses a signal peptide sequence, a galabiose-binding domain, and tandem repeat domains at its N-terminus. The C-terminus features a Leu-Pro-X-Thr-Gly (LPXTG) cell wall-anchoring motif. SadP specifically binds to the Galα1–4Gal oligosaccharides contained in glycolipid receptors that are prevalent on diverse cell surfaces [18, 19]. Based on unique recognition patterns of galabiose-containing carbohydrates in hemagglutination assays, SadP has been categorized into two subtypes—P_N and P_O—with P_N being the more prevalent and dominant subtype [20]. Madar et al. mechanistically detailed the molecular-level interaction mechanism between SadP and the globo-series glycolipids known as globotriaosylceramide (Gb3) and globotetraosylceramide (Gb4) [19]. Receptors containing these glycolipids are extensively expressed across various tissues and organs, including the pig brain, and deletion of *sadP* significantly reduces the ability of *S. suis* to bind to multiple endothelial cell types [19, 21]. SadP likely plays a crucial adhesive role in the systemic infection process of *S. suis*.

SsPepO

S. suis protein endopeptidase O (SsPepO), characterized by a putative secretion signal at its N-terminus, induces a robust humoral immune response in both mouse and pig models, demonstrating notable immunoprotective effects [22]. SsPepO binds with high affinity to FN and plasminogen (PG) in the extracellular matrix (ECM),

