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The molecular determination of emm genotypes in non-group a beta-hemolytic streptococci isolated from clinical samples

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Abstract

Objective: M protein is an important marker in epidemiological and phylogenetic surveillance of Beta-Hemolytic Streptococci.

The aim of this study is to determine *emm* types and their distribution among the Group C/G streptococci (GCG/GGS) strains isolated from clinical samples.

Material and methods: The study includes 98 β-hemolytic streptococcus strains isolated from clinical samples in Çukurova University/Balcalı Hospital. 67 of these isolates, defined as serologic, of which 56 were identified as *Streptococcus dysgalactiae subsp. equisimilis*, 8 as *S. anginosus* and 3 as *S.equi subsp.zooepedimicus*, have been included in the study. These strains were also confirmed by the Vitek 2GP-ID system. These isolates were confirmed by the polymerase chain reaction (PCR) method with primers based on *groESL* sequences. PCR-sequence analysis method developed by the Centers for Disease Control (CDC) was used for typing according to *emm* polymorphism.

Results: 56/67 (83.6%) isolates in which *emm*-PCR was determined as positive were typed by sequence analysis. The findings were identified using the CDC database, and the most common type was determined to be stG485 (51.85%), stG643 (12.96%) and stG6 (9.25%).

Conclusion: Consequently, GCS/GGS should be treated more seriously in our country as in the whole world.

Key words: *emm*, PCR, GCS, GGS

Introduction

Beta hemolytic streptococci (BHS) are often the commensal flora members of various body regions such as upper respiratory tracts, gastrointestinal system, lower genital system and skin in humans. They cause local or systemic infections and complications frequently in the pyogenic character in patients with suppressed cellular and humoral immunity, such as children, overweight individuals, diabetic patients, and pregnant women. It is known that BHS serogroups and their types responsible for infections are as effective as host-related factors in the prognosis of infections. In this context, tonsillopharyngitis and its complications and skin and soft tissue infections such as erysipelas, impetigo, cellulitis and necrotizing fasciitis are mostly associated with Group A Beta-hemolytic streptococcal infections, while C and G groups are associated with more benign upper respiratory tract infections as well as uncomplicated skin-soft tissue infections and gastrointestinal system infections. It has been shown that *Streptococcus agalactiae, Streptococcus dysgalactiae subsp. equisimilis* (SDSE) and *S. anginosus*, located in the flora of female genital tract as opportunistic commensals, may lead to mortal invasive and non-invasive infections such as puerperal sepsis, bacteremia, endocarditis, meningitis, arthritis, osteomyelitis, pneumonia, toxic shock-like syndrome, and rhabdomyolysis in mother and neonate after delivery through vaginal delivery [1-5]. Today, the genotypic surveillance of SDSE strains is gradually gaining importance due to the similarity of *S. pyogenes* infections with the infections they cause in terms of the clinical and epidemiological characteristics [6,7].

The most important virulence factor of microorganism that affects the prognosis of infection in pyogenic streptococci is M protein, which plays a role in adhesion and immune system evasion. The M protein encoded in the *emm* in the chromosome is an antigenic protein found on the cell surface. This protein, which has many serotypes with phase and size variations, is responsible for the evasion of the host immune system by the microorganism, the formation of reinfections and reactivations. On the other hand, it has

been claimed that *emm* genotypes might be associated with different clinical manifestations. Thus, a database containing *emm* homologous sequences has been established in the CDC data bank among streptococci strains of Group C/G, like *emm* polymorphism database used in the surveillance of *S. pyogenes* strains. The DNA sequence analysis method is used in genotypic surveillance studies based on *emm* polymorphism [8-10].

Streptococcal infections and related sequelae are important health problems in our country as well as all over the world. The detection of M serotypes, the major virulence factor on the surface of streptococci, and their effects on clinical prognosis would provide the chance of early and rational intervention for infections and complications. There are no studies on the *emm* types of group C and G streptococci in our country. Therefore, information about group C and G streptococcal infections and pathogenesis is also limited, and this project is the first study in Turkey.

The aim of this study is to investigate the *emm* types and their distribution among the GCS/GGS strains isolated from clinical samples.

