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The role of glutathione for oxidative stress and pathogenicity of *Streptococcus suis*

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ABSTRACT

Streptococcus suis is an important zoonotic pathogen that threatens human and pig health. During infection, the host can impose oxidative stress to resist pathogen invasion. Resistance to oxidative toxicity is an important factor for pathogens. Glutathione synthesis contributes to reactive oxygen species (ROS) detoxification in bacterial cells. Little is known about the roles of glutathione synthesis and transport in *S. suis*. In this study, we demonstrated that glutathione treatment increased oxidative stress tolerance in *S. suis*. GshAB and GshT were found in *S. suis* glutathione synthesis and import by bioinformatics. In vitro, inactivation of *gshAB* and *gshT* led to increased sensitivity to oxidative stress. Inactivation of *gshT* led to growth defects in the medium. The intracellular glutathione content of *gshAB* or *gshT* deletion mutants was lower than that of wild type (WT) strain. The phagocytic resistance of *gshAB* and *gshT* mutants was lower than that of the WT strain. Moreover, the virulence of *gshAB* and *gshT* deletion mutants was significantly lower than that of the WT strain in mouse survival and tissue loading experiments. In conclusion, these results revealed the functions of GshAB and GshT in the pathogenesis of *S. suis*. These findings enhance our understanding of bacterial virulence mechanisms and may provide a new avenue for therapeutic intervention aimed at curbing *S. suis* infections.

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Streptococcus suis; glutathione; oxidative stress; GshAB; GshT; virulence

Introduction

Streptococcus suis (*S. suis*) serotype 2 is an important zoonotic pathogen that can infect swine and humans [1]. *S. suis* can infect humans through direct contact with contaminated raw pork products or infected pigs and can cause streptococcal toxic shock-like syndrome [2]. Two huge outbreaks of *S. suis* infecting humans occurred in 1998 and 2005 in China [3]. Currently, *S. suis* is divided into 29 serotypes, and the main types of *S. suis* infecting humans are type 2 and type 9 in China [4,5].

The host uses oxidative stress as a strategy to defend against invading pathogens, and pathogens develop various mechanisms to protect themselves from oxidative stress, thus evading the host's immune defence [6]. Reactive oxygen species (ROS), including superoxide ions (O^{2-}), hydroxyl radicals (HO.), and hydrogen peroxide (H_2O_2), are effector molecules of oxidative stress applied by the host and cause severe damage to DNA, proteins, and lipids [7]. Bacterial cells detoxify ROS by synthesizing several enzymes, including superoxide dismutases [8], alpha-1-microglobulin [9], and glutathione peroxidases [10]. The ability of bacteria to resist oxidative stress helps them survive in their host [6].

Glutathione (GSH) is the most abundant low-molecular-weight thiol compound that can remove excess ROS [11]. Bacteria can import and synthesize GSH to maintain intracellular GSH content. The ABC transporter substrate binding protein named GshT has been reported to import GSH [12]. GSH synthesis is catalysed by glutamylcysteine synthetase (*gshA*) and glutathione synthetase (*gshB*) [13]. In the presence of H_2O_2, glutathione peroxidase, a selenoprotein oxidoreductase, catalyzes the conversion of GSH to glutathione disulphide (GSSG). This activity maintains cellular redox homoeostasis and protects cells from the deleterious effects of ROS [14,15]. In *Streptococcus pyogenes*, impaired import of glutathione induces oxidative stress [16]. In *Streptococcus agalactiae*, it has been demonstrated that GshAB plays a crucial role in

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oxidative stress tolerance and pathogenesis. The *gshAB* mutant effectively reduced bacterial loads in the blood and had a higher survival rate in a sepsis model [17]. In *Streptococcus mutans*, the *gshAB* mutant exhibited reduced tolerance to H_2O_2 [18]. In *Listeria monocytogenes*, the GSH synthase gene *gshF* contributes to oxidative stress tolerance and virulence [19]. In *Salmonella enterica*, the lack of *gshA* attenuates virulence in an acute model of *Salmonella* infection [20]. GSH transport is important for bacteria, and only a few gram-positive bacteria are capable of producing glutathione. GSH import has been an alternative strategy employed by some bacteria, such as *Streptococcus mutans* and *Streptococcus pneumoniae* [12,21]. Thus, GSH may play an important role in host immune defence by increasing the oxidative stress tolerance of bacteria.

In this study, we investigated the role of GshAB and GshT in *S. suis*. This study revealed that GSH can improve oxidative stress tolerance in *S. suis*. Through bioinformatics analysis, the homology of GshAB and GshT in *S. suis* was identified and found to be highly conserved in different *S. suis* isolates. The inactivation of GshAB and GshT reduced intracellular GSH levels. Finally, we determined that GshAB and GshT play important roles in the virulence of *S. suis*. These results demonstrated that GshAB and GshT contribute to *S. suis* pathogenesis.

Materials and methods

Bacterial strains, plasmids, primers, and growth conditions

The bacterial strains, plasmids, and primers used in this study are listed in Tables S1 and S2 in the supplementary material. *S. suis* strains were cultured in tryptic soy broth (TSB, BD) or tryptic soy agar (TSA, BD) containing 10% (vol/vol) newborn bovine serum (EVERY GREEN, China) at 37°C. *Escherichia coli* DH5α competent cells were grown in Luria-Bertani (LB) broth or on LB agar at 37°C.

