

Original Research Articles

Comparative analysis of *Streptococcus agalactiae* S03 and S07 isolated from *Schizothorax* spp. with different antibiotic resistance and virulence

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Streptococcus agalactiae (GBS) is an important Gram-positive pathogen of fish aquaculture worldwide. In this study, we performed a comparative analysis of GBS S03 (serotype III) and S07 (serotype Ia) isolated from *Schizothorax* spp.. We explored the association between phenotypic antibiotic resistance, virulence, and genomic characteristics. Antimicrobial sensitivity tests on 12 common antibiotics using the disc diffusion method revealed that the GBS S03 resisted seven antibiotics, while GBS S07 showed sensitivity to eleven antibiotics. Pathogenicity analysis demonstrated greater virulence of GBS S07 than S03. Then, the occurrence of antibiotic resistance and virulence genes were identified using the whole-genome sequence (WGS) of GBS S03 and GBS S07. Besides just GBS S03 had *gyrA* and *parC* gene mutations, there were *mefE*, *tetO*, *lnuB*, *lsaE*, *APH3'*, and *sat-4* resistance genes present only in GBS S03 genome. In addition to 51 virulence genes in both GBS S03 and S07 genomes, GBS S07 carried virulence genes associated with invasion, such as *SAN_1519*, *rfbA* and *cylE* genes. There was complete concordance between genotypic evidence and phenotypic characteristics. Virulence factors and phylogenetic analysis showed that GBS S03 and human sources shared an extremely close evolutionary relationship. Our findings also provide important proof for using WGS as an effective tool for phenotypic predictions of GBS.

INTRODUCTION

Streptococcus agalactiae, or group B *streptococcus* (GBS), is an important Gram-positive pathogen responsible for morbidity and mortality in fish aquaculture worldwide.¹⁻³ GBS is also an emerging pathogen associated with neonatal meningitis in humans, mastitis in cows, and hematosepsis in rabbits.^{2,4,5,6} GBS can be classified into ten serotypes (Ia, Ib, and II to IX). The serotypes Ia, Ib, and III are regarded as the most predominant in fish GBS infection.^{7,8} In fish farms, transmission of GBS is possible through direct contact among individuals with cohabitation or indirectly through immersion in contaminated water of culture systems.⁹

Whole-genome sequencing (WGS) technology has become a fast and affordable tool that assists research in genetics, microbiology, ecology, and public health surveillance and response.¹⁰ WGS captures the full extent of bacterial genomic diversification and allows for genome-wide

comparisons of clinical isolates with each other.¹¹ Comparative genome analysis between bacterial strains, which are greatly different in host specificity or virulence, may help to rapidly screen for dispensable genes, gene deletions or mutations, and differentially-expressed proteins.¹² Thus, WGS is an effective way of studying the mechanisms of cross-host infection, immunogenicity, pathogenicity, and resistance genotypes of bacteria. At present, hundreds of complete genomes of GBS have been sequenced. However, our understanding of the piscine GBS at the whole genome level is currently limited. Although some complete genome sequences and draft genome sequences of piscine GBS isolates have been recorded so far,^{9,13-17} the correlation between WGS analysis and phenotypic characteristics has not been reported. Here, this is the first report on draft genomes of GBS S03 and S07 isolated from *Schizothorax* spp. with different pathogenicity and drug resistance. Draft genomes of GBS S03 and S07 are important additions to the GenBank database of piscine GBS.

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In the current study, antimicrobial susceptibility testing, experimental infection, and WGS analysis were carried out to compare two GBS clinical strains isolated from *Schizothorax* spp. Phylogenetic analysis was performed to identify their evolutionary relationships in GBS. We attempted to assess the use of WGS to evaluate the genomic diversity and genotypic prediction of drug-resistant phenotype and pathogenicity of them.

MATERIALS AND METHODS

BACTERIAL STRAINS

GBS S03 and S07 were isolated from diseased *Schizothorax* spp., with characteristic clinical and pathogenic meningoencephalitis. These strains were preserved in the College of Veterinary Medicine, Sichuan Agricultural University, China. Serotyping assigned GBS S03 to serotype III and GBS S07 to serotype Ia by cps cluster as described previously.¹⁸

ANTIMICROBIAL SUSCEPTIBILITY TESTS

Antimicrobial susceptibility to vancomycin (VAN), penicillin (PEN), cephalexin (CFX), enrofloxacin (ENR), ofloxacin (OFX), norfloxacin (NOR), tetracycline (TET), doxycycline (DOX), erythromycin (ERY), gentamicin (GEN), florfenicol (FLO) and clindamycin (CLIN) was performed by the disc diffusion method. Antimicrobial susceptibility test results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints.¹⁹ *Escherichia coli* (ATCC 25922) was used as the reference strain for quality control according to CLSI guidelines. The results were collected from three repeated independent tests.