Material and methods

This study was carried out with the approval of Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 01.04.2016 and Decision No: 2016/19). 98 BHS isolates isolated from different clinical samples in Çukurova University/ Balcalı Hospital and identified with Vitek 2GP-ID system were included in the study between November 2015 and January 2017. The Streptococcal Grouping Kit (Oxoid) was used for the serotypic identification of these isolates. The phenotypically confirmed *S. dysgalactiae subsp. equisimilis and S. anginosus* strains were genotypically evaluated by the PCR method with EL-F (5'ACTCTTGTGTTAAATAAAATCC-3') and EL-R (5'ACGCAGCATTTTGAAGRGCA-3') primers based on *groESL* sequences [11]. The PCR conditions were performed in 30 cycles, each cycle being 1 minute at 94 ° C, 30 seconds at 53 ° C and 1 minute at 72 ° C and then 7 minutes at 72 ° C. The PCR products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide and photographed under UV light.

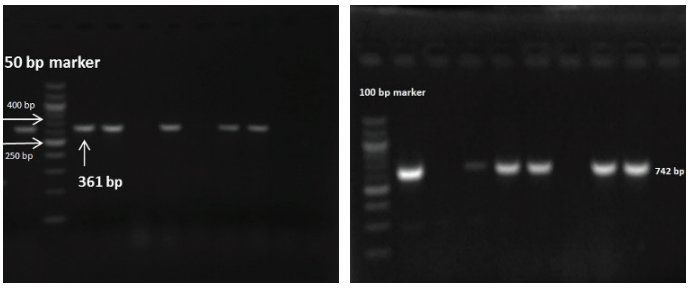
The clinical isolates were evaluated by *emm* type-specific PCR and Sequence analysis methods in respect of genotypic characteristics. For the detection of M protein in streptococci and replication of the *emm* region, the protocol that the CDC applied was taken into consideration and the CDC database was used for *emm* typing [9].

Results

In the study, 67 isolates were found to be included in C/G groups in the serotypic identification of 98 BHS isolates with the Streptococcal Grouping Kit (Oxoid). Of these strains, a total of 67 isolates including SDSE (56), *S. anginosus (8), S. equi subsp. zooepedimicus* (3), identified phenotypically by Vitek 2GP ID system, were included in the study. *SDSE (56) and S. anginosus* (8) isolates were confirmed by the PCR method with primers based on *groESL* sequences (Figure 1).

The determination and prevelance of *emm* types of a total of 67 isolates, which were SDSE (56), *S. equi subsp. zooepedimicus (3) and S. anginosus (8),* were examined. As a result of *emm*-PCR carried out for this purpose, in which emmSeq primer sequences were used, the *emm* gene was detected in 54 of 56 SDSE isolates, while presence of no *emm* gene was observed in 2 isolates. The *emm* gene was detected in only 2 of *S. equi subsp.*

Figure 1 - SDSE (361bp) and *S. anginosus* (742 bp) isolates gel electrophoresis of *groESL*-PCR products.



zooepedimicus isolates. However, no *emm* gene was detected in 8 *S. anginosus* isolates, either (Table 1).

54 SDSE isolates and 2 *S.equi subsp.zooepedimicus, emm* gene of which was detected in consequence of *emm*-PCR, were evaluated for *emm* typing. When the patient material from which SDSE strains taken under evaluation were isolated was examined, it was seen that the strains were isolated from the blood samples (31) the most and from the abscess material (3) the least (Table 2).

Table 1 Detection of the emm gene in SDSE and *S. anginosus* isolates.

Type	Number	emm-PCR (+)	emm-PCR (-)
SDSE	56	54	2
S. anginosus	8	0	8
S. equi subsp zooepedimicus	3	2	1

Table 2 Distribution of SDSE samples according to the patient material.

