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Review Article

# How *Streptococcus suis* serotype 2 attempts to avoid attack by host immune defenses

**Xiaojing Xia [a,b,c,1], Wanhai Qin [d,1], Huili Zhu [a], Xin Wang [e], Jinqing Jiang [a,**], Jianhe Hu [a,b,*]**

[a] *College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang, China*
[b] *Postdoctoral Research Base, Henan Institute of Science and Technology, Xinxiang, China*
[c] *Postdoctoral Research Station, Henan Agriculture University, Zhengzhou, China*
[d] *Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam Infection & Immunity, Meibergdreef 9, 1105AZ Amsterdam, Netherlands*
[e] *College of Agriculture and Forestry Science, Linyi University, Linyi, China*

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**Abstract** *Streptococcus suis* (*S. suis*) type 2 (SS2) is an important zoonotic pathogen that causes swine streptococcosis, a widespread infectious disease that occurs in pig production areas worldwide and causes serious economic losses in the pork industry. Hosts recognize pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) to activate both innate and acquired immune responses. However, *S. suis* has evolved multiple mechanisms to escape host defenses. Pathogenic proteins, such as enolase, double-component regulatory systems, factor H-combining proteins and other pathogenic and virulence factors, contribute to immune escape by evading host phagocytosis, reactive oxygen species (ROS), complement-mediated immune destruction, etc. SS2 can prevent neutrophil extracellular trap (NET) formation to avoid being trapped by porcine neutrophils and disintegrate host immunoglobulins via IgA1 hydrolases and IgM proteases. Currently, the pathogenesis of arthritis and meningitis caused by SS2 infection remains unclear, and further studies are necessary to elucidate it. Understanding immune evasion mechanisms after SS2 infection is important for developing high-efficiency vaccines and targeted drugs.


* Corresponding author. College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, No. 90, Hualan Street, Xinxiang, Henan, 453003, China.
** Corresponding author. College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, No. 90, Hualan Street, Xinxiang, Henan, 453003, China.
*E-mail addresses:* jinqingjiang73@163.com, jjq5678@126.com (J. Jiang), jianhehu@yeah.net, xxjianhe@126.com (J. Hu).
[1] Equal contribution to the author.

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## Introduction

*Streptococcus suis* (*S. suis*) is an important pathogen that infects humans and pigs and is distributed worldwide. *S. suis* causes meningitis, pneumonia, endocarditis, septicemia, arthritis and other symptoms in pigs, as well as meningitis, endocarditis, septicemia, permanent deafness, and even death in humans. *S. suis* can be divided into 35 serotypes based on differences in its capsule antigens.[1] In 2005, serotypes 34 and 32 were removed from the *S. suis* taxon, and serotypes 33, 26, 22 and 20 were suggested to be classified as *Streptococcus orisratti*.[2–4] Currently, 29 verified *S. suis* serotypes exist, among which the *S. suis* type 2 (SS2) is the most widespread. The first report of human infection was in 1968, and more than 1600 SS2 infection cases have been reported since then, mainly in Southeast Asia.[5,6] Outbreaks of human SS2 infection occurred in Jiangsu Province in 1998 and Sichuan Province in 2005 in China, causing 14 and 38 deaths, respectively.[7,8] Therefore, SS2 is a serious danger to public health and has aroused widespread concern among researchers and the public.

SS2 is present in the respiratory tracts of normal pigs; however, the SS2 infection incidence in pigs is not directly related to the bacterial carrier rate but to the pathogenicity of the bacterial strains.[9] Differences in SS2 strain virulence are related to virulence factors or the infectious sites.[9] Comparative genomics, transcriptomics, proteomics, selective capture transcriptomics, suppression subtractive hybridization and other methods have been used to identify over 70 virulence factors in *S. suis*. Most are bacterial surface components, surface proteins, extracellular proteins, enzymes, or regulatory factors and are directly or indirectly involved in host cell adherence, in vivo survival, and immune escape.[10] During infection, SS2 can directly affect and evade host immune responses using multiple strategies. Factor H-combining protein (Fhb) can suppress activation of alternative complement pathways by binding to complement factor H.[11] SS2 secretes IgM protease and IgA1 hydrolase, which can directly disintegrate host immunoglobulins. Recent studies have shown that virulent strains of SS2 can inhibit NET formation by host neutrophils.[12] SS2's capsule can suppress sphingosine-dependent immune discrimination/surveillance,[12] while its superoxide dismutase can protect against reactive oxygen species (ROS) produced by host immune cells.[13] Therefore, studying the mechanisms behind the bacteria-host interaction contributes to understanding SS2 pathogenesis.

## Main virulence factors of *S. suis* serotype 2

### Surface proteins

Bacterial surface elements, especially surface proteins, are directly related to the external environment and closely related to pathogen viability and pathogenicity. Surface proteins on gram-positive bacteria such as SS2 anchor on the bacterial cell wall by covalently binding to peptidoglycan after being cut by sorting enzymes. These surface proteins are generally important virulence factors or immunogenic proteins, most of which are essential for pathogens to infect their hosts by contributing to bacterial adhesion to cells, blood invasion, blood clotting, immune evasion, and transmembrane nutrient delivery.[10,14,15]

### Enolase

Enolase is a 45 kDa key glycolytic enzyme located in the cytoplasm and cell wall. Enolase is present in Streptococcus and in various serotypes and has the significant ability to combine with plasminogen. Both its C-terminal lysine residue and N-terminal region are key plasminogen binding sites. Enolase is involved in various pathological processes and autoimmune diseases after a streptococcal infection.[16] Eagles found that enolase on the surface of Streptococcus can combine with plasminogen and fibronectin dose-dependently. *S. suis* $\alpha$-enolase (SsEno) may contribute to streptococcal virulence. Huo et al. used an antibody-blocking assay in a human blood bactericidal model and found that SsEno is a potential antiphagocytic factor of *S. suis*.[17] Our study found that Eno protein expression increased after *S. suis* interacted with porcine brain microvascular endothelial cells (PBMECs) and astrocytes (ACs) and promoted interleukin (IL)-8 release, thus helping *S. suis* to penetrate the blood-brain barrier.[18] Furthermore, the SsEno antibody can inhibit streptococcal invasion and adhesion to brain microvascular endothelial cells.[19] The SsEno protein is also an immunogenic protein. SsEno can elicit multiple IgG subtype antibody responses via subcutaneous immunization in mice.[20] More importantly, SS2 enolase can stimulate humoral immune responses and protect against SS2 and streptococcus type 7 in BALB/c mice.[21]

### Surface antigen one (Sao)

The Sao protein is a 110 kDa protein that anchors to the bacterial surface via its C-terminus. Li discovered Sao while searching for *S. suis* functional proteins in 2006. Sao exists in most *S. suis* serotypes and has the typical characteristics of gram-positive membrane-anchoring proteins such as muramidase-released protein (MRP) and LPXTG membrane-anchoring structures. The Sao protein has 3 variations: Sao-L, Sao-M and Sao-S. Sao-M is the most common, accounting for approximately 80% of these proteins. A high concentration of anti-Sao antibodies was found in the serum of convalescent pigs, indicating that Sao is immunogenic and expressed during *S. suis* infection.[22] Furthermore, ELISA detection based on Sao-M has reportedly been used to successfully monitor *S. suis* infection.[6,25] Since Sao is a cell wall-anchoring protein, Roy et al. assumed that it could invade and adhere to epithelial cells as a first step in colonizing pathogenic hosts, but experimental results using whole blood showed that Sao was not an antiphagocytic factor.[23] Some studies have shown that Sao is a protective antigen but not an important virulence factor of SS2.[24] Immunization with recombinant Sao protein induces humoral reactions in pigs and mice, which weakens clinical symptoms, slows the spread of pathogens, reduces mortality, and produces cross-serotype protection.

