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# Investigation of Virulence Factors in Group A Streptococci Strains Isolated from Various Clinical Samples by Whole Genome Sequence Analysis

## Farklı Klinik Örneklerden İzole Edilen Grup A Streptokok Suşlarında Tüm Genom Sekans Analiziyle Virülans Faktörlerinin Araştırılması

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### ABSTRACT

***Introduction:*** *The increase in the prevalence of group A streptococcal (GAS) infection in the 2022-2023 period and the increase in invasive cases have raised concerns. Fatal cases have been reported in the United States of America and European countries. To investigate this situation, whole genome sequence analysis was performed on GAS strains that were isolated from three different clinical samples (pleural fluid, blood, urine) which were sent to our laboratory, using next-generation sequencing methods, and were compared with the virulence factors of National Center for Biotechnology Information (NCBI) GenBank throat swab isolates.*

***Materials and Methods:*** *Bacteria-specific DNA isolation was performed by isolation of DNA from culture and removal of host DNA from blood. The genomic DNAs were then sequenced with Oxford Nanopore, a long reading platform. Longer contigs were created using the de novo assembly tool Flye (v2.9.2) and scanned in the nucleotide (nt) database using NCBI Basic Local Alignment Search Tool (v2.14.0). To find the closest GAS strain, a new database was created from the NCBI database with all the genomes of this species. The reads of the samples were then aligned to the GAS strains in the newly created database with the alignment tool Minimap2 (v2.26) used for long reads. After the closest GAS strain was determined, it was scanned with the VFanalyzer using the virulence factor database and the virulence factors were investigated. The virulence factors of Streptococcus pyogenes CP000259.1, CP000262.1, CP013838.1, AE009949.1, and CP013839.1 throat swab strains obtained from the NCBI GenBank library were compared.*

***Results:*** *When the virulence factors of the isolates were compared; Sortase A, Streptococcal lipoprotein rotamase A, Streptococcal complement inhibitor, Streptococcal pyrogenic exotoxin J, Streptolysin S were either absent or detected in at most one of the throat swab isolates, but at least two of the three isolates examined in the study. emm serotypes of the examined isolates were determined as emm1-43 for pleural fluid and urine isolates, and emm141-2 for blood isolates. Antibiotic susceptibility testing was conducted on pleural fluid and blood isolates. While both isolates were sensitive to penicillin; isolates grown from the pleural fluid sample were resistant to erythromycin and levofloxacin.*

***Conclusion:*** *emm serotypes may be evaluated alongside virulence factors to predict the clinical severity of GAS infections. It was concluded that the variation in the pathways used by virulence factors in different cell types, as well as the epigenetic modifications that may occur in virulence factors, may affect the behavior of the isolates.*

***Key Words:*** *Virulence factor; Group A streptococcus; Whole genom sequence*

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## ÖZ

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***Giriş:*** *2022-2023 döneminde grup A streptokok (GAS) infeksiyonu prevalansındaki artışla beraber invaziv seyreden vakaların da artması endişe uyandırmıştır. Amerika Birleşik Devletleri ve Avrupa ülkelerinde mortal seyreden vakalar bildirilmiştir. Bu durumu araştırmak amacıyla laboratuvarımıza gelen üç farklı klinik örnekten (plevra mayii, kan, idrar) izole edilen GAS suşuna yeni nesil dizileme yöntemleri kullanılarak tüm genom sekans analizi yapılmış ve National Center for Biotechnology Information (NCBI) GenBank boğaz sürüntüsü izolatlarına ait virülans faktörleri ile karşılaştırmalı olarak incelenmiştir.*

***Materyal ve Metod:*** *Kültürden DNA izolasyonu; bakteri spesifik DNA izolasyon kitiyle yapılmıştır. Ardından genomik DNA'lar bir uzun okuma platformu olan Oxford Nanopore ile dizilenmiştir. Elde edilen diziler de novo assembly aracı olan Flye (v2.9.2) kullanılarak daha uzun contigler oluşturulmuş ve NCBI Basic Local Alignment Search Tool (v2.14.0) kullanılarak nükleotid veri tabanında taratılmıştır. En yakın olduğu GAS suşunu bulmak için NCBI veri tabanından bu türe ait genomların hepsiyle yeni bir veri tabanı oluşturulmuştur. Ardından örneklere ait okumalar, uzun okumalar için kullanılan hizalama aracı Minimap2 (v2.26) ile yeni oluşturulan veri tabanındaki GAS suşlarına hizalanmıştır. En yakın GAS suşu belirlendikten sonra virülans faktör veri tabanı kullanılarak VFanalyzer ile taratılmış ve virülans faktörleri araştırılmıştır. National Center for Biotechnology Information GenBank kütüphanesinden edinilen Streptococcus pyogenes CP000259.1, CP000262.1, CP013838.1, AE009949.1, CP013839.1 boğaz sürüntüsü suşlarına ait virülans faktörleriyle karşılaştırılmıştır.*