Strain no	Material	Strain no	Material	Strain no	Material
S1	Blood	S19	Throat	S37	Throat
S2	Throat	S20	Blood	S38	Blood
S3	Blood	S21	Blood	S39	Blood
S4	Abscess	S22	Abscess	S40	Blood
S5	Blood	S23	Throat	S41	Blood
S6	Throat	S24	Abscess	S42	Throat
S7	Blood	S25	Blood	S43	Blood
S8	Blood	S26	Blood	S44	Blood
S9	Throat	S27	Throat	S45	Throat
S10	Blood	S28	Wound	S46	Blood
S11	Throat	S29	Wound	S47	Blood
S12	Blood	S30	Blood	S48	Blood
S13	Throat	S31	Blood	S49	Throat
S14	Wound	S32	Wound	S50	Blood
S15	Blood	S33	Blood	S51	Blood
S16	Throat	S34	Blood	S52	Blood
S17	Blood	S35	Throat	S53	Blood
S18	Blood	S36	Wound	S54	Blood

Table 3 ***emm*** types detected in SDSE isolates.

emm type	Number (54 isolates)	Percent (%)
stG485	28	51.85
stG643	7	12.96
stG6	5	9.25
stG480	4	7.40
stG840	4	7.40
stG6792	3	5.55
stG6.1	2	3.70
stG4222	1	1.85

In the *emm* typing of SDSE isolates using the CDC database, 8 different *emm* types were identified, and it was found that the most frequent types were stG485 (%51.85), stG643 (%12.96), stG6 (%9.25), respectively, and the least frequent type was *stG4222 (%1.85). Additionally, stG6*792 type was identified in 2 S.equi subsp.zooepedimicus isolates (Table 3).

When the distribution of the detected *emm* types according to the patient material was examined, it was determined that stG485 type was most frequently isolated from blood material and stG643 type was most frequently isolated from throat material. The distribution of other SDSE strains according to their *emm* and material types is given in the table below (Table 4).

Table 4 Distribution of SDSE strains according to their *emm* and material types.

Strain no	Material	emm type	Strain no	Material	emm type	Strain no	Material	emm type
S1	Blood	stG485	S19	Throat	stG485	S37	Throat	stG643
S2	Throat	stG643	S20	Blood	stG6	S38	Blood	StG485
S3	Blood	stG6	S21	Blood	stG485	S39	Blood	StG485
S4	Abscess	stG480	S22	Abscess	stG485	S40	Blood	stG643
S5	Blood	StG485	S23	Throat	stG643	S41	Blood	stG480
S6	Throat	stG840	S24	Abscess	stG4222	S42	Throat	StG485
S7	Blood	stG485	S25	Blood	stG485	S43	Blood	stG840
S8	Blood	stG485	S26	Blood	stG485	S44	Blood	stG6.1
S9	Throat	stG6792	S27	Throat	stG643	S45	Throat	stG643
S10	Blood	stG6792	S28	Wound	stG480	S46	Blood	StG485
S11	Throat	StG485	S29	Wound	stG840	S47	Blood	StG485
S12	Blood	stG840	S30	Blood	StG485	S48	Blood	StG485
S13	Throat	stG485	S31	Blood	stG6792	S49	Throat	StG485
S14	Wound	stG6	S32	Wound	stG6.1	S50	Blood	StG485
S15	Blood	stG485	S33	Blood	StG485	S51	Blood	StG485
S16	Throat	stG6	S34	Blood	StG485	S52	Blood	StG485
S17	Blood	stG485	S35	Throat	stG643	S53	Blood	StG485
S18	Blood	stG6	S36	Wound	stG480	S54	Blood	StG485

Discussion

BHSs are a temporary part of normal flora in humans, and recent studies have reported that they increasingly may lead to various invasive and non-invasive infections such as pharyngitis, cellulitis, sepsis, meningitis, endocarditis, acute rheumatic fever, and poststreptococcal glomerulonephritis. GCS/GGS-related infections have been associated with predisposing factors such as bacteremia, alcoholism, diabetes mellitus, malignancy, intravenous substance use, or rupture of the skin. It has been carefully emphasized in recent studies that the major factor in BHS infections is the M protein. However, in many regions of the world, regarding GAS infections, importance is attached to the detection, prevalence, surveillance and epidemiological studies of emm type, while there is not a sufficient number of studies about GCS/GGS. Currently, the global burden of disease caused by SDSE is still not known in many parts of the world because it is not a common practice to identify Group C and G streptococci at the species level in clinical laboratories [12].