Construction of deletion and complemented strains of S. suis

To obtain a markerless deletion mutant strain, the upstream and downstream regions of the target gene were amplified using PCR. Then, the PCR products were cloned into a pSET4s vector following digestion with the corresponding restriction enzymes. The recombinant plasmid was transformed into *S. suis*. Then, the mutant strain was obtained as previously described [22]. After two rounds of allelic exchange at 28°C, spectinomycin-sensitive clones were selected, and the mutants were identified by PCR using both external and internal primers. To obtain complemented strain, the target gene, including its promoter region, was cloned into the plasmid pSET2. The recombinant plasmid was then introduced into the deletion mutant strain by electroporation. The complemented strain was selected using spectinomycin and confirmed by PCR.

Growth curves

Overnight cultures (wild type (WT), Δ*gshAB*, Δ*gshT*, CΔ*gshAB*, and CΔ*gshT* strains) were diluted 100-fold in fresh TSB containing 10% (vol/vol) newborn bovine serum with or without 0.1 mm H_2O_2 or GSH (0.1 mm or 0.5 mm) (BioFroxx, Germany) at 37°C. OD_{600} was measured using an Eppendorf Biophotometer.

H_2O_2 survival assay

The WT, Δ*gshAB*, and CΔ*gshAB* strains were cultured to the mid-log phase. Bacterial solutions were harvested by centrifugation and resuspended in phosphate-buffered saline (PBS). The bacterial solution was then serially diluted 10-fold up to a 10^{-6} dilution, and 5 µL of each dilution was spotted onto TSA plates supplemented with 10% (vol/vol) newborn bovine serum and H_2O_2 (0, 1, or 3 mm). The plates were then incubated at 37°C for 12 h.

Determination of GSH levels

To determine GSH levels in *S. suis*, the WT, Δ*gshAB*, CΔ*gshAB*, Δ*gshT*, and CΔ*gshT* strains were cultured to the mid-log phase. Cells were harvested by centrifugation and resuspended in PBS. The cells were then broken using ultrasonic waves. GSH levels were determined using a routine GSH assay kit (Solarbio, China).

Macrophage anti-phagocytosis experiment

We tested whether GshAB and GshT are involved in the survival of *S. suis* within RAW 264.7 cell line during infection. RAW 264.7 was used to measure the intracellular number of survival WT, Δ*gshAB*, CΔ*gshAB*, Δ*gshT*, and CΔ*gshT* strains. Cells were seeded in 12-well plates, and all *S. suis* strains were added to the cell monolayers at a multiplicity of infection (MOI) of 100. After incubation for 1.5 h, the suspensions were removed and the cells were washed three times with PBS. Then, medium with gentamicin (100 µg/mL) and penicillin-G (5 µg/mL) was used to kill extracellular bacteria. After 40 min, the cells were

washed three times with PBS. The number of viable bacterial cells in the macrophages was calculated. The phagocytosis rate was calculated as follow: number of bacteria per 1 mL cells (3×10^6).

Animal assays

All animal assays were approved by the Animal Care and Utilization Committee of Hubei University of Medicine (Approval No. SYXK2019–0031) in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals. Mice were monitored and euthanized in accordance with ethical animal protocols. A total of 21 female BALB/c mice (5 weeks old) were randomly divided into three groups. For each group, 2×10^8 CFU of bacteria per mouse were injected into the abdominal cavity. Mice were observed daily for symptoms, and monitored and euthanized as required by animal ethics protocols. The mortality rates for each group were recorded daily.

To test the colonization ability of *S. suis* strains in mice, 21 female BALB/c mice (5 weeks old) were randomly separated into three groups. The WT, Δ*gshAB*, and Δ*gshT* strains (5×10^7 CFU bacteria per mouse) were injected into the abdominal cavity. After 24 h, the blood from each mouse was removed via cardiac perfusion. The heart, liver, spleen, lungs, kidneys, and brain were aseptically excised. Homogenized tissues and blood were plated on TSA plates supplemented with 10% (v/v) newborn bovine serum to determine the bacterial loads.

Statistical analysis

The data were analysed using GraphPad Prism 8 software. The Kaplan-Meier survival curve analysis was performed to test for significant differences among the different groups. The statistical significance of differences between the means of three independent groups was compared via one-way analysis of variance. The Student's t test for analysis of variance was used to analyse the results between experimental and control groups. The following significance levels were adopted: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$; ns indicates no significant difference.

Results

GSH increases the ability of S. suis to tolerate oxidative stress

Glutathione (GSH) plays an important role in antioxidative stress in streptococci [23]. To explore the role of GSH in oxidative stress tolerance of *S. suis*, we used H_2O_2 to elucidate the role of GSH in oxidative stress resistance. As shown in Figure 1, GSH improved the H_2O_2 tolerance of *S. suis*. These results demonstrate that glutathione plays a critical role in oxidative stress tolerance during *S. suis* infection.