## Glutamate dehydrogenase (GDH)

The 45 kDa protein, GDH, resides on the surface of *S. suis*.[26,27] GDH is an important functional protein in bacterial metabolism, playing important roles in bacterial carbon and nitrogen metabolism. The *gdh* gene is highly conserved and has been cloned and expressed. A fusion protein has been purified to prepare a multiantibody that can react with proteins of the same molecular weight from other isolates, demonstrating that this protein is commonly expressed and immunogenic in different stains. The protein can also specifically react with anti-SS2 serum, indicating that it can be used as an antigen for diagnosing *S. suis* infection.[5,28] Moreover, *S. suis* can be divided into avirulent, attenuated and virulent strains using nondenaturing gel electrophoresis on the GDH protein, indicating that GDH is a virulence factor of *S. suis*.

## Evaluation of elongation factor Tu (EF-Tu)

EF-Tu is a conserved multifunctional protein involved in various cellular processes and diseases, including viral replication, apoptosis, cytoskeletal organization, signal transduction, and translation control.[29–31] EF-Tu enables amino acid tRNAs to enter specific parts of the ribosome. Barbier showed that EF-Tu may have a role as a bacterial virulence factor and significantly affects promoting early *Pseudomonas aeruginosa* colonization.[32] Cabeen and Losick reported a sudden demand for EF-Tu associated with Tse6 toxicity, which could destroy the recipient cells' core metabolism.[33] An immunoproteomics study found that EF-Tu from *S. suis* could specifically combine with human fibronectin (FN) and laminin (LN), and EF-Tu-FN or EF-Tu-LN interactions play important roles in bacterial adhesion and colonization.[34,35] In our previous studies, we found that recombinant EF-Tu could be used to identify *S. suis* antisera. Immunizing New Zealand rabbits with EF-Tu elicited significant immune responses and protected the rabbits against lethal SS2 infective doses, indicating that EF-Tu can be used as an effective subunit vaccine candidate antigen for SS2.[6]

## Sortases

Sortases are commonly expressed in gram-positive bacteria and are known as transpeptidases. Their main physiological function is to anchor the bacterial surface proteins to peptidoglycans on the cell wall, depending on the C-terminus sorting signal (CWS) domain, which recognizes LPXTG and other related motifs, thereby covalently binding those motifs containing secreted proteins to the peptidoglycan on the cell wall.[36,37] SS2 sortases include sortases A, B, C, D, E, and F.[38,39] Sortase A can be isolated and detected in most *S. suis* serum and is highly homologous in SS2 and *Streptococcus gordonii*.[40] Vanier found that sortase A knockout SS2 strains had significantly reduced adhesion to and invasion of cerebral microvascular endothelial cells, indicating that the C-terminus CWS contributes to *S. suis* invasion processes.[41] In addition, sortase A knockout partially decreased *S. suis* from adhering to fibronectin and plasma, suggesting that other sortases may also participate in bacterial adhesion. Although sortase A (SrtA) has long been considered an ideal drug target for treating gram-positive pathogens, the effect of sortase A on *S. suis* virulence and pathogenicity is controversial. Several studies used CD1 mice as model animals for toxicity attack tests and showed that the virulence was nearly the same between the sortase A gene knockout and wild-type strains.[41] However, sortase A gene knockout in the Chinese isolate, O5ZYH33, resulted in decreased pathogenicity in pigs compared with the parent strain.[39]

## Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

GAPDH in SS2 is a glycolytic enzyme that phosphorylates glyceraldehyde dehydrogenase to produce glycerol-1,3-diphosphate (DPGN), which is mainly found in bacterial cytoplasm.[42] Madureira et al. and Zhu et al. showed that the GAPDH protein is expressed on the surfaces of *Streptococcus pyogenes*, *Erysipelothrix rhusiopathiae* and other species.[43,44] Except the nonvirulent strain, SS2T15, 36 streptococcal strains, including 7 strains of *Streptococcus equi*, 2 strains of SS1, 24 strains of SS2, 2 strains of SS9 and 1 strain of SS7, have the *gapdh* gene. Brassard showed that *S. pyogenes* GAPDH had more than 95% amino acid sequence homogeneity with other streptococcal GAPDHs. Sichuan isolate ZY05719 and Jiangsu isolate HA9801 showed more than a 99.8% nucleotide sequence homogeneity.[47] GAPDH has many functions, including effects on membrane fusion, nuclear export, microtubule assembly, and protein phosphotransferase/kinase reactions. Eukaryotic GAPDH has been reported to regulate cell attachment and play a role in enhancing the cytoskeletal structure.[43] Jobin et al. studied SS2's plasminogen binding capacity and found that the GAPDH receptor could bind to and activate plasminogen, thus promoting fibronectin degradation.[45] Additionally, GAPDH deficiency in streptococcus S735 decreases bacterial binding to plasminogen by 25%.[45] Furthermore, pretreating pig tracheal ring cells with recombinant GAPDH protein increases streptococcal adhesion to cells, indicating that the streptococcal GAPDH protein is associated with bacterial adhesion to host cells.[46,47]

## Factor H-combining protein (Fhb)

Several studies have demonstrated that bacterial cell surface proteins play important roles in regulating the host's complement system, mainly by manipulating complement regulation factor H (FH). In *S. suis*, factor H-combining protein (Fhb) can specifically bind with the negative regulator H factor of the alternative pathway, inhibiting alternative pathway activation, thus helping *S. suis* to avoid complement-mediated phagocytosis and allowing its survival in the blood.[11] Li et al. found that Fhb (domain II) can also bind to C3b/C3d via electrostatic and hydrophobic interactions to form a large immune complex with factor H (FH), thereby enhancing bacterial antiphagocytosis by polymorphonuclear leukocytes.[48] The H-binding protein receptor binding domain (Fhb RBD) binds to the Gb2 receptor mainly via hydrogen and hydrophobic interactions.[49] Fhb is also a ligand for host cell surface proteins and

enhances the ability of *S. suis* to adhere to host cells by recognizing oligosaccharides.[50] In addition, Kong et al. confirmed for the first time that Fhb contributes to *S. suis* penetrating the human blood-brain barrier.[51] Via immunoproteomic analysis, Fhb was shown to have good immunogenicity and can be used as a candidate vaccine molecule.[52]

## Metabolic and regulatory virulence elements

Pathogens produce relevant signal-regulating molecules to cope with stress from the bacterial population and the external environment while growing and infecting the host. Through these signal-regulating molecules, pathogens can quickly adjust their structure and physiological behavior to survive and proliferate in new environments. These signal-regulating molecules include energy metabolism regulators, stress-related regulators, bacterial quorum-sensing signaling molecules, bacterial units, binary regulatory systems and bacterial virulence posttranslational modification elements.[53] Although these factors do not directly express virulence proteins, most can regulate bacterial survival in the host, environmental tolerance and expression of virulence factors. Therefore, these molecules are important virulence-related bacterial factors and are also important targets for vaccine and novel antibacterial drug research.

