



Invasive Infection With *emm3*/ST15 *Streptococcus pyogenes*: The First Case Report From China and Complete Genome Analysis

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Streptococcus pyogenes (GAS) may cause severe invasive disease with a high fatality rate, especially M3-type strains, which are less common in China. Here, we report the first *emm3*/ST15 invasive GAS infection case in China. The patient was diagnosed with severe skin and soft tissue infection (SSTI) and septicaemia caused by one GAS strain. Antibiotic susceptibility tests showed that the isolate was susceptible to all tested drugs. Antimicrobial therapy was then applied, and the patient fully recovered and was discharged from the hospital on Day 43. Whole-genome sequencing was carried out using the Illumina and Oxford Nanopore platforms and revealed this to be the first *emm3*/ST15-type GAS invasive infection in China. The closely related *emm3*/ST15-type GAS strains are MGAS315 from the United States and M3-b from Japan. Our finding is a warning that we should pay attention to invasive M3-type GAS infections in China and indicates the global spread of the highly virulent *emm3*/ST15 GAS strain.

Keywords: *Streptococcus pyogenes*, M type, *emm3*/ST15, invasive infection, whole-genome sequencing (WGS)

INTRODUCTION

Streptococcus pyogenes (Group A *Streptococcus*, GAS) is a common Gram-positive pathogenic bacterium that may induce various diseases, including minor ones, such as pharyngitis and scarlet fever, and serious ones, such as pneumonia and toxic shock syndrome (1). Severe GAS infections are less common but present a high fatality rate and have been reported worldwide (2–4). For example, the symptoms of GAS pharyngitis include fever, throat pain, and chills, while streptococcal toxic shock syndrome patients would develop multiorgan failure within a short time, leading to death (5, 6). It was reported a combined antibiotic therapy of penicillin and clindamycin would improve the outcomes of severe GAS infections (7).

The transmission of GAS would be through direct person-to-person *via* the inhalation of respiratory droplets, or through direct contact with contaminated objects (8). The increment in the incidence of GAS invasive infections has been associated with particular clones and varies by time

and region, which may reflect a population's susceptibility to specific strains (9, 10). GAS strains can be serologically separated into M protein serotypes based on a surface protein encoded by the *emm* gene (1). In clinical epidemiological studies, M1 and 3 are the most common GAS serotypes of invasive and toxic streptococcal diseases. Among the different sequence types of M3 strains, *emm3*/ST15-induced toxic shock syndrome and other invasive diseases have often been reported in Japan, United Kingdom, and United States (11).

In the past, we considered that M3 GAS infections were rare in China, but a recent large-scale epidemiological study indicated that M3 GAS scarlet fever has increased substantially in China since 2018 (12) and became one of the prevalent *emm* types that induced scarlet fever in 2019 (13). Here, we report the first case of a severe GAS infection caused by the *emm3*/ST15 GAS strain SHZ-1 in China. The complete genome sequence of this strain was also analyzed in the current study.

METHODS

Bacterial Isolation and Antimicrobial Susceptibility Testing

Wound secretion specimens were submitted to the clinical laboratory of Zhejiang local hospital for slide microscopy and bacterial culture using 5% sheep blood agar plates. A GAS strain was obtained and identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany) (14). Antimicrobial susceptibility testing (AST) of levofloxacin, chloramphenicol, clindamycin, ceftriaxone, erythromycin, penicillin, tetracycline, and vancomycin against the identified GAS strain, named SHZ-1, was performed using a disk diffusion method, and the results were interpreted according to the Clinical and Laboratory Standards Institute (15).