PATHOGENICITY EXPERIMENTS

To confirm the pathogenicity and virulence of GBS S03 and S07, we injected the two strain suspensions into healthy *Schizothorax* spp. and *Danio rerio*, intraperitoneally. Healthy *Schizothorax* spp. (11.5 ± 1.2 g) and *Danio rerio* (0.4 ± 0.05 g) were purchased from Sichuan Ya-fish Company and Model Animal Research Center of Nanjing University, respectively. 260 *Schizothorax* and *Danio rerio* were kept in 13 groups equally, respectively, in 120-L plastic tanks ($n = 20$ /tank or group) with aeration and were acclimatized at $24 \pm 1^\circ\text{C}$ for 7 days. The *Schizothorax* of 6 experiment groups was challenged with 0.1 ml of bacterial suspensions of the GBS S03 and the other 6 groups were injected with the same volume of GBS S07 suspensions in sterile 0.8% NaCl solution at concentrations of 1.0×10^4 , 1.0×10^5 , 1.0×10^6 , 1.0×10^7 , 1.0×10^8 , 1.0×10^9 cfu·ml⁻¹. The control fish ($n = 20$) were injected with 0.1 ml 0.8% NaCl solution. Similarly, the *Danio rerio* of test groups were injected intraperitoneally with 0.05 mL of bacterial suspensions of the GBS S03 and S07 in sterile 0.8% NaCl solution at the abovementioned concentrations. The control animals were injected with 0.05 mL 0.8% NaCl solution. Mortality was recorded daily for 21 d and mean lethal dose (LD50) values were calculated. All the fish were euthanized by using 300 mg·L⁻¹ MS222 (yuanye Bio. Co. Ltd., Shanghai, China).

WHOLE GENOME SEQUENCING AND ANNOTATION

To explore further differences between GBS S03 and S07, their draft genomes were sequenced and analyzed at whole genome level. The genomic DNA of the two strains were extracted using the TIANamp bacteria DNA kit (Tiangen, Beijing, China) and applied for library preparation using the Nextera DNA sample preparation kit (Illumina, San Diego, USA). The samples were accessed for the genome sequencing on an Illumina HiSeq™ 2000 (Illumina Inc., San Diego, USA) using a paired-end 2×100 bp protocol. The whole genome sequences were assembled using CLC Genomics Workbench 10.0 software (QIAGEN, Hilden, Germany). Contigs were initially annotated using Rapid Annotations using Subsystems Technology (RAST) and then manually checked. Resistance-related genes of the two strains were analyzed using the ResFinder 2.1 server.²⁰ Gene clusters were classified into COG (Cluster of Orthologous Groups of proteins) categories (<http://www.ncbi.nlm.nih.gov/COG/>) according to BLAST annotations.

IDENTIFICATION OF ANTIBIOTIC RESISTANCE GENES AND VIRULENCE FACTORS

To find antibiotic-resistant genes in two GBS strains genomes, known antibiotic resistance genes were downloaded from the Comprehensive Antibiotic Resistance Database (CARD) and aligned with all coding sequences (CDSs) of those genomes using BLAST (<https://card.mcmaster.ca/>). A similar method has been applied to identify virulence genes from the Virulence Factor Database (VFDB) (<http://www.mgc.ac.cn/VFs/>).

PHYLOGENETIC TREE

A phylogenetic tree of GBS strains was constructed based on seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkt*) as previously described.²¹ The alignments of these genes were concatenated into a single sequence alignment and a maximum likelihood tree was reconstructed using MEGA (version 10.0). The allele number and sequence type (ST) were assigned by the multilocus sequence typing (MLST) website (<https://pubmlst.org/organisms/streptococcus-agalactiae>).

RESULTS

GENERAL FEATURES OF THE GBS S03 AND S07 GENOMES

Characteristics of the two genomes are summarized in **Table 1**. The circular maps of the two strains showed that the genome of GBS S03 was 2,156,979 bp in length (**Figure 1a**), while that of GBS S07 was 1,790,578 bp (**Figure 1b**). The genome sequence of both strains showed 35% G+C contents. RAST server predicted the 2,160 and 1,641 CDSs for GBS S03 and S07 strains, respectively. Based on the COG classification of GBS S03 and S07, 23.7% and 22.6% of CDSs in GBS S03 were responsible for information storage and processing, whereas 37.5% and 39.4% of CDSs in GBS S07