Bioinformatics analysis of the gshAB gene in S. suis

GshAB synthesizes GSH and is associated with bacterial pathogenicity [17]. The amino acid sequence of RS09230 in *S. suis* exhibited approximately 47.18%, 68.59%, 64.56%, and 65.68% amino acid identity with GshAB from *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus agalactiae*, and *Streptococcus thermophiles* (Figure S1). *RS09230* was named *gshAB*, and we examined the conformance of *gshAB* to different *S. suis* isolates. As shown in Figure 2, *gshAB* in different *S. suis* isolates was highly conserved. To explore the function of *gshAB* in *S. suis*, a deletion

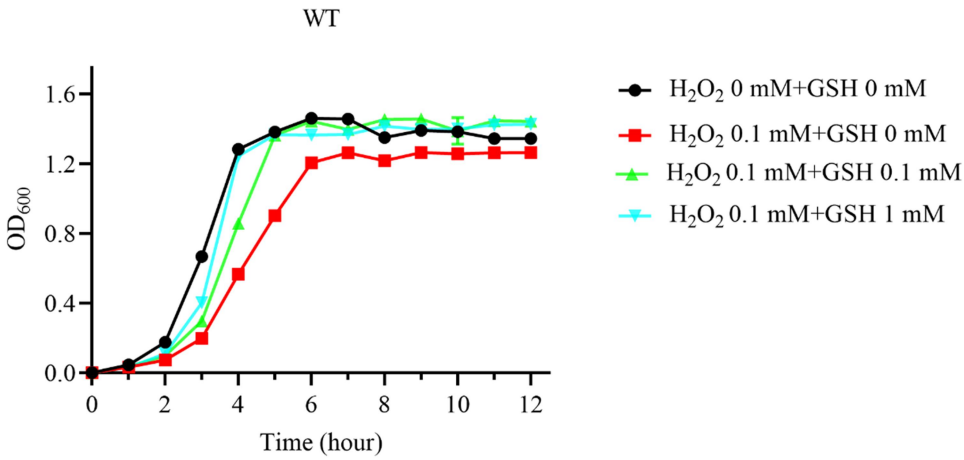

Figure 1. The growth curves of wild-type (WT) strain supplementary with or without H_2O_2 or GSH. The data are expressed as mean ± standard deviations of three independent experiments.

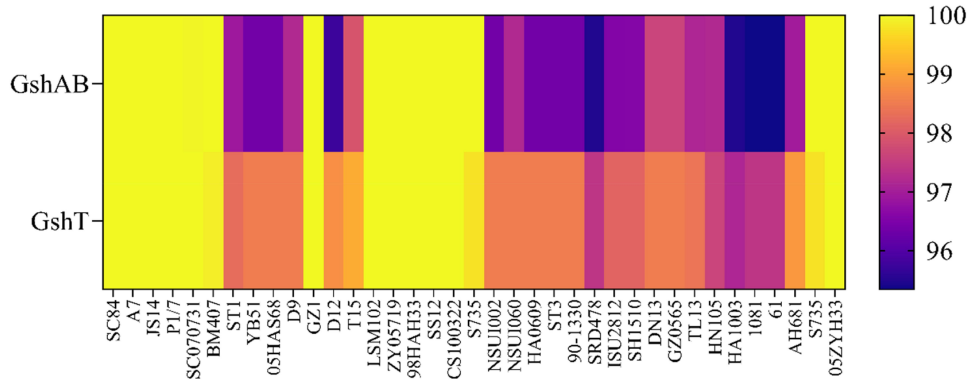

Figure 2. Conservation of GshAB and GshT in different strains of *S. suis*.

mutant strain of *gshAB* was constructed using a marker-less system (Figure S2A-B).

GshAB contributes to resistance to H_2O_2 of S. suis

To verify the role of *gshAB* in oxidative stress tolerance in *S. suis*, the WT, Δ*gshAB*, and CΔ*gshAB* strains were cultured with or without H_2O_2. As shown in Figure 3a, the growth curves of all three strains were almost identical in the medium without supplementary H_2O_2. The Δ*gshAB* strain exhibited a growth defect in the medium with 0.1 mm H_2O_2 compared with the WT and CΔ*gshAB* strains (Figure 3b). Similar results were obtained in spot dilution assays: the Δ*gshAB* strain formed fewer colonies in the presence of 1 mm and 3 mm H_2O_2 (Figure 3c-e). These results indicated that GshAB played an important role in the oxidative stress tolerance of *S. suis*.

GshT contributes to the growth and resistance to H_2O_2 of S. suis

Through bioinformatic analysis, the gene *RS09710* encoding an amino acid ABC transporter substrate-binding protein was identified. The amino acid sequence of *RS09710* in *S. suis* exhibited approximately 31.41% and 28.82% amino acid identity with GshT from *Streptococcus pneumoniae* and *Streptococcus mutans* (Figure S3). *RS09710* was named *gshT*, and we examined the conformance of *gshT* to different *S. suis* isolates. As shown in Figure 2, *gshT* in different *S. suis* isolates was highly conserved among different isolates. A *gshT* deletion mutant was constructed (Figure S2C), and the deletion mutant exhibited growth restriction in the medium (Figure 4a). After adding GSH to the medium, the growth of *gshT* deletion mutant was restored. These results indicate that GshT may be related to growth in a low-GSH environment.

To verify the role of *gshT* in oxidative stress tolerance in *S. suis*, the WT, Δ*gshT*, and CΔ*gshT* strains were cultured with or without H_2O_2. As shown in Figure 4b-c, the Δ*gshT* strain exhibited a growth defect in the medium with 0.1 mm H_2O_2 compared with the WT and CΔ*gshT* strains. Similar results were obtained in spot dilution assays (Figure 4d). These results indicated that GshT was related to growth and played an important role in the oxidative stress tolerance of *S. suis*.

GshAB and GshT improves the intracellular GSH level of S. suis

To evaluate the importance of GSH in *S. suis*, the total GSH levels in WT, Δ*gshAB*, Δ*gshT*, CΔ*gshAB*, and CΔ*gshT* were measured. As expected, the *gshAB* and *gshT* deletion strains had lower GSH levels than the WT, CΔ*gshAB*, and CΔ*gshT* strains (Figure 5). These data revealed that GshAB and GshT contributed to an increase in the GSH content of *S. suis*.