Although the number of studies on GAS *emm* typing is limited in our country, there is no study regarding the *emm* typing of GCS/GGS. In this study, our aim was the *emm* detection of a total of 67 isolates including *S. dysgalactiae subsp. equisimilis* (56), *S. anginosus* (8) and *S. equi subsp. zooepedimicus* (3), isolated from the patients presented with various clinical complaints to clinics/outpatient clinics during a period of approximately 14 months. As a result of *emm*-PCR carried out for this purpose, no presence of *emm* in 2 of 56 SDSE isolates, 1 of 3 *S. equi subsp. zooepedimicus* isolates and 8 *S. anginosus* isolates was detected, and only 56 SDSE isolates and 1 S. *equi subsp. zooepedimicus* isolate were included in the study. Similarly, Reibmann S. et al. determined in their study published in 2010 that 2 of 254 SDSE isolates and all of 59 *S. anginosus* isolates had negative *emm*-PCR result [13]. In their study, Rantala S. et al. also stated that *emm* was negative in 3 out of 140 SDSE isolates [10].

In our study, 8 different *emm* types were identified, and it was observed that stG485 (51.85%), stG643 (12.96%), stG6 (9.25%) were the most common types, respectively, while stG4222 (1.85%) was the least common type. In addition, stG6792 (2/3) type was determined in *S. equi subsp. zooepedimicus* isolate. In their study, Kittang B. R. et al. found that stG485, stG643 and stG6 types were more prevalent in the emm typing of 76 SDSE isolates isolated from invasive and non-invasive infections [14].

Poradosu et al. reported that 13 different *emm* types were present in the sequence typing of 56 GGS isolates and that the predominant type was stG48512. When Tseng et al. in Taiwan examined the distribution of emm types in 274 SDSE isolates, they reported that stG485 (45/274) was the most common type, followed by stG6.1 (43/274), stC839 (32/274), stG652 (24/274) and stG652.1 (17/274) types, which they reported to be common [15].

In their study carried out in South India, Reibmann et al. identified 44 different *emm* types in 252 SDSE isolates and reported that the most common types were stG245 (32), stG6792 (18), stG643 (17), stG6 (14), respectively [13].

When we compared the emm types detected in our study with those of other studies, we observed that they were similar to the *emm* types seen in different regions. Furthermore, in our study, upon examining the distribution of *emm* types according to the patient material, we identified stG485 type most frequently in SDSE isolates isolated from blood samples. In blood samples taken from patients with septicemia, Rantala et al., and in SDSE isolates isolated from the blood culture obtained from various

clinics, Poradosu et al. found that the most common type was stG485, and the most common type was determined to be stG6.1 and the second most common type was determined to be stG485 by Tseng et al. in blood samples in their study conducted in Taiwan [10-12-15].

In our study, the second most common stG643 *emm* type was detected in SDSE isolates isolated from the throat material. Anand et al. determined that stG643 type was predominant in SDSE isolates isolated from the throat material [16].

Consequently, identification of the GCS/GGS strain will enrich epidemiological data. Additionally, the definition of GCS/GGS-associated infections is important for the treatment of patients. We think that the relationship between emm types and GCS / GGS infections should be examined with future studies. In addition, this study will guide researchers in phenotypic-genotypic identification in future studies with Non-Group A Beta-Hemolytic Streptococci.

This study has limitations. Types of *emm* and their relationship with infection should also be examined. Examining the relationship between *emm* types and infection in streptococci will contribute to epidemiological studies.

Conclusion

As a result, today, GGS and GSS, which cause invasive and non-invasive infections, are still not treated seriously across the world, and there is no sufficient number of studies on the infection load, virulence factors, epidemiological and surveillance studies of these microorganisms. In the near future, these ignored microorganisms will probably increase in patients with chronic disease and many predisposing factors.

Specifically, the identification of *emm* types remains important for identifying the incidence, relation with infections and rapid changes in species distribution, which may lead to an increase in mortality. In this context, further studies on the relationship between *emm* types and infections should be demanded to be done. We think that this study, which is the first in our country, will be a reference for other studies to be conducted and the emm types should be examined with studies attended by multicentre and large groups related to GGS/GSS that cause serious infections.

Authors' Contributions: SK collected samples, cultured the isolates, and performed DNA extraction, PCR, *emm* sequencing. FK and SK designed the study. FK and SK supervised the practical work and data management. SK, CÖG and FK wrote the manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate: This study was carried out with the approval of Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 01.04.2016 and Decision No: 2016/19).

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