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CASE REPORT

A Case of Necrotizing Fasciitis/Myositis and Streptococcal Toxic Shock Syndrome Caused by *emm*22/ST46 Strain of *Streptococcus pyogenes*

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Abstract: *Streptococcus pyogenes*, also known as Group A *Streptococcus* (GAS), can cause severe invasive diseases with high fatality rates. We report a case of necrotizing fasciitis and myositis complicated by Streptococcal Toxic Shock-Like Syndrome (STSS) caused by the invasive *emm*22/ST46 strain of *Streptococcus pyogenes* in China. A previously healthy 57-year-old Chinese Canadian man presented with right calf pain and ulceration following a hike in the Gobi Desert, which progressed to unconsciousness and severe infection. Despite initial treatment, his condition deteriorated, leading to his transfer to our intensive care unit. Metagenomic Next-Generation Sequencing identified *Streptococcus pyogenes*, and antimicrobial susceptibility testing revealed resistance to erythromycin, tetracycline, and clindamycin. Despite broad-spectrum antimicrobial therapy, debridement, and supportive measures, the patient's condition necessitated amputation of the right lower limb. He recovered and was discharged from the hospital on Day 43. Whole-genome sequencing of the isolate identified 15 multiple virulence factors. Phylogenetic analysis revealed that the closest relative of the isolate was a strain identified in China. This case underscores the importance of early recognition and treatment of invasive GAS infections to prevent severe outcomes, and we should pay attention to invasive *emm*22/ST46 GAS infections in China.

Keywords: group A *Streptococcus*, streptococcal toxic shock-like syndrome, amputation, superantigens, metagenomic next-generation sequencing

Introduction

Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS), is a Gram-positive, host-adapted bacterial pathogen. It causes a wide spectrum of diseases, from non-invasive infections like pharyngitis, scarlet fever and impetigo, to invasive conditions such as septicemia, necrotizing fasciitis, myositis and streptococcal toxic shock-like syndrome (STSS).[1] Necrotizing fasciitis, a rare yet severe form of soft-tissue invasive GAS (iGAS) infection, accounts for about 50% of cases associated with STSS. It has a mortality rate of 30–50%, and is particularly high in the presence of STSS.[2,3]

GAS can be classified into more than 150 *emm* types based on the sequence typing of the 5′end of the M protein (*emm*) gene.[4,5] The common *emm* types responsible for invasive GAS disease vary by country. For instance, a study on the epidemiology of invasive group A streptococcal infections in Ireland from 2012 to 2015 highlighted *emm*1, *emm*3, *emm*28, *emm*12, and *emm*89 as predominant.[6] Another study from Spain covering the period from 2007 to 2019 indicated that the most prevalent *emm* types were *emm*1, *emm*89, *emm*3, *emm*4, *emm*12, and *emm*6.[7] Invasive infections caused by

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the *emm*22 type are less common compared to those caused by the most frequent serotypes. Among *emm*22 isolates, the most common sequence type (ST) is ST46.

In this paper, we present a severe case of necrotizing fasciitis/myositis complicated by STSS induced by the invasive *emm*22/ST46 strain of *Streptococcus pyogenes* in China. Additionally, we provide an analysis of the complete genome sequence of this strain.

Case Presentation

A previously healthy 57-year-old Chinese Canadian was transported to our hospital with a 4-day history of right calf pain and ulceration, followed by 3 days of unconsciousness. The pain in his right lower limb started after hiking in the Gobi Desert of Dunhuang, China. No obvious external trauma or skin lesions were found at the location of the pain. He experienced dizziness, nausea, and vomiting, prompting rapid transfer to the ICU of the local hospital due to unconsciousness and hypotension (60/30mmHg). Concurrently, his right lower limb developed oedema and exhibited a purplish discoloured lesion, measuring 10×8 cm, accompanied by significant tenderness. The area expanded quickly within 2 days, accompanied by fever (38.3°C). Despite initial treatment and supportive measures at a local hospital, his condition deteriorated. He was transported to the intensive care unit of our hospital.