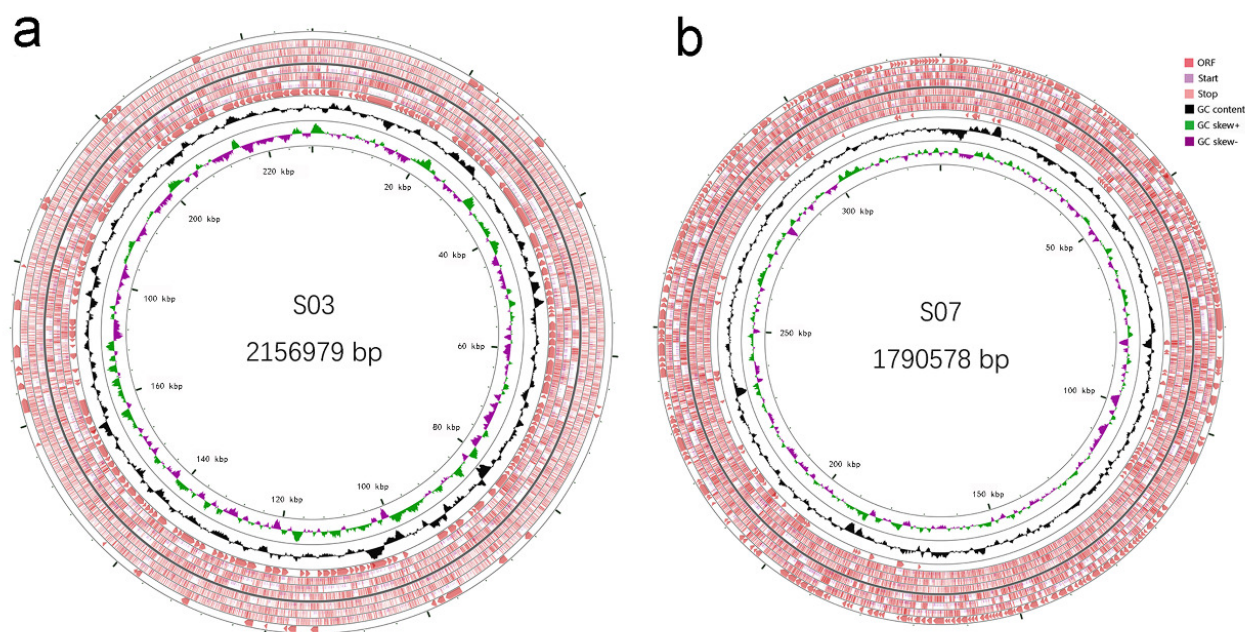


Figure 1. Circular maps of the GBS S03 (a) and GBS S07 (b) genome, generated using cgview (version 2.2.2); from outside to inside, the tracks display the following information: ORFs of S03/S07 on the + strand (1-4); ORFs of GBS S03/S07 on the - strand (5-8); GC content (9), GC skew with + value (green) and - value (purple).

Table 1. General features of GBS S03 and S07

Features	S03	S07
Host	<i>Schizothorax prenati</i>	<i>Schizothorax pylzovi</i>
Sequence type	ST19	ST891
Serotyping	III	Ia
Genome size (bp)	2,156,979	1,790,578
G + C content (%)	35	35
Total CDSs	2,160	1,641
CDSs responsible for information storage and processing (%)	23.7	22.6
CDSs responsible for metabolism (%)	37.5	39.4
Transfer RNA	42	29

were for metabolism (Figure 2). The genome sequences of GBS S03 and S07 have been deposited in the NCBI GenBank database under the accession numbers NAPX000000000 and NHZY000000000, respectively.

ANTIBIOTIC RESISTANCE PROFILE AND ANTIBIOTICS RESISTANCE GENES

Results of three repeated antimicrobial susceptibility tests for GBS S03 and S07 are given in Table 2. Antimicrobial tests revealed that GBS S03 showed drug resistance against macrolide, fluoroquinolone and tetracycline. In this study, a set of 12 antimicrobials was employed, and it was shown that S03 exhibited resistance to 7 of these antimicrobials, resulting in a resistance rate of 58.3%. By contrast, GBS S07 was sensitive to 11 tested antibiotics except enrofloxacin (intermediate), the resistance rate in this study was 0. The

results indicated that the drug resistance of GBS S03 was stronger than that of S07 and had multiple drug resistance.

The genomes of GBS S03 and S07 showed that GBS S03 had 20 resistance genes, while GBS S07 had 14 resistance genes (Table 3). In addition to two macrolide resistant genes (*macB* and *ermB*) and one tetracycline resistant gene *tetM*, GBS S03 also had *mefE* and *tetO* genes. Meanwhile, GBS S03 had additionally *lnuB*, *lsaE*, *APH3'*, and *sat-4* genes not present in GBS S07. Both strains carried genes encoding resistance to fluoroquinolone: *parC*, *gyrA*, *gyrB*, *norA* and *norB*, however, just GBS S03 had chromosomal *gyrA* [81:S-L] and *parC* [79:S-Y] mutations (Figure 3).