***Bulgular:*** *İzolatlara ait virülans faktörleri karşılaştırıldığında Sortase A, Streptokokal lipoprotein rotamase A, Streptokokal kompleman inhibitör, Streptokokal pirojenik ekzotoksin J, Streptolizin S'nin boğaz sürüntüsü izolatlarında ya hiç bulunmadığı ya da en fazla birinde olduğu, buna karşılık çalışmada incelenen üç izolattan en az ikisinde saptandığı görülmüştür. İncelenen izolatların emm serotipleri plevra mayii ve idrar izolatları için emm1-43, kan izolatı için emm141-2 olarak saptanmıştır. Plevra mayii ve kan izolatlarına antibiyotik duyarlılık testi çalışılmıştır. Her iki izolat da penisilin duyarlı saptanırken; plevral örnekten üreyen izolat eritromisin ve levofloksasin dirençli, kan kültür setlerinden üreyen izolatlar eritromisin, klindamisin ve tetrasiklin dirençli tespit edilmiştir.*

***Sonuç:*** *Grup A streptokok infeksiyonlarının klinik şiddetini öngörmek için emm serotiplerinin virülans faktörleri birlikte değerlendirilebilir. Virülans faktörlerinin farklı hücre tiplerinde kullandığı yolakların ve epigenetik modifikasyonların da izolat davranışlarını etkileyebileceği sonucuna varılmıştır.*



***Anahtar Kelimeler:*** *Virülans faktörü; Grup A streptokok; Tüm genom dizileme*


## INTRODUCTION

During the coronavirus diseaes-2019 (COVID-19) pandemic, the prevalence of group A streptococcal (GAS) infection decreased, probably due to community isolation[1]. Since December 2022, GAS cases have increased 4-fold in the United Kingdom (UK) compared to the pre-pandemic period. Reports on the increase in prevalence have been published in European countries and the United States of America (USA), with two of the 34 cases identified in the USA being reported as fatal[1,2]. GAS usually causes non-invasive infections but can rarely cause life-threatening diseases such as bacteremia and streptococcal toxic shock syndrome[3]. With recent advances in whole genome sequencing (WGS) technology, it has become possible to analyze serotypes encoded by the *emm* gene, exotoxin genes, virulence factors, antimicrobial resistance genes (AMR), and other factors that may contribute to this condition[4]. In the 2022-2023 period, WGS analyses regarding the causes of the increase in severe GAS cases were also conducted in other countries, and it was investigated whether genomic changes in bacteria contributed to this increase[1,2,5]. In this study, virulence factors were investigated by WGS analysis of GAS strains isolated from three different clinical samples received by our laboratory.

## MATERIALS and METHODS

Three GAS strains isolated from pleural fluid, blood, and catheter urine samples at the Bacteriology Laboratory of the Ankara Bilkent City Hospital Department of Medical Microbiology between January-February 2023 were included in the study. While including these isolates in the study, it was taken into consideration that the isolates exhibited unexpected sites of involvement outside the throat. Another inclusion criterion was that the isolates were obtained from different patients and different types of clinical samples. The pleural fluid sample was obtained by thoracentesis from a previously healthy six-year-old male patient and a series of blood cultures were taken from the patient simultaneously. The blood sample was obtained from an 86-year-old male patient with malignant neoplasm of the lip and atherosclerotic heart disease, collected in two series of blood culture bottles. A series of urine cultures was also requested from the patient simultaneously. The catheter urine sample was obtained from a previously healthy six-year-old female patient with symptoms of urinary tract infection. The pleural fluid sample was cultured on 5% sheep blood agar, chocolate agar, and MacConkey agar. The blood sample was incubated in the BACT/ALERT® device in blood culture bottles and a positive signal was detected in both series at six hours. Gram staining of the bottles revealed gram-positive cocci, which were subsequently cultured on 5% sheep blood agar. Urine samples were cultured on 5% sheep blood agar and chromogenic agar. After incubation of the media at 35-37 °C for 18-24 hours, colonies were identified with MALDI-TOF mass spectrum (bioMérieux, France), and antibiotic susceptibility testing (AST) was performed with VITEK-2 (bioMérieux, France) according to European Committee on Antimicrobial Susceptibility Testing standards. One isolate was collected from each patient and stored in cryotubes at -80 °C. WGS analysis was then performed using third-generation sequencing methods.