On admission, the patient presented with fever and unstable arterial pressure, which was managed using norepinephrine. The physical examination showed skin redness and swelling in the right lower limb, along with extensive cyanotic lesions covering the shin, inner and posterior aspects of the thigh, extending to the ankle region and dorsum of the foot (Figure 1A). Initial lab tests indicated infection, metabolic alkalosis, respiratory acidosis and coagulation dysfunction. Further assessments of other organ functions revealed elevated alanine aminotransferase, urea, and creatinine levels. Low albumin and high creatine kinase were also observed. Results of his initial investigations are presented in Table 1. Accordingly, the patient was considered for the diagnosis of necrotizing soft tissue infection complicating septic shock, concurrent with rhabdomyolysis and hemolytic uremic syndrome. Considering the life-threatening condition, we implemented an empirical regimen of broad-spectrum antimicrobial treatment, incorporating penicillin, ceftriaxone, and meropenem. Before antibiotic treatment, samples were obtained from pustules on the right lower extremity, deep soft tissue, and blood from both arms for pathogen detection using Metagenomic Next-Generation Sequencing (mNGS). To further characterize the pathogen, samples were inoculated onto blood agar plates and incubated 20h at 37°C. Simultaneously, intravenous immunoglobulin (IVIG), plasma, and platelet transfusions were also administered, along with continuous renal replacement therapy. Furthermore, due to the severe infection, we performed debridement of necrotic tissue and fascial incision and drainage on the right lower limb (Figure 1B).

On the second day after admission, the mNGS analysis identified *Streptococcus pyogenes* in the pus sample. On the same day, the blood agar plates revealed the presence of small, round, and translucent colonies. These colonies exhibited beta-hemolysis, characterized by a clear zone of complete red blood cell lysis surrounding the colonies (Supplementary Figure 1). The subsequent specimen culture on day three also affirmed its presence. Thus, the patient was diagnosed with STSS and necrotizing fasciitis/myositis. Antimicrobial susceptibility testing (AST) (Supplementary Table 1) revealed that the bacterial isolate was susceptible to penicillin, cefotaxime, vancomycin, quinupristin/dalfopristin, linezolid, levofloxacin and chloramphenicol, but resistant to erythromycin, tetracycline and clindamycin. Based on this, we discontinued ceftriaxone and retained penicillin to specifically eradicate *Streptococcus pyogenes* and meropenem for other mixed bacterial infections.

Despite robust anti-infective measures and debridement and drainage of the lesion, the condition of the right lower extremity remains insufficient improvement. To prevent the infection from worsening further, the right lower limb was amputated on day 12 (Figure 1C). On the 2nd postoperative day, the patient's temperature normalized, infection indicators gradually decreased, and organ function gradually recovered. On the 26th day of hospitalization, the patient regained consciousness from the deep coma and he was discharged on the 51st day of hospitalization.

Materials and Methods

Whole-genome sequencing (WGS) of *Streptococcus pyogenes* strain CQ01 was performed using Oxford Nanopore MinION, generating 68528 high-quality reads. De novo assembly using Canu v2.0 yielded a single chromosome and an extra plasmid. We searched the National Center for Biotechnology Information (NCBI) genome database and downloaded 2928 GAS genome assemblies. Genome sequences were then used for in silico M protein gene (*emm*) typing by BLAST against CDC *Streptococcus*