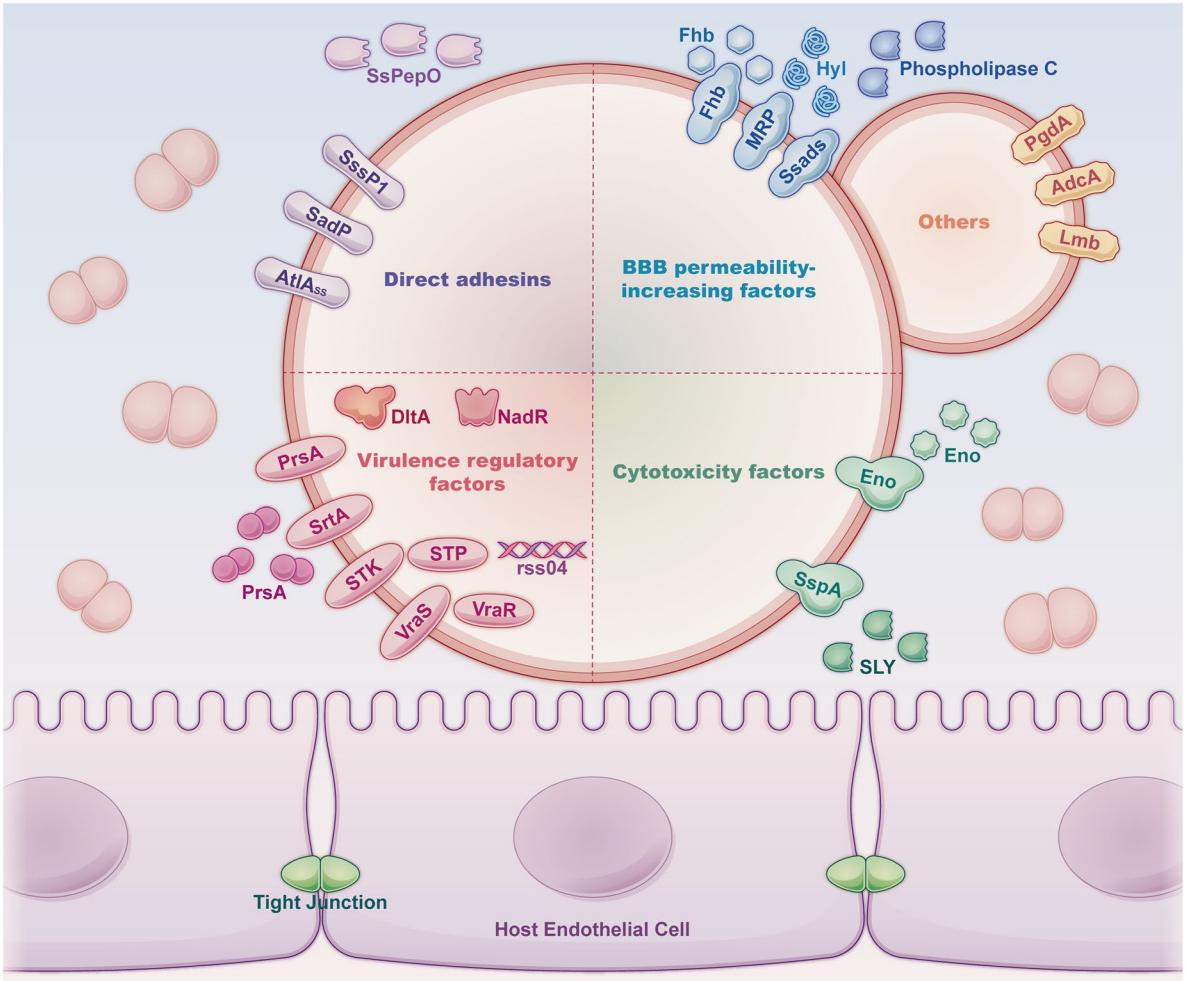

Fig. 1 Potential virulence-related factors involved in the process of *S. suis*-mediated blood-brain barrier disruption

facilitating enhanced adhesion of *S. suis* to BMECs. This serves as a prerequisite for the subsequent bacterial disruption and increased permeability of the BBB, and accelerated development of meningitis. The absence of SsPepO significantly reduces the pathogenicity of *S. suis*, although not completely [23]. FN and PG bind to SsPepO non-competitively, showcasing its efficient ECM-binding capability. Furthermore, upon binding to SsPepO, PG is converted into the protease plasmin, which then cleaves and hydrolyzes the complement protein C3b. These findings indicate that SsPepO also contributes to immune evasion [24].

SssP1

SssP1, a serine-rich repeat (SRR) glycoprotein spanning 4,647 amino acids (aa), assembles on the surface of the bacterium to form fimbria-like structures. Zhang and colleagues initially identified SssP1 within the 50-kb genomic island of the *S. suis* strain CZ130302, discovering that it is transported via the SecY2/A2 secretion system [25]. *S. suis* leverages the immunoglobulin (Ig)-like domains of SssP1 to bind to sialic acid on the host cell surface, thereby enhancing its adhesion to BMECs. Deleting *sssP1* significantly impairs the ability of *S. suis* to penetrate and damage the in vitro BBB model [25, 26]. In 2022, Pan et al. employed pull-down assays to further demonstrate that vimentin on the surface of BMECs mediates adhesion of SssP1 to host cells [26]. Antibody-mediated blocking of vimentin reduces the adhesion capability of *S. suis*, with vimentin sialylation playing a crucial role in this process.

Virulence regulatory factors

DltA

The *S. suis* gene *dltA* encodes a subunit of a D-alanine–poly (phosphoribitol) ligase with a total length of 1,536 bp (strain BM407). Deletion of *dltA* inhibits D-alanylation of lipoteichoic acid (LTA) in *S. suis*. D-alanylation notably increases surface hydrophobicity, facilitating

enhanced bacterial adhesion and invasion when encountering BMECs. In addition, D-alanylation contributes to the resistance of *S. suis* to cationic antimicrobial peptides and immune attacks, ultimately influencing its virulence in animal infection models [27, 28].

NadR

NadR, a cytoplasmic nicotinamide-nucleotide adenylyltransferase, is involved in regulating and facilitating the bacterial nicotinamide adenine dinucleotide synthesis pathway. Pan-genome analysis indicates that *nadR* is unique to virulent *S. suis* isolates, and is particularly associated with the virulence of serotype 2, serving as a transcriptional regulator [29]. Mutation of *nadR* significantly impairs the adhesion and invasion of BMECs by *S. suis*, leading to reduced virulence in animal models, including the eradication of meningitis pathology. Additionally, RNA sequencing results suggest that NadR functions as a global transcription regulator, affecting a wide range of *S. suis* life processes, such as cell growth and defense mechanisms [30].

