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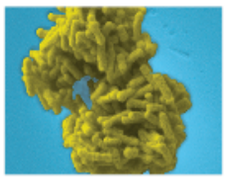

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Streptococcus suis infection

An emerging/reemerging challenge of bacterial infectious diseases?

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Streptococcus suis (*S. suis*) is a family of pathogenic gram-positive bacterial strains that represents a primary health problem in the swine industry worldwide. *S. suis* is also an emerging zoonotic pathogen that causes severe human infections clinically featuring with varied diseases/syndromes (such as meningitis, septicemia, and arthritis). Over the past few decades, continued efforts have made significant progress toward better understanding this zoonotic infectious entity, contributing in part to the elucidation of the molecular mechanism underlying its high pathogenicity. This review is aimed at presenting an updated overview of this pathogen from the perspective of molecular epidemiology, clinical diagnosis and typing, virulence mechanism, and protective antigens contributing to its zoonosis.

Introduction

Streptococcosis is regarded as a leading infectious disease in the swine industry, that clinically features with meningitis, septicemia, or arthritis and annually results in significant economic loss worldwide.[1] *Streptococcus suis* (*S. suis*) that was initially reported in 1954[2] has been demonstrated as an etiological agent for this kind of frequently-occurring bacterial infection.[1,3] Indeed, *S. suis*, a complex population consisting of heterogeneous strains,[4] can be classified into 35 serotypes (1–34, 1/2) based on the differentiation of capsule antigens.[1,3] Based on the varied virulence of these bacteria, they may be categorized into highly-pathogenic, weakly-pathogenic (hypo-virulent), and nonpathogenic (avirulent) strains.[1] Generally, serotype 2 of *S. suis* (SS2) is considered to be the most virulent, and is frequently isolated from clinically-diseased piglets.[1] In fact, serotype 9 of *S. suis* is also one of the most important serotypes in several countries. Of particular note, SS2 seems to be a previously neglected but recently emerging human pathogen,[5] whose infection has become increasingly potent, especially in the southeast Asian countries like Thailand,[6] Vietnam,[7] and China.[8,9]

As the primary agent of meningitis, septicemia, arthritis and as an opportunistic pathogen in the case of pneumonia,[1,5] *S. suis* have been reported to have spread over 30 countries and/or regions (**Fig. 1**) and has claimed no less than 1600 human cases, some of which were fatal.[2] Also, similar clinical symptoms including bacterial meningitis, septicemia, and arthritis are frequently observed in human SS2 infections.[2,3] Occasionally, serotypes other than SS2, including SS1,[10] SS4,[10] SS5,[11,12] SS14,[13,14] SS16,[15,16] and SS24[11] can also be found to function as the causative agents responsible for sporadic cases of human *S. suis* infection.[3] Of note, two big outbreaks of human SS2 endemics which occurred in China, in 1998 and 2005, respectively,[9,17,18] have raised serious concerns in public health and have challenged the conventional opinion that human SS2 infections are only present in sporadic cases.[2,8,19] Unfortunately, no specific/effective human therapeutics or vaccine against SS2 infections is available thus far. Considering the severity (high mortality and modality) of SS2 infection in humans,[5,8] it is important to develop a method for convenient and quick diagnosis, which can be applied toward local SS2 detection.[4,18]

Over the past four decades, significant progress has been made toward better understanding the highly infectious clones of *S. suis*. At the time of formulating this review, 1104 articles were available in PubMed regarding *S. suis* (http://www.ncbi.nlm.nih.gov/pubmed/?term=Streptococcus+suis).Totally, over 20 bacterial virulence-associated factors have been identified that include capsular polysaccharides (CPS),[20] Muramidase-released protein (MRP),[21] and Suilysin (SLY).[22] To date, genomic sequences of a collection of *S. suis* strains are available (**Fig. 2**), the majority of which are derived from SS2 species,[23,24] except two newly-released genomes which correspond to SS3[25] and SS14,[26] respectively. Genomic mining combined with bacterial genetics have elucidated that Chinese epidemic strains of highly pathogenic *S. suis* 2 carry a specific 89K PAI (pathogenicity island).[23,27] Further studies suggested that 89K PAI with a transposon-like essence can

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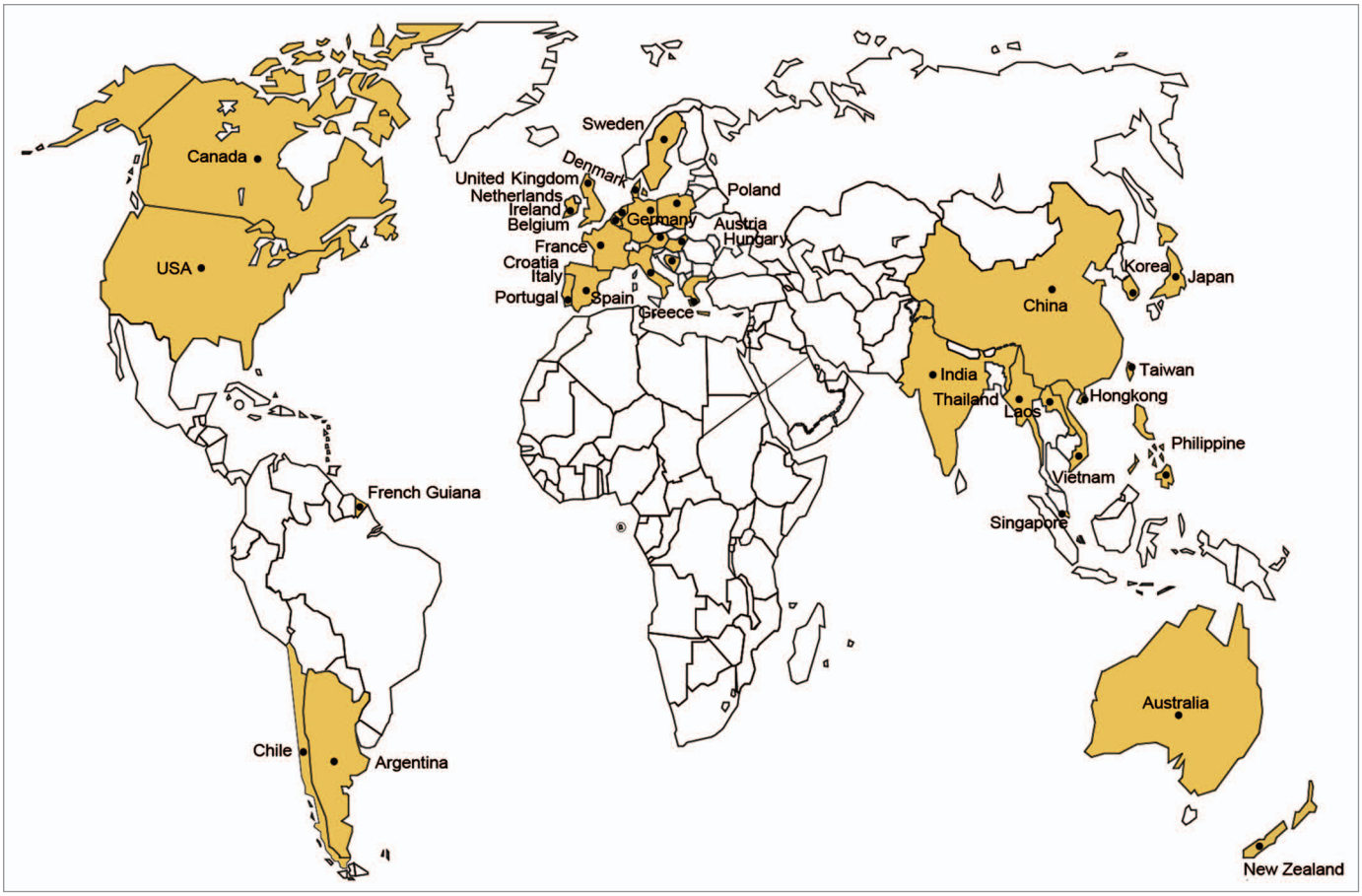

Figure 1. Global epidemiology of human SS2 infections. Countries/regions with human cases of SS2 infections were labeled and highlighted in yellow. Adapted from references 2 and 48 with permission.

undergo GI-type T4SS-mediated horizontal transfer in epidemic SS2 species.[28] The systematic elucidation of the of *S. suis* pathogenesis in the Omics Era was illustrated by functional definition of a collection of other new genes or putative orthologs (such as Zur, a zinc uptake regulator,[29] CovR, an orphan response regulator,[30] and Rgg-like transcription factor[31]) following the release of the genome sequence of SS2 (e.g., 05ZYH33).[23] Although we have gained a partial glimpse of the molecular mechanism underlying the high pathogenicity of SS2 itself, we are still lacking further insights into the interface between the SS2 pathogen and the host it infects.[3,8]

In this review, we aim to describe an updated but partial picture of SS2 as an emerging infectious agent, which centers on five aspects: global epidemiology/distribution, clinical diagnostics/typing, pathogenesis, protective antigen/candidate vaccine, and zoonotic potential.