### DNA Isolation

Group A streptococcal colonies growing on culture media were collected under sterile conditions, and bacterial DNA was isolated by following the gram-positive bacterial DNA isolation workflow using the all-in-one DNA isolation kit (DA101, MobiomX, Türkiye). Total bacterial DNA concentrations were measured using the Qubit spectrofluorometer (Thermo Fischer Scientific, USA).

### Library Preparation and Sequencing

GAS isolates were prepared using third-generation long-read platform Oxford Nanopore Technologies (ONT) ligation-based library preparation kits and sequenced with the mk1C sequencer. Library preparation of DNA isolated from pure culture was performed using the SQK-LSK109 library preparation kit and the EXP-NBD104 and EXP-NBD114 kits, which enable simultaneous sequencing of multiple samples in a single flow cell. For end repair and DNA repair, 3 µg of genomic DNA was prepared using the above-mentioned enzymes and protocol in a final volume of 48 µl. Samples were purified using magnetic beads, and barcode sequences were ligated to the fragments with NEB Blunt/TA Ligase Master Mix (M0367, NEB, UK) by incubation for 10 minutes at room temperature. The samples were once again purified with magnetic beads and their concentrations were measured using a Qubit spectrofluorometer. Equal amounts of barcoded DNAs from each sample were pipetted into a single tube with a final volume of 35 µl. Ligation adapters were incubated at 30 °C for 10 minutes to bind to barcode-linked DNA fragments with Rapid T4 DNA Ligase (MobiomX, Türkiye), and the final purification step was carried out. The library was prepared by adding sequencing buffer and library beads to the resulting DNA, and it was loaded into the FLO-MIN106D (ONT, UK) R9.4.1 spot-on flow cell and sequenced with the Mk1C sequencing device for 72 hours or until a sufficient amount of data was produced.

### Bioinformatics Method

Group A streptococcal isolates sequenced with ONT, which has long-read technology, were subjected to bioinformatics analysis using different tools for strain determination, obtaining polymorphism results, and finding antibiotic resistance genes and virulence factors.

First, longer contigs were created from the reads obtained from each sample using the de novo assembly tool Flye (v2.9.2) and scanned in the nt database using National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (v2.14.0). The reads obtained were found to belong to the GAS species and further genetic analyses were continued.

To find the closest GAS strain to the reads obtained from the isolates, a new database was created with all genomes of this species from the NCBI database. Then, the reads of the samples were aligned to the GAS strains in the newly created database with the alignment tool Minimap2 (v2.26) used for long reads.

The obtained isolates were scanned with VFanalyzer using the virulence factor database to investigate the virulence factors that may be present in their entire genomes and the data were compared with the virulence factors of *Streptococcus pyogenes* CP000259.1, CP000262.1, CP013838.1, AE009949.1, CP013839.1 throat swab strains obtained from the NCBI GenBank library.

## RESULTS

### Culture and AST Results

The isolate identified as GAS, grown from the pleural fluid sample, was found to be sensitive to penicillin and resistant to erythromycin and levofloxacin. There was no growth in the blood culture taken simultaneously. Both isolates grown from blood culture sets were identified as GAS and were found to be resistant to erythromycin, clindamycin, and tetracycline, and sensitive to penicillin. No growth was detected in the urine culture obtained simultaneously. Finally, the isolate grown from the urine culture sample was identified as GAS; however, AST was not performed.

### Bioinformatics Analysis Result

After alignment, it was observed that the isolates (pleural fluid, blood, urine) were best aligned to GAS strain MGAS2221 (NCBI GenBank ID: CP043530.1), strain MGAS2221 (NCBI GenBank ID: CP043530.1), strain S119 (NCBI GenBank ID: LR031521.1), respectively. Some important statistical information obtained after alignment were as follows: 100% coverage rate of the reads to the reference genome for all three isolates; pairwise similarity rates were 85.1%, 85%, 84.3%; average coverage values were 257X, 42X, 307X; total number of reads were 803.180, 650.902, 2.004.324; average read length was 6140 base pair (bp), 6200 bp, 5320 bp; maximum read length was 142383 bp, 198588 bp, 143301 bp; average quality score was 20, 19 and 20, respectively. The software used for quality control of reads is Geneious Prime (v.2022), Trimmomatic, FastQC. The threshold quality score was set at 15 (Phred score).

Circular representations of the consensus genomes obtained by aligning isolates (pleural fluid, blood, and urine) to the reference genome are shown in Figure 1, Figure 2, and Figure 3, respectively.

In addition, the reads obtained from the isolates were aligned to the database of "*emm*" genes used by the Centers for Disease Control and Prevention for serotyping GAS bacteria. This alignment was performed using Minimap2, a long-read alignment tool, within the Geneious Prime® (v2022.2.2) application. It was observed that the isolates were best aligned to the "*emm*1-43", "*emm*141-2", and "*emm*1-43" genes, respectively. Reference genome coverage of all isolates was 100%; pairwise similarity rates were 86.9%, 82%, and 85.4% and average coverage values were 67X, 207X, and 118X, respectively.