PrsA

Parvulin-type peptidyl-prolyl isomerase (PrsA), a ubiquitous chaperone protein in Gram-positive bacteria, is present both on the cell surface and in culture supernatants and has a gene length of 1,002 bp (strain BM407). Initially identified by Jiang et al. in a transcriptome analysis of a type IV secretion system (T4SS) in the *virD4* mutant strain, PrsA demonstrates significant dose-dependent cytotoxicity when applied to brain-derived endothelial cells (bEnd.3) at concentrations exceeding 75 μg/mL [31]. Furthermore, PrsA influences the adhesion and invasion of *S. suis* to bEnd.3 and modulates the secretion or translocation of virulence factors, such as Suilysin (SLY), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and enolase at the post-translational level, thereby augmenting the pathogenicity and invasiveness of *S. suis* [32].

rss04

The small RNA rss04 exhibits significantly increased expression in pig cerebrospinal fluid than in blood or in vitro. Wu and colleagues identified *rss04* in *S. suis* strain P1/7 through transcriptome analysis, and it is speculated to play a role in the pathogenesis of meningitis [33]. Subsequent studies have shown that *rss04* regulates the expression of more than 10 virulence-related factors and inhibits the synthesis of CPS [34]. The decapsulation process enhances the exposure of surface proteins, increasing the likelihood of interactions between surface-expressed virulence-related factors and host cells. In vitro experiments show that the adhesion and invasion capacity of the Δ*rss04* strain towards bEnd.3 is significantly reduced compared with that of *S. suis* P1/7. In a mouse intracranial subarachnoid infection model, Δ*rss04* infection leads to a notable reduction in the release of cytokines (including Toll-like receptor 2 (TLR2), C-C motif ligand 2 (CCL2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)) in the brain, along with improved survival rates, underscoring its specific virulence regulatory ability.

SrtA

SrtA, a member of Class A of the sortase family, specializes in covalently anchoring proteins with a C-terminal LPXTG motif to cell wall peptidoglycan. Its homologs are widespread across various Gram-positive bacteria, playing key roles in the processing, modification, and localization of surface proteins, including Sao and muramidase-released protein (MRP), making it an important virulence-related factor [35–37]. Mutation of *srtA* hinders the adhesion and invasion of *S. suis* into BMECs [38], reduces adhesion to ECM proteins, and decreases the distribution and load of bacteria in tissues, including the central nervous system (CNS) and lungs, severely impairing virulence [37, 39]. However, the essential adhesion factors regulated by SrtA during the interaction between bacteria and host cells remain to be determined.

STK/STP

S. suis serine/threonine protein kinase (STK) and phosphatase (STP) serve as crucial global modulators of bacterial virulence. This signal transduction system, found in numerous prokaryotic organisms, swiftly responds to external stimuli by phosphorylating target proteins via ATP hydrolysis. It influences various bacterial activities, including CPS synthesis, cell division, metabolism, and oxidative stress resistance, at the post-translational level [40, 41]. The STK and STP proteins are 665 aa and 246 aa in length, respectively (strain SS2-1) [42]. STK includes a cytoplasmic kinase domain, an extracellular domain with four penicillin-binding proteins, and a Ser/Thr kinase-associated domain [41], while STP features a protein phosphatase 2C domain. STK controls *S. suis* cell division by phosphorylating the Thr-199 residue on the cell division initiation protein DivIVA [43]. Li et al. employed phosphoproteomic analysis to reveal that *S. suis* phosphoglucosamine mutase (GlmM) can be phosphorylated by STK, controlling cell wall peptidoglycan synthesis and assisting *S. suis* in evading the host immune response [44]. Mutation of *stk* or *stp1* significantly impairs the adhesion and invasion capabilities of *S. suis* towards BMECs and bEnd.3, hindering its transmission to animal brain tissue. This phenomenon may be attributable to