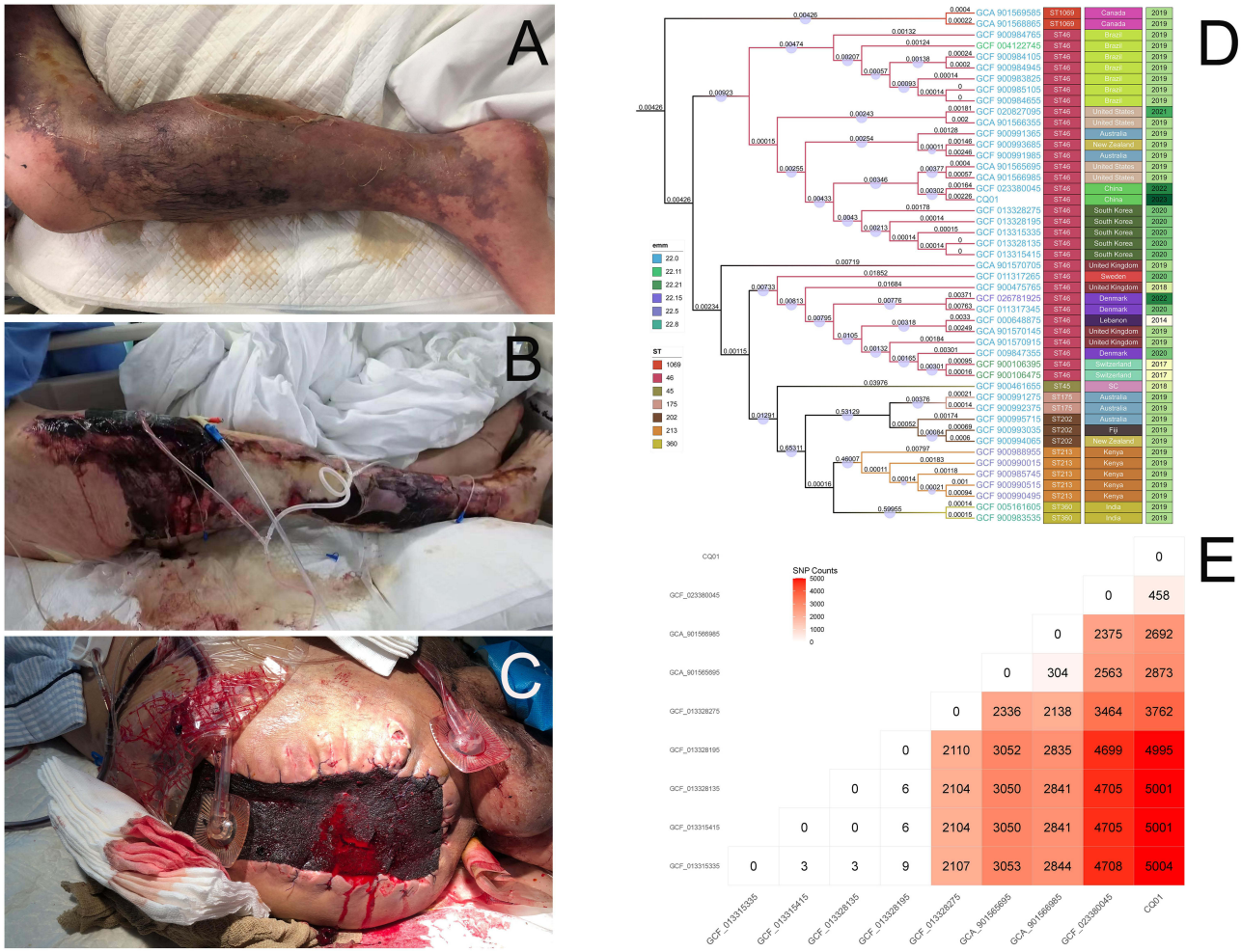

Figure 1 (**A**) the right lower limb of the patient exhibited dusky skin, swelling and hemorrhagic bullae; (**B**) Debridement of necrotic tissue and fascial incision and drainage were performed on the right lower limb; (**C**) Amputation of the right lower limb; (**D**) Maximum parsimony phylogenetic tree of 47 GAS *emm*22 strains. Node labels are color-coded according to the *emm* subtype. The strip aligned with node labels display the MLST. The location information, if available, of the assembly submitter (SC denotes Sanger Welcome Institute Center) and the year are listed after. Circular size on branches represents the support level (0.8 to 1, those below 0.8 are omitted). The length of the branches is indicated above the branch line. (**E**) SNP counts between each relative GAS strains.

Laboratory *emm* database.[8] The multi-locus sequence typing (MLST) was made by BLAST against the PubMLST database.[4,9] All 47 GAS *emm*22 strains, including ST46 and others, were filtered out as input genomes for phylogenetic analysis. kSNP4 was used to build the SNP matrix and parsimony tree.[10] The paired genetic distance of CQ01 and related strains were calculated based on the SNP matrix and represented by SNP counts. The annotated genome was then used for virulence factor detection against the Virulence Factors of Pathogenic Bacteria (VFDB).[11] To improve the completeness of virulence factors in VFDB, we obtained virulence-related gene sequences from public databases and utilized NCBI BLAST for comparative analysis to identify potential additional known virulence factors. To identify antibiotic resistance genes, we utilized the ResFinder tool available at the Center for Genomic Epidemiology. To elucidate the potential underlying mechanisms of why the CQ01 strain caused such severe clinical manifestations, we examined genes related to the major virulence regulator *covR/covS* in the WGS data. The genes analyzed included *rocA, covS, covR*, and *rpoB*. We specifically looked for mutations in these genes. *RocA* gene sequences were validated by Sanger sequencing. Method details are in the Supplementary materials.