PATHOGENICITY EXPERIMENTS AND VIRULENCE GENES

At 21 days post-infection (dpi), GBS S07 induced higher levels of mortality and revealed a greater virulence than GBS

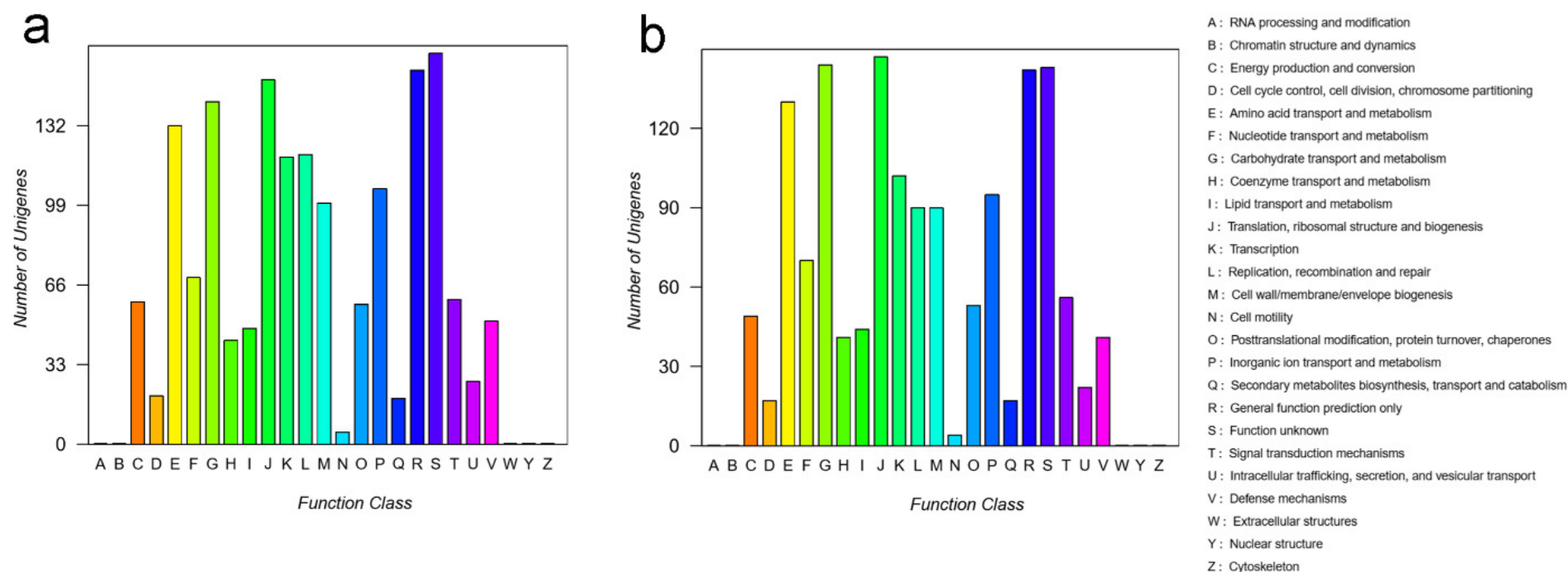


Figure 2. The distribution of accessory gene number and gene functional classification of GBS S03 (a) and GBS S07 (b).

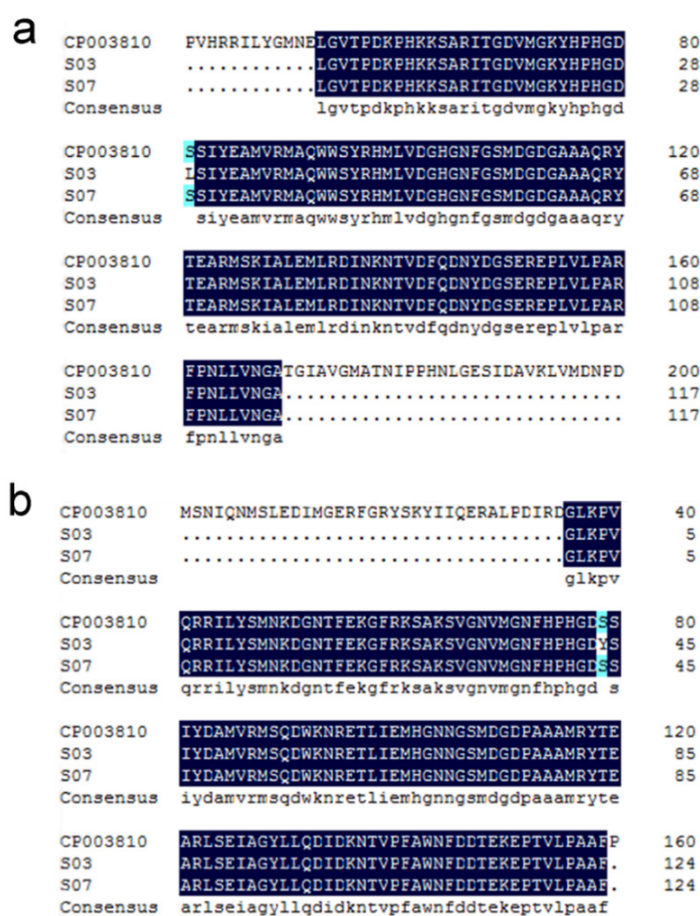
Table 2. The results of antibiotic resistance of GBS S03 and S07