An Overview of *S. suis*

General microbiology of *S. suis*

S. suis is a group of heterogeneous gram-positive bacteria that were earlier classified into Lancefield groups R, S, and T (**Fig. 3**).[1] These bacteria are facultative anaerobes with a spherical/ovoid shape which exist in pairs and/or short chains (**Fig. 3B and C**). Generally, these microorganisms show either α-hemolysis when growing on selective plates of horse blood agar[1] (**Fig. 3A**). Given the variation in their CPS antigens, 35 serotypes have been proposed for *S. suis* population.[1] Very few studies of pathogenicity have been done for serotypes other than serotypes 2, 1, and 7.[1] Among them, SS2 is recognized as the most virulent species that is frequently associated with diseased pigs and often causes an opportunistic infection of adults having occupational contact with pig carcass or pork-related products.[2,5]

S. suis, an important animal pathogen, naturally inhabits in the upper respiratory (particularly the tonsils and nasal cavities), genital, and alimentary tracts of piglets.[1,32] In addition to the natural host swine (**Fig. 3D**), this pathogen has been suggested to be isolated from a wide range of other animals, such as horses, dogs, and cats.[1,33] Of note, some variants of *S. suis* probably have evolved into highly infectious zoonotic agents that can cause meningitis, septicemia, arthritis, and even streptococcal toxic shock-like syndrome (which can cause rapid death) in humans.[9,17,18,34] Soon after the big outbreak of human SS2 infections in China, in 2005, serious concerns from both the public health and scientific community have been raised.[18] Toward better understanding

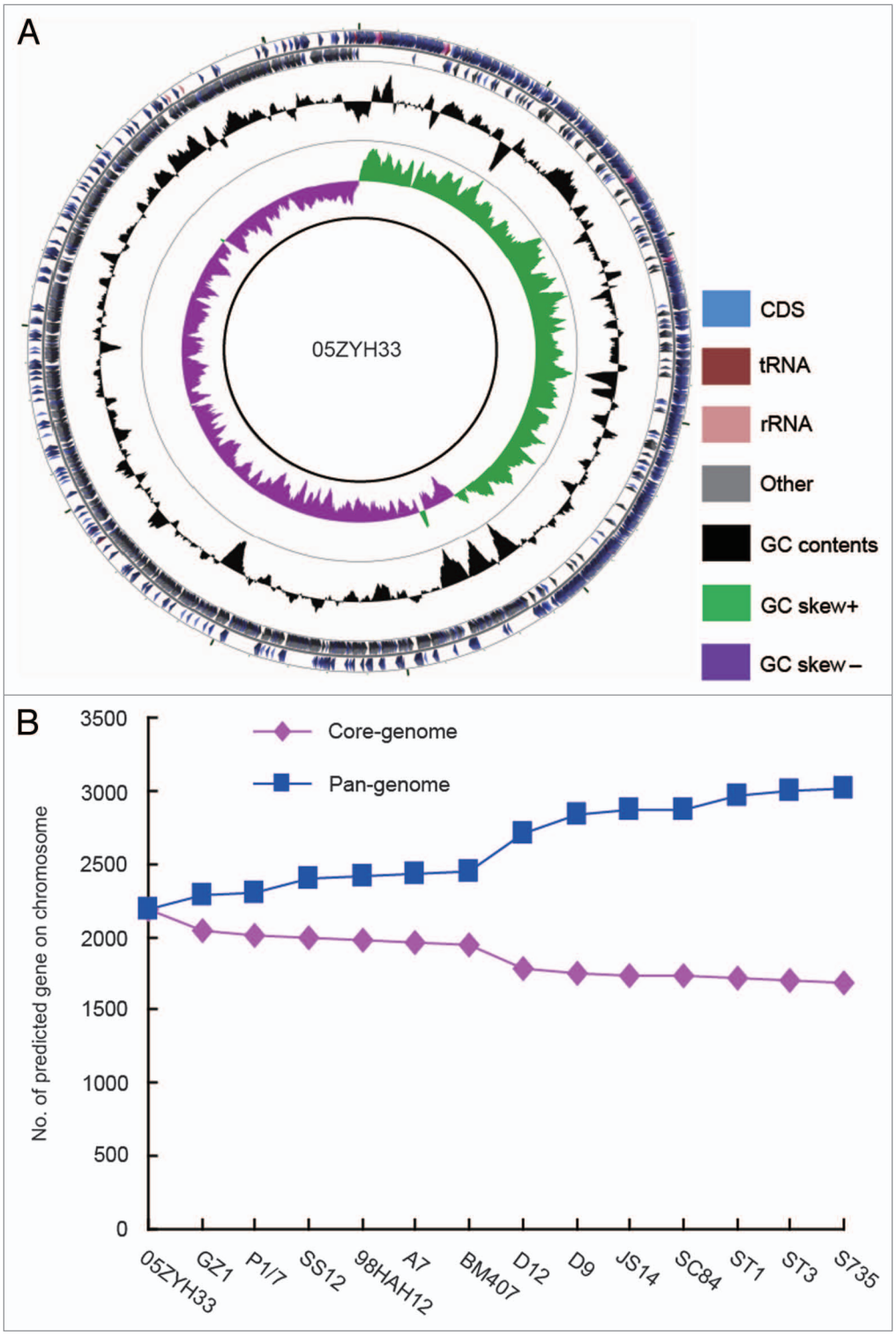

Figure 2. Circular diagram and pan-genome analyses of SS2 genome. (**A**) Circular diagram of representative SS2 genome. (**B**) Pan-genome analyses of *S. suis* 2 species.

and prevention/control of SS2 infections, multiple lines of new bacterial virulence determinants (such as *salK–salR* two component system,[27] FeoB transporter,[35] and Rgg regulator[31]) have been identified, and fast assays using PCR-based molecular detection[18] as well as ELISA (enzyme-linked immunosorbent assay)-guided diagnostics were established[36] (**Table 1**). Most recently, epidemiological investigations conducted in Vietnam proposed that (1) pig population in slaughterhouses is a major reservoir of SS2

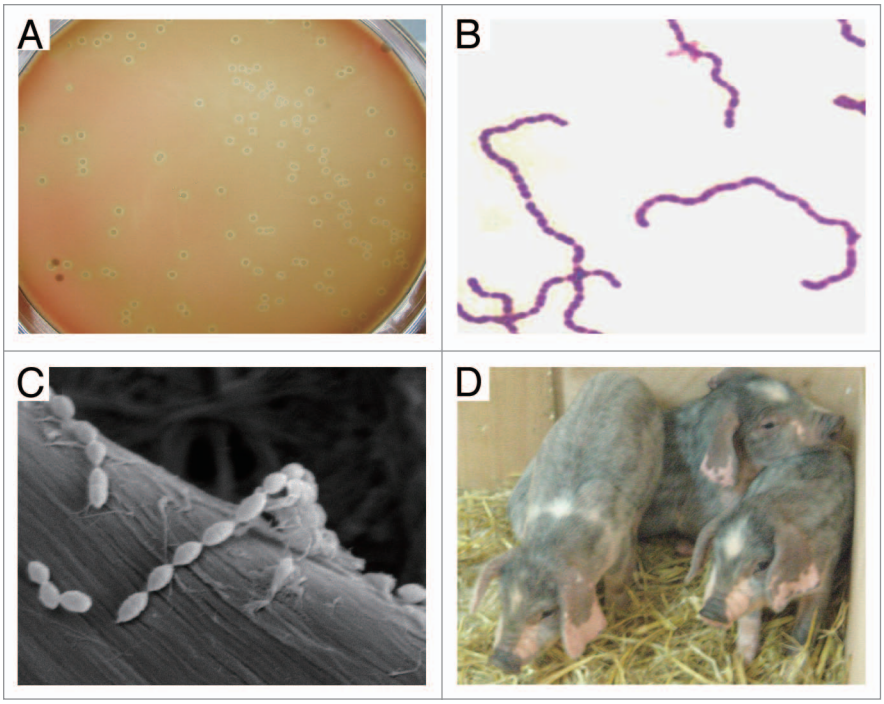

Figure 3. Characterization of *S. suis* and its natural host piglets. (**A**) Colony phenotype of *S. suis* serotype 2 grown on THB plate with 5% sheep blood. (**B**) Gram staining analyses of *S. suis* serotype 2 grown in liquid THB media. It was adapted from reference 18 with permission. (**C**) Scanning electronic microscopic analyses of *S. suis* serotype 2 collected from overnight culture in THB media. (**D**) Phenotypic characterization of the reservoir of *S. suis* serotype 2, piglets maintained in backyard.

with a capacity to cause human infections[37] and (2) the most important risk factors of human *S. suis* infections are consecutively eating "high risk" pork-derived dishes, occupational exposure to pigs and pork-related products, and preparation of pork in the presence of skin lesions.[38] The availability of the epidemiological knowledge on human SS2 infections is critical to improve the current situation of public awareness of SS2 infections and to effectively prevent the potential occupational infections caused by SS2.

Genomics/proteomics-based glimpse of *S. suis*

Genomics/proteomics approaches to probe *S. suis* have yielded unprecedented comprehensive information/knowledge about this pathogen.[8,39] At the time of writing this review, no less than 14 genomes of *S. suis* strains have been available in PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome&cmd=DetailsSearch&term=streptococcus+suis&save_search=false), most of which were completed by the research groups in China (**Fig. 2**). Genomic sequence analyses of *S. suis* showed that (1) all the sequenced genomes feature with the nearly same average GC content, ~41%, indicating slight evolutionary conservation; (2) genome size varies markedly from 1 640 446 nt (05HAS68, NZ_AARD00000000) to 2 146 229 nt (BM407, NC_012926), reflecting the genomic flexibility present in these species (**Fig. 2B**); (3) the number of putative protein-encoding genes is dramatically different (1559 for 05HAS68, and 1932 for BM407), suggesting that nearly 1/5–1/6 of total genes are not essential (redundant) for bacterial viability, and might confer some new/unknown functions to adapt to varied environmental niches. Functional/comparative genomics of *S. suis* has defined a series of new infection/virulence-related determinants such as ArgR regulator,[40] SspA, a subtilisin-like protease,[41] CiaRH two-component regulatory system,[42] and so on.