GshAB and GshT contribute to S. suis resisting phagocytosis against macrophages

In order to test the anti-macrophage phagocytosis ability of deletion mutants. In this study, we tested the ability of *S. suis* to resist phagocytosis by RAW 264.7. As shown in Figure 6, the number of Δ*gshAB* and Δ*gshT* strains entering the RAW 264.7 were higher than those of the WT and complemented strains. These results revealed that GshAB and GshT contributed to phagocytosis resistance of *S. suis*.

GshAB and GshT contributes to the virulence of S. suis in a mouse model

To determine whether GshAB and GshT in *S. suis* are involved in virulence, a mouse model was used to test

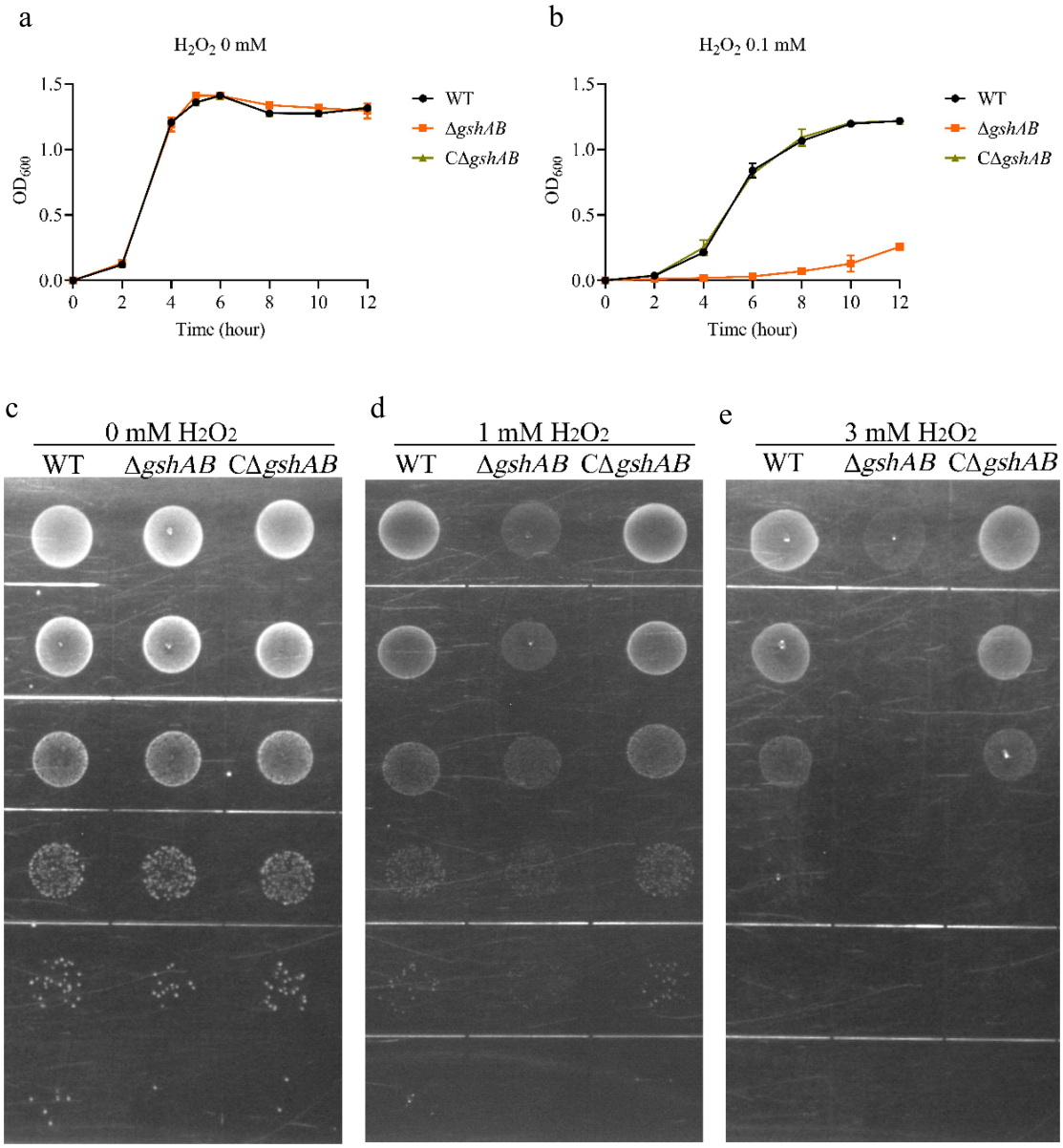

Figure 3. (a-b) growth curves of WT, Δ*gshAB*, and CΔ*gshAB* strains in the absence or presence of 0.1 mm H_2O_2. The data are expressed as mean ± standard deviations of three independent experiments. (c-e) spot dilution assays of WT, Δ*gshAB*, and CΔ*gshAB* strains with or without 1 or 3 mm H_2O_2.

the virulence of *gshAB* and *gshT* mutants. As shown in Figure 7a, the survival rates of animals infected with Δ*gshAB* and Δ*gshT* were 42.86% and 57.14%, respectively, while the survival rate of animals infected with the WT strain was 0%. Furthermore, the colonization abilities of these strains in the blood, heart, liver, spleen, lung, kidney, and brain tissues were tested. After 24 h of infection, the bacterial counts of Δ*gshAB* and Δ*gshT* were significantly lower than those in the WT-infected group (Figure 7b-h). These results demonstrate that GshAB and GshT contribute to the virulence of *S. suis* in the mouse model.