The virulence factors of the three isolates analyzed by WGS were compared with five throat isolates previously sequenced and available in NCBI GenBank (Table 1). The virulence factors that were present in at least two of the three isolates analyzed by WGS within the scope of our study, and were present in at most one of the throat isolates obtained from NCBI GenBank, were Sortase A, Streptococcal lipoprotein rotamase A (SlrA), Streptococcal complement inhibitor (SIC), Streptococcal pyrogenic exotoxin J (SpeJ), Streptolysin S (SLS).

Additionally, data on the isolates (pleural fluid, blood, and urine) were submitted to the NCBI GenBank, with the accession numbers CP148744, CP150790, and CP147828, respectively.

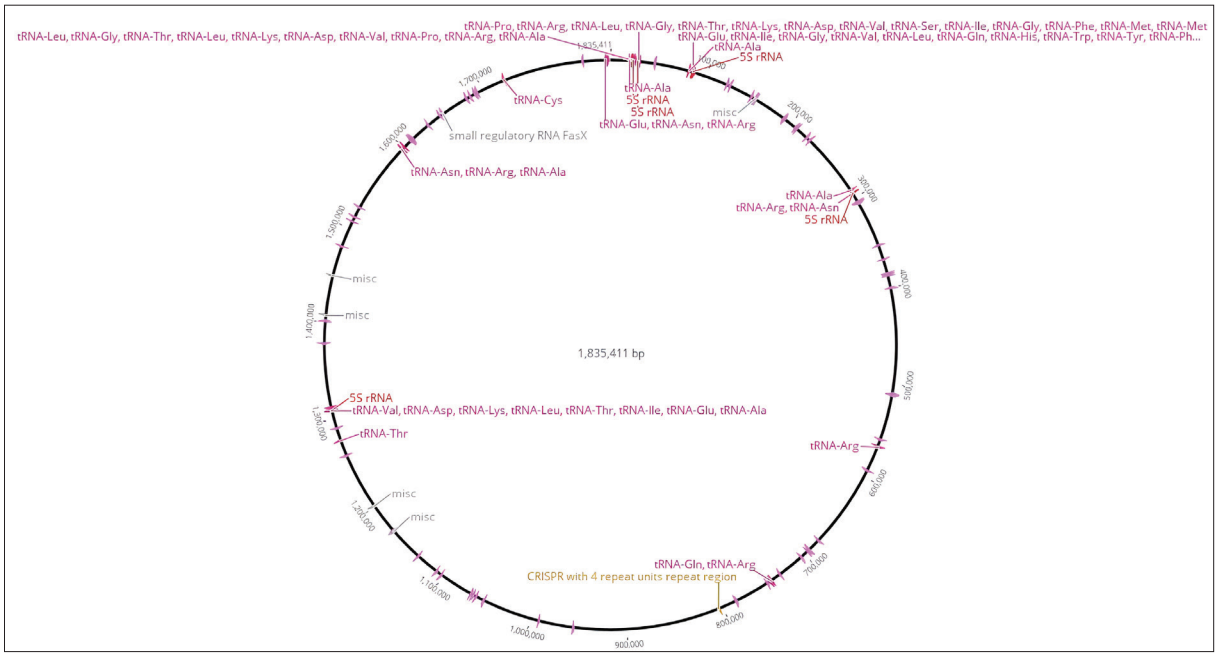


**Figure 1.** Circular representation of the consensus genome obtained by aligning the pleural fluid isolate to the reference genome of *Streptococcus pyogenes* strain MGAS2221 (NCBI GenBank ID: CP043530.1).