Class	Antibiotics	Dosage (μ g)	The standard of the inhibition zone diameters (mm)			S03		S07	
			Resistant	Intermediate	Sensitive	inhibition zone(mm)	result	inhibition zone(mm)	result
Macrolide	CLIN	15	≤ 16	16-18	≥ 18	15.10	R	18.70	S
	ERY	16	≤ 14	14-22	≥ 22	000	R	37.17	S
Fluoroquinolone	ENR	10	≤ 22	22-28	≥ 28	0.00	R	25.40	I
	NOR	10	≤ 13	13-16	≥ 16	11.43	R	17.00	S
	OFX	5	≤ 13	13-17	≥ 17	10.73	R	18.43	S
Tetracycline	TET	30	≤ 15	15-18	≥ 18	8.53	R	35.10	S
	DOX	30	≤ 13	13-15	≥ 15	13.47	R	16.07	S
β -lactam	PEN	10	≤ 20	20-27	≥ 27	37.80	S	47.83	S
	CFX	30	≤ 19	19-25	≥ 25	25.40	S	27.37	S
Others	GEN	10	≤ 13	13-14	≥ 14	14.63	S	16.10	S
	VAN	30	≤ 15	15-16	≥ 16	28.77	S	32.13	S
	FLO	30	≤ 13	13-17	≥ 17	24.80	S	32.80	S

CLIN, clindamycin; ERY, erythromycin; ENR, enrofloxacin; NOR, norfloxacin; OFX, ofloxacin; TET, tetracycline; DOX, doxycycline; PEN, penicillin; CFX, cephalexin; GEN, gentamicin; VAN, vancomycin; FLO, florfenicol; S, sensitive; I, intermediate; R, resistant.

Table 3. Resistance genes of GBS S03 and S07.

Resistance genes	S03	S07
Beta-lactam resistance	<i>PBP1a, PBP1b, PBP2b, PBP2x</i>	<i>PBP1a, PBP1b, PBP2b, PBP2x</i>
Fluoroquinolone resistance	<i>parC, gyrA, gyrB, norA, norB</i>	<i>parC, gyrA, gyrB, norA, norB</i>
Macrolide resistance	<i>mefE, macB, ermB</i>	<i>macB, ermB</i>
Lincosamide resistance	<i>lnuB, lsaE</i>	
Tetracycline resistance	<i>tetM, tetO</i>	<i>tetM</i>
Rifampin resistance	<i>rpoB</i>	<i>rpoB</i>
Aminoglycoside	<i>APH3'</i>	
Variant or mutant	<i>mprF</i>	<i>mprF</i>
Antibiotic inactivation enzyme	<i>sat-4</i>	

**Figure 3. Comparison of the deduced amino acid sequences of *gyrA* (a) and *parC* (b) from GBS S03 and S07. GBS GD201008-001 (accession number: CP003810) was used as a reference strain for comparative analysis.**

S03 in the pathogenicity experiments. The mortality rates of infected fish are shown in Table 4. Concretely, the LD₅₀ value of GBS S03 for *Schizothorax* spp. and *Danio rerio* was 5.27×10^8 cfu·ml⁻¹ and 7.09×10^7 cfu·ml⁻¹ respectively, while that of GBS S07 was 8.9×10^3 cfu·ml⁻¹ for *Schizothorax* spp. and 1.88×10^3 cfu·ml⁻¹ for *Danio rerio*. There was no mortality in the control groups during the observation period.

Fifty-one virulence genes were identified in both genomes (Table 5). Among these, *bibA*, *dltA*, *fbsA*, *pavA*,

psaA, *srtA*, *fbsB*, *pavB*, *lap*, *lep*, *lmb* and *srr-1* were involved in attachment; *CLL_A 2400*, *hylB*, *lgt*, *mf3*, and *sugC* were involved in invasion; *cfb*, *cpsA-L*, *cpsA-Y*, *manA*, *neuA-D*, *uppS*, *rgpA*, *rmlA*, *scpA*, *rgpB*, *scpB*, *oppF*, *rgpG*, *sip*, *BC5263*, *EFD32*, *EFD32_0765* and *SMU .322c* were involved in evading/destroying host defenses; and other virulence genes, included were *coxK2*, *clpC*, *clpE*, *clpP*, *cppA*, *msbA*, *nanA*, *gbpB*, *fhuC*, *licD*, *lisR*, *lplA1*, *groEL*, *htrA/deg*, *CT396* and *stp*. Comparison of the virulence genes between GBS S03 and

Table 4. Mortality rates (%) of *Schizothorax spp.* and *Danio rerio* after challenge with different concentrations of GBS S03 and S07.