On the other hand, proteomics analyses of Chinese epidemic SS2 strain 05ZYH33 identified 373 proteins in total from 834 processed spots.[43] Using an immune-proteomic approach, Lu's group revealed 11 membrane-related proteins from Chinese vaccine strain SS2-HA9801[44] and 9 extracellular antigenic proteins from the virulent Chinese SS2 strain ZY05719,[45] respectively. Using a similar strategy, Wu and coauthors from the same group addressed two strains of SS9, another prevalent serotype of *S. suis* (GZ0565 and SH040197), and observed 13 candidate proteins, five of which are virulence-associated factors.[46] Additionally, eight immunogenic proteins localized on bacterial surface were determined in strain GZ0565, including extracellular solute-binding protein.[47] These findings might provide a solid/reasonable basis for development of subunit vaccine candidates.[39]

Epidemiology of *S. suis* Infection

Geographic distribution of human SS2 infections

As a swine pathogen, *S. suis* was first reported by a vegetarian in 1954,[2] while its zoonotic role could be traced to a human SS2 meningitis case in Denmark, in 1968.[1] In light of the available literature with human *S. suis* infection recorded thus far, we expect that *S. suis* infections have been involved in no less than 30 countries and/or regions (**Fig. 1**), and resulted in around 1600 cases of severe human infections.[2,8,48] In North America (United States[49,50] and Canada[51,52]) and the South American countries (Argentina,[53,54] Chile,[55] and French Guiana[56]) only a very few cases of human SS2 infections were reported. Human SS2 infections are featuring with sporadic cases in Europe (Ireland,[3] the United Kingdom [UK],[3] France[3], Spain,[57] Netherlands,[58] Belgium,[3] Poland,[61] Sweden,[3] Denmark,[1] Germany,[62] Hungary,[3] Austria,[63] Croatia,[64] Italy[65-67], Greece,[68] and Portugal[1]), some Asian countries (Laos,[1] Singapore,[33,69] India,[2,3,70] Korea,[71-73] Japan,[74] Hong Kong,[75-80] Taiwan,[33,81,82] and Philippine[2,3]), Australia,[83,84] and New Zealand.[85,86] So far, endemics of human SS2 infections was only observed in two Asian counties Vietnam[2,7,16,37,38] and Thailand.[6,11,14,87] Of particular note, coexistence of sporadic cases and epidemics of human SS2 infections were present in China.[4,8,9,17-19,33,48] It seemed true that the majority of human SS2 infection cases occurred in southeast Asia (especially Vietnam, Thailand, and China), indicating an obvious geographic tropism (**Fig. 1**).

Table 1. Approaches for detection, identification and typing of *Streptococcus suis*

Different approaches	Description	Year	References
Microbiological methods			
Selective medium-based cultivation	A modified Todd-Hewitt Broth agar containing 5% defibrinated sheep blood and crystal violet	1991	193
Molecular tests			
Conventional PCR	Only *gdh* as target gene	2003	194
Swabs PCR	Only *epf* as target gene	2005	195
Nested PCR	To differentiate *S. suis* from other bacteria like *Hemophilus parasuis*	2012	196
Single-tube LAMP assay	Loop-mediated isothermal amplification (LAMP) with 100–1000 times higher sensitivity than the conventional PCR assay	2012/2013	197 and 198
Real-time PCR	*cps2J,* glutamate dehydrogenase *(gdh)*	2010/2011	199 and 200
Multiplex-PCR	*mrp*, *epf*	1998/	201 and 202
	cps, epf	2002	203
	mrp, epf, sly	2000/2003	204 and 205
	cps, *epf*, *mrp, sly, arcA*, *gdh*	2006/2013	206 and 207
	16s, *cps2J*	2004	208
RFLP	Used for ribotyping	1995	209
PFGE	Pulsed-field gel electrophoresis	2002–2011	37 and 210–217
ISR-RFLP	PCP amplification of 16S-23S rDNA intergenic spacer region (ISR) that was followed by restriction fragment length polymorphism (RFLP) analysis (ISR-RFLP)	2006/2007	210 and 218
MLST	Multi-Locus Sequence Typing	2002/2007–2012	37, 67, 211, 213, and 219–221
MLVA	multiple-locus variable tandem repeat number analysis	2010	222
RAPD	randomly amplified polymorphic DNA (RAPD)	1999	223
Immunological assays			
An enzyme-based in situ hybridization method	*16S rRNA* as target gene	2000/2001	224 and 225
MRP/EF-based ELISA	MRP/EF protein as capture antigen	1993	226
CPS-based indirect ELISA	CPS as capture antigen	1996	227 and 228
Sao-based ELISA	SAO protein as capture antigen	2007	36
SERS	surface enhanced Raman scattering with MRP protein as capture antigen	2012	229
Indirect immunofluorescence assay	/	2000	225
Peroxidase-antiperoxidase method	/	2000	225
ICS (Immunochromatographic strip)	To detect anti-CPS antibody	2007	98
Colloidal gold immunochromatographic strips	To direct detection of the *S. suis* serotype 2 antigen	2010	97
Electrochemiluminescence (ECL) immunosensor	It is based on l-cysteine combined with mimicking bi-enzyme synergetic catalysis	2012	99

/, not listed

Although we are not quite sure what mechanism can explain such kind of tropism, we anticipate that the following factors are probably correlated with frequent occurrence of human SS2 infections in above countries, which include (1) similar local climates and/or environments; (2) backyard cultivation of pigs; and (3) popular consumption of raw pork sold in the wet market.

Current situation of human *S. suis* infections in Europe and North America

During the past 40 years, around 100 cases of human *S. suis* infections were estimated in European countries. Among them, the top three countries in the history of human SS2 infections recorded are Netherlands (41 cases), United Kingdom (15 cases),

and Denmark (12 cases).[2] The rest of the European countries (such as France and Germany) had less than ten sporadic cases of human infection.[2] Moreover, the prevalent type of clinical disease caused by *S. suis* infections in these countries is bacterial meningitis.

Although human *S. suis* infections are considered sporadic cases in most countries,[2,5,8] it is unbelievable that its zoonotic infectious events are rarely reported in North America (Canada and USA), two huge countries with numerous, large, and frequent swine operations.[3,5] To the best of our knowledge, only three human SS2 meningitis cases were confirmed in USA,[49,50,89] and three cases were diagnosed in Canada (one case was due to SS14, and the other 2 cases were caused by SS2[51,52]). Gottschalk and coworkers[3] believe that this small number of reported cases from these two huge countries with a big industry for pig cultivation might be attributed to the following two major reasons: (1) Clinical under-diagnosis and/or misdiagnosis of *S. suis* infection, rather than true absence of this infectious disease, (2) *S. suis* isolates in North America are less virulent, relative to those from Europe and Asia. A pilot study conducted by Smith et al.[90] recently indicated that human infection with *S. suis* is more common in the United States than what it is generally thought. The reason might lie in underdiagnosis or misdiagnosis, rather than a real lacking of disease.[3,91,92] On this issue, we doubt the current situation of *S. suis* infections as well, though we agree that good hygiene conditions and prevention strategies during the whole process of pork operation (e.g., wearing gloves) might secure the major route of bacterial entry into blood by small cuts in the skins, and greatly decrease the incidents of *S. suis* infections in North America. Therefore, we believe that it is necessary to employ combined specific approaches (like multiplex PCR plus ELISA) for the re-evaluation of the epidemiological aspects of human *S. suis* infections in North America. Interestingly, Schmid et al.[93] recently demonstrated that American SS2 isolates do not carry 89K PAI, a DNA fragment present in Chinese epidemic strain, which might be helpful for development of an American SS2 strain-specific PCR detection assay.

Situation of human *S. suis* infections in southeast Asia

Accumulated epidemiological data suggests that over 85% of total cases have occurred in Asian countries.[2] The cases of human SS2 infections in the mainland of China and Vietnam are comparable.[2,8,16] Relative to the above 2 countries, the human cases clinically infected by *S. suis* are second.[6,14,87] Of being noteworthy, SS2 has been recognized as a pathogen with the mostly-relevance to human bacterial meningitis in southern Vietnam.[16] In Hongkong, the specialized administrative region of China, the first human case of *S. suis* infection was recorded in 1983,[78,80] and the accumulated number of *S. suis* infections were estimated to be about 60 cases.[75,78] In particular, two big outbreaks of human SS2 infections in China (1998 and 2005) seriously challenged public health.[9,18] In the 1998 epidemic, 14 out of 25 SS2-infected persons died along with an estimated 80 000 pigs.[18] In the 2005 epidemic, totally 215 patients had SS2 infections, 38 of which are dead. A similar scenario was also observed when more than 600 pigs were demonstrated to be infected by SS2.[9,18] The causative agents of the both epidemics were subsequently determined to be highly invasive clones of strong virulent SS2 strain that seemed to have acquired a new pathogenicity island 89K.[8,23,94] Additionally, sporadic human meningitis cases caused by SS2 infections were observed in three other cities of China (Shenzhen City, Chongqing City, and Nanjing City) in 2007,[4,95] implying that the situation of SS2 infection in China is complicated. This could be due to variants of *S. suis* 2 identified in subsequent investigations. It might be of much interest to unveil the possible evolutionary relationship of the Chinese epidemic strain with those of the neighboring country like Vietnam through comparative genomics.