Discussion

Most streptococci are opportunistic and would encounter situations of oxidative stress during infection [24]. Reducing intracellular ROS levels is important in bacteria. The goal of this study was to investigate how *S. suis* resists oxidative stress via glutathione (GSH). GSH, an antioxidant, can maintain intracellular redox homoeostasis and is the most abundant antioxidant molecule in cells [25,26]. Here, we revealed that *S. suis* encodes a gene named *gshAB* that synthesizes GSH and a gene named *gshT* that transports GSH.

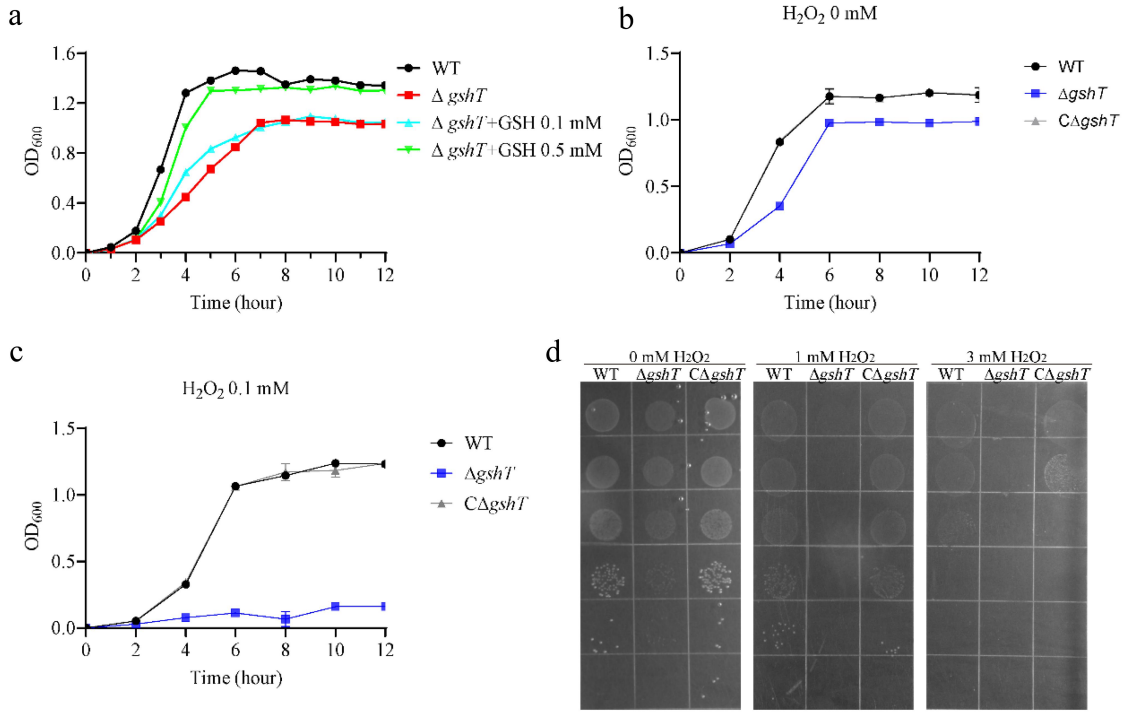

Figure 4. (a) growth curves of WT and Δ*gshT* in the absence or presence of 0.1 or 0.5 mm GSH. (b-c) growth curves of WT, Δ*gshT*, and CΔ*gshT* strains in the absence or presence of 0.1 mm H_2O_2. (d) Spot dilution assays of the WT, Δ*gshT*, and CΔ*gshT* strains with or without 1 or 3 mm H_2O_2. The data are expressed as mean ± standard deviations of three independent experiments.

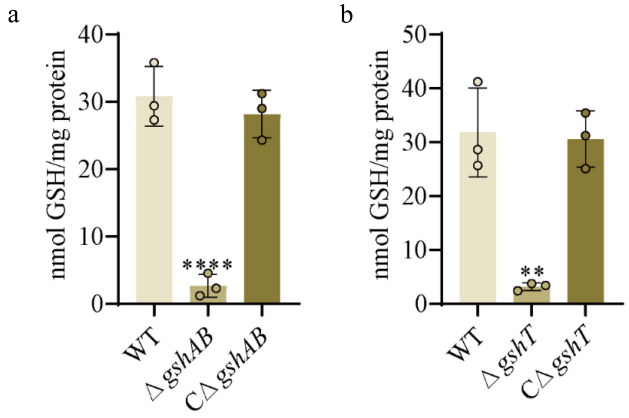

Figure 5. Quantification of intracellular levels of total GSH in WT, Δ*gshAB*, CΔ*gshAB*, Δ*gshT*, and CΔ*gshT* strains. The data are expressed as mean ± standard deviations of three independent experiments. Statistically significant differences were determined via one-way analysis of variance (**$p < 0.01$, ****$p < 0.0001$).

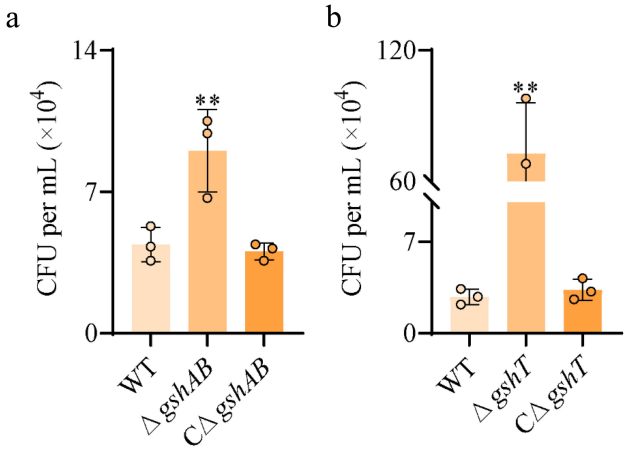

Figure 6. (a-b) the intracellular number of *S. suis* in RAW 264.7. The data are expressed as mean ± standard deviations of three independent experiments. Statistically significant differences were determined via one-way analysis of variance (**$p < 0.01$).