## Catabolite control protein A (CcpA)

A set of molecular mechanisms closely regulates the intake and energy metabolism of sugars in bacteria. For example, when a bacterium ingests and metabolizes glucose, other energy sources are inhibited. This phenomenon is called carbohydrate activation and inhibition and is very important for bacterial growth. CcpA is a key factor in inhibiting carbohydrates in a multifunctional environment or in excessive sugars by regulating related gene expression at the transcriptional level. This factor is an important bacterial virulence regulator in other streptococci.[54] Lang et al. found that the metabolic products of CcpA mutant SS2 strains differed from those of the wild strains. Furthermore, the bacterial virulence of the CcpA-deficient strains was significantly decreased,[55] possibly due to downregulated bacteriolysin, enolase, bacterial adhesion and invasion-related factors, suggesting that CcpA is also an important virulence regulator.[13] Xiao et al. confirmed for the first time that *S. suis* meningitis strain CcpA can act as a transcription factor and regulate sRNA as an sRNA-binding protein. For example, CcpA regulates capsule expression through rSSO4, promoting bacterial adherence and invading mouse brain microvascular endothelial cells, thus causing meningitis in mice.[56]

## S-ribosylhomocysteinase (LuxS)

To effectively resist the host immune defense system, pathogenic bacteria are pathogenic to the host only when they multiply at the infection site and reach a certain population density. Quorum-sensing controls various biological processes in bacterial populations, such as fluorescent protein production, biofilm formation, and virulence factor expression.[57] The key factors for quorum sensing in both intraspecific and interspecific bacterial colonies are the autotrophic peptides, AI-1 (Autoinducer-1) and AI-2, and LuxS (S-ribosylhomocysteinase), the key rate-limiting enzyme for AI-2 synthesis. Wang and Cao et al. reported the effects of a LuxS SS2 knockout on inducing quorum sensing and bacterial virulence, respectively. Their results showed that LuxS is an essential factor for AI-2 release in *S. suis*. LuxS knockout increases bacterial capsule thickness and H2O2 stress resistance but decreases adhesion, hemolytic activity and biofilm formation abilities on epithelial cells and reduces pathogenicity in piglets and zebrafish. A transcriptional analysis found that related virulence genes, such as GDH, CPS, GAPDH, Sly, FBPS and EF, were all downregulated to varying degrees.[58,59]

## Arginine deiminase system (ADS)

The ADS is conserved in different bacterial species. This system can catalyze the production of carbon dioxide, ammonia and ornithine from arginine, which produces 1 mol of ATP per 1 mol of arginine. However, activating the ADS can be affected by carbon sources, oxidative stress, temperature and changes of the arginine substrate directly or indirectly. ADS has been studied in many bacteria. ADS in bacteria such as *Listeria monocytogenes* can affect immune evasion and environmental stress responses both in vitro and in vivo, and initiating ADS metabolism in the host cells' phagolysosomes can prolong bacterial survival in cells.[60] The *S. suis* ADS contains an arcABC gene cluster, the transporter, arcD, and the system regulator, arcR.[61] Gruening et al. studied the SS2 arcABC gene cluster and searched for the promoter recognition region of its regulated genes. Gruening's group also found that the arcABC function was inactivated after deleting arcA, resulting in significantly decreased cell viability under acidic stress.[62] Marcus Fulde et al. showed that mutation of the arginine transporter, arcD, in SS2 significantly reduced arginine intake and the ability to neutralize acid and decreased the epithelial intracellular survival rate, suggesting that arcD may play an important role in adapting to the environment and regulating virulence in *S. suis*.[63] arcR is the only regulator of the arcABC gene cluster, and it activates the arcABC promoter by binding to the upstream transcription start site of the arcABC cluster genes.[61] However, no effect of arcR has been reported on bacterial pathogenicity.

## Two-component signal transduction systems (TCSTs)

Pathogenic bacteria have many signal transduction pathways, and two-component signal transduction systems (TCSTs) are the most common. The classic TCSTs consist of a histidine protein kinase located in the cell membrane that recognizes and transmits external environmental signals as well as a response element in the cytoplasm.[53] Five dual regulatory elements have been reported in SS2 TCSTs. Wang et al. found that the dual regulator SalK/SalR was involved in regulating the secretion of antibiotic suicin antimicrobial peptides.[64] Li et al. reported that the ciaK/ciaR dual

regulator is involved in regulating SS2 strain virulence. Knocking out this bacterial regulator weakens the bacteria's growth response, reduceds the bacteria's capacity to resist antioxidant stress, and decreases bacterial growth and viability in acidic environments.[65] Han et al. discovered that the SS2 Ihk/Irr binary system was involved in bacterial adhesion, acid resistance and antioxidative stress. Further, a deletion mutant was less virulent and pathogenic, the expression of metabolically relevant proteins was downregulated, and superoxide dismutase (SOD) was suppressed.[66] Wang and Xu et al. reported the two-component systems, VirR/VirS and NisK/nISR, respectively. A mutant strain containing a deletion of the corresponding regulatory subgenes showed weakened virulence that was easily cleared by the host, reduced adhesion and invasiveness, and a weakened ability to resist oxidative stress. The bacteria's morphological characteristics were changed, but no underlying mechanisms were analyzed.[67,68]

### Secretory virulence factor

The secretory virulence factor of *S. suis* is secreted independent of an interaction between the bacteria and the host cell but remain involved in enhancing bacterial infection, invasion and pathogenicity. *S. suis* is reported to secrete nearly 20 biologically functional protein types. Of those secretory virulence factors, suilysin (Sly) has been the most thoroughly studied. Extracellular factor (EF) is another highly studied secretory virulence factor of *S. suis*. Other *S. suis*-secreted proteins include the endonucleases, SsnA, IgG-binding proteins, phospholipase C and bacteriocin-like inhibition factors.[10,14,69]

### S. suis-*secreted nuclease A (SsnA)*

Bacteria interact with neutrophils to induce PMN cell degranulation to form NETs, thereby promoting the release of nucleic acid substances and granular proteins, which can form a localized high concentration of antibacterial molecules and capture and kill various pathogens in a limited area. *S. suis*-secreted nuclease A (SsnA) and other nuclease homologs, such as EndAsuis, are endonucleases that are secreted by the bacteria while *S. suis* interacts with the host cell. SsnA can hydrolyze and destroy NET particle formation by degrading NET's important component DNA, but its activity depends on a calcium or magnesium ionic environment.[70] SsnA is distributed in SS1-SS9 and can be detected in several host organs such as brain tissue and joint cavities after infection. Moreover, SsnA is an immunogenic candidate, and its immunization together with an aluminum gel adjuvant mediates protection against the same *S. suis* infection type in mouse models.[71]

### S. suis *IgG-binding protein (SSPG)*

SS2-secreted IgG-binding proteins (SSPGs) bind nonspecifically to the Fc-end of most mammalian IgG and IgA molecules and influence antibody activity. Some scholars consider that SSPGs likely act as pathogenic factors and play important roles in streptococcal infections.[72] Experimental studies have also shown that the SSPG in group G streptococci cause bacterial aggregation, and this aggregation destroys activation of the complement pathway and complement protein-mediated opsonization.[73,74] SSPG was listed as an important candidate in the latest review of *S. suis* virulence-related factors. Liu et al. found that SSPG induces a strong humoral immune response and provides better immune protection in animals.[75] However, reports concerning the biological function of SSPG remain limited, and SSPG's interaction with the host requires further study.

Fig. 1 summarizes the identified histories of most *S. suis* virulence factors.