Detection and Typing of *S. suis*

Fast/effective detection and analyses of *S. suis* is critical for the prevention and/or diagnosis of endemic *S. suis* 2 infection in the swine industry as well as for *S. suis* infected patients. Three types of experimental approaches are available thus far that consist of (1) selective media-based microbiological cultivation, (2) molecular tests, and (3) immunological assays (**Table 1**). Given its advantage in sensitivity and fastness, the second method has been experimentally developed into two subgroups (PCR-based detection plus typing-oriented analyses like pulsed-field gel electrophoresis [PFGE] and restriction fragment length polymorphism [RFLP]) and might be potential in clinical re-confirmation and/or re-valiadtion. Generally, PCR assays can be classified into six kinds among which multiplex-PCR (using sets of specific primers including *epf*, *mrp*, and *gdh*) is appreciably-valid approach to assay SS2 and applied in some countries (**Table 1**). The major four kinds of typing methods include PFGE, RFLP, multi-locus sequence typing (MLST) and random amplified polymorphic DNA (RAPD), some of which have derivatives such as ISR-RFLP (**Table 1**). In general, the three experimental methods (PFGE, RFLP, and RAPD) can return clues about genomic differences between different strains/serotypes. Given direct sequencing of multiple loci (*cpn60*, *dpr*, *recA*, *aroA*, *thrA*, *gki*, and *mutS*), MLST can directly capture the nucleotide sequence deviation used for typing purpose. Among the immunological approaches, ELISA could be the most popular way to address *S. suis* infection in some experimental tests (**Table 1**). In fact, different versions of ELISA have been developed that are based on various capture antigens identified to be specifically against *S. suis* 2. We have also established two kinds ELISA assays (one is based on SAO protein,[4,36] the other is based on Enolase surface antigen[96]), both of which work well in our trials during field screening and clinical detections. Recently, two more new derivative methods were reported, which are immunochromatographic strip[97,98] and electrochemiluminescence immunosensor,[99] respectively (**Table 1**).

Molecular Mechanism for *Streptococcus suis* Infection

Bacterial virulence determinants

The clinical consequence of *S. suis* infection is determined by the complicated interplay between this zoonotic pathogen and

its host. A series of bacterial components as well as a collection of host cell factors contribute to this pathogenesis-related process. So far, almost 60 bacterial components have been identified to be involved in the infection and/or pathogenicity of *S. suis* (**Table 2**). Of particular note, Wilson and coworkers[100] developed a powerful signature-tagged mutagenesis (STM) system for *S. suis* and identified nearly 20 potential virulence associate elements through screening the library consisting of approximately 2600 mutants (**Table 2**). However, exact roles of these genes needed further verification. According to their general roles in the context of bacterial life cycles, these bacterial virulence-associated factors were temporarily classified into the following three sub-groups (of note some genes probably can be attributed to two different classifications due to their dual characteristics): (1) surface/secreted elements; (2) enzymes/proteases; (3) transcription factors/regulatory systems; and (4) others (transporters/secretion systems) (**Table 2**).

Surface/secreted components

For Subgroup 1, a total of 17 genes/gene clusters have been determined thus far to contribute to bacterial pathogenicity (**Table 2**). In addition to six previously identified elements (capsular polysaccharides [CPS],[20,101] extracellular protein factor [EF],[102] fibronectin binding factor [FBP],[103] muramidase-released protein [MRP],[102] a protein of 38 kDa localized on bacterial surface [abbreviated 38 kDa],[104] and thio-activated hemolysin with known crystal structure [Suilysin, SLY])[105,106] plus implication into bacterial meningitis,[107] 11 more members have been supplemented into this group that include SspA, the surface-associated subtilisin-like serine protease,[41,108,109] HtpS, a novel immunogenic cell surface-exposed protein,[110] and Sat surface protein[111,112](**Table 2**). Like FBP,[103] the gene *SSU05_1311* with unknown function was determined to be one more surface anchored fibronectin-binding protein. More importantly, this surface protein functions in vivo in crossing the mucosal epithelia to disseminate, suggesting it is a novel virulence factor.[113]

Two independent research groups (one from Canada[41] and another from China[114]) identified the subtilisin-like protease (SspA)-encoding gene (*sspA*, SSU0757) from the *S. suis* organism by screening the mutant library and genomic expression library, respectively. SspA proteinase possesses a typical cell wall anchoring signal, LPXTG, at its C-terminus, implying it is a cell surface-displayed protein.[108] Infection assays have demonstrated that SspA protein plays critical roles in SS2 pathogenicity.[108,109,114] A subsequent study further revealed that *S. suis* SspA protease might modulate cytokine secretion by macrophages, and thus trigger central nervous system inflammation associated with bacterial meningitis, frequently observed in SS2-infected patients as well as piglets.[41]

HP0197 is a new a surface protective antigen that was originally identified by Jin's group in 2009. This immunogenic antigen can elicit obvious humoral antibody response and confer efficient protection against SS2 challenge in the infection models of both mice and pigs.[115] Subsequently, HP0197 protein was found to interact with host cell surface glycosaminoglycans (GAGs), and the binding sites were further proved by solving the X-ray structure of N-terminal GAG-binding domain combined with site-directed mutagenesis plus indirect immunofluorescence assay.[116] Very recently, the same research group reported that HP0197 is involved in bacterial virulence by evaluating the performance of the isogenic mutant Δ*hp0197* in both mice and pigs.[117] A reasonable interpretation would be that virulence attenuation is due to easier clearance of the Δ*hp0197* mutant by host immunological system during the stage of infection, and the fast clearance is attributed to the reduced CPS thickness and decreased resistance to phagocytosis.[117] Further comparative transcriptomics-based analyses elucidated that expression of CPS synthetic operon is downregulated in the Δ*hp0197* mutant relative to the wild-type strain.[117] Also the introduction of plasmid-borne *hp0197* gene into the Δ*hp0197* mutant can restore the decreased expression level of *cps* operon to those seen with the wild type strain.[117] This observation is consistent with the fact that CcpA, a global carbon catabolite regulator, contributes to bacterial infectivity/pathogenicity.[118,119] Preliminary evidence pointed out that HP0197 might determine the Ser-46 phosphorylation level of phospho carrier protein (HPr-46), a partner protein of CcpA in binding the catabolite-responsive elements (cre) of the target operons. Together, it suggested that integration of the posttranslational modification of HPr-46 and CcpA-mediated transcriptional regulation is linked to regulatory network of bacterial virulence.

The serum opacity factor of *S. suis* (OFS) is a putative member belonging to the family of MSCRAMM (**M**icrobial **S**urface **C**omponents **R**ecognizing **A**dhesive **M**atrix **M**olecules).[120] N-terminal region of OFS exhibits similarity to the serum opacity factor of *Streptococcus pyogenes* and fibronectin-binding protein A (FnBA) of *Streptococcus dysgalactiae*, and its C-terminus harbors repetitive sequence elements. Crude extract of *S. suis* and the recombinant OFS protein both possessed serum opacification activity. Experimental infections demonstrated that the deletion of the *ofs* gene severely impairs *S. suis* virulence.[120]

Sao is a surface antigen first identified by Li et al.,[121] which can react with convalescent-phase sera from pigs clinically infected by *S. suis* type 2. Subsequently recombinant Sao formulated with Quil A was found to induce potent opsonizing antibody responses, and confer cross-protection of mice against challenges of virulent heterogeneous *S. suis*.[122] We also discovered that three allelic variants of the *sao* gene (namely *sao-S*, *sao-M*, and *sao-L*) are present in *S. suis* population, and we have developed an efficient ELISA method in which Sao-M protein serves as the capture antigen.[36] Recent further genetic study suggested that SAO protein is only a minor virulence factor[123]

Sat (HP272) is another newly-identified surface protein from the *S. suis* serotype 2,[111,112] and two different research groups have shown evidence that recombinant Sat protein can confer effective immuno-protection of mice against SS2 infections, implying that it is a vaccine molecule candidate.[111,112] In addition, HtpS, a putative member of histidine triad protein family, was determined to be cell surface-associated protein that was expressed during the infection of Chinese SS2 strain 05ZYH33.[110] Moreover, recombinant HtpS protein was demonstrated to function as a protective antigen against SS2 infections.[110] Similarly, Zhang et al.[124] recently defined another new infection-associated

Table 2. Bacterial and host components associated with *Streptococcus suis* infectivity

Gene	Functional annotation	Structural information	SS2/host	References
Bacterial virulence-associated determinants				
Surface/secreted components (17)				
cps	Capsular polysaccharide (CPS)	Known	Strain S735 (Netherlands)	20 and 101
epf	Extra-cellular protein factor (EF)	Unknown	Pig isolate (Netherlands)	102
fbp	Fibronectin binding protein (FBP)	Unknown	Pig isolate (Netherlands)	103
mrp	Muramidase-released protein (MRP)	Unknown	Pig isolate (Netherlands)	102
38 kDa	A protein of 38 kDa localized on bacterial surface and/or cell wall	Unknown	An avirulent Strain 1933 (Kansas, USA)	104
sly	Suilysin, thio-activated hemolysin	X-ray crystal structure at 2.85 A	Strain P1/7 (Netherlands)	105–107, and 230
SspA	A surface-associated subtilisin-like serine protease (SspA), SSU0757	Unknown	SC-19 (China) and P1/7 (Canada)	41 and 114
103	Zinc-binding lipoprotein 103	Unknown	P1/7 (Canada)	126
SSU05_1311	A surface anchored fibronectin-binding protein	Unknown	SC-19 (China)	113
HP0197	A surface protective antigen	Crystal structure	ZYS (China)	115–117
htpS	A histine triad surface protein	Unknown	05ZYH33 (China)	110
hp272 or *sat*	HP272 or Sat surface protein	Unknown	Strain P1/7 (Netherlands); Strain 05ZYH33 (China)	
trag	Trag antigen	Unknown	Strain HA9801 (China)	124
ofs	OFS, a novel serum opacity factor of *S. suis*	Unknown	Strain 10 (Netherlands)	120 and 231
sao	Surface antigen protein (SAO), a minor virulence factor	Unknown	Strains 89/1591 (Canada) and 05ZYH33 (China)	36, 121, and 123
ssu05_0473	PAPI-2b, a surface protein as an ancillary pilus subunit	Unknown	Strain 235/02 (pig isolate in Spain)	125
/	HP0245	Unknown	SC-19 (China)	232
Enzymes/protease (22)				
Ssads	Adenosine synthase	Unknown	05ZYH33 (China)	147
SsnA	DNase	Unknown	Strain 10 (Netherlands)	233
endo D	Endo-β-N-acetylglucosaminidase	Unknown	Strain S735 (Netherlands)	100
gtfA	Sucrose phosphorylase	Unknown	Strain S735 (Netherlands)	100
purA	Adenylosuccinate synthetase	Unknown	Strain S735 (Netherlands)	100
purD	Phosphoribosylamine-glycine ligase	Unknown	Strain S735 (Netherlands)	100
scrB	Sucrose-6phosphate hydrolase	Unknown	Strain S735 (Netherlands)	100
cdd	Cytidine deaminase	Unknown	Strain S735 (Netherlands)	100
neuB	Sialic acid synthase	Modeled structure	O5ZYH33 (China)	100 and 141
neuC	UDP *N*-Acetylglucosamine 2-Epimerase	Unknown	P1/7 (Canada)	142