S. suis uses GshAB and GshT to respond to oxidative stress and contribute to its virulence.

Innate immunity is the first line of host defence against pathogens and is initiated along with ROS production. An increased ROS-induced oxidative stress response plays a critical role in the host defence against antimicrobial activity [6]. After being engulfed by phagocytes, pathogens are targeted by ROS [27]. Neutrophils use an "oxidative burst" to kill pathogenic bacteria during host-pathogen interactions, which is very important for bacteria to resist oxidative stress [28]. Many strategies, such as antioxidant enzymes and the repair of oxidized proteins, are used to counteract host oxidative pressure [29]. Our previous study revealed that in the presence of Mn, *S. suis* is capable of fighting against the deleterious consequences of oxidants [30]. Additionally, the intake of

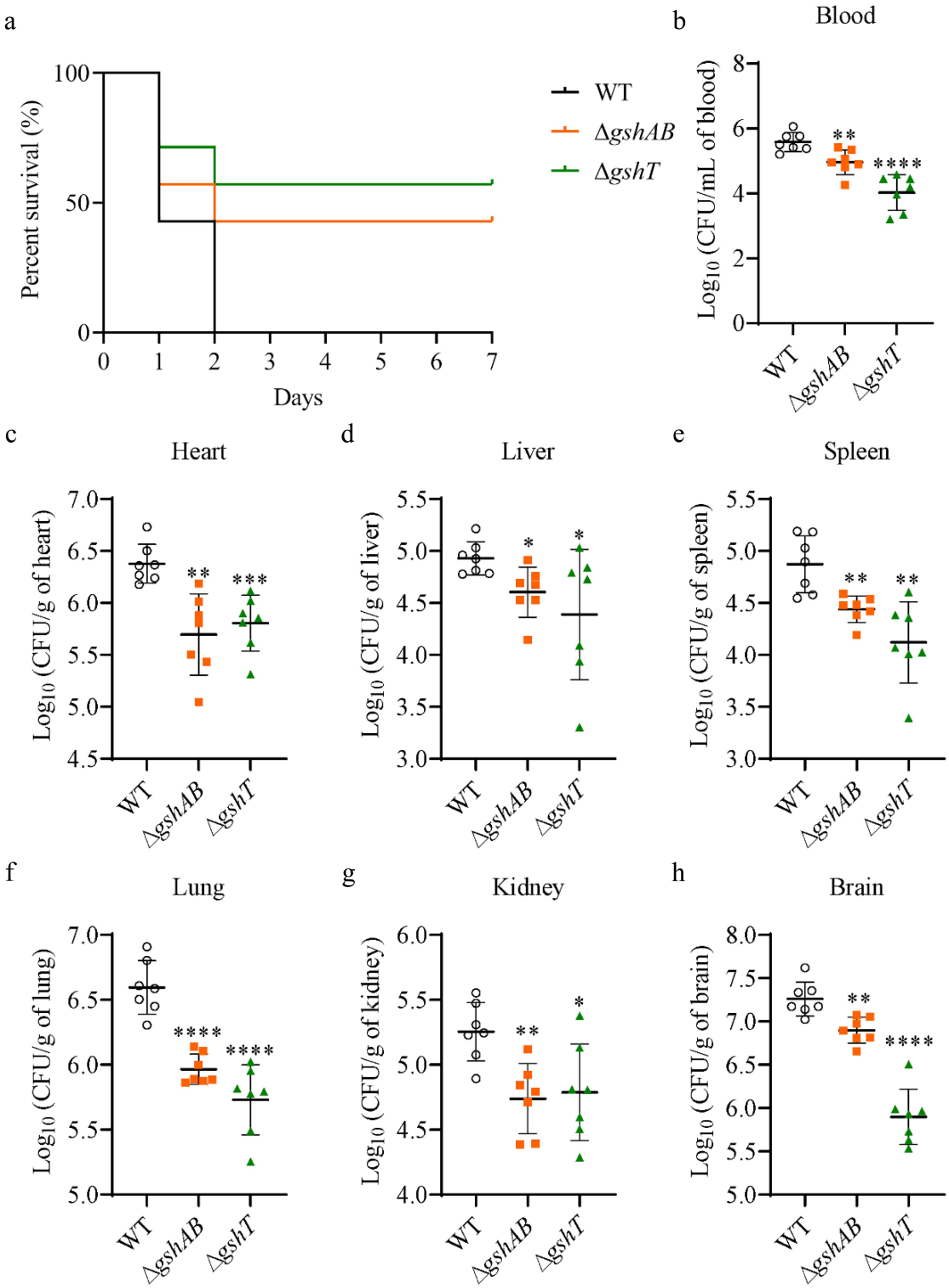

Figure 7. GshAB and GshT contributed to virulence of *S. suis*. (a) The animal survival experiment in mouse. (b-h) the animal tissue bacterial load experiment in mouse. The Kaplan-Meier survival curve analysis was performed to test for significant differences among the different groups. Statistically significant differences of bacterial load were determined via Student's *t* test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

GSH from the host is regarded as a new mechanism for combating redox stress caused by innate immune attacks [16]. The GSH/GSSG couple can be considered the main redox buffer for cells [31]. In this study, we found that GSH increased the ability of oxidative stress tolerance of *S. suis*.