Whole-Genome Sequencing and Analysis

Genomic DNA of strain SHZ-1 (single colony) was prepared using a DNA mini kit (Qiagen, Valencia, CA, United States) and submitted to next-generation sequencing (NGS) and long-read sequencing using the Illumina HiSeq2000TM (Illumina Inc., San Diego, CA, United States) and Oxford Nanopore MinION platforms (Oxford Nanopore Technologies, Oxford, United Kingdom), respectively. The Illumina sequencing generated 9.1M reads with an average of ~150 bp per read and 200 X coverage. The nanopore sequencing of this strain generated 139 contigs with an N50 of 1329619 bp and a GC content of 39.22%. Both short and long reads were used for *de novo* assembly via Unicycler version 1.0 (15) in a hybrid assembly model with default parameters. The genome was then used as input for *in silico* multilocus sequence typing (MLST) via BLAST against the PubMLST database (16), *emm* typing against the CDC *emm* type and subtype database tsem (17). The annotation of SHZ-1 was performed via Prokka against *S. pyogenes* database (18). The annotated genome was then used for virulence factor detection against the VFDB database (19). Both SNP and cgMLST strategies

were performed in the BacWGSTdb server (20) for bacterial source tracking. An *ad hoc* cgMLST scheme for *S. pyogenes* (1,170 target genes) was designed to characterize the gene-by-gene allelic profile of *S. pyogenes* strains. The identified *S. pyogenes* strain SHZ-1 was used as the reference genome in this analysis. SNP distance matrices were calculated using snp-dists 0.6.3 (21).

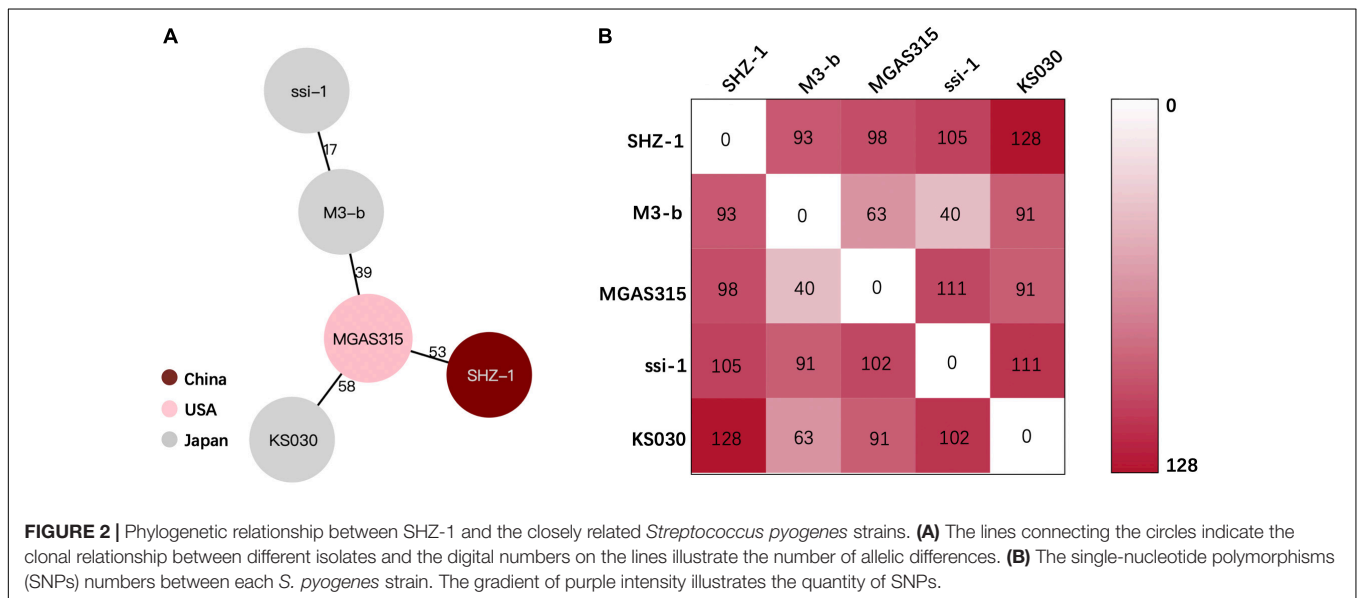
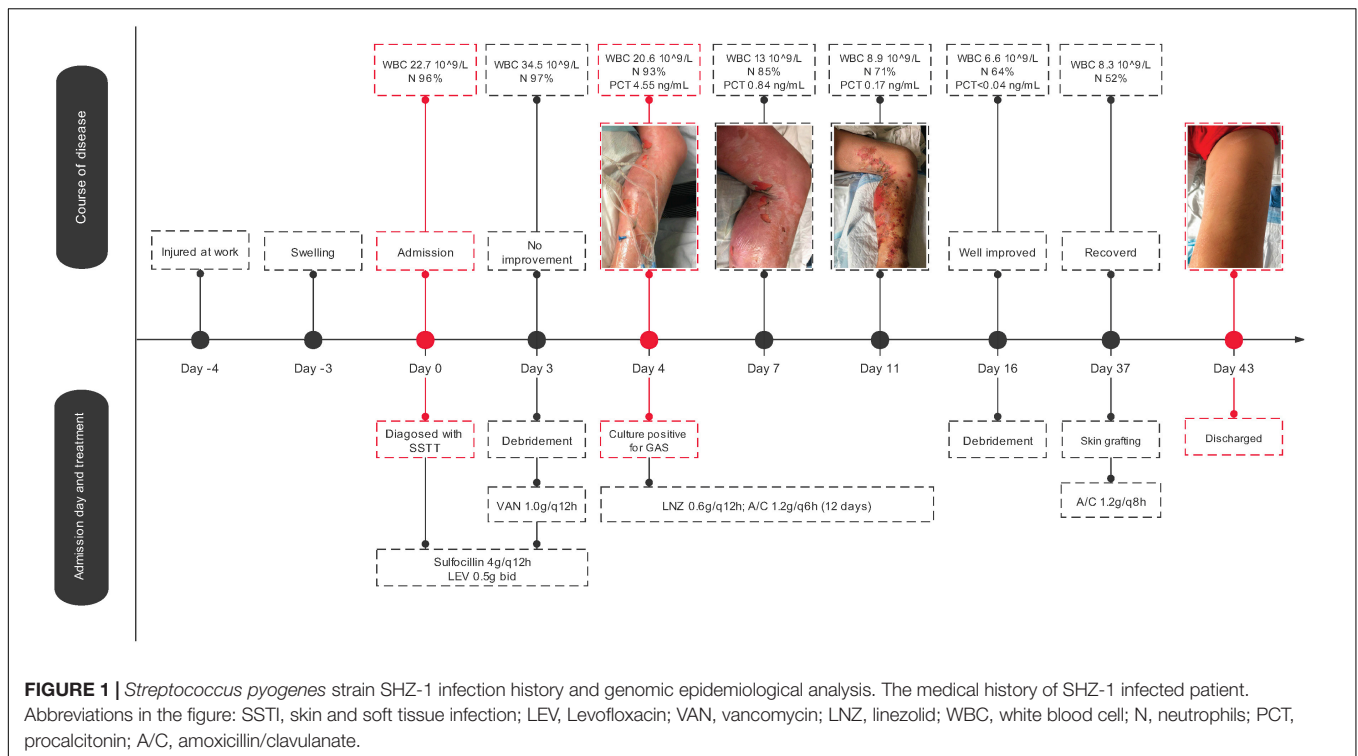
RESULTS