/, not listed

Table 2. Bacterial and host components associated with *Streptococcus suis* infectivity (continued)

Gene	Functional annotation	Structural information	SS2/host	References
luxS	S-ribosylhomocysteinase	Modeled structure	HA9801, 05ZYH33 (China)	139 and 140
igA1	IgA1 protease	Unknown	05ZYS (China)	138
glnA	Glutamine synthetase	Unknown	SC19[a] (China)	127
apuA	A multifunctional α-glucan-degrading enzyme	Unknown	Strain 10 (Netherlands)	134
gdh	Glutamate dehydrogenase (GDH)	Unknown	Strain 1933 (USA)	128
srtA	Transpeptidase mediating covalent linkage of surface proteins to peptidoglycan	Unknown	Strains NCTC10234 (Canada) and 05ZYH33 (China)	136 and 137
dltA	Enzyme catalyzing lipoteichoic acid D-alanylation	Unknown	Strain 31533 (France)	132
pgdA	Peptidoglycan N-acetylglucosamine deacetylase	Unknown	Strain 31533 (France)	133
dppIV	Di-peptidyl peptidase IV	Unknown	Strain 05ZYH33 (China)	135
eno	Enolase for dehydration of 2-phosphoglycerate to phosphoenolpyruvate (Eno)	Crystal structure (2.4A)	Strain 166 (France) and 05ZYH33 (China)	96, 129, 143, and 144
lmpdh	Inosine 5-monophosphate dehydrogenase	Unknown	Strain SS2-H[a] (China)	131
arcABC	An operon encoding arginine deiminase system (ADS)	Unknown		146
Transcriptional factors/two component signal transduction systems (17)				
ccpA	Catabolite control protein A	Unknown	Strain 10 (Netherlands) and ZJJX081101 (China)	118 and 119
adcR	AdcR, a pleiotropic regulator	Unknown	Strain P1/7 (Netherlands)	149
05SSU0053	A predicted transcription factor that is similar to *S. mutans* SMU_61	Unknown	Strain S735 (Netherlands)	100
perR	PerR, a Fur-like regulator	Unknown	Strain SC-19 (China)	150
argR	An ADS-associated repressor of the ArgR/AhrC arginine family	Unknown	Strain 10 (Netherlands)	40
rgg	Rgg transcription factor	Unknown	05ZYH33 (China)	31
treR	Transcriptional factor	Unknown	Strain S735 (Netherlands)	100
nadR	Transcriptional factor	Unknown	Strain S735 (Netherlands)	100
scrR	A repressor for sucrose operon	Unknown	Strain S735 (Netherlands)	100
nisK-nisR	A two-component system	Unknown	05ZYH33 (China)	154
salK-salR (*suiK-suiR*)	A two-component system of the 89K PAI regulating the bioactive lantibiotic suicin production	Unknown	05ZYH33 (China)	27, 151, and 158
ciaR-ciaH	A two-component system	Unknown	SC19 (China)	42
ihk-ihr	A two-component system	Unknown	05ZYH33 (China)	152
virR/virS	A two-component system	Unknown	05ZYH33 (China)	153
covR	Orphan response regulator (CovR)	Unknown	Strain 05ZYH33 (China)	30
revSC21	Orphan response regulator RevSC21	Unknown	Strain SC21 (China)	155
revS	Orphan response regulator	Unknown	Strain 10 (Netherlands)	156
Others (5)				
05SSU0660	An uncharacterized protein homologous to *S. pneumonia spr*1018	Unknown	Strain S735 (Netherlands)	100
feoB	FeoB transporter	Unknown	Strain P1/7 (Netherlands)	35

/, not listed

Table 2. Bacterial and host components associated with *Streptococcus suis* infectivity (continued)

Gene	Functional annotation	Structural information	SS2/host	References
virD4-virB4	Two elements of the T4SS-like system(VirD4–89K/VirB4–89K)	Unknown	05ZYH33 (China)	160
virA	VirA, virulence factor	Unknown	Strain ZY458 (China)	122
Tig	Trigger factor	Unknown	SC21 (China)	159
Host immunological/inflammatory factors				
all	Novel murine ribonuclease, angiogenin inhibitor 1 (AI1), interacting with hyaluronidase (Hyl) of *Streptococcus suis* serotype 2	/	Murine brain	167
	Pro-inflammatory cytokines tumor necrosis factor α (TNF-α), interleukin-1 (IL-1); IL-6, the chemokines IL-8 and monocyte chemotactic protein-1 (MCP-1)	/	Human brain microvascular endothelial cells	161
tlr2	Toll-like receptor (TLR)2	/	Murine infection model	165
/	NFκB and MAP-kinases	/	3D4 porcine alveolar macrophages cell line	166
cd14	CD14	/		234
il-8	Interleukin-8 (IL-8)	/	Porcine brain microvascular endothelial cell	234 and 235
/	Balance between the increased arachidonic acid, a proinflammatory ω-6 polyunsaturated fatty acid (PUFA) and decreased docosahexaenoic acid, an anti-inflammatory ω-3 PUFA	/	Macrophage	236

/, not listed

antigen protein, Trag, using in vivo-induced antigen technology (IVIAT). An inactivation of the *trag* gene was observed to attenuate full virulence of Chinese SS2 strain in the experimental model of Zebrafish.[124] As a surface protein, PAPI-2 was found to constitute an ancillary pilus subunit and exhibit ability to confer protection of mice against serious challenge of *S. suis*.[125]

Although we have never reported evidence that Zur, a zinc uptake regulator is essential for *S. suis* virulence,[29] Aranda et al. very recently reported that the zinc-binding lipoprotein 103, the structural component of zinc uptake system is associated with the infectivity of *Streptococcus suis*, implying that zinc uptake system might be involved into bacterial pathogenesis.[126] This discrepancy may be attributed to the fact that the deletion of *zur* only partially affect expression of zinc uptake system genes, whereas inactivation of "103" completely impairs zinc uptake system. That is why the latter could more seriously disrupt the normal zinc metabolism, and in turn lead to virulence attenuation in this sick bacterium.

Enzymes and proteinases

Accumulated data has suggested that no less than 20 bacterial enzymes might be implicated in the manifestation of *S. suis* virulence (**Table 2**). Among them, eight enzymes are proposed to be virulence factors by Wilson et al.[100] using the system of signature-tagged mutagenesis (**Table 2**). Four of them are generally regarded as enzymes of central metabolism, which separately correspond to (1) GlnA, glutamine synthetase,[127] (2) Gdh, glutamate dehydrogenase,[128] (3) enolase catalyzing dehydration of 2-phosphoglycerate to phosphor-enolpyruvate,[96,129,130] and (4) Impdh, inosine 5-monophosphate dehydrogenase.[131] Five of these enzymes are directly or indirectly related to synthesis and/or modification of bacterial surface structure, including DltA, an enzyme catalyzing lipoteichoic D-alanylation,[132] PgdA, peptidoglycan N-acetylglucosamine deacetylase,[133] ApuA, a bifunctional amylopullulanase,[134] DPP IV, di-peptidyl peptidase IV,[135] and sortase A, a transpeptidase.[136,137] One of the remaining two enzymes is IgA1 protease, which has highly immune-reactive activity to convalescent sera,[138] and the other is S-ribosylhomocycteinase (LuxS) catalyzing synthesis of auto-inducer 2 (AI-2) utilized in interspecies quorum sensing.[139,140] As follows, we will discuss the roles of these enzymes in *S. suis* pathogenesis.