the role of STK in the ubiquitination and degradation of the tight junction (TJ) protein claudin-5, which leads to increased BBB permeability. Furthermore, STK and STP are involved in regulating the secretion and translocation of virulence factors such as SLY and GAPDH [45, 46]. Recent studies suggest that STK regulates CPS synthesis by initiating the phosphorylation of CcpS. Once phosphorylated, CcpS modulates the activity of CpsB, affecting the phosphorylation level of CpsD and ultimately mediating CPS synthesis [47]. Research on the *stp1* mutation has shown that, while the mutant strain demonstrates enhanced adhesion to bEnd.3 and increased resistance to reactive oxygen species, its virulence in mouse models is notably reduced [48].

VraSR

The VraSR system in *S. suis* is a two-component signaling system (TCSS) with a nucleotide sequence that is highly conserved across different serotypes and genotypes of *S. suis* [49]. VraSR comprises a membrane-bound sensor histidine kinase (VraS) and a cytoplasmic response regulator (VraR). VraS is characterized by a dimerization and histidine phosphotransfer (DHp) domain, a catalytic and ATP-binding (CA) domain, and a HAMP structural domain, potentially facilitating transmembrane signaling. VraR features a receiver domain for phosphorylation catalysis and a helix-turn-helix (HTH) domain for DNA binding [50]. Mutation of *vraSR* indirectly affects expression of the CPS biosynthesis gene cluster, resulting in a marked reduction in CPS thickness and an enhanced adhesion to BMECs and bEnd.3. As a virulence regulatory factor, *vraSR* also plays a role in the BMECs infection process and affects the expression of TJ proteins (ZO-1, β-catenin, Occludin, and Claudin-5) through an unknown pathway, leading to increased paracellular permeability. This accelerates the spread and translocation of *S. suis*, damages the BBB, and ultimately triggers an inflammatory response [50, 51]. Furthermore, VraSR plays roles in the resistance to macrophage-mediated killing and oxidative stress, and is involved in the development of antibiotic drug resistance. However, elucidation of the downstream regulatory signaling regulation network of VraSR, particularly its mechanism for regulating the CPS biosynthesis gene cluster, requires further investigation [49].

BBB permeability-increasing factors

Fhb

The *fhb* gene, spanning 2,094 bp (strain 05ZYH33), encodes the factor H (FH)-binding protein, and is widespread across various serotypes of *S. suis*, with slight variations in size [52]. Fhb is found in culture supernatants and on the surface of *S. suis*, and features an LPXTG motif at the C-terminus, two proline-rich repeat sequences, and XPZ domains [52, 53]. Crystal structure analysis has shown that the Fhb surface is rich in negatively charged amino acids, likely accounting for its affinity for FH binding [54]. Fhb plays a role in immune evasion, directly binding to complement C3b/C3d via aa 134–244. This interaction protects against killing by polymorphonuclear leukocytes (PMNs) [53]. The N-terminus of Fhb can binds to the glycolipid receptor Gb3, enhancing adhesion capabilities and facilitating *S. suis* penetration through the human cerebral microvascular endothelial cell (hCMEC/D3) barrier. The interaction of Fhb with Gb3 activates the host cell's Rho/ROCK signaling pathway, leading to the phosphorylation of myosin light chain 2 (MLC2) and consequently increasing endothelial cell permeability. Deficiency in genes related to Gb3 can significantly reduce the invasion and damage caused by *S. suis* in animal brain tissues [18].