Dose of bacteria received (cfu·ml ⁻¹)	GBS S03		GBS S07	
	<i>Schizothorax spp.</i>	<i>Danio rerio</i>	<i>Schizothorax spp.</i>	<i>Danio rerio</i>
1.0×10 ⁴	0	0	55	70
1.0×10 ⁵	0	0	75	85
1.0×10 ⁶	0	20	95	95
1.0×10 ⁷	0	35	100	100
1.0×10 ⁸	25	55	100	100
1.0×10 ⁹	60	75	100	100

Table 5. Virulence genes of GBS S03 and S07.

Class	S03	S07
Attachment	<i>bibA, dltA, fbsA, pavA, psaA, srtA, fbsB, pavB, lap, lep, lmb, srr-1, rib, SAG1404, SAG1405, SAG1406, SAG1407, SAG1408, SAK_0778, SAK_0779, gbs0628, gbs0629</i>	<i>bibA, dltA, fbsA, pavA, psaA, srtA, fbsB, pavB, lap, lep, lmb, srr-1</i>
Invasion	<i>CLL_A 2400, hylB, lgt, mf3, sugC</i>	<i>CLL_A 2400, hylB, lgt, mf3, sugC, SAN_1519, rfbA, cylE</i>
Evading/destroying host defenses	<i>Cfb, cpsA-L, cpsA-Y, manA, neuA-D, uppS, rgpA, rmlA, scpA, rgpB, scpB, oppF, rgpG, sip, BC5263, EFD32, EFD32_0765, SMU.322c</i>	<i>Cfb, cpsA-L, cpsA-Y, manA, neuA-D, uppS, rgpA, rmlA, scpA, rgpB, scpB, oppF, rgpG, sip, BC5263, EFD32, EFD32_0765, SMU.322c</i>
Other virulence genes	<i>coxK2, clpC, clpE, clpP, cppA, msbA, nanA, gbpB, fhuC, licD, lisR, lplA1, groEL, htrA/deg, CT396, stp</i>	<i>coxK2, clpC, clpE, clpP, cppA, msbA, nanA, gbpB, fhuC, licD, lisR, lplA1, groEL, htrA/deg, CT396, stp</i>

S07 showed differences. GBS S03 carried *SAG1404-1408, rib, SAK_0778-0779* and *gbs0628-0629* associated with attachment genes, while GBS S07 were carrying *SAN_1519, rfbA* and *cylE* involved in invasion genes.

PHYLOGENETIC ANALYSIS

By MLST, GBS S03 was determined to be ST19, while GBS S07 was ST891. To investigate the phylogenetic relationships of our two isolates to other GBS strains, we used the maximum likelihood method to construct a phylogenetic tree (Figure 4). Phylogenetic analysis was performed using 38 reported GBS strains. Interestingly, GBS S03 was closely related to Sag158, H002 and SG-M25 strains isolated from human while GBS S07 was grouped separately.

DISCUSSION

Since 2008, GBS has been present and emerging in tilapia in China. Zhang et al.²² and Liu et al.¹⁴ reported the first complete genome sequence of the piscine GBS strain isolated from cultured tilapia in China. In this study, draft genomes of GBS S03 and S07 isolated from *Schizothorax spp.* have been recorded. The genome size of GBS S07 was approximately 1.79 Mbp and approximately 0.37 Mbp smaller than GBS S03. There were more predicted CDSs in the GBS S03 genome than in GBS S07. Genome size is the result of a balance between amplification and loss of DNA, which evolves toward an optimal state for organismal fitness.^{23,24} Fur-

thermore, genomic changes leading to streamlined genomes with increased pathogenicity and virulence are important.²⁵ They might involve fragment recombination and contribute to the different virulence of GBS S03 and S07.

Antimicrobial tests revealed that the GBS S03, showed multidrug resistance, but GBS S07, showed drug-sensitive to eleven antibiotics except fluoroquinolones. In contrast to GBS S07, GBS S03 exhibited a stronger resistance towards macrolides, fluoroquinolones, and tetracycline antibiotics. In comparison to the WGS results of the two strains, it was shown that GBS S03 exhibited a greater abundance and complexity of resistance genes, especially those associated with drug resistance of macrolides, lincosamides, tetracyclines and aminoglycosides. The phenotypes of the two strains were in concordance with genomic characteristics revealed by WGS. Fluoroquinolones exert antibacterial activity by inhibiting DNA gyrase (DNA gyrase) and Topoisomerase IV (Topo IV). DNA helicase is composed of *gyrA* and *gyrB* subunits, and DNA topoisomerase IV is composed of *parC* and *parE* subunits.²⁶ Two strains had fluoroquinolone genes: *parC, gyrA, gyrB, norA* and *norB*, however, just GBS S03 had chromosomal *gyrA* [81:S-L] and *parC* [79:S-Y] mutations, changing the target structure of fluoroquinolones in GBS. Therefore, GBS S03 has a high level of fluoroquinolone resistance.²⁷⁻²⁹ Current investigations have proven that there are two mechanisms of macrolide drug resistance: *ermB* encoding ribosome methylase modifies the binding site, *mefA* and *mefE* can change the active