With the exceptions of *neuB* and *neuC*, the remaining six among the eight enzymes proposed by Wilson (including endo-β-N-acetylglucosaminidase [*endo D*], sucrose phosphorylase [*gtfA*], adenylosuccinate synthetase [*purA*], phosphoribosylamine-glycine ligase [*purD*], sucrose-6phosphate hydrolase [*scrB*], cytidine deaminase [*cdd*]) are poorly addressed and require further experimental validation.[100] NeuB is a sialic acid synthase that catalyzes the last committed step of the de novo biosynthetic pathway of sialic acid, a major element of bacterial surface structure. Recently, we systematically addressed its molecular and immunological role in bacterial virulence and claimed that an altered architecture of *S. suis* surface attenuates its virulence.[100,141] Similarly, *neuC* that encodes UDP *N*-Acetylglucosamine 2-Epimerase with an involvement in sialic acid biosynthesis is also found to be essential for capsule production and required for virulence in a mouse infection model.[142]

Gdh, the glutamine dehydrogenase-encoding gene, was originally known as a virulence factor. It has been widely applied

to develop effective methods for differentiate and detect virulent *Streptococcus suis* species.[128] Si et al.[127] demonstrated that glutamine synthetase, the *glnA*-encoding product, is associated with *S. suis* virulence using a mouse model. We and two other research groups[96,129,143] have reported that *S. suis* enolase acts as an octamer,[144] and can exported to the bacterial surface with capability of binding to host fibronectin, indicating its possible role in crosstalk between pathogen and host. However, the protective efficiency of recombinant enolase with different bacterial origins does not seem consistent.[96,129,130] Similarly, the gene encoding Inosine 5-monophosphate dehydrogenase, a nucleotide metabolism-related enzyme, was initially cloned by Lu's research group, and was subsequently suggested to be involved in full virulence of SS2-H, a Chinese strain in the infection model of piglets.[131]

Modification of the bacterial surface is critical for successful invasion and entry of pathogens into host cells. Gottschalk's group systemically evaluated contributions of two kinds of modification systems to *S. suis* pathogenicity: lipoteichoic acid (LTA)-D-alanylation, and peptidoglycan (PG) N-deacetylation, respectively.[132,133] The Δ*dltA* mutant with defection in LTA D-alanylation was found to be attenuated in its virulence, which can be correlated with its diminished adherence/invasion of porcine brain microvascular endothelial cells, and decreased capacity to escape immune clearance or killing by porcine neutrophils.[132] Fittipaldi et al.[133] observed that the expression level of the *pgdA* gene can be induced upon interaction of SS2 with neutrophils in vitro and infected mice in vivo, implying that PG N-deacetylation is tightly involved in SS2 infections. This hypothesis was further validated by virulence attenuation of the Δ*pgdA* mutant of SS2. Not only does ApuA behave like a bacterial surface protein with a LPKTGE cell-wall-anchoring motif at C-terminus, but it also functions as a bi-functional amylopullulanase.[134] Further genetic study showed that the multifunctional α-glucan-degrading enzyme ApuA can promote adhesion to porcine epithelium and mucus, which might link bacterial carbohydrate utilization to its capability of colonization and invasiveness into hosts. We and the other research group demonstrated that Sortase A (SrtA, originally referred to as a transpeptidase in *Staphylococcus aureus*) is essential for full virulence of SS2.[136,137] However, other sortase paralogs SrtBCD are not associated with bacterial virulence.[145] In addition, we reported the functional definition of di-peptidyl peptidase IV (DPP IV) in *S. suis* 2, and also confirmed that it does contribute greatly to bacterial virulence.[135]

Quorum sensing is a method of bacterial cell density-dependent communication, using secreted chemical molecules (like the auto-inducer) as a form of "language". The *luxS* gene product is the synthetase of autoinducer 2 (AI-2) that is required for interspecies communication. Recently, we and Lu's group both defined a functional LuxS member present in Chinese isolates of *S. suis* 2, and demonstrated its relevance to bacterial virulence.[139,140] Zhang et al. identified that IgA1 protease, an immune-dominant antigen, is necessary for full virulence of *S. suis* 2.[138] Additionally, Gruening et al.[146] proved that the arginine deiminase system (ADS), encoded by *arcABC* operon, is necessary for *S. suis* survival in acidic stress, indicating its possibility of correlating with bacterial successful infection.

Very recently, it was discovered that the adenosine synthase functions as an effector in evasion of PMNs-mediated innate immunity[147]. It might suggest possibility that cyclic AMP (cAMP)-dependent signaling is linked to streptococcal infectivity. Given the fact that CadD enzyme, cAMP deaminase from *Leptospira*,[148] can quench cAMP-dependent signaling, it would be of interest to test the hypothesis that in vivo expression of CadD protein in *S. suis* modulate bacterial pathogenesis or not?

Transcriptional factors/regulators

No less than 16 pleiotropic regulators have been suggested to be involved in modulation of *S. suis* virulence, which consist of nine transcription factors (five well-studied ones [*adcR*,[149] *ccpA*,[118,119] *argR*,[40] *rgg*,[31] a Fur-like repressor PerR[150]] plus four poorly-known transcription factors such as 00SSU0053, *treR*, *nadR*, and *scrR*[100]), five TCS systems (*sal*K-*sal*R [renamed as *suiK-suiR*],[27,151] *cia*R-*cia*H,[42] *ihk-ihr*,[152] *virR/virS*,[153] and *nisK-nisR*[154]), and three orphan regulators (CovR,[30] RevSC21,[155] and RevS[156,157]).

AdcR is a regulator controlling zinc transport in *S. suis*. Aranda and coauthors observed that disruption of this transcription factor can attenuate bacterial virulence in mouse model.[149] In contrast, Zur, the other zinc uptake regulator from 05ZYH33 strain of *S. suis* 2 is not essential for strong pathogenicity in porcine models.[29] Driven by the idea that host environment is critical for expression of bacterial virulence factors during the process of infection, Willenborg et al.[118] evaluated the effect of the sugar metabolism regulator catabolite control protein A (CcpA) on *S. suis* pathogenesis. As anticipated, expression levels of several virulence factors (such as ArcB, Sao, and Enolase) were altered in the Δ*ccpA* mutant. Of particular note, a recent study implied that Sao protective antigen plays a limited role in bacterial virulence.[123] Moreover, the deletion of *ccpA* led to significant reduction of both capsule thickness and resistance to killing by porcine neutrophils[118] and impaired bacterial virulence.[119] ArgR, a member of ArgR/AhrC arginine repressor family, was recently proved to regulate expression of *arcABC* operon encoding an arginine deiminase system that is recognized as a putative virulence factor.[40,146] Therefore, it is of interest to test the role of *argR* in *S. suis* virulence. Similar to the scenario observed with Rgg regulators present in other gram-positive pathogen, Zheng et al. defined an *rgg*-like ortholog in *S. suis* 05ZYH33, and observed its multiple roles in bacterial metabolism. More importantly, it was verified to be a virulence determinant of *S. suis* 2 in experimental models of piglets.[31] Interestingly, an H_2O_2-responsive Fur-like regulator, PerR was confirmed to determine bacterial virulence through regulating expression of both *dpr*, a Dps-like peroxide resistance protein-encoding gene, and *metQIN* encoding a methionine transporter.[150]

Among the 15 putative two-component signal transduction systems in Chinese virulent strain of *S. suis* 2,[23] five have been proposed to be correlated with manifestation of strong virulence.[27,42,152,153] In 2008, we reported the *salK-salR* system present in the 89K pathogenicity island. The deletion of this TCS system resulted in significant downregulation of 26 genes' expression level, and increased its susceptibility to polymorphonuclear leukocyte (PMN)-mediated killing. Consequently, the virulence

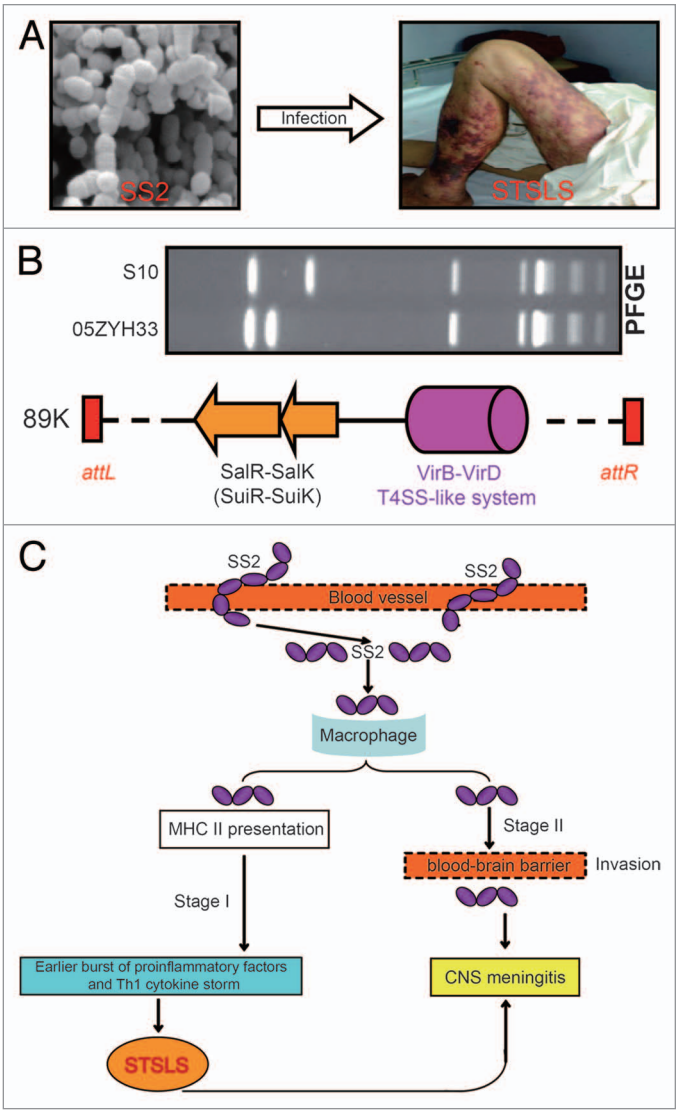

Figure 4. Clinical syndromes, genetic basis and working model for streptococcal toxic shock-like syndrome (STSLS) caused by *S. suis* 2. (**A**) Clinical visualization of a representative SS2-infected patient with streptococcal toxic shock-like syndrome. It clearly shows purpura and gangrenous changes on this patient's legs. Partially adapted from reference 9 with permission. (**B**) Genetic evidence that 89K pathogenicity island carries elements associated with bacterial virulence and the clinical consequence of streptococcal toxic shock-like syndrome. PFGE assay reveals Chinese epidemic strain 05ZYH33 is distinct from the international strain S10.[4] The two genetic elements of the transposable 89K pathogenicity island[23,28] have been functionally defined: one is SalK-SalR (renamed as SuiK-SuiR[151]) TCS with requirement for full virulence,[27] and other is VirD4-VirB4 T4SS-like system associated with clinical manifestation of STSLS in mouse model.[160] (**C**) The proposed model of the two-stage hypothesis for STSLS. SS2 gets into blood vessels in stage I and results in an early burst of proinflammatory factors and Th1 cytokine storms. Such kind of inflammatory super-responses lead to STSLS with death as early as 13 h after SS2 infection. In stage II (i.e., several days post-infection), SS2 might use virulence factors like suilysin to cause disease, particularly meningitis.[94] CNS, central nervous system. Integrated and modified from references 4, 23, 27, 28, 94, and 160 with permission.