## Research progress on the immune evasion/ escape mechanisms of pathogenic bacteria

The innate immune system includes highly integrated and networked cells and effector molecules that continuously help the host resist microbial infections in complex environments. This system includes the natural barrier of the skin and mucosal epithelium, ecological competition and antibacterial products produced by the resident flora, as well as soluble antibacterial molecules such as antimicrobial peptides, complement proteins and reactive oxygen species. The innate immune system recognizes the microbes and produces inflammatory responses after being activated by these microbes. Neutrophils and macrophages migrate to the bacterial infection sites to phagocytize and clear pathogenic microorganisms. However, pathogenic bacteria have evolved strategies to resist the innate defense mechanisms and promote spreading into the circulation and deeper into host tissues and organs. Pathogens can escape phagocytosis and elimination by neutrophils and macrophages. In conclusion, pathogenic bacteria use different mechanisms to evade the innate immune system and clearance from the host.

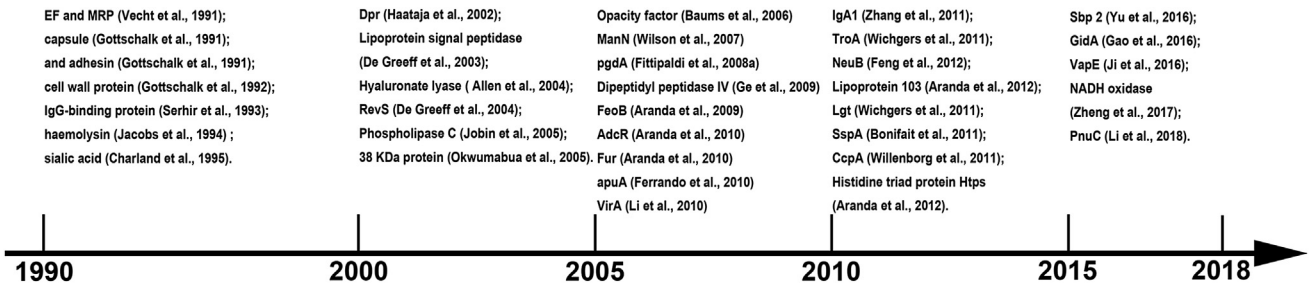


**Figure 1.** Timeline summary of the history of *S. suis* virulence factors.

## Escape from neutrophil extracellular traps

Neutrophils are important to the host's innate immune system. Neutrophils are the first cells recruited to infection sites to clear pathogenic microorganisms by phagocytosis and degranulation. Brinkmann discovered that PMNs can kill pathogens by a third mechanism mediated by NET.[76] NETs are composed of extracellular fibrous structures formed by a DNA skeleton, antimicrobial peptides, histones and neutrophil granular proteins and are produced by activated neutrophils.[77] NET is considered broad-spectrum and highly efficient, causing little tissue damage when killing pathogenic microorganisms.[78,79] However, pathogens have evolved several mechanisms to escape/evade the bactericidal effects of NET. Beiter found that *Streptococcus pneumoniae* was not killed after being trapped in NET. This anti-NET phenomena was largely mediated by the endolytic endoenzyme, End A, on the surface of *Streptococcus pneumoniae*, which degrades the DNA skeleton of the NET, allowing the bacteria to escape the NET.[80] Zhao et al. reported that SS2 induced NET in a mouse infection model, but nuclease treatment destroyed the NET and increased the bacterial load and TNF-a content, indicating that NET play a vital role in eliminating SS2 and regulating inflammatory responses.[81] De Buhr et al. reported that virulent SS2 strains only induce a low degree of NET in porcine neutrophils or even inhibit NET formation.[82] This may be one reason why virulent SS2 strains infect humans and pigs causing extensive bacteremia. Biofilm formation mediates SS2 escape/evasion from phagocytosis by phagocytic cells during infection but has a limited effect on NET-mediated clearance. However, biofilm formation inhibits NET formation, thus enabling SS2 to proliferate in the blood and produce extensive bacteremia.[83]

## Escape from complement deposition and activation

The complement system is the first line of defense against microbial invasion. The complement delineates innate and adaptive immunity and is a special focus of pathogenic immune escape strategies.[84] Although this enzymatic cascade of finely regulated enzymes, protein complexes, and receptors can rapidly identify and eliminate foreign objects, it also provides many intervention sites that can disrupt this balanced protein interaction network. Common strategies that pathogens use to escape the complement system include expressing proteases that cleave complement factors, producing complement inhibitory factors, and recruiting or simulating complement regulatory factors.[85] In the $C3^{-/-}$ experimental mouse model, the complement system was demonstrated to be crucial in preventing morbidity and mortality caused by meningitis.[86] This finding indicates that escaping complement activation may be necessary for *S. suis* to survive in the host.[87] Two H-binding proteins (FHB and SUS0186) found in *S. suis* are homologous to *Streptococcus pneumoniae* pneumococcal surface protein (PspC).[11,88] Seele et al. demonstrated IdeSsuis as a new complement escape factor.[87] Immunoglobulin M-degrading enzyme of *S. suis* (IdeSsuis) specifically cleaves porcine IgM in vivo to allow escape from opsonization and complement-mediated killing in the blood flow, which is critical to bacterial adaption to the immune response and bacterial survival at the early phase of infection.[87] The *SS2 05ZYH33* strain has 4 open reading frames that encode the proteins, NeuD, NeuC, NeuB and NeuA, which are involved in metabolizing sialic acid. Destruction of NeuB leads to capsule membrane damage, which is characteristic of decreased sialic acid content, capsule thinning, increased pH sensitivity, and increased adhesion and invasion in Hep-2 cells. Deleting NeuB leads to greater interleukin-8 secretion, increased neutrophilic chemotaxis and activation, and enhanced $CD8^{+}$ and $CD4^{+}$ T cell activation. Therefore, bacterial sialic acid can decrease neutrophilic recruitment by chemokines and dampen the host immune response to SS2.[89] NeuC deletion in SS2 affects capsule synthesis, rendering the bacteria more susceptible to phagocytosis.[90] How the capsular sialic acid and sialic acid modifications affect SS2 relative to host immune response evasion remains unclear, and further research is necessary.

## Invasion of the central nervous system (CNS)

Two major entrances to the CNS (central nervous system) are the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF)-barrier (BCSFB). The predominant cells in the BBB are the brain microvascular cells.[91] SS2 invasion and intrusion into porcine brain microvascular cells depends on adhesins and invasins and cell wall components, such as lipoteichoic acid (LTA), as well as host cell extracellular matrix proteins such as fibronectin. Cell wall-anchoring proteins and enolase may play the same roles as adhesins and invasins via fibronectin adhesion.[91] We found that Eno protein expression in *S. suis* interacts with porcine brain microvascular endothelial cells (PBMEC) and astrocytes (AC), promoting the release of cytokines, such as interleukin IL-8, and thus helping *S. suis* penetrate the BBB.[21] In addition, Eno can induce PBMEC apoptosis, which plays an important role in destroying the blood-brain barrier during SS2 infection both in vitro and in mouse experiments. Eno also promotes SS2 adhesion to the PBMEC (unpublished observations) and increases the permeability of the blood-brain barrier by inhibiting expression of the intercellular tight connexin, occludin. Hemolysin is another factor that destroys the blood-brain barrier via cytotoxic effects. The invasion and penetration of *S. suis* through the blood-cerebrospinal fluid barrier has also been demonstrated. *S. suis* can adhere to choroid plexus epithelial cells (CPEC), indicating that such adhesion is necessary to directly enter the extracellular matrix. In addition, *S. suis* can induce CPEC necrosis and may play a role in apoptosis.[92] Capsular polysaccharides can significantly impede bacterial invasion of CPEC, suggesting that this process requires cytoderm components and surface proteins. However, the functions of these adhesins and invasins remain largely unknown. *S. suis* can also affect the blood-cerebrospinal fluid barrier, further promoting bacterial and leukocyte transport function and integrity. Other soluble factors, such as hemolysin, may also be involved in influencing the barrier function of the blood-cerebrospinal fluid.[93]