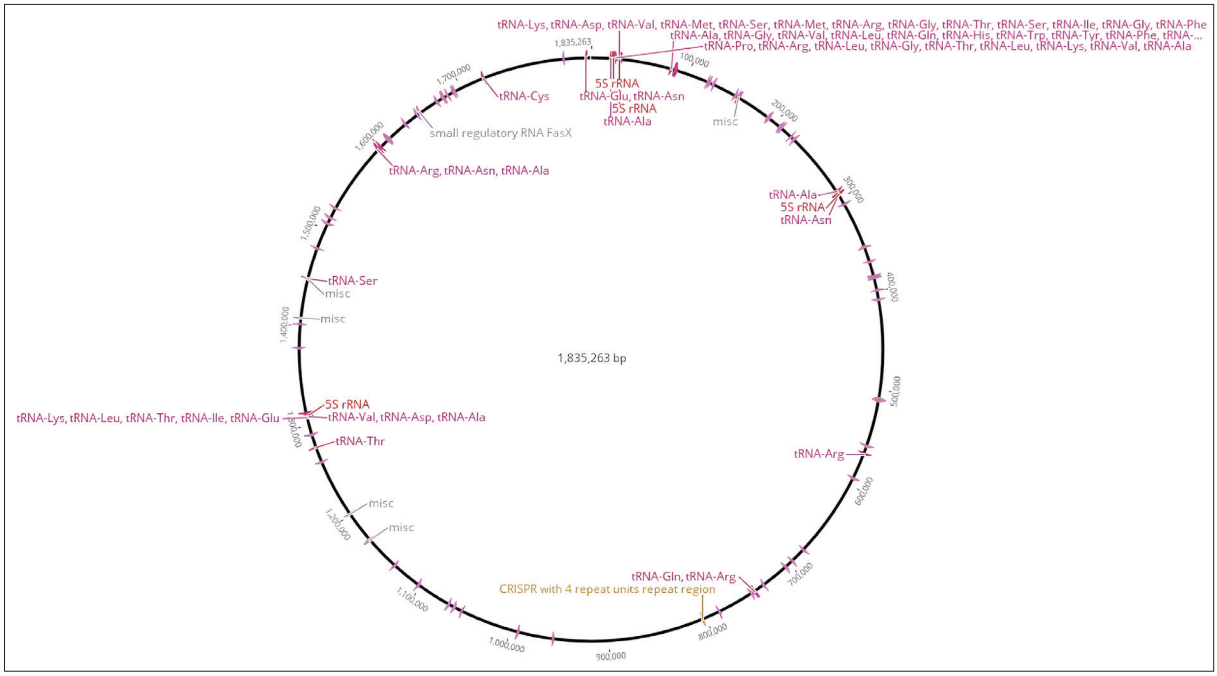


**Figure 2.** Circular representation of the consensus genome obtained by aligning the blood isolate to the reference genome of *Streptococcus pyogenes* strain MGAS2221 (NCBI GenBank ID: CP043530.1).

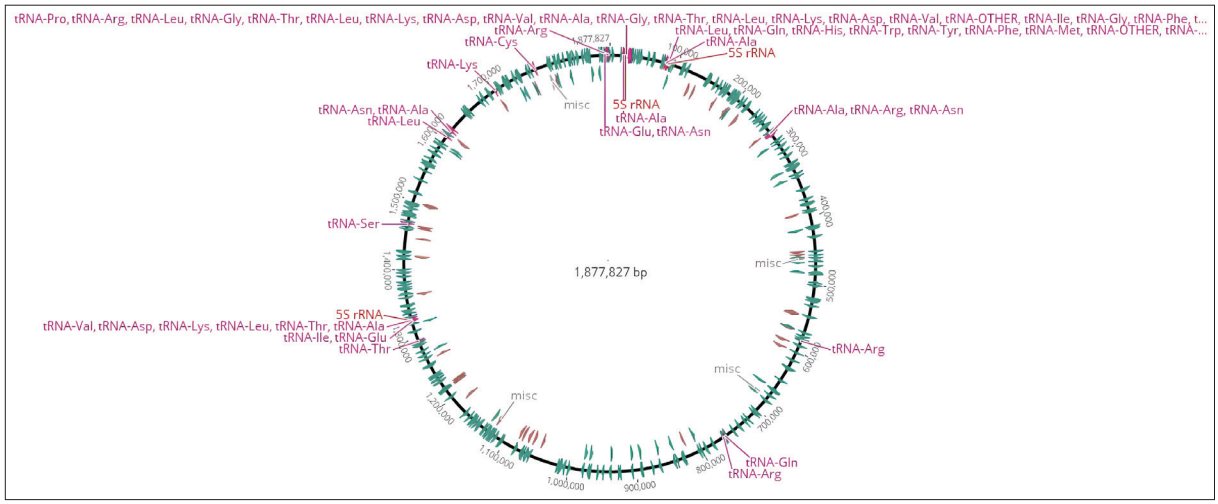


**Figure 3.** Circular representation of the consensus genome obtained by aligning the urine isolate to the reference genome of *Streptococcus pyogenes* strain S119 (NCBI GenBank ID: LR031521.1).