of the Δ*salK-R* mutant was seriously attenuated.[27] Shen et al.[158] conducted a follow-up study to address the regulatory network of SalK-SalR TCS system. As expected, proteomics-based investigation revealed 14 downregulated proteins and 1 upregulated protein, which partially agreed with our former microarray analysis.[158] To our much surprise, Zhong's group very recently elucidated the physiological role of this SalK-SalR TCS in regulating a bioactive lantibiotic suicin production, thereby replaced it with SuiK-SuiR.[151]

Li and coworkers[42] reported the second TCS system, *ciaK-ciaR*, which is required for pathogenicity of SS2 in the infection models of both CD1 mice and piglets. Han et al. showed that Ihk/Irr TCS contributes to full virulence of *Streptococcus suis* serotype 2 strain 05ZYH33 via regulating bacterial cell metabolism.[152] Very recently, two more TCS (VirR/VirS[153] and NisK/R[154]) was demonstrated to be essential for SS2 pathogenicity.

Also, an orphan response regulator revSC21 was determined by the same research group.[155] This regulator revSC21 positively modulates expression levels of virulence factors (e.g., *mrp*, *sly*, and *cps*) and is required for bacterial pathogenesis.[155] In contrast, Pan et al. reported another orphan response regulator CovR with an opposite effect on *S. suis* pathogenicity.[30] The *covR*-defective (Δ*covR*) mutant displayed thicker capsules and increased hemolytic activity. Furthermore, adherence of this mutant to epithelial cells was greatly increased, as well as its resistance to phagocytosis and killing by neutrophils and monocytes. Eventually, the removal of *covR* gene was found to be correlated with increased lethality of piglets, relative to those inoculated with its parent virulent strain 05ZYH33.[30]

Others

VirA is a newly-determined virulence factor that is exclusively present in virulent SS2 strain.[122] Trigger factor is also a virulence determinant which acts by controlling expression of a collection of known virulence factors, such as *cps*, *mrp*, and *sly*.[159] *FeoBA* encoding an iron transporter system might represent the first example of the fact that the machinery of a transporter can be involved in *S. suis* pathogenicity.[35] After Li et al.[28] defined transposal characteristics of 89K PAI in epidemic strain of Chinese virulent SS2, Hu's group recently obtained further insights into 89K PAI. In addition to virulence-determining element, *salK-salR* TCS system, 89K PAI also carried a second virulence factor, *virD4-virB4*, a type IV-like secretion system. Unexpectedly, this T4SS-like system is responsible for stimulating host immune reaction observed in mice infected with Chinese streptococcal toxic shock-like syndrome (STSLS)-causing strain of *S. suis* 2 [160], raising a possible relationship of 89K to molecular/immunological machinery by which this non-group A streptococcus (GAS), SS2 to cause STSLS in infected patients (**Fig. 4**). Finally, Wilson et al. also suggested that 05SSU0660, an uncharacterized protein homologous to *S. pneumonia spr*1018 might be a virulence factor.[100]

Host Immunological/Inflammatory Factors

Pathogens have evolved capacity to recruit/hijack host cell factors for their successful infections/invasions. So far, the majority

Table 3. List of protective antigens and/or candidate vaccine molecules from *Streptococcus suis*

Candidate vaccine molecules	Properties	Animal model	Year	References
EF	Extra-cellular factor	Pigs	2001	171
38 kDa	A protein of 38 kDa localized on bacterial surface and/or cell wall	Pigs	2005	104
SLY	Suilysin	Mice	2009/2013	96, 172, and 230
MRP	Muramidase-released protein	Pigs, CD1 mice	2001, 2012	171 and 237
SAO	Surface antigen protein	Mice and piglets	2007	121 and 122
6PGD	6-Phosphogluconate dehydrogenase (6PGD) localized on cell surface	Piglets	2009	238
Enolase	A surface-localized enzyme of central metabolism, catalyzing the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate	Mice	2009	96, 129, 130, and 143
RfeA	RTX family exoprotein A	Mice	2009	172
ESA	Epidermal surface antigen	Mice	2009	172
IBP	Immunoglobulin G (IgG)-binding protein	Mice	2009	172
PAPI-2b	Pilus subunit	Mice	2010	125
SsPepO	A secretary immunogenic protein discovered by immuno-proteomic techniques	Mice/pigs	2011	173
HP0197	An immunogenic protein	Mice/piglets	2009	115
HP0245	in vivo-induced protein located on the cell surface	Mice	2011	239
Lmb	A surface protein	Mice	2013	240
Sat (HP0272)	Immunogenic surface protein Sat	Mice	2010	112 and 174

of host cell factors identified to be involved in *S. suis* pathogenesis are immunological/apoptotic/inflammatory factors.

Nearly ten years ago, Gottschalk et al.[161] had observed that cell wall components of *S. suis* can induce releases of interleukin-1 (IL-1), IL-8, monocyte chemotactic protein-1 (MCP-1) in human brain microvascular endothelial cells (BMEC), which might increase the permeability of the blood-brain barrier. Using the experimental model of CD1 mice, the same group observed that (1) ~20% animals with sudden death exhibit high levels of systemic TNF-α, IL-6, IL-12, IFN-γ, CCL2, CXCL1, and CCL5 24 h after infection; (2) infected mice that survived the early sepsis later developed clinical signs of meningitis present in the transcriptional activation of TLR2, TLR3, CD14, NFκB, IL-1β, CCL2, and TNF-α, mainly in myeloid cells located in affected cerebral structures.[162] Apparently, the inflammatory response plays important roles in *S. suis* infection of CD1 mice. Similar observation was also reported by Scherk et al.[163] that *S. suis* infections are correlated with the release of pro-inflammatory cytokines and chemokines (e.g., IL6 and IL8). Gottschalk et al. found that the toll-like receptor 2 (TLR-2)-deficient exhibit significantly reduced production of astrocytes,[164] and proposed that bacterial polysaccharides probably modulates TLR2-dependent recognition of *S. suis* entry/invasiveness into CD1 mice.[165]

Using a transcriptomic approach, de Greeff et al.[166] identified macrophage-specific genes (IL-1-β, MIP-2-α, and TNF-α) with significantly different expression upon *S. suis* infection, suggesting that MAP-kinase signaling pathway and NFκB signaling are implicated into response of porcine alveolar macrophages to *S. suis* infections. Additionally, Wu et al.[167] verified that a novel murine ribonuclease, angiogenin inhibitor 1 (AI1) can bind to *S. suis* hyaluronidase (Hyl), and hypothesized that this interaction between host AI1 partner and bacterial Hyl protein might contribute to *S. suis* meningitis.

Protective Antigens

Development of a safe and efficient vaccine is a useful strategy to combat against *S. suis* infection. In contrast to conventional killed/live whole-bacteria vaccines,[168-170] engineering of subunit vaccine exhibits significant advantage in its safety and its large-scale producibility.[171] Identification of protective antigens is a prerequisite for identifying candidate vaccine molecules. Totally, there are no less than 15 protective antigens identified thus far (**Table 3**). Among them, four molecules of protective antigen are well-known virulence associated factors and are MRP, EF, 38 kDa, and SLY (**Table 3**). Of note, most of the remaining 10 protein antigens were elucidated by research groups in China. In 2009, in addition to identification of the known protective antigen SLY, Liu's report verified three more new protective antigens that are RTX family exoprotein A (RfeA), epidermal surface antigen (ESA), and immunoglobulin G (IgG)-binding protein (IBP).[172] Two different research groups from China confirmed that enolase, an enzyme of central metabolism, acts as a protective antigen displayed on bacterial surface.[96,129] However, Esgleas and coworker[130] reported an opposite result regarding the protective efficiency of enolase in mice. This discrepancy could be due to different versions of recombinant protein, different strains plus deviations in animal

vaccination protocols. In particular note, all the three newly-identified immunogenic antigens (6PGD, HP0197, and HP245) are elucidated by the same Chen's research group in China (**Table 3**). Interestingly, proteomics also facilitated to discover three new immunogenic proteins SsPepO,[173] Sat (HP0272),[112,174] and the pilus subunit PAPI-2b[125] (**Table 3**). In disagreement with their former observation,[121] Li and coworkers[122] demonstrated that SAO surface antigen can confer efficient protection in both mice and piglets against virulent SS2 infection (**Table 3**).

Zoonotic Potential of *S. suis*

Clinical consequence of human SS2 infections

It is accepted that *S. suis* has developed into a significant human pathogen, especia in southeast Asia, posing a great challenge to public health.[2] Clinically, a collection of disease types can be observed in those patients with *S. suis* infections, including meningitis, septicemia, and pneumonia. In terms of epidemiological/clinical statistics, bacterial meningitis is the most prevalent symptom caused by *S. suis* infection.