Remarkably, not all bacteria can synthesize GSH; therefore, they need to benefit from host-derived GSH, which is used to combat oxidative stress [16,17]. In this study, we revealed that *S. suis* used GshT and GshAB to maintain intracellular GSH content. Among the different *S. suis* isolates, the *gshAB* and *gshT* DNA sequences were highly conserved. This may be a common antioxidant stress mechanism in *S. suis*. GshAB catalyzes GSH synthesis, and GSH is then oxidized to GSSG under the catalysis of glutathione peroxidase, accompanied by the reduction of toxic peroxides to non-toxic hydroxyl compounds [15,16]. GshT plays an anti-oxidative stress role by transporting GSH [16]. The mutant of *gshT* showed a marked growth defect and was unable to reach a culture density equivalent to that of WT [16], the similar result was observation in this study. GSH supplementation was sufficient to restore growth of Δ*gshT*, there might be other low affinity GSH import systems.

Previous studies have reported that GshAB and GshT play critical roles in oxidative stress tolerance [16,32]. In *Streptococcus mutans*, the deletion of *gshAB* affects the S-glutathionylation of many proteins involved in various oxidoreductase and peroxidase activities [33]. In this study, deletion of *gshAB* and *gshT* decreased the intracellular GSH content of *S. suis*. These results are in accordance with those reported for other streptococci [17,18]. To provide definitive insights into the significance of GSH in *S. suis* fitness and pathogenesis, we tried to constructed the the double deletion mutant of *gshAB* and *gshT*. Unfortunately, we failed to obtain the double deletion mutant under the current experimental conditions.

GSH has been reported as a virulence switch in bacteria [34]. As an important redox system for maintaining metabolism and homoeostasis in many bacteria, GSH maintains optimal bacterial growth and survival and contributes to bacterial pathogenicity [34]. To determine whether GSH synthesis affected the virulence of *S. suis*, we used a mouse model to evaluate the virulence of the Δ*gshAB* and Δ*gshT* strains. Similar results were observed in this study. The *gshAB* and *gshT* mutants exhibited low lethality and tissue load in a mouse model.

Collectively, these results revealed that GSH increased the ability of *S. suis*. Our results offer fundamental insights into the antioxidative stress mechanism that enables extracellular bacterial pathogens to utilize GSH. This provides a fundamental idea for *S. suis* to adapt to its host.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

XY and WB conceived and designed the experiments. WP and QJ performed experiments, interpreted data, and wrote the manuscript. YW and LH performed experiments. BL performed interpreted data. All authors approved the final version to be published and agreed to be accountable for all aspects of this work.

Data availability statement

The data supporting the findings of this study are available at Figshare https://doi.org/10.6084/m9.figshare.27297762

Ethical statements

All animal assays were approved by the Animal Care and Utilization Committee of Hubei University of Medicine (Approval No. SYXK2019–0031) in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals. A complete ARRIVE guidelines checklist appears in supplementary materials the ARRIVE Guidelines checklist.

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References

[1] Bonifait L, Veillette M, Letourneau V, et al. Detection of *Streptococcus suis* in bioaerosols of swine

confinement buildings. Appl Environ Microbiol. 2014;80(11):3296–3304. doi: 10.1128/AEM.04167-13

[2] Segura M, Fittipaldi N, Calzas C, et al. Critical *streptococcus suis* virulence factors: are they all really critical? Trends Microbiol. 2017;25(7):585–599. doi: 10.1016/j.tim.2017.02.005

[3] Yu H, Jing H, Chen Z, et al. Groups *Streptococcus suis* study, human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis. 2006;12(6):914–920. doi: 10.3201/eid1206.051194

[4] Okura M, Osaki M, Nomoto R, et al. Current taxonomical situation of *Streptococcus suis*. Pathogens. 2016;5(3):45. doi: 10.3390/pathogens5030045

[5] Yang H, Huang J, Hu X, et al. Comparative genome analysis of *Streptococcus suis* serotype 9 isolates from China, the Netherland, and the U.K. Life (Basel). 2021;11(12):1324. doi: 10.3390/life11121324

[6] Lushchak VI. Adaptive response to oxidative stress: bacteria, fungi, plants and animals. Comp Biochem Physiol C Toxicol Pharmacol. 2011;153(2):175–190. doi: 10.1016/j.cbpc.2010.10.004

[7] Xia X, Qin W, Zhu H, et al. How *Streptococcus suis* serotype 2 attempts to avoid attack by host immune defenses. J Microbiol Immunol Infect. 2019;52(4):516–525. doi: 10.1016/j.jmii.2019.03.003

[8] Lynch M, Kuramitsu H. Expression and role of superoxide dismutases (SOD) in pathogenic bacteria. Microbes Infect. 2000;2(10):1245–1255. doi: 10.1016/S1286-4579(00)01278-8

[9] Okumura CYM, Anderson EL, Doehrmann S, et al. IgG protease Mac/IdeS is not essential for phagocyte resistance or mouse virulence of M1T1 group a streptococcus. MBio. 2013;4(4):e00499–13. doi: 10.1128/mBio.00499-13

[10] Moore TD, Sparling PF. Isolation and identification of a glutathione peroxidase homolog gene, *gpxA*, present in *Neisseria meningitidis* but absent in *Neisseria gonorrhoeae*. Infect Immun. 1995;63(4):1603–1607. doi: 10.1128/iai.63.4.1603-1607.1995