**Table 1. Comparison of virulence factors obtained by WGS analysis of NCBI GenBank throat swab GAS isolates and GAS isolates analyzed within the scope of the study**

| | | NCBI GenBank Samples (Throat swab isolates) | | | | | Study Samples | | |
|---|---|---|---|---|---|---|---|---|---|
| Virulence factor | Related Genes | CP00025 9.1 | CP0026 2.1 | CP01383 8.1 | AE00994 9.1 | CP01383 9.1 | Blood isolate | Pleural fluid isolate | Urine isolate |
| Sortase A | *str*A | | | | | | | x | x |
| Streptococcal lipoprotein rotamase A | *slr*A | | | | | | x | x | x |
| Streptococcal complement inhibitor (SIC) | *sic* | x | | | | | x | | x |
| Streptococcal pyrogenic exotoxin J | *spe*J | | | | | | x | x | x |
| Streptolysin S | *sag*A | | | | x | | x | x | x |

## DISCUSSION

In this study, WGS analysis was performed on three GAS strains isolated from different clinical samples in our laboratory during a period of increased and fatal invasive GAS infections. These strains were examined in comparison with throat isolates for which WGS analysis had previously been conducted by the NCBI. The presented patients were selected as they showed regions of involvement that are not commonly seen with GAS. This study aimed to examine the virulence factors; Sortase A, Streptococcal lipoprotein rotamase A (SlrA), Streptolysin S (SLS), Streptococcal pyrogenic exotoxin J (SpeJ), which differ among the isolates.

Sortase A was identified as one of the virulence factors present in two of the three GAS isolates analyzed in our study (pleural fluid and urine) but was absent in the throat isolates available in the NCBI GenBank database. Sortase A is a group of enzymes found in the cell membrane and is involved in the fixation of M protein to the cell wall through the covalent bonds it forms[6]. Studies have shown that strains lacking Sortase A can survive but become more susceptible to phagocytic killing[7]. In addition, it has been shown that the loss of Sortase A leads to the disruption of membrane integrity and changes in cellular morphology in bacteria, thus making them more susceptible to external factors[8]. Based on these studies, it

can be inferred that the presence of Sortase A is significant for both bacterial stability and resistance to eradication, potentially contributing to the differing sites of involvement and clinical progression observed in patients.

Another notable virulence factor was Streptococcal lipoprotein rotamase A (SlrA). It was detected in all three isolates analyzed but not in the throat isolates compared. Studies on *Streptococcus pneumonia* strains, regarding this virulence factor, showed that SlrA mutant isolates were rapidly eliminated from the upper respiratory tract, but no significant differences were found in elimination from the lungs, blood, or spleen. This virulence factor is thought to play a role in colonization but does not significantly contribute to invasive pneumococcal disease[9]. Further studies are needed regarding GAS, to understand the role of this virulence factor in invasive disease.

Streptococcal complement inhibitor (SIC), another virulence factor examined, is a protein that interferes with the membrane attack complex, inhibits antibacterial peptides, and triggers the release of proinflammatory cytokines through the pathways it activates[10,11]. Streptococcal complement inhibitor was detected in the blood and urine isolates analyzed by WGS but was found in only one of the NCBI throat isolates used for comparison. This virulence factor may help explain the clinical presentation of the patient with bacteremia. However, the gene for this factor was either not expressed in the urine isolate or may contribute to the current clinical presentation through different mechanisms that have not yet been identified. Further large-scale studies are needed to explore this topic.

It was known that virulence factors could activate different pathways depending on their environment. A study found that Streptolysin S (SLS) utilizes a different pathway in keratinocytes compared to erythrocytes, leading to programmed cell death[3]. This virulence factor was identified in all three isolates (pleural fluid, blood, and urine) but was present in only one of the NCBI GenBank throat isolates. The clinical samples and disease progression of the patients from whom these three isolates were obtained varied. It is conceivable that alternative pathways may have been activated in different contexts for the GAS strains studied, resulting in altered clinical progression.

Streptococcal pyrogenic exotoxins (Spe) are superantigens believed to be responsible for the excessive cytokine release observed in streptococcal toxic shock syndrome. Various Spe have been linked to invasive disease in different studies[12]. Within the scope of our study, SpeJ was particularly notable, as it was detected in all three isolates we analyzed but was absent in any of the NCBI GenBank throat isolates. In a recent study by Adela et al. on invasive GAS infections, SpeA and SpeJ were significantly higher in invasive GAS isolates compared to non-invasive GAS isolates[1]. Based on other studies, SpeJ may be considered one of the virulence factors potentially responsible for invasive disease.