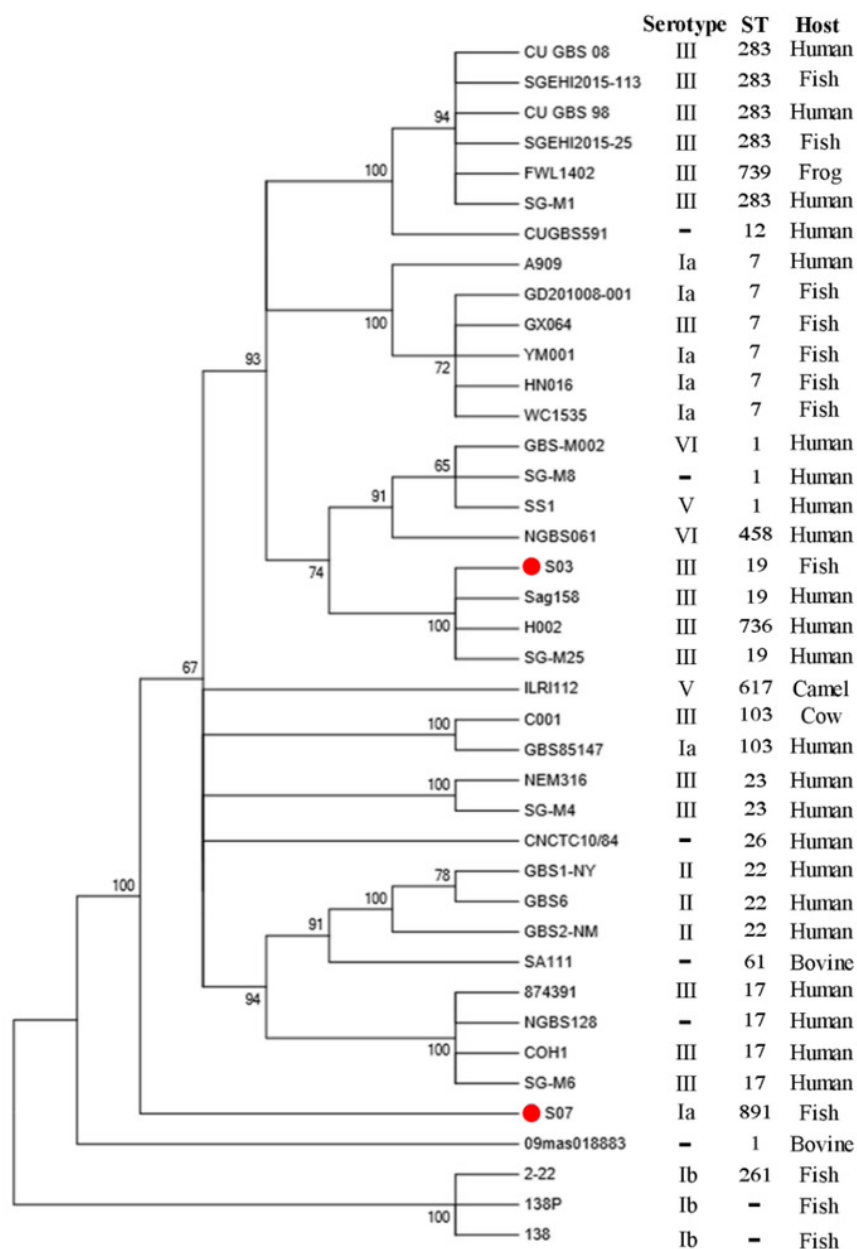


Figure 4. Molecular phylogenetic tree of the evolutionary relationship among different GBS strains using the Neighbor-Joining algorithm and 1000 bootstraps.

drug efflux system by changing the encoding efflux pump protein. In addition to two macrolide resistant genes (*macB* and *ermB*), GBS S03 additionally had *mefE*, which contributes to high-level macrolide resistance.²⁸ The phenomenon of tetracycline resistance is attributed to the encoding of ribosome protection proteins, which effectively hinder the binding of ribosomes.³⁰ The genes *tetM* and *tetO* are associated with the synthesis of ribosome-protective proteins. The tetracycline resistance of GBS S03 is notably enhanced compared to GBS S07 due to the presence of the *tetO* gene in addition to *tetM*.^{28,31} Meanwhile, GBS S03 had additional *lnuB*, *lsaE*, *APH3'*, and *sat-4* genes not present in GBS S07 strain, the first two of which play an important

regulatory role in lincosamide resistance.³² The gene *lnuB* is responsible for encoding a nucleotide transferase enzyme that causes clindamycin resistance.³³ *lsaE* is a multi-drug resistance gene that can encode ABC transporter and mediate drug resistance of three drugs with different chemical structures: linkeramines, truncated laterin and streptomycin A.³⁴ The combination of *APH3'* and *sat-4*, associated with transposon Tn5405 among Gram-positive bacteria, encodes resistance to aminoglycosides (except gentamicin) and streptothricins.^{35,36} These results reveal a trend towards concordance between resistance phenotype and genotypic evidence for antibiotic resistance.