## Resistance to killing via reactive oxygen species

Phagocytosis of bacteria can induce oxidation reactions and oxygen consumption to produce ROS, which kills bacteria. This process occurs inside the cell, first via activating and operating NOX complexes to produce superoxide ($O^{2-}$) or $H_2O_2$. Both these substances can dehydrate enzymes' iron-sulfur clusters and release free iron ions (iron reaction). The iron ions can combine with $H_2O_2$ to produce hydroxyl radicals that kill bacteria.[94] Second, $O^{2-}$ and $H_2O_2$ are converted by MPO to produce HOCl, and all HOCl produced by ROS is mainly bactericidal. Gram-positive bacteria can encode a bacterial SOD,[95,96] which can accelerate the conversion of $O^{2-}$ and $H_2O_2$ and can convert $H_2O_2$ into smaller molecules. Gram-negative bacteria can use catalase to convert $H_2O_2$ into $O_2$ and $H_2O$. However, all *S. suis* are catalase-negative, so other systems must be employed to hydrolyze $H_2O_2$.[97] $H_2O_2$ degradation can reduce HOCl production by MPO, thus promoting bacterial resistance to ROS-mediated killing. *Streptococcus pyogenes* produces iron-binding proteins, and *Staphylococcus aureus* produces NO, both of which can inhibit the iron reaction. Certain gram-positive bacteria can enhance the antioxidant capacity to resist oxidation-mediated killing, and *Streptococcus agalactiae* produces carotenoids that hinder phagocytosis and killing.[98] Thus, bacteria resist being killed by host ROS largely by reducing the host's ROS production and increasing bacterial antioxidant production.

## N-deacetylation of peptidoglycan

Peptidoglycan is the major cell wall component of gram-positive bacteria, ensuring cell wall rigidity and stability. Peptidoglycan is a comparatively conserved polysaccharide chain that helps the host to recognize the minimal peptidoglycan fragments released by lysozyme hydrolysis or the peptides released during bacterial cell wall turnover via the NOD1 and NOD2 proteins.[99,100] However, gram-positive bacteria, including *S. suis*, have acquired various effective mechanisms to escape the host immune response by mediating peptidoglycan. SS2 pgdA expression is increased when SS2 interacts with PBMEC or neutrophils in vitro and during in vivo infection processes, indicating that peptidoglycans may undergo N-deacetylation during infection.[101] Virulence of the SS2 PGDA-deficient strain was reduced both in murine and swine models. Interleukin-6 and interferon-γ release was markedly impaired in PGDA-deletion mutant-infected animals compared with animals infected with the wild-type strain. This decrease may be attributed to the enhanced sensitivity of the PGDA-deletion mutant to neutrophils. Interestingly, neither the PGDA-deletion mutants nor the wild-type strain differed in antipolymyxin B, maltogenic protein II, colistin, human neutrophil peptides (HNP-1, HNP-2) or other cationic antimicrobial peptides, suggesting that PGDA may improve N-deacetylation of bacterial peptidoglycans and enhance the antineutrophilic lysozyme's effect. These results show that the PGDA transcription level is enhanced in vivo, resulting in increased N-deacetylation of peptidoglycan and greatly contributing to SS2 virulence.[102]

SS2 appears to have developed several strategies to escape the host immune system, and some of these strategies are summarized in Fig. 2.[10,103,104]

## Summary and prospect

The *S. suis* immune evasion process is highly complex. This process likely occurs due to the synergistic result of several mechanisms. Many genes that contribute to SS2 immune invasion have been identified by taking advantage of the newly discovered SS2 complete genome sequence, functional genomic studies and advanced experimental techniques, thus providing important information for finding effective strategies to kill SS2 and develop new vaccines. Seeking therapeutic targets in host cells against SS2 from a host perspective has become a new research area. The latest study also found that SS2 can form biofilms to escape phagocytosis by phagocytic cells during infection and inhibit host NET formation, although biofilms cannot protect SS2 against the direct killing and clearance mediated by NET. This finding explains why SS2 survives and proliferates in the blood, causing extensive bacteremia. In murine models, the host can remove bacteria in the biofilm state via NET; eventually, the surviving mice can become immune to SS2 reinfection. This finding suggests that new perspectives and new strategies targeted at both host and bacterial aspects are needed to counter SS2 evasion mechanisms to effectively control and prevent *S. suis* diseases.

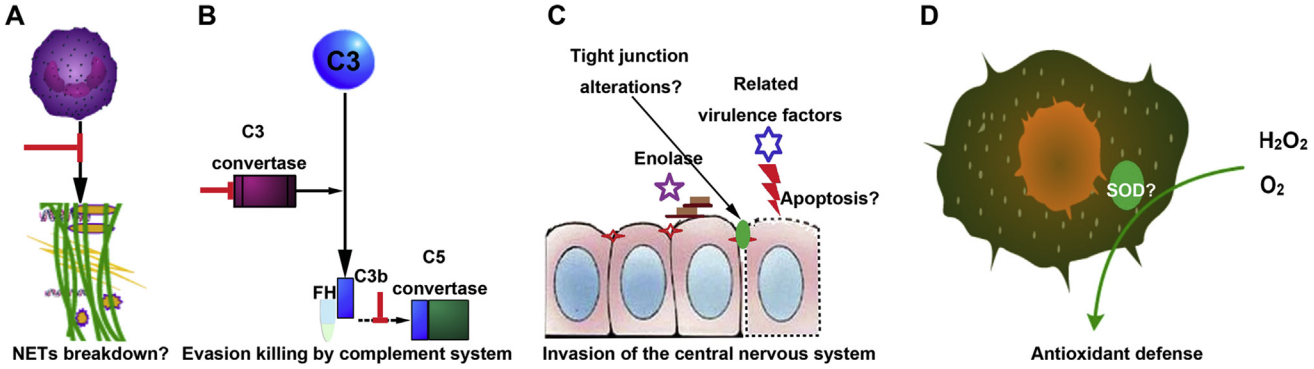


**Figure 2. Evasion of host's innate immune response.** (A) SS2 breaks down NET; (B) SS2 evades being killed by the complement system; (C) SS2 invades the central nervous system; (D) Antioxidant defense from SS2.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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## References