Results

The genome size was 1.95 Mb with a GC content 38.57%. The isolate is classified as an *emm*22 (*emm*-type) and ST46 (MLST) strain. This whole genome has been deposited in GenBank under the accession no. CP141675-CP141676. The phylogenetic analysis revealed that the CQ01's closest relative was GCF_023380045, also identified in China. Beside,

Table 1 Results of Laboratory Investigations

Investigation	Result	Normal range
White blood cell count ($\times10^9$/L)	28.49	3.5–9.5
Neutrophil count ($\times10^9$/L)	25.93	1.8–6.3
Lymphocyte count ($\times10^9$/L)	0.57	1.1–3.2
Red blood cell counts ($\times10^{12}$/L)	3.34	4.3–5.8
Platelet count ($\times10^9$/L)	20	85–303
Urea (mmol/L)	12.7	3.2–7.1
Creatinine (μmol/L)	200	58–110
Alanine aminotransferase (U/L)	59	<50
Aspartate aminotransferase (U/L)	155	17–59
Albumin (g/L)	26	35–50
Activated partial thromboplastin time (seconds)	45.2	28.0–44.0
Prothrombin time (seconds)	17.7	11–14.5
D-dimer (mg/L FEU)	27.53	0–0.55
Creatine kinase levels (U/L)	701	55–170
Procalcitonin (ng/mL)	>100	0–0.05
C-reactive protein (mg/L)	479	0–8
Interleukin-6 (pg/mL)	>1000	0–3.4
Arterial blood PH	7.51	7.35–7.45

another two closest neighbors form the sister clade were GCA 901565695 and GCA 901566985, both from the United States (Figure 1D). The single nucleotide polymorphism (SNP) calling indicated that the smallest SNP number of 458 was identified between the CQ01 and GCF_023380045 strains from China (Figure 1E).

The detection of virulence factors revealed that CQ01 encompasses 15 virulence factors. It not only retains all the virulence factors identified in its closest relative strain, GCF_023380045, but also comprises additional factors such as *mf2* and *speC* (Table 2). Among these, *speC, speA, speG, smeZ* and *ssa* are superantigen virulence gene. Antimicrobial resistance genes identified in the CQ01 strain include erm(B) and tet(M). A single base deletion was identified in *rocA* which was further examined via Sanger sequencing, resulting in no observed deletion at the specific site.

Table 2 Virulence Factors Detection of CQ01 and GCF023380045 Strains

Database	Gene	Product	Accession	CQ01 (%Co;%Id)	GCF023380045 (%Co;%Id)
vfdb	*scpA/scpB*	(scpA/scpB)streptococcal C5a peptidase	WP_010922711	100;98	100;98
vfdb	*hylA*	(hylA)hyaluronidase	WP_010922241	100;98	100;98
vfdb	*prtF2*	(prtF2)Cna B-type domain-containing protein	WP_011284442	100;97	100;97
vfdb	*fbp54*	(fbp54)fibronectin-bing protein Fbp54	WP_002991968	100;98	100;98
vfdb	*slo*	(slo)streptolysin O precursor	WP_010921831	100;98	100;98
vfdb	*speB*	(speB)pyrogenic exotoxin B	WP_010922720	100;99	100;99
vfdb	*ideS/mac*	(ideS/mac)immunoglobulin G-degrading enzyme	WP_010922160	100;98	100;98
vfdb	*lmb*	(lmb)laminin-binding surface protein	WP_000715197	100;98	100;98
vfdb	*sfbX*	(sfbX)LPXTG cell wall anchor domain-containing protein	WP_011285229	100;98	100;98
vfdb	*mf2*	(mf2)deoxyribonuclease, phage associated	WP_002985324	100;100	ND
vfdb	*speC*	(speC)streptococcal exotoxin C precursor, phage associated	WP_002988478	100;99	ND
ncbi	*speA*	(speA) streptococcal exotoxin A precursor - phage associated	WP_159317283	100;96	100;98
ncbi	*speG*	(speG) streptococcal exotoxin G precursor	WP_010921857	100;99	100;99
ncbi	*smeZ*	(smeZ) streptococcal mitogenic exotoxin Z	WP_010922705	100;96	100;96
ncbi	*ssa*	(ssa) streptococcal superantigen SSA-phage associated	NP_795487	100;99	100;99

Abbreviations: %Co, percentage of coverage; %Id, percentage of identity; ND, not determined.