Case Presentation

A 37-year-old man injured his left foot at work 4 days prior to admission. He immediately felt pain, but it was tolerable and he had no open wounds; hence, he ignored the symptoms. The injured foot developed skin redness, swelling, and obvious tenderness 1 day later. The patient developed a conscious fever accompanied by chills, dizziness, abdominal pain, and distension. The symptoms worsened after another 2 days, and the patient was then admitted to the local hospital, Zhejiang, China. His lab results showed leukocyte counts of $22.3 \times 10^9/L$ with 92.3% neutrophils, and an X-ray showed no fracture. Based on this, the diagnoses of severe skin and soft tissue infection (SSTI) was made. Three days of sulbenicillin (4 g/q12 h) and levofloxacin (0.5 g bid) treatment showed no improvement. After debridement, postoperative anti-infection treatment with vancomycin (1.0/q8 h) was applied. Leukocyte counts were still high with elevated levels of procalcitonin, and the patient was diagnosed with septicemia. X-ray confirmed no migrating lung lesions. On the fourth day of admission, a specimen culture was confirmed to be positive for GAS. AST showed that the bacterial isolate was susceptible to all tested drugs (Supplementary Table 2). Antimicrobial therapy was then applied with linezolid (0.6 g q12 h) and amoxicillin/clavulanate potassium (1.2 g q6 h). Declines in all inflammatory indices were then observed. Thereafter, the patient improved from GAS infection after 11 days of treatment and was transferred to debridement, suture, and skin grafting. The patient was treated with amoxicillin/clavulanate potassium (1.2 g q8 h) for another 6 days since Day 37 due to GAS culture positivity. The patient fully recovered and was discharged from the hospital on Day 43 (Figure 1).