[11] Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009;30(1–2):1–12. doi: 10.1016/j.mam.2008.08.006

[12] Potter AJ, Trappetti C, Paton JC. *Streptococcus pneumoniae* uses glutathione to defend against oxidative stress and metal ion toxicity. J Bacteriol. 2012;194(22):6248–6254. doi: 10.1128/JB.01393-12

[13] Masip L, Veeravalli K, Georgiou G. The many faces of glutathione in bacteria. Antioxid Redox Signal. 2006;8(5–6):753–762. doi: 10.1089/ars.2006.8.753

[14] Arthur JR. The glutathione peroxidases. Cell Mol Life Sci. 2000;57(13):1825–1835. doi: 10.1007/PL00000664

[15] Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. Methods Enzymol. 2002;348:93–112.

[16] Brouwer S, Jespersen MG, Ong CY, et al. *Streptococcus pyogenes* hijacks host glutathione for growth and innate immune evasion. MBio. 2022;13(3):e0067622. doi: 10.1128/mbio.00676-22

[17] Walker EA, Port GC, Caparon MG, et al. Glutathione synthesis contributes to virulence of *Streptococcus agalactiae* in a murine model of sepsis. J Bacteriol. 2019;201(20):e00367–19. doi: 10.1128/JB.00367-19

[18] Zheng X, Zhang K, Zhou X, et al. Involvement of *gshAB* in the interspecies competition within oral biofilm. J Dent Res. 2013;92(9):819–824. doi: 10.1177/0022034513498598

[19] Reniere ML, Whiteley AT, Hamilton KL, et al. Glutathione activates virulence gene expression of an intracellular pathogen. Nature. 2015;517(7533):170–173. doi: 10.1038/nature14029

[20] Song M, Husain M, Jones-Carson J, et al. Low-molecular-weight thiol-dependent antioxidant and antinitrosative defences in *Salmonella* pathogenesis. Mol Microbiol. 2013;87(3):609–622. doi: 10.1111/mmi.12119

[21] Vergauwen B, Verstraete K, Senadheera DB, et al. Molecular and structural basis of glutathione import in gram-positive bacteria via GshT and the cystine ABC importer TcyBC of *Streptococcus mutans*. Mol Microbiol. 2013;89(2):288–303. doi: 10.1111/mmi.12274

[22] Takamatsu D, Osaki M, Sekizaki T. Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. Plasmid. 2001;46(2):140–148. doi: 10.1006/plas.2001.1532

[23] Kusuhara S, Ito M, Sato T, et al. Intracellular GSH of *Streptococcus thermophilus* shows anti-oxidative activity against low-density lipoprotein oxidation in vitro and in a hyperlipidaemic hamster model. Benef Microbes. 2018;9(1):143–152. doi: 10.3920/BM2017.0065

[24] Sader HS, Streit JM, Fritsche TR, et al. Antimicrobial susceptibility of gram-positive bacteria isolated from European medical centres: results of the daptomycin surveillance programme (2002–2004). Clin Microbiol Infect. 2006;12(9):844–852. doi: 10.1111/j.1469-0691.2006.01550.x

[25] Henningham A, Dohrmann S, Nizet V, et al. Mechanisms of group a *Streptococcus* resistance to reactive oxygen species. FEMS Microbiol Rev. 2015;39(4):488–508. doi: 10.1093/femsre/fuu009

[26] Jamieson DJ. Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. Yeast. 1998;14(16):1511–1527. doi: 10.1002/(SICI)1097-0061(199812)14:16<1511::AID-YEA356>3.0.CO;2-S

[27] Lauridsen C. From oxidative stress to inflammation: redox balance and immune system. Poult Sci. 2019;98(10):4240–4246. doi: 10.3382/ps/pey407

[28] Mortaz E, Alipoor SD, Adcock IM, et al. Update on neutrophil function in severe inflammation. Front Immunol. 2018;9:2171. doi: 10.3389/fimmu.2018.02171

[29] Beavers WN, Skaar EP, Napier B. Neutrophil-generated oxidative stress and protein damage in *staphylococcus aureus*. Pathog Dis. 2016;74(6):ftw060. doi: 10.1093/femspd/ftw060

[30] Peng W, Yang X, Wang Y, et al. Mn uptake system affects the virulence of *Streptococcus suis* by mediating

oxidative stress. Vet Microbiol. 2022;272:109518. doi: 10.1016/j.vetmic.2022.109518

[31] Stewart LJ, Ong CY, Zhang MM, et al. Role of glutathione in buffering excess intracellular copper in *Streptococcus pyogenes*. MBio. 2020;11(6):11. doi: 10.1128/mBio.02804-20

[32] Lin J, Xie J, Luo L, et al. Characterization of GshAB of *tetragenococcus halophilus*: a two-domain glutathione synthetase. Appl Microbiol Biotechnol. 2023;107(9):2997–3008. doi: 10.1007/s00253-023-12497-1

[33] Li Z, Zhang C, Li C, et al. S-glutathionylation proteome profiling reveals a crucial role of a thioredoxin-like protein in interspecies competition and cariogenecity of *Streptococcus mutans*. PLOS Pathog. 2020;16(7):e1008774. doi: 10.1371/journal.ppat.1008774

[34] Ku JWK, Gan YH. New roles for glutathione: modulators of bacterial virulence and pathogenesis. Redox Biol. 2021;44:102012. doi: 10.1016/j.redox.2021.102012