1. Staats JJ, Feder I, Okwumabua O, Chengappa MM. *Streptococcus suis*: past and present. *Vet Res Commun* 1997;**21**:381—407.
2. Hill JE, Gottschalk M, Brousseau R, Harel J, Hemmingsen SM, Goh SH. Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. *Vet Microbiol* 2005;**107**:63—9.
3. Nomoto R, Maruyama F, Ishida S, Tohya M, Sekizaki T, Osawa R. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22 and 26: *Streptococcus parasuis* sp. nov. *Int J Syst Evol Microbiol* 2015;**65**:438—43.
4. Tien LH, Nishibori T, Nishitani Y, Nomoto R, Osawa R. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22, 26, and 33 based on DNA-DNA homology and sodA and recN phylogenies. *Vet Microbiol* 2013;**162**:842—9.
5. Xia X, Wang L, Shen Z, Qin W, Hu J, Jiang S, et al. Development of an Indirect Dot-PPA-ELISA using glutamate dehydrogenase as a diagnostic antigen for the rapid and specific detection of *Streptococcus suis* and its application to clinical specimens. *Anton Leeuw Int J G* 2017;**110**:585—92.
6. Xia X, Zhang H, Cheng L, Zhang S, Wang L, Li S, et al. Development of PPA-ELISA for diagnosing *Streptococcus suis* infection using recombinant Sao-M protein as diagnostic antigen. *Kafkas Univ Vet Fak* 2017;**23**:989—96.
7. Tang J, Wang C, Feng Y, Yang W, Song H, Chen Z, et al. Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. *PLoS Med* 2006;**3**:e151.
8. Yao H, Chen G, Lu C. Identification of isolates of swine Streptococcus in Jiangsu Province during 1998. *J Nanjing Agric Univ* 1999;**22**:70—3.
9. Gottschalk M, Segura M, Xu J. *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. *Anim Health Res Rev* 2007;**8**:29—45.
10. 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**:259—79.
11. Pian Y, Gan S, Wang S, Guo J, Wang P, Zheng Y, et al. Fhb, a novel factor H-binding surface protein, contributes to the antiphagocytic ability and virulence of *Streptococcus suis*. *Infect Immun* 2012;**80**:2402—13.
12. Houde M, Gottschalk M, Gagnon F, Van Calsteren MR, Segura M. *Streptococcus suis* capsular polysaccharide inhibits phagocytosis through destabilization of lipid microdomains and prevents lactosylceramide-dependent recognition. *Infect Immun* 2012;**80**:506—17.
13. Tang Y, Zhang X, Wu W, Lu Z, Fang W. Inactivation of the sodA gene of *Streptococcus suis* type 2 encoding superoxide dismutase leads to reduced virulence to mice. *Vet Microbiol* 2012;**158**:360—6.
14. Baums CG, Valentin-Weigand P. Surface-associated and secreted factors of *Streptococcus suis* in epidemiology, pathogenesis and vaccine development. *Anim Health Res Rev* 2009;**10**:65—83.
15. Schneewind O, Missiakas DM. Protein secretion and surface display in Gram-positive bacteria. *Philos Trans R Soc Lond B Biol Sci* 2012;**367**:1123—39.
16. Pancholi V, Fischetti VA. Alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. *J Biol Chem* 1998;**273**:14503—15.
17. 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—9.
18. Sun Y, Li N, Zhang J, Liu H, Liu J, Xia X, et al. Enolase of *Streptococcus suis* serotype 2 enhances blood-brain barrier permeability by inducing IL-8 release. *Inflammation* 2016;**39**:718—26.
19. Esgleas M, Li Y, Hancock MA, Harel J, Dubreuil JD, Gottschalk M. Isolation and characterization of alpha-enolase, a novel fibronectin-binding protein from *Streptococcus suis*. *Microbiology* 2008;**154**:2668—79.
20. Esgleas M, Dominguez-Punaro ML, Li Y, Harel J, Dubreuil JD, Gottschalk M. Immunization with SsEno fails to protect mice against challenge with *Streptococcus suis* serotype 2. *Fems Microbiol Lett* 2009;**294**:82—8.
21. Zhang A, Chen B, Mu X, Li R, Zheng P, Zhao Y, et al. Identification and characterization of a novel protective antigen, Enolase of *Streptococcus suis* serotype 2. *Vaccine* 2009;**27**:1348—53.
22. Li Y, Martinez G, Gottschalk M, Lacouture S, Willson P, Dubreuil JD, et al. Identification of a surface protein of *Streptococcus suis* and evaluation of its immunogenic and protective capacity in pigs. *Infect Immun* 2006;**74**:305—12.
23. Roy D, Fittipaldi N, Dumesnil A, Lacouture S, Gottschalk M. The protective protein Sao (surface antigen one) is not a critical virulence factor for *Streptococcus suis* serotype 2. *Microb Pathog* 2014;**67—68**:31—5.
24. Lecours MP, Segura M, Lachance C, Mussa T, Surprenant C, Montoya M, et al. Characterization of porcine dendritic cell response to *Streptococcus suis*. *Vet Res* 2011;**42**:72.
25. Feng Y, Zheng F, Pan X, Sun W, Wang C, Dong Y, et al. Existence and characterization of allelic variants of Sao, a newly identified surface protein from *Streptococcus suis*. *Fems Microbiol Lett* 2007;**275**:80—8.
26. Brunhuber NM, Blanchard JS. The biochemistry and enzymology of amino acid dehydrogenases. *Crit Rev Biochem Mol* 1994;**29**:415—67.
27. Okwumabua O, Persaud JS, Reddy PG. Cloning and characterization of the gene encoding the glutamate dehydrogenase of *Streptococcus suis* serotype 2. *Clin Diagn Lab Immunol* 2001;**8**:251—7.
28. Hammer BA, Johnson EA. Purification, properties, and metabolic roles of $NAD^+$-glutamate dehydrogenase in Clostridium botulinum 113B. *Arch Microbiol* 1988;**150**:460—4.
29. Jonak J. Bacterial elongation factors EF-Tu, their mutants, chimeric forms, and domains: isolation and purification. *J Chromatogr B Anal Technol Biomed Life Sci* 2007;**849**:141—53.
30. Mayer F. Cytoskeletons in prokaryotes. *Cell Biol Int* 2003;**27**:429—38.
31. Zhang X, Chu Y, Zhao P, Gao P, He Y, Wang N, et al. Characterization of elongation factor tu of *Mycoplasma ovipneumoniae*. *Pak Vet J* 2013;**33**:515—9.
32. Barbier M, Owings JP, Martinez-Ramos I, Heath Damron F, Gomila R, Blazquez J, et al. Lysine trimethylation of EF-Tu mimics platelet-activating factor to initiate *Pseudomonas aeruginosa* pneumonia. *mBio* 2013;**4**:e00207—13.