While the relationship between invasive GAS cases and *emm* serotypes was more commonly studied before the COVID-19 pandemic, research focusing on whether the virulence factors of the bacteria contribute to the severe clinical conditions observed in the post-pandemic period has become more prevalent. In a study by Gonzalez-Abad et al. evaluating the increase in invasive GAS infections between 2011-2018 and its relationship with *emm* serotypes, isolates from various clinical samples (blood, pleura, synovial fluid, cerebrospinal fluid) were analyzed. M1 and M3 were found to be the most frequent[13]. In the study by Villalon et al. covering the period between 2007 and 2019, *emm*1, *emm*89, *emm*3, *emm*12, and *emm*76 were the most frequently identified serotypes in invasive GAS infections, and SpeA was associated with severe GAS cases[14]. In our cases, *emm*1-43 was identified in GAS isolates from the pleural fluid and urine samples, while *emm*141-2 was identified in the GAS isolate from the blood sample. Although the clinical courses of the patients from whom the pleural fluid and urine GAS isolates were obtained differed, their serotypes were found to be similar. From this perspective, it can be concluded that *emm* serotyping alone is insufficient to establish a correlation with the severity of the clinical presentation, as other virulence factors also play a significant role.

Previous AST studies on invasive GAS isolates have consistently shown universal susceptibility to penicillin, while resistance to macrolide antibiotics has been reported at rates ranging from 2.0-39.8%; to tetracyclines, 9.8-27.0%; to clindamycin, 0.7-9.0%; to trimethoprim-sulfamethoxazole, 1.9-6.3%; and to levofloxacin, 0.5-1.3%[14-19]. In the pleural fluid and blood isolates we analyzed by WGS, AST revealed that both isolates were susceptible to penicillin, trimethoprim-sulfamethoxazole, tigecycline, vancomycin, and teicoplanin. However, the pleural fluid isolate showed resistance to erythromycin and levofloxacin, while the blood sample isolate exhibited resistance to erythromycin, clindamycin, and tetracycline. These resistance profiles align with those reported in previous studies[14-19].

In this study, which aimed to investigate the reasons for the unexpected increase in prevalence and mortality of invasive GAS infections, various clinical samples from patients with differing clinical courses were analyzed using WGS and compared with the virulence factors of throat isolates available in the NCBI GenBank database. In predicting the clinical severity of GAS infections, evaluating *emm* serotypes in combination with virulence factors may be more informative and useful than considering *emm* serotypes alone. We believe that further research into the pathways utilized by virulence factors in different cell types, as well as changes in gene expression resulting from epigenetic modifications, could provide valuable insights.

The limitations of our study include the focus on the presence and absence of virulence factors in the isolates, without conducting any analysis on gene expression. Additionally, throat cultures were not obtained from the patients, which prevents any conclusions regarding GAS carriage or colonization.

## ETHICS COMMITTEE APPROVAL

This study was approved by the Ankara Bilkent City Hospital Number 2 Clinical Research Ethics Committee (Decision no: E2-23-5340, Date: 25.10.2023).

## CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## AUTHORSHIP CONTRIBUTIONS

Concept and Design: GK, AZUE, NÇ, BD

Analysis/Interpretation: UG, NK, GG, AZUE

Data Collection or Processing: UG, NK, GG, AZUE

Writing: GK, NÇ, BD, AZUE

Review and Correction: GK, NÇ, BD, AZUE

Final Approval: All of authors

## REFERENCES


1. *Alcolea-Medina A, Snell LB, Alder C, Charalampous T, Williams TGS; Synnovis Microbiology Laboratory Group; Tan MKI, Al-Yaakoubi N, Humayun G, Newsholme W, Goldenberg S, Nebbia G, et al. The ongoing Streptococcus pyogenes (Group A Streptococcus) outbreak in London, United Kingdom, in December 2022: A molecular epidemiology study. Clin Microbiol Infect 2023;29:887-90. https://doi.org/10.1016/j.cmi.2023.03.001*
2. *Barnes M, Youngkin E, Zipprich J, Bilski K, Gregory CJ, Dominguez SR, et al. Notes from the field: Increase in pediatric invasive group A Streptococcus infections - Colorado and Minnesota, October-December 2022. MMWR Morb Mortal Wkly Rep 2023;72:265-7. https://doi.org/10.15585/mmwr.mm7210a4*
3. *Hammers DE, Donahue DL, Tucker ZD, Ashfeld BL, Ploplis VA, Castellino FJ, et al. Streptolysin S targets the sodium-bicarbonate cotransporter NBCn1 to induce inflammation and cytotoxicity in human keratinocytes during Group A Streptococcal infection. Front Cell Infect Microbiol 2022;12:1002230. https://doi.org/10.3389/fcimb.2022.1002230*
4. *Li Y, Rivers J, Mathis S, Li Z, Velusamy S, Nanduri SA, et al. Genomic Surveillance of Streptococcus pyogenes Strains Causing Invasive Disease, United States, 2016-2017. Front Microbiol 2020;11:1547. https://doi.org/10.3389/fmicb.2020.01547*
5. *Garancini N, Ricci G, Ghezzi M, Tommasi P, Zunica F, Mandelli A, et al. Invasive group A streptococcal infections: are we facing a new outbreak? A case series with the experience of a single tertiary center. Ital J Pediatr 2023;49:88. https://doi.org/10.1186/s13052-023-01494-9*
6. *Wójcik M, Eleftheriadis N, Zwinderman MRH, Dömling ASS, Dekker FJ, Boersma YL. Identification of potential antivirulence agents by substitution-oriented screening for inhibitors of Streptococcus pyogenes sortase A. Eur J Med Chem 2019;161:93-100. https://doi.org/10.1016/j.ejmech.2018.10.027*