Meningitis

Meningitis is medically defined as an inflammation of the lining that covers the brain and spinal cord (the meninges).[10,16,60] This kind of inflammation usually initiates with a brief influenza-like prodroma and results in hearing impairment or loss.[77,175] Generally, meningitis can be grouped into two types, bacterial and non-bacterial (e.g., viral or fungal meningitis).[77] For the former, no less than 10 kinds of bacterial pathogens (such as *Mycobacteria tuberculosis*, *Streptococcus pneumonia*, and *Staphylococcus aureus*) have been determined as the causatives of development of human meningitis.[33,77]

Although SS2 is a swine pathogen, we have come to know that SS2 can also be a severe agent for human bacterial meningitis.[60,88] The first case of human SS2 infection worldwide was recorded in Denmark in 1968.[1] Hui et al. declared that SS2 would be another leading cause of the so-called community-acquired meningitis, which is only inferior to *M. tuberculosis* and *S. pneumonia*.[77] A systematic survey of bacterial meningitis in Vietnamese adults recently suggested that (1) SS2 with multiple virulence factors (e.g., EPF, SLY, MRP) is the most common pathogen and (2) its mortality is relatively low (2.6%), but hearing loss occurs at high percentage (66.4%).[16] Moreover, Hoa et al.[176] addressed the antimicrobial susceptibility profile of *S. suis* strains from meningitis patients, an important question, using comprehensive approaches to analyze bacterial isolates in Vietnam from 1997 to 2008. As result, they found that (1) multidrug resistance in *S. suis* 2 causing meningitis in southern Vietnam has increased over the 11-y period studied; (2) the *tet*(L) carried in these bacteria is functionally expressed, and multiple other genes are probably co-expressed.[176] These findings alerted us again that it is important to minimize abuse of antibiotics in treatment of bacterial infectious diseases, and that the development of new therapeutics against SS2 infections are in great demand. Fortunately, Ho et al.[177] reported the largest prospective epidemiological survey of human SS2 meningitis in Vietnam, and pointed out that three important risk factors associated with human SS2 meningitis included (1) eating "high risk" dishes popular in southeast Asia, (2) occupational exposure to pigs and/or pork-related products, and (3) preparation of pork in the presence of skin lesions. Further investigations from the same research group demonstrated that slaughterhouse pigs are a major reservoir of SS2 that led to human infections of SS2 meningitis in southern Vietnam.[178] It highlighted (1) the importance of an improved hygiene at pork processing facilities, and (2) the necessity for education programs concerning food safety and proper handling of pork.[178]

Similar findings were also noted in the Netherlands[59] and New Zealand.[86,179] In the Netherlands, researchers have clearly indicated that the risk of developing SS2 meningitis among abattoir workers, butchers, and pig breeders are above 1000 times higher than that among persons without close contact with pigs or their unprocessed pork products.[59] To our surprise, a study performed in New Zealand revealed that a high ratio of farmers and meat inspectors in markets were sero-positive to SS2, suggesting the presence of human sub-clinical SS2 infections to a small extent.[86,179]

Septicemia

Septicemia is a serious and life-threatening infection, in which a large amount of bacteria are present in the blood. It is commonly referred to as "blood poisoning" or "bacteremia with sepsis".[1] This disease often causes multi-organ failure by reducing the amount of blood reaching vital organs such as the liver and kidneys.[1,48,75] In addition to the known bacterial pathogens (such as *Staphylococcus*) with capability of leading to septicemia with about 25% lethal rate,[1,180] SS2 is also a causative agent of this disease.[1] In general, SS2-caused septicemia arises as a result of the localized infection in the body, especially cut skin.[1] In patients with septicemia, some of the following symptoms can be observed: fever and chills, rising heart/respiratory rate, cold and clammy feeling, fallen blood pressure, paled ,and petechial skin, and ultimately even unconsciousness. SS2 most likely releases some toxins into the blood that break down the walls of blood vessels which allows blood to leak out under the skin (it is this leaking that causes the rash or petechiae).[33] In some cases, SS2 infects the bloodstream and the meninges at the same time, causing both septicemia and meningitis.[33,181] Due to the rapidly progressive condition of septicemia, it can evolve into an irreversible toxic shock and even acute death if sufferers do not receive urgent treatment.[33,181,182] Certainly, an antibiotic remedy could be altered quickly to treat the target bacteria agents, when medical tests have identified which bacteria cause the septicemia and which antibiotics are best effective.[33,182] Of note, some medical treatments themselves are the inducers of septicemia, e.g., dental treatment, long-term use of intravenous needles, a colostomy, and so on.[33,175] Collectively, septicemia is so serious and complex that it deserves a lot of attention worldwide.

Others

In addition to the above two major forms of SS2-caused diseases, there are some other clinical types that occur less frequently in the cases of human SS2 infections, including arthritis, endocarditis, streptococcal toxic shock-like syndrome (STSLS),

etc. Since STSLS is a newly-described disease form of SS2 infections (**Fig. 4**), here we have discussed it in detail. Toxic-shock syndrome refers to a highly invasive infection of deep tissues, and can be correlated with production of bacterial super-antigens (e.g., staphylococcal and streptococcal exotoxins).[183,184] Before Tang et al.[18] formally proposed that SS2 is another bacterial non-GAS agent responsible for STSLS, we had been aware that *Staphylococcus*[185,186] and *Streptococcus pyogenes*, a group A streptococcus (GAS)[183,184] are both leading pathogens with a capability of causing STSLS. The clinical criteria for diagnosis of STSLS disease can be described as follows: (1) clear erythematous blanching rash (**Fig. 4**), (2) sudden onset of high fever, (3) hypotension diarrhea, (4) blood spots and petechiae, and (5) dysfunction of multiple organs (e.g., disseminated intravascular coagulation and acute renal failure).[8,9,18] According to above clinical criteria, two independent research groups in China reported two big outbreaks of human SS2-caused STSLS, which occurred in Jiangsu Province, 1998, and Sichuan Province, 2005, respectively.[9,17-19,34] Two models have been proposed to explain the molecular mechanism by which SS2 triggers STSLS in its infected patients, one of which is a two-stage hypothesis,[94] the other is a specific 89K PAI.[23,160] Although we came to know that similar findings were also recorded in Thailand,[187] France,[188] and Australia,[84] we did not gain any information on difference of these SS2 strains abroad in comparison with Chinese STSLS-causing isolates. Given that the above two models are based on evidence from studying the Chinese SS2 strains, we can't conclude presently that they represent common or strain-specific mechanisms.

Two kinds of non-SS2 serotypes have been determined in sporadic cases of human *S. suis* infections: One of these is SS14,[13] and the other is SS16.[16] Poggenborg and coworker[189] reported a pig-farm worker with SS14 meningitis and septicaemia complicated with thoracal and lumbar spine spondylodiscitis. Similarly, Ahmed et al.[13] also documented a meningitis case caused by SS14 infections, in a female patient with occupational exposure to piglets each day. Of particular note, totally 12 cases of human SS14 infections were identified in Thailand during 2006–2008, and their clinical presentations included meningitis, septic arthritis and sepsis.[14] Further analyses demonstrated that 11 of the 12 SS14 isolates from these patients belonged to the multilocus sequence types (ST) 105, suggesting clonal dissemination of ST105 strains in Thailand. In another neighboring Asian country, Vietnam, a fatal case of human SS16 infection was revealed by Schultsz's research group,[16] indicating multiple serotypes of *S. suis* are developing into human pathogens circulating in this country. Together, non-SS2 serotypes of *S. suis* have also exhibited its zoonotic potential, highlighting a great demand for monitoring their epidemiology and development of relevant approaches applied toward prevention and therapeutics.

Concluding Remarks and Perspectives

As a zoonotic agent, *S. suis* is gathering increasing accumulated attention from public health officials as well as the relevant academic community.[2,3,8] With respect to *S. suis* epidemiology, we believe that current situation of human SS2 infections should be re-evaluated using comprehensive approaches. To minimize the occupational infections by *S. suis*, it is suggested that advances be made to improve public awareness of *S. suis* infection by science education and popularization. Although progress has been obtained toward understanding *S. suis* pathogenicity (especially identifying a group of bacterial virulence factors), it still sounds fragmentary, and lacks an insightful dissection of integrated regulatory networks of bacterial virulence. In the future, an important direction would be to link posttranscriptional regulation (e.g., ncRNA and riboswitch) and modification (such as acetylation and de-acetylation) to the bacterial virulence of *Streptococcus suis*.[190] Moreover, structural information on these virulence factors is very limited (**Table 2**). We therefore believe it is necessary to strengthen the study of pathogenesis-related structural biology, which could establish a solid basis for design of small molecule drugs targeting bacterial virulence factors.

It is of interest to search for other virulence-related or immunological regulatory elements, different from 89K PAI, because of recent exciting findings on this issue.[160] Safe and effective vaccines that can be used for patients is still not available, which is due to lack of comprehensive knowledge of *S. suis* infections. Description of a full picture of *S. suis* surface antigen proteins might be helpful to screen and/or design vaccine molecules candidate. The fact that non-SS2 serotypes of *S. suis* can affect humans and even lead to fatal infections, has put the situation of *S. suis* infection in a much more complicated and serious status in public health. This might be an alternate way to decode the genome sequences of all 35 kinds of different serotypes, providing new insights into evolution and diversity of heterogeneous species, as well as distinct clues for preventing and controlling severe infections by these pathogens.[191,192] Systematic proteomic approaches are also encouraged to further address this question, which can complement genomics-based explorations.[138]

In summary, our understanding and response to the situation of *S. suis* infections occurring especially in southeast Asia is not satisfactory. It would be helpful to integrate representative virulent/avirulent strains from different countries/regions for collaborative investigations. Therefore, there is a long way to go toward the complete conquest of *S. suis*, an emerging human pathogen.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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