MRP

MRP in *S. suis* is a 136-kDa protein that is anchored to the cell wall via an LPXTG motif. MRP is also present as a secreted protein into the culture supernatants [55]. While the C-terminus of MRP is highly conserved, its N-terminus varies among different *S. suis* strains [56]. Many studies have identified MRP as a significant virulence marker of *S. suis* serotype 2, and mrp^+ isolates are often suspected to be of high virulence in epidemiological studies. However, given the variability in virulence of mrp^- and mrp^+ *S. suis* isolates across animal models, the current consensus is that MRP may not be a key virulence factor, and its role in virulence remains under debate [57]. The N-terminus of MRP binds to the Aα and Bβ chains of the D fragment of human FG, enhancing resistance to phagocytosis by PMNs in an αXβ2 integrin-dependent manner. This interaction aids *S. suis* in surviving in the bloodstream [58, 59] and disrupts p120-catenin in hCMEC/D3, altering intercellular connections among endothelial cells, promoting adhesion and facilitating the development of meningitis [60]. Additionally, while MRP specifically binds to human FN and FH, and pig IgG, further research is needed to determine the precise roles of these interactions. Mutation of *mrp* diminishes the survival of *S. suis* within macrophages and reduces its virulence in mouse infection models [56].

Phospholipase C

Currently, research on phospholipase C in *S. suis* is limited. Existing studies suggest that *S. suis* releases phospholipase C upon contact with BMECs. The hydrolysis product, diacylglycerol, can prompt host cells to release arachidonic acid, in turn increasing the permeability of BBB vessels [61].

Ssads

Ssads functions as an adenosine synthase in *S. suis*, converting AMP into adenosine. Ssads contains an LPXTG motif and is anchored to the cell wall. The adenosine produced by Ssads binds to the A1 and A2A adenosine receptors on host cells, mediating multiple virulence effects. Ssads enhances survival in the bloodstream by activating the adenosine synthesis-A2A receptor-cAMP pathway, reducing PMN oxidative activity and degranulation, thereby evading PMNs-mediated killing [62]. Ssads also plays a critical role in breaching the BBB by engaging the A1 adenosine receptors on mouse BMECs. This triggers the rearrangement of the actin cytoskeleton and junctional proteins (JPs), facilitating the rapid infection and spread of *S. suis* into the CNS. Deleting *ssads* diminishes the virulence of *S. suis*, hindering its translocation to the brain [63].

Hyl

The *hyl* in *S. suis* encodes hyaluronidase, an enzyme that degrades hyaluronic acid (HA)— a key component of the ECM — into unsaturated disaccharides. This process enhances tissue permeability, facilitating diffusion and dissemination of the pathogen [64]. Recent studies have revealed that, in highly virulent variants of *S. suis*, the normal intact *hyl* is fragmented into four truncated, co-transcribed genes. This altered form of the *hyl* exhibits a loss in hyaluronidase activity but significantly enhanced its complement-binding capability [65]. Wu et al. confirmed that Hyl interacts with angiogenin inhibitor 1 (AI1) within the mouse brain in vitro [66]. The potential of Hyl to prompt new blood vessel formation upon binding with AI1 could contribute to the increased permeability of the BBB.

Cytotoxicity factors

Eno

Enolase (Eno), an enzyme of ~ 52 kDa in size, is found in the culture supernatants and cell wall and cytoplasmic fractions of *S. suis*, with minimal presence in the cell membrane. Eno is devoid of a signal peptide and an LPXTG motif. In vitro, Eno binds to FG and PG in a dose-dependent manner, facilitating adherence to BMECs [67]. In an in vitro BBB model comprising BMECs and astrocytes, Eno significantly induces IL-8 release, promoting the permeability of the BBB and compromising its integrity [68]. Two research groups have shown that *S. suis* Eno binds to the 40S ribosomal protein SA (RPSA) on the host cell surface, activating the p38/ERK-eIF4E signaling pathways and leading to phosphorylation. This process facilitates the translocation and increased expression of the heat shock protein family D member, HSPD1. HSPD1 interacts with β-actin in the cytoplasm, ultimately resulting in the cleavage of pro-caspase-3 and inducing cellular apoptosis [69, 70]. However, recent studies have revealed an additional cytotoxic mechanism of Eno, wherein its binding with RPSA on the cell membrane significantly stimulates vimentin expression, leading to the formation of an RPSA-vimentin complex. This pathological outcome leads to mitochondrial swelling and a significant increases in both mitochondrial and intracellular Ca^{2+} levels, which, as expected, triggers cellular apoptosis [71].