33. Cabeen MT, Losick R. Bacterial backstabbing: EF-Tu, brute? *Cell* 2015;**163**:537—9.
34. Li Q, Liu H, Du D, Yu Y, Ma C, Jiao F, et al. Identification of novel laminin- and fibronectin-binding proteins by far-western blot: capturing the adhesins of *Streptococcus suis* type 2. *Front Cell Infect Microbiol* 2015;**5**:82.
35. Zhang W, Lu CP. Immunoproteomics of extracellular proteins of Chinese virulent strains of *Streptococcus suis* type 2. *Proteomics* 2007;**7**:4468—76.
36. Ilangovan U, Ton-That H, Iwahara J, Schneewind O, Clubb RT. Structure of sortase, the transpeptidase that anchors proteins to the cell wall of *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 2001;**98**:6056—61.
37. Mazmanian SK, Liu G, Ton-That H, Schneewind O. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science* 1999;**285**:760—3.
38. Osaki M, Takamatsu D, Shimoji Y, Sekizaki T. Characterization of *Streptococcus suis* genes encoding proteins homologous to sortase of gram-positive bacteria. *J Bacteriol* 2002;**184**:971—82.
39. Wang C, Li M, Feng Y, Zheng F, Dong Y, Pan X, et al. The involvement of sortase A in high virulence of STSS-causing *Streptococcus suis* serotype 2. *Arch Microbiol* 2009;**191**:23—33.
40. Dramsi S, Trieu-Cuot P, Bierne H. Sorting sortases: a nomenclature proposal for the various sortases of Gram-positive bacteria. *Res Microbiol* 2005;**156**:289—97.
41. Vanier G, Sekizaki T, Dominguez-Punaro MC, Esgleas M, Osaki M, Takamatsu D, et al. Disruption of srtA gene in *Streptococcus suis* results in decreased interactions with endothelial cells and extracellular matrix proteins. *Vet Microbiol* 2008;**127**:417—24.
42. Sheokand N, Malhotra H, Kumar S, Tillu VA, Chauhan AS, Raje CI, et al. Moonlighting cell-surface GAPDH recruits apotransferrin to effect iron egress from mammalian cells. *J Cell Sci* 2014;**127**:4279—91.
43. Madureira P, Baptista M, Vieira M, Magalhaes V, Camelo A, Oliveira L, et al. Streptococcus agalactiae GAPDH is a virulence-associated immunomodulatory protein. *J Immunol* 2007;**178**:1379—87.
44. Zhu W, Zhang Q, Li J, Wei Y, Cai C, Liu L, et al. Glyceraldehyde-3-phosphate dehydrogenase acts as an adhesin in Erysipelothrix rhusiopathiae adhesion to porcine endothelial cells and as a receptor in recruitment of host fibronectin and plasminogen. *Vet Res* 2017;**48**:16.
45. Jobin MC, Brassard J, Quessy S, Gottschalk M, Grenier D. Acquisition of host plasmin activity by the Swine pathogen *Streptococcus suis* serotype 2. *Infect Immun* 2004;**72**:606—10.
46. 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**:87—94.
47. 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**:207—9.
48. Li X, Liu P, Gan S, Zhang C, Zheng Y, Jiang Y, et al. Mechanisms of host-pathogen protein complex formation and bacterial immune evasion of *Streptococcus suis* protein Fhb. *J Biol Chem* 2016;**291**:17122—32.
49. Zhang C, Hao H, Yu Y, Kong D, Chen S, Jiang H, et al. Structural basis of the interaction between the meningitis pathogen *Streptococcus suis* adhesin Fhb and its human receptor. *FEBS Lett* 2016;**590**:1384—92.
50. Kouki A, Haataja S, Loimaranta V, Pulliainen AT, Nilsson UJ, Finne J. Identification of a novel streptococcal adhesin P (SadP) protein recognizing galactosyl-alpha1-4-galactose-containing glycoconjugates: convergent evolution of bacterial pathogens to binding of the same host receptor. *J Biol Chem* 2011;**286**:38854—64.
51. Kong D, Chen Z, Wang J, Lv Q, Jiang H, Zheng Y, et al. Interaction of factor H-binding protein of *Streptococcus suis* with globotriaosylceramide promotes the development of meningitis. *Virulence* 2017;**8**:1290—302.
52. Geng H, Zhu L, Yuan Y, Zhang W, Li W, Wang J, et al. Identification and characterization of novel immunogenic proteins of *Streptococcus suis* serotype 2. *J Proteome Res* 2008;**7**:4132—42.
53. Fulde M, Valentin-Weigand P. Epidemiology and pathogenicity of zoonotic streptococci. In: Chhatwal G, editor. *Host-pathogen interactions in streptococcal diseases*. Berlin, Heidelberg: Springer; 2012. p. 49—81.
54. Willenborg J, Fulde M, de Greeff A, Rohde M, Smith HE, Valentin-Weigand P, et al. Role of glucose and CcpA in capsule expression and virulence of *Streptococcus suis*. *Microbiology* 2011;**157**:1823—33.
55. Lang X, Wan Z, Pan Y, Wang X, Wang X, Bu Z, et al. Investigation into the role of catabolite control protein A in the metabolic regulation of *Streptococcus suis* serotype 2 using gene expression profile analysis. *Exp Ther Med* 2015;**10**:127—32.
56. Xiao G, Tang H, Zhang S, Ren H, Dai J, Lai L, et al. *Streptococcus suis* small RNA rss04 contributes to the induction of meningitis by regulating capsule synthesis and by inducing biofilm formation in a mouse infection model. *Vet Microbiol* 2017;**199**:111—9.
57. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 2005;**21**:319—46.
58. Cao M, Feng Y, Wang C, Zheng F, Li M, Liao H, et al. Functional definition of LuxS, an autoinducer-2 (AI-2) synthase and its role in full virulence of *Streptococcus suis* serotype 2. *J Microbiol* 2011;**49**:1000—11.
59. Wang Y, Zhang W, Wu Z, Zhu X, Lu C. Functional analysis of luxS in *Streptococcus suis* reveals a key role in biofilm formation and virulence. *Vet Microbiol* 2011;**152**:151—60.
60. Ryan S, Begley M, Gahan CG, Hill C. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. *Environ Microbiol* 2009;**11**:432—45.
61. Fulde M, Willenborg J, de Greeff A, Benga L, Smith HE, Valentin-Weigand P, et al. ArgR is an essential local transcriptional regulator of the arcABC operon in *Streptococcus suis* and is crucial for biological fitness in an acidic environment. *Microbiol-Sgm* 2011;**157**:572—82.
62. Gruening P, Fulde M, Valentin-Weigand P, Goethe R. Structure, regulation, and putative function of the arginine deiminase system of *Streptococcus suis*. *J Bacteriol* 2006;**188**:361—9.
63. Fulde M, Willenborg J, Huber C, Hitzmann A, Willms D, Seitz M, et al. The arginine-ornithine antiporter ArcD contributes to biological fitness of *Streptococcus suis*. *Front Cell Infect Microbiol* 2014;**4**:107.
64. Wang J, Gao Y, Teng K, Zhang J, Sun S, Zhong J. Restoration of bioactive lantibiotic suicin from a remnant lan locus of pathogenic *Streptococcus suis* serotype 2. *Appl Environ Microbiol* 2014;**80**:1062—71.
65. Li J, Tan C, Zhou Y, Fu S, Hu L, Chen H, et al. The two-component regulatory system CiaRH contributes to the virulence of *Streptococcus suis* 2. *Vet Microbiol* 2011;**148**:99—104.
66. Han H, Liu C, Wang Q, Xuan C, Zheng B, Tang J, et al. The two-component system Ihk/Irr contributes to the virulence of *Streptococcus suis* serotype 2 strain 05ZYH33 through alteration of the bacterial cell metabolism. *Microbiology* 2012;**158**:1852—66.
67. Wang H, Shen X, Zhao Y, Wang M, Zhong Q, Chen T, et al. Identification and proteome analysis of the two-component VirR/VirS system in epidemic *Streptococcus suis* serotype 2. *Fems Microbiol Lett* 2012;**333**:160—8.