Whole-Genome Analysis

Hypervirulent invasive GAS infections are rare in China. To better understand the pathogen in the current case, the strain was sent for whole-genome sequencing. The complete chromosome data of SHZ-1 were submitted to NCBI (Genbank No.CP072523.1). After the hybrid assembly, we obtained a 1819973 bp complete circular genome of GAS strain SHZ-1 with a GC content of 38.6%. We identified SHZ-1 as an *emm3*/ST15-type GAS strain via *emm* typing and MLST analysis (Supplementary Figure). Then we downloaded the complete genome of *emm3*/ST15 GAS strains that were isolated from different regions. Using BacWGSTdb to determine the clonal relationship among these isolates according to the pairwise comparison of the cgMLST alleles or SNP differences. In



the end, the phylogenetic relatedness showed that the closest strain to SHZ-1 was MGAS315 from the United States, which has 53 core gene differences (Figure 2A). The SNP calling results indicated that the smallest SNP number of 93 was determined between strains SHZ-1 and M3-b from Japan (Figure 2B). Virulence factor detection against VFDB showed that SHZ-1 had all the virulence factors found in the other two *emm3*/ST15 strains (MGA315 and M3-b) except the streptococcal superantigen SSA-encoding gene *ssa* (Supplementary Table 2).

DISCUSSION

Streptococcus pyogenes serotype M3 strain-induced invasive infections have been widely reported worldwide, although they have not been common in recent years in China. We report the first case of the *emm3*/ST15 GAS strain causing severe invasive infection in China.

Recent large-scale epidemiological studies have reported an M-type shift of scarlet fever GAS isolates in China, which clearly showed that M3 GAS has become one of the most prevalent

strains in Beijing, China, since 2018 (1, 2). Our reported severe infection case indicates the emergence of widespread M3 strains in China, which belong to the most virulent strain *emm3/ST15*. Whole-genome analysis of SHZ-1 showed that the closest strain was MGAS315 from United States (22) and then M3-b from Japan (23). Both strains are invasive isolates from patients with streptococcal toxic shock syndrome in the late 1980s and 1994, respectively. Later, whole-genome analysis of these two and other *emm3/ST15* strains showed that their association with severe infections was due to their distinct arrays of virulence factors, for example, *speA*-encoded streptococcal pyrogenic exotoxin A and *ssa*-encoded streptococcal superantigen A (22). The latter is a phage-associated streptococcal superantigen that is missing in our reported strain SHZ-1 but is encoded in both the MGAS315 and M2-b genomes. *ssa* has been detected in toxic shock syndrome-related M3 isolates (24), but only 25% of *emm3*-type GAS isolates are from Chinese patients (13). In our reported case, strain SHZ-1 that lacked the *ssa* gene still presented a strong pathogenesis in the host. We believe that genetically related *emm/ST15* GAS strains may have geographic characteristics for their virulence, and a large-scale molecular epidemiology study of invasive GAS infection in China is needed.

In conclusion, we report the first *emm3/ST15* invasive GAS infection in China. The complete genome analysis of pathogenic GAS strain SHZ-1 indicates the emergence of the global spread of highly virulent *emm3/ST15*-type GAS. This is a warning that attention should be paid to the epidemiology of M3-type GAS in China to prevent severe invasive GAS infections.

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ETHICS STATEMENT

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XM, JZ, and LS collected the clinical data. YW and SZ did the laboratory work. XW and YY designed the study and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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