SspA

The subtilisin-like serine protease (SspA) of *S. suis* strain SC19 is 1,962 aa long and has a deduced molecular mass of 187 kDa, localizing to the cell wall and featuring the catalytic triad characteristic of subtilase family proteases. SspA also contains a signal peptide, a C-terminal LPXTG motif, and a hydrophobic structural domain enriched with positively charged residues for cell wall anchoring [72]. SspA specifically induces macrophages to release significant quantities of cytokines, including IL-1β, TNF-α, IL-6, C-X-C motif chemokine ligand 8 (CXCL8), and CCL5 [73]. SspA can cleave and degrade the Aα chain of FG, thereby inhibiting thrombin-mediated FG polymerization. The exogenous addition of SspA significantly reduces the vitality of BMECs, indicating its pronounced cytotoxic effects [74]. Research by Yin et al. found that SspA-1 from *S. suis* 05ZYH33 is secreted through the T4SS encoded by the 89-kb pathogenicity island, contributing to the maintenance of its high-virulence phenotype and the overactivation of inflammatory factors in mouse infection models [75].

SLY

SLY, a cholesterol-dependent cytolysin, exhibits pore-forming activity along with cytotoxic and hemolytic properties. SLY is among the most extensively studied virulence factors in *S. suis*. This 497-aa secretory protein forms transmembrane pores in cholesterol-rich cell membranes, resulting in cell lysis [76]. SLY-mediated damage to BMECs, alongside the substantial release of cytokines, is considered a key factor in *S. suis* invasion of the CNS and pathogenesis [77, 78]. Research has demonstrated that deletion *sly* significantly diminishes the invasiveness of *S. suis* towards astrocytes [79]. Meanwhile, SLY markedly induces the expression of group III secretory phospholipase A2 (PLA2G3) in hCMEC/D3 via a TNF-α-dependent mechanism, leading to cell apoptosis

and compromising the integrity of the BBB [80]. Furthermore, SLY at sublytic concentrations exhibits additional functions beyond its pore-forming toxicity, notably through activation of Rac1 and RhoA, which remodel the actin cytoskeleton of BMECs and promote *S. suis* translocation [81]. The pore-forming ability of SLY also affects platelets, causing aggregation and thrombus formation, which can lead to tissue necrosis and damage [76].

Uncharacterized factors

PgdA

PgdA, a member of the polysaccharide deacetylase family, mediates the N-deacetylation of peptidoglycan in *S. suis*. The *pgdA* gene in *S. suis* spans 1,398 bp and exhibits high homology with its counterparts in *Streptococcus pneumoniae* and *Listeria monocytogenes*. Fittipaldi et al. employed selective capture of transcribed sequences (SCOTS) to identify genes preferentially expressed in *S. suis* during its interaction with BMECs, revealing significant *pgdA* upregulation [82]. Deletion of *pgdA* results in a marked reduction in *S. suis* virulence in both mouse and piglet infection models, leading to its rapid clearance by the host. Furthermore, PgdA is implicated in the resistance to neutrophil-mediated damage and the induced release of key inflammatory factors [83]. However, the mechanism and contribution of N-deacetylation of N-deacetylation of *S. suis* peptidoglycan in the progression of meningitis remain unclear, necessitating further research to fully characterize the function of PgdA.

AdcA and Lmb

The zinc-binding lipoproteins AdcA and Lmb facilitate zinc acquisition in *S. suis*, enhancing survival in zinc-restricted environments. Protein sequence analysis has revealed the high homology of these two genes within the *Streptococcus* genus. The adhesion and invasion capabilities of the Δ*adcA*Δ*lmb* strain towards BMECs are significantly reduced, but the individual deletion of either gene does not significantly affect these capabilities, confirming that *adcA* and *lmb* may have functional complementarity. The double knockout strain exhibits a loss of virulence in mouse infection models, resulting in significantly reduced damage to brain tissue. Additionally, AdcA and Lmb contribute to biofilm formation and influence bacterial morphology [84].