7. *Readnour BM, Ayinuola YA, Russo BT, Liang Z, Lee SW, Ploplis VA, et al. Evolution of Streptococcus pyogenes has maximized the efficiency of the Sortase A cleavage motif for cell wall transpeptidation. J Biol Chem 2022;298:101940. https://doi.org/10.1016/j.jbc.2022.101940*

8. *Raz A, Tanasescu AM, Zhao AM, Serrano A, Alston T, Sol A, et al. Streptococcus pyogenes sortase mutants are highly susceptible to killing by host factors due to aberrant envelope physiology. PLoS One 2015;10:e0140784. https://doi.org/10.1371/journal.pone.0140784*

9. *Hermans PW, Adrian PV, Albert C, Estevão S, Hoogenboezem T, Luijendijk IH, et al. The streptococcal lipoprotein rotamase A (SlrA) is a functional peptidyl-prolyl isomerase involved in pneumococcal colonization. J Biol Chem 2006;281:968-76. https://doi.org/10.1074/jbc.M510014200*

10. *Neumann A, Happonen L, Karlsson C, Bahnan W, Frick IM, Björck L. Streptococcal protein SIC activates monocytes and induces inflammation. iScience 2021;24:102339. https://doi.org/10.1016/j.isci.2021.102339*

11. *Frick IM, Shannon O, Åkesson P, Mörgelin M, Collin M, Schmidtchen A, et al. Antibacterial activity of the contact and complement systems is blocked by SIC, a protein secreted by Streptococcus pyogenes. J Biol Chem 2011;286:1331-40. https://doi.org/10.1074/jbc.M110.178350*

12. *Barnett TC, Cole JN, Rivera-Hernandez T, Henningham A, Paton JC, Nizet V, et al. Streptococcal toxins: Role in pathogenesis and disease. Cell Microbiol 2015;17:1721-41. https://doi.org/10.1111/cmi.12531*

13. *González-Abad MJ, Alonso Sanz M. Invasive Streptococcus pyogenes infections (2011-2018): EMM-type and clinical presentation. An Pediatr (Engl Ed) 2020;92:351-8. https://doi.org/10.1016/j.anpedi.2019.10.014*

14. *Villalón P, Sáez-Nieto JA, Rubio-López V, Medina-Pascual MJ, Garrido N, Carrasco G, et al. Invasive Streptococcus pyogenes disease in Spain: A microbiological and epidemiological study covering the period 2007-2019. Eur J Clin Microbiol Infect Dis 2021;40:2295-303. https://doi.org/10.1007/s10096-021-04279-2*

15. *Imöhl M, van der Linden M. Antimicrobial susceptibility of invasive Streptococcus pyogenes isolates in Germany during 2003-2013. PLoS One 2015;10:e0137313. https://doi.org/10.1371/journal.pone.0137313*

16. *Ikebe T, Hirasawa K, Suzuki R, Isobe J, Tanaka D, Katsukawa C, et al. Antimicrobial susceptibility survey of Streptococcus pyogenes isolated in Japan from patients with severe invasive group A streptococcal infections. Antimicrob Agents Chemother 2005;49:788-90. https://doi.org/10.1128/AAC.49.2.788-790.2005*

17. *Vigliarolo L, Gazzeli L, Bonofiglio L, Mollerach M, Lopardo H. [Susceptibility of Streptococcus pyogenes isolated from invasive infections to trimethoprim-sulfamethoxazole]. Medicina (B Aires) 2018;78:311-4.*

18. *Nir-Paz R, Block C, Shasha D, Korenman Z, Gorodnitzky Z, Jaffe J, et al. Macrolide, lincosamide and tetracycline susceptibility and emm characterisation of invasive Streptococcus pyogenes isolates in Israel. Int J Antimicrob Agents 2006;28:313-9. https://doi.org/10.1016/j.ijantimicag.2006.07.005*

19. *Ikebe T, Okuno R, Kanda Y, Sasaki M, Yamaguchi T, Otsuka H, et al. Molecular characterization and antimicrobial resistance of group A streptococcus isolates in streptococcal toxic shock syndrome cases in Japan from 2013 to 2018. Int J Med Microbiol 2021;311:151496. https://doi.org/10.1016/j.ijmm.2021.151496*

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