68. Xu J, Fu S, Liu M, Xu Q, Bei W, Chen H, et al. The two-component system NisK/NisR contributes to the virulence of *Streptococcus suis* serotype 2. *Microbiol Res* 2014;**169**:541—6.
69. Segura M. Streptococcus suis vaccines: candidate antigens and progress. *Expert Rev Vaccines* 2015;**14**:1587—608.
70. de Buhr N, Stehr M, Neumann A, Naim HY, Valentin-Weigand P, von Kockritz-Blickwede M, et al. Identification of a novel DNase of *Streptococcus suis* (EndAsuis) important for neutrophil extracellular trap degradation during exponential growth. *Microbiology* 2015;**161**:838—50.
71. Fontaine MC, Perez-Casal J, Willson PJ. Investigation of a novel DNase of *Streptococcus suis* serotype 2. *Infect Immun* 2004;**72**:774—81.
72. Serhir B, Higgins R, Foiry B, Jacques M. Detection of immunoglobulin-G-binding proteins in *Streptococcus suis*. *J Gen Microbiol* 1993;**139**:2953—8.
73. Nitsche-Schmitz DP, Johansson HM, Sastalla I, Reissmann S, Frick IM, Chhatwal GS. Group G streptococcal IgG binding molecules FOG and protein G have different impacts on opsonization by C1q. *J Biol Chem* 2007;**282**:17530—6.
74. Serhir B, Dubreuil D, Higgins R, Jacques M. Purification and characterization of a 52-kilodalton immunoglobulin G-binding protein from *Streptococcus suis* capsular type 2. *J Bacteriol* 1995;**177**:3830—6.
75. Liu L, Cheng G, Wang C, Pan X, Cong Y, Pan Q, et al. Identification and experimental verification of protective antigens against *Streptococcus suis* serotype 2 based on genome sequence analysis. *Curr Microbiol* 2009;**58**:11—7.
76. Desai J, Kumar SV, Mulay SR, Konrad L, Romoli S, Schauer C, et al. PMA and crystal-induced neutrophil extracellular trap formation involves RIPK1-RIPK3-MLKL signaling. *Eur J Immunol* 2016;**46**:223—9.
77. Naccache PH, Fernandes MJ. Challenges in the characterization of neutrophil extracellular traps: the truth is in the details. *Eur J Immunol* 2016;**46**:52—5.
78. Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. *Nat Rev Immunol* 2017;**17**:151—64.
79. Papayannopoulos V, Zychlinsky A. NETs: a new strategy for using old weapons. *Trends Immunol* 2009;**30**:513—21.
80. Beiter K, Wartha F, Albiger B, Normark S, Zychlinsky A, Henriques-Normark B. An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps. *Curr Biol* 2006;**16**:401—7.
81. Zhao J, Lin L, Fu L, Han L, Zhang A. Neutrophil extracellular Taps play an important role in clearance of *Streptococcus suis* in vivo. *Microbiol Immunol* 2016;**60**:228—33.
82. de Buhr N, Neumann A, Jerjomiceva N, von Kockritz-Blickwede M, Baums CG. *Streptococcus suis* DNase SsnA contributes to degradation of neutrophil extracellular traps (NETs) and evasion of NET-mediated antimicrobial activity. *Microbiology* 2014;**160**:385—95.
83. Ma F, Yi L, Yu N, Wang G, Ma Z, Lin H, et al. *Streptococcus suis* serotype 2 biofilms inhibit the formation of neutrophil extracellular traps. *Front Cell Infect Microbiol* 2017;**7**:86.
84. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 2010;**11**:785—97.
85. Hovingh ES, van den Broek B, Jongerius I. Hijacking complement regulatory proteins for bacterial immune evasion. *Front Microbiol* 2016;**7**:2004.
86. Seitz M, Beineke A, Singpiel A, Willenborg J, Dutow P, Goethe R, et al. Role of capsule and suilysin in mucosal infection of complement-deficient mice with *Streptococcus suis*. *Infect Immun* 2014;**82**:2460—71.
87. Seele J, Beineke A, Hillermann LM, Jaschok-Kentner B, von Pawel-Rammingen U, Valentin-Weigand P, et al. The immunoglobulin M-degrading enzyme of *Streptococcus suis*, IdeSsuis, is involved in complement evasion. *Vet Res* 2015;**46**:45.
88. Vaillancourt K, Bonifait L, Grignon L, Frenette M, Gottschalk M, Grenier D. Identification and characterization of a new cell surface protein possessing factor H-binding activity in the swine pathogen and zoonotic agent *Streptococcus suis*. *J Med Microbiol* 2013;**62**:1073—80.
89. Feng Y, Cao M, Shi J, Zhang H, Hu D, Zhu J, et al. Attenuation of *Streptococcus suis* virulence by the alteration of bacterial surface architecture. *Sci Rep* 2012;**2**:710.
90. Lecours MP, Fittipaldi N, Takamatsu D, Okura M, Segura M, Goyette-Desjardins G, et al. Sialylation of *Streptococcus suis* serotype 2 is essential for capsule expression but is not responsible for the main capsular epitope. *Microbes Infect* 2012;**14**:941—50.
91. Vanier G, Segura M, Friedl P, Lacouture S, Gottschalk M. Invasion of porcine brain microvascular endothelial cells by *Streptococcus suis* serotype 2. *Infect Immun* 2004;**72**:1441—9.
92. Tenenbaum T, Essmann F, Adam R, Seibt A, Janicke RU, Novotny GE, et al. Cell death, caspase activation, and HMGB1 release of porcine choroid plexus epithelial cells during *Streptococcus suis* infection in vitro. *Brain Res* 2006;**1100**:1—12.
93. Vadeboncoeur N, Segura M, Al-Numani D, Vanier G, Gottschalk M. Pro-inflammatory cytokine and chemokine release by human brain microvascular endothelial cells stimulated by *Streptococcus suis* serotype 2. *FEMS Immunol Med Microbiol* 2003;**35**:49—58.
94. Winterbourn CC, Kettle AJ. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid Redox Signal* 2013;**18**:642—60.
95. Karavolos MH, Horsburgh MJ, Ingham E, Foster SJ. Role and regulation of the superoxide dismutases of *Staphylococcus aureus*. *Microbiology* 2003;**149**:2749—58.
96. Yesilkaya H, Andisi VF, Andrew PW, Bijlsma JJ. *Streptococcus pneumoniae* and reactive oxygen species: an unusual approach to living with radicals. *Trends Microbiol* 2013;**21**:187—95.
97. Yesilkaya H, Kadioglu A, Gingles N, Alexander JE, Mitchell TJ, Andrew PW. Role of manganese-containing superoxide dismutase in oxidative stress and virulence of *Streptococcus pneumoniae*. *Infect Immun* 2000;**68**:2819—26.
98. Liu CI, Liu GY, Song Y, Yin F, Hensler ME, Jeng WY, et al. A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence. *Science* 2008;**319**:1391—4.
99. Chaput C, Boneca IG. Peptidoglycan detection by mammals and flies. *Microbes Infect* 2007;**9**:637—47.
100. Melnyk JE, Mohanan V, Schaefer AK, Hou CW, Grimes CL. Peptidoglycan modifications tune the stability and function of the innate immune receptor Nod2. *J Am Chem Soc* 2015;**137**:6987—90.
101. Vollmer W, Tomasz A. The pgdA gene encodes for a peptidoglycan N-acetylglucosamine deacetylase in *Streptococcus pneumoniae*. *J Biol Chem* 2000;**275**:20496—501.
102. Fittipaldi N, Sekizaki T, Takamatsu D, de la Cruz DM, Harel J, Bui NK, et al. Significant contribution of the pgdA gene to the virulence of *Streptococcus suis*. *Mol Microbiol* 2008;**70**:1120—35.
103. FAN H. Advances in pathogenesis of Streptococcus suis serotype 2. *J Integr Agric* 2017;**16**:2834—47.
104. 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**:3772—99.


## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2019.03.003.