Beyond the factors already discussed, researchers have utilized techniques including SCOTS, quantitative proteomic analysis, and far-western blotting assays to identify numerous *S. suis* genes with preferential or high expression during host cell (or protein) interactions. These genes play roles in envelope modification, interaction with ECM proteins (e.g., laminin and FN), and metabolism. Further investigation and detailed research are essential to elucidate the roles of these candidate genes [58, 82, 85, 86].

Discussion

The impact of *S. suis* meningitis on humans is profound, resulting in lifelong trauma and the potential for recurrent episodes. Notably, East and Southeast Asia are the primary regions reporting *S. suis* meningitis cases. Although antibiotic treatments are available, specific medications and vaccines for *S. suis* infection have not been developed, causing incalculable damage to the global pig farming industry. During the invasion and subsequent destruction of the CNS, *S. suis* induces the expression of various virulence-related factors. These factors have diverse and sometimes overlapping functions, including the promotion of adhesion and invasion, regulation of virulence, enhancement of BBB permeability, induction of host cell death, and evasion of the immune response (Table 1).

The BBB is a selectively permeable physiological barrier, comprising capillary endothelial cells, basement membranes, TJ proteins, pericytes, and astrocytes. Shielding the brain from foreign objects and toxins while allowing nutrient entry, the BBB is crucial for maintaining the internal environment and functionality of the brain. The existence of a singular mechanism or pathway that enables *S. suis* to traverse the BBB remains uncertain.

The relative contributions of pathogenic mechanisms such as toxin-mediated cell death, disruption of TJ proteins, and inflammation-mediated increases in BBB permeability are poorly understood. While the CPS comprise the most important and extensively studied virulence factor of *S. suis*, this dense, effective 'armor' impedes the interactions of many surface proteins with the host, and is thus not covered in this review. Diagnosis of *S. suis* infection using metagenomic next-generation sequencing (mNGS) represents a novel diagnostic approach in clinical medicine, enabling the identification of complicated or rare pathogens. Unlike traditional isolation and pathogen cultivation, mNGS is characterized by its speed, broad detection range, and high specificity. Furthermore, it enables dynamic infection monitoring and the analysis of pathogen resistance genes.

Despite extensive global research on *S. suis* meningitis, identification of the core virulence-related factors remains elusive. The potent immune evasion and cross-tissue translocation abilities of *S. suis* likely involve a

Table 1 Virulence-related factors involved in BBB disruption

Factors	Description	Putative or confirmed function during BBB disruption	Putative or confirmed targets	References
$AtlA_{SS}$	Autolysin protein	Adhesin Modulating chain length	BMECs, FN, FG	[17]
SadP	Streptococcal adhesin P	Adhesin	Gb3, Gb4	[19]
SsPepO	Fibronectin binding protein	Adhesin Increasing the permeability of BBB Immune evasion	BMECs, FN, PG	[23, 24]
SssP1	Fimbria-like protein	Adhesin Promoting the capacity of invasion Promoting cytokine expression	BMECs, Vimentin, Sialic acids	[25, 26]
DltA	Lipoteichoic acid (LTA) D-alanylation	Promoting the capacity of adherence and invasion	BMECs	[27]
NadR	Nicotinamide-nucleotide adenylyl-transferase	Promoting the capacity of adherence and invasion	BMECs	[30]
PrsA	Peptidyl-prolyl isomerase	Cytotoxicity Affecting secretion or translocation of virulence factors Immune evasion	bEnd.3	[31, 32]
rss04	Small RNA	Promoting the capacity of adherence and invasion Regulating transcription and virulence factors expression Regulating biofilm formation Promoting cytokine expression	bEnd.3, CcpA	[34]
SrtA	Sortase A	Promoting the capacity of adherence and invasion Protein sorting and anchoring	BMECs	[37–39]
STK/STP	Serine-threonine kinase/phosphatase	Phosphorylation and Dephosphorylation Promoting the capacity of adherence and invasion Affecting secretion or translocation of virulence factors Modulating chain length and surface structures Involving in cell division Inducing disruption of the JPs Affecting the expression of E3 ubiquitin ligase	BMECs, bEnd.3, GlmM, DivIVA	[43–46, 48, 86]
VraSR	Two-component signal transduction system	Promoting bacterial adherence Increasing the permeability of BBB Downregulating the expression of JPs Regulating the CPS biosynthesis Immune evasion	BMECs, bEnd.3	[49–51]
Fhb	Factor H binding protein	Promoting the capacity of adherence and invasion Immune evasion	BMECs, Gb3, FH	[53, 87]
MRP	Muraminidase released protein	Promoting the capacity of adherence and invasion Increasing the permeability of BBB Destroying the stability of p120-Catenin Immune evasion	BMECs, bEnd.3, FN, FG, FH, IgG	[56, 58–60]
Phospholipase C	Hydrolyzing phosphatidylcholine	Inducing the release of arachidonic acid	BMECs	[61]
Ssads	Adenosine synthase	Adenosine production Increasing the permeability of BBB Inducing cytoskeletal reorganization and JPs redistribution Immune evasion	BMECs, A1 AR, A2A AR	[62, 63]
Hyl	Hyaluronidase	Increasing the permeability of BBB	AI1	[66]