Discussion

To the best of our knowledge, this is the first complete comprehensive case reports documenting necrotizing fasciitis and myositis complicated by STSS caused by *emm*22/ST46 GAS in immunocompetent patient, along with sequencing analysis of the strains.

The progression of iGAS infections is rapid, as observed in this patient. Such infections can lead to fatal outcomes, even in individuals who have previously been perfectly healthy. Therefore, prompt diagnosis and timely treatment are crucial for enhancing patient outcomes. In this case, prior to definitive pathogen identification, a broad-spectrum antimicrobial regimen targeting Gram-positive and Gram-negative bacteria, including anaerobes, was initiated alongside supportive care. In the patient's right lower limb, the appearance of violaceous bullae and rapidly progressing swelling suggested the possibility of a deeper soft tissue infection such as necrotizing fasciitis or myositis.[12] Consequently, immediate debridement of all necrotic tissue and fasciotomy were performed.

After isolating GAS, a 10 to 14-day course of penicillin combined with clindamycin is generally recommended.[13] However, in this case, the strain demonstrated resistance to macrolide/lincosamide and tetracycline antibiotics. Consequently, clindamycin was not administered. Literature indicates that most macrolide resistance genes are identified as *erm(A)*, while *erm(B)* is more common in China.[14] The correlation between resistance and *emm* types affects the efficacy of macrolides and tetracyclines. In *emm*22 type, resistance to tetracyclines is more prevalent than resistance to macrolides.[15,16] Besides antibiotics, IVIG, an additional therapy for STSS, functions by enhancing phagocytic killing, neutralizing toxins and exhibiting broad anti-inflammatory effects.[17] Given these benefits, it was administered to the patient. Notably, when strong anti-infection and debridement cannot achieve good results, the affected limb should be amputated in time, as in this case. The aggressive therapeutic interventions led to the patient regaining consciousness and ultimately surviving.

To elucidate why this bacterial strain caused severe necrotizing fasciitis/myositis and STSS, its phylogenetic relationships and virulence genes were analyzed. The phylogenetic analysis revealed that CQ01's closest relative was GCF_023380045, which was also identified in China. This suggests a possible geographical linkage within China for these strains. However, given the significant genetic differences observed (>400 SNPs), determining whether these strains share a common origin remains challenging and warrants further investigation and require more in-depth investigation. In terms of virulence, GAS is capable of producing numerous virulence factors, which contribute to its pathogenicity. This particular strain contains 15 virulence genes, likely contributing to its high pathogenicity. Streptococcal superantigens, the secreted virulence factors, are associated with several human diseases, most notably STSS and scarlet fever. This strain tested positive for five superantigen genes: *smeZ, speA, speC, speG* and *ssa*. Notably, *speA, speC* and *ssa* have been linked with increased fitness and virulence of contemporary GAS strains causing invasive disease.[1] The *speB* gene, also positive in this strain, is associated with localized tissue damage. Its wide substrate specificity can cleave multiple host and bacterial proteins, including intercellular barrier proteins at epithelial junctions. Although speB is a constitutive gene present in all GAS strains, it plays a critical role in severe iGAS infections, making it a significant virulence factor.[1] The *covR/covS* system in GAS is a well-studied two-component signal transduction system. Mutations in *covR/covS* affect the expression of various virulence factors crucial to GAS infection.[18] Although *rocA* mutations have been shown to alter virulence factor expression and increase invasiveness in other GAS serotypes,[19–21] they have not been identified in the *emm*22 serotype. The patient's strain showed a *rocA* mutation in the WGS result, but Sanger sequencing failed to confirm this mutation. This discrepancy suggests the WGS result may be a false positive, implying other mechanisms might contribute to the strain's elevated invasiveness.

In conclusion, early diagnosis, adequate antibiotic therapy, aggressive surgical intervention, IVIG therapy and supportive care are crucial to the survival of patients suffering from STSS with severe, extended necrotizing fasciitis and myositis. Additionally, attention should be paid to the global dissemination of *emm*22/ST46, which has the potential to cause invasive disease.

Ethics Approval and Consent to Participate

Ethical approval was not required by the local ethical committee, as this is a case report. Its details have been anonymized and do not contain any personally identifiable information.

Informed Consent Statement

Written informed consent for the publication of case details and associated images was obtained from the patient.

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Disclosure

The authors report no conflicts of interest in this work.

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