The pathogenicity of the two strains was compared by intraperitoneal injection in *Schizothorax* spp. or *Danio rerio*, which demonstrated that GBS S07 showed much greater virulence than GBS S03. GBS S07 also carried more virulence genes associated with invasion, such as *SAN_1519*, *rfaA* and *cylE* genes. However, it was counter to the previous report on GBS from tilapia in Thailand,¹⁶ which is most likely related to the diversity in host, area and strain. Fifty-one virulence genes in both GBS S03 and S07 involved in attachment, persistence, evading host defenses, tissue penetration, and toxin-mediated diseases had been identified in the GBS genomes, providing evidence of the pathogenicity of GBS.³⁷⁻³⁹ Differences in virulence genes between the two strains are mainly reflected in the genes encoding pili, including *SAG_1404-1408*, *gbs0628-0629* and *SAN_1519*. Genes encoding pili in GBS are located within two distinct loci, denoted pilus islands 1 and 2 (PI-1 and PI-2), and comparative analysis of available genomes revealed two variants of PI-2, designated PI-2a and PI-2b.⁴⁰ *Gbs0628-0629* gene, *SAG_1404-1408* gene, and *SAN_1519* gene are located in PI-1, PI-2a and PI-2b, respectively.^{40, 41} Pili are supposed to be important virulence factors.⁴¹ In GBS, pilus 1 is related to the immune escape, pilus 2a confers formation of biofilms, and pilus 2b is linked to the ability of attachment and invasion.^{42,43} In this study, GBS S03 carried PI-1 plus PI-2a type pilus islands while GBS S07 carried PI-2b type pilus island, which suggested there was concordance between pathogenicity and WGS result. Previous studies showed that most of the fish-sources strains carried PI-2a type, PI-2b type or PI-1 plus PI-2b type pilus islands, while in human strains, a great number of GBS carried PI-1 plus PI-2a type or PI-1 plus PI-2b type.⁴⁴

In summary, based on genome-wide analysis combined with phenotypic experiments, we demonstrated a correlation between the two. The phenotypic findings of our study indicate that the GBS S03 strain exhibited greater resistance, whereas the GBS S07 strain had higher virulence. Correspondingly, in the results of whole genome sequencing, the drug resistance gene of GBS S03 strain was more complex. GBS S03 exhibited the presence of genes that govern resistance to multiple drugs. In order to prevent the future enhancement of GBS resistance mechanisms, it is imperative to minimize the overuse of antibiotics or explore other antibacterial ingredients (traditional Chinese medicine monomer) in practical aquaculture. The results of phenotypic testing indicate that GBS S07 exhibits greater virulence compared to GBS S03. The WGS result reveals a higher correlation between the pilus islands encoded by the virulence gene of GBS S07 and attachment and invasion, hence contributing to its greater virulence. However,

whether our two strains in this study will exchange and transfer resistance genes and virulence genes in nature, with the appearance of new strains with great toxicity and extreme drug resistance, deserves further research and attention. Additionally, phylogenetic analysis showed that GBS S03 was closely related to human sources (Sag158, H002, and SG-M25) while GBS S07 was grouped separately. Combined with genome size and phenotypic characteristics, we hypothesized that they had different origins and evolutionary directions.

Further, bestowed pili types and phylogenetic analysis, we hypothesized that GBS S03 had a common source with human GBS or the possibility of cross-infection with human strains. Unfortunately, we do not have adequate epidemiological information to confirm this hypothesis. Although the knowledge about the ability of GBS to cross the interspecies barrier and allow human-derived strains to infect animals, or vice versa, is poorly understood,⁴⁵ demonstrated that GBS strains from human and bovine origins could infect fish. Priyanka et al.⁴⁶ found that fish-isolated GBS could infect humans by consuming raw fish. Therefore, we should attach great importance to cross-host transmission of GBS and its potential threat to public health security.

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AUTHORS' CONTRIBUTION PER CREDIT

Writing – original draft: Yihao Wang (Lead). Validation: Yu Yuan (Lead). Formal Analysis: Kun Peng (Lead). Data curation: Yilin Wang (Lead). Project administration: Longjun Deng (Lead). Supervision: Tiancai Li (Lead). Investigation: Defang Chen (Lead). Conceptualization: Ping Ouyang (Lead). Funding acquisition: Xiaoli Huang (Lead). Resources: Hongrui Guo (Lead). Visualization: Huidan Deng (Lead). Methodology: Weiming Lai (Lead). Writing – review & editing: Yi Geng (Lead).

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