Table 1 (continued)

Factors	Description	Putative or confirmed function during BBB disruption	Putative or confirmed targets	References
Eno	Enolase	Promoting bacterial adherence Increasing the permeability of BBB Promoting cytokine expression Inducing cell apoptosis	BMECs, FN, PG, RPSA	[67–71, 88]
SspA	Serine-associated subtilisin-like protease	Cytotoxicity Promoting cytokine expression	BMECs	[73–75]
Suilysin	Hemolysin	Cytotoxicity Increasing the permeability of BBB Inducing intravascular thrombosis Inducing cytoskeletal reorganization Promoting cytokine expression and degradationInducing cell apoptosis	BMECs, Platelet, Astrocytes, Rac1, RhoA	[76–81]
PgdA	Peptidoglycan N-acetylglucosamine deacetylase	Unknown	BMECs, Peptidoglycan	[82, 83]
AdcA, Lmb	Zn-binding lipoproteins	Promoting the capacity of adherence and invasion	BMECs	[84]

complex interplay among multiple factors. We propose that researchers urgently work to reveal the virulence-related factors preferentially expressed by *S. suis* across various infection stages, exploring highly immunogenic proteins at the whole-genome level and conducting comprehensive mechanistic analyses to decipher the complex underlying regulatory networks.

Abbreviations

AI1	Angiogenin inhibitor 1
AR	Adenosine receptor
bEnd.3	Mouse brain microvascular endothelial cells
BBB	Blood–brain barrier
BMECs	Brain microvascular endothelial cells
CcpA	Catabolite control protein A
CNS	Central nervous system
CPS	Capsular polysaccharide
ECM	Extracellular matrix
FG	Fibrinogen
FH	Factor H
FN	Fibronectin
Gb3	Globotriaosylceramide
Gb4	Globotetraosylceramide
GlmM	Phosphoglucosamine mutase
hCMEC/D3	Human cerebral microvascular endothelial cell D3 clone
JPs	Junctional proteins
LTA	Lipoteichoic acid
mNGS	Metagenomic next-generation sequencing
MRP	Muramidase-released protein
PG	Plasminogen
PMN	Polymorphonuclear leukocyte
RPSA	40S ribosomal protein SA
SCOTS	Selective capture of transcribed sequences
TJ	Tight junction

Authors' contributions

Z.S. designed the review, provided discussion and revised the manuscript; G.Z. conducted references search, wrote the manuscript and drew the figure; L.Y. revised the figure and provided discussion; Y.S., Y.D. and M.F. performed literature search and synthesized information. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no potential conflicts of interest.

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