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Original Article

Investigation of *Streptococcus agalactiae* serotypes in clinical samples

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Abstract

Objective: A key contributor to neonatal early-onset sepsis, postpartum maternal sepsis, and infant late-onset invasive infections is Streptococcus agalactiae (group B Streptococcus; GBS). GBS may also result in bone and joint infections, bacteremia, endocarditis, pneumonia, and infections of the skin and soft tissues in adults. The bacterial polysaccharide capsule may distinguish between ten GBS serotypes (Ia, Ib, and II–IX). In order to reduce maternal colonization and prevent transmission to neonates, GBS capsular polysaccharide vaccines have been investigated. We aimed to detect the differences of the polysaccharide capsules among GBS isolates.

Methods: This study used GBS isolates from several clinical specimens in the Microbiology Laboratory at Marmara University Hospital in Istanbul. Fifty of the isolates were colonized (37 genital site, 13 throat swab), and 50 of the isolates were infectious (33 urine, 7 blood, 2 respiratory specimens, and 4 wound swab specimens, 4 sterile body fluids). The serotypes of GBS isolates were determined by detecting visible agglutination when specific GBS capsule antigens reacted with serotype monospecific antibodies.

Results: Colonized strain distribution values for serotype Ia, serotype II, serotype III, serotype Ic, serotype R, serotype V, serotype IV, serotype II/R, serotype III/R, and non-serotyping stains were 12 (24%), 11 (22%), 7 (14%), 3 (6%), 3 (6%) , 2 (4%), 1 (2%), 2 (4%), 1 (2%), 8 (16%) respectively. Causative agents distribution values for serotype II, serotype Ia, serotype III, serotype R, serotypes IV and serotype VIII, II / R, III / R, and non-serotyping stains were 15 (30%), 13 (26%), 10 (20%), 2 (4%), 1 (2%), 1 (2%), 3 (6%), 2 (4%) and 2 (4%) respectively.

Conclusion: Serotype Ia, serotype II, and serotype III were the common serotypes in our clinical isolates but molecular-based studies associated with GBS population are needed in our country.

Keywords: Infection, colonization, serotype, *Streptococcus agalactiae*.

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INTRODUCTION

Streptococcus agalactiae (group B Streptococcus; GBS) exists in ordinary human genitourinary and gastrointestinal flora. GBS is the significant cause of early-onset sepsis, late-onset invasive infections in babies, post-partum maternal sepsis, skin and soft tissue infections, pneumonia, bone and joint infections, bloodstream infections, and endocarditis in non-pregnant adults with underlying medical conditions (1,2).

The polysaccharide capsule of the bacteria classified ten GBS serotypes (Ia, Ib, and II-IX). The diversity of polysaccharide capsules is a major virulence factor that helps the microorganism evade the host's defense mechanisms. To decrease maternal colonization and avoid infection to newborns, a clinical trial of a GBS polysaccharide conjugate vaccine targeting serotypes Ia, Ib, II, III, and V was conducted in 2017. Followingly, because of the rise of infections caused by serotype IV, serotype IV was included to compose a hexavalent vaccine (Ia, Ib, II, III, IV, and V), that covered at minimum 98% of GBS isolates causing neonatal invasive disease. However, α-like protein (Alp) family which contains α, Rib, Alp2, Alp3/R28, Alp4, and ε proteins, encoded by the allelic bca, rib, alp2, alp3, alp4, and alp1/alp5 genes respectively, presented on the pathogenicity island IV has a significant role in GBS pathogenesis and is also a potential vaccine candidates (3-7). The β-protein, encoded by the bac gene, together with α forms so-called C-protein, the first known surface antigen of GBS (2,5).

The serotypes commonly causing neonatal GBS disease are Ia, III, and V worldwide (8-10). However, the predominant serotypes isolated from different clinical samples may vary between countries (11). In Turkey, epidemiological data related with GBS serotypes is limited. For this reason, the purpose of this study is to identify the differences of the polysaccharide capsules among GBS strains isolated in Marmara University Hospital Central Laboratory.

MATERIALS AND METHODS

Strains

This study investigated GBS strains that were the isolated from a Marmara University Hospital Microbiology Laboratory variety of clinical specimens. Fifty of the strains were colonized strains (37 genital site, 13 throat swab), and 50 of the strains were infectious strains (33 urine, 7 blood, 2 respiratory specimens, and 4 wound swab specimens, 4 sterile body fluids). Isolated strains were stored at -20°C. The isolates were subcultured on 5% sheep blood agar (Becton Dickinson, USA). Plates were incubated at 37ºC for 24 hours. The next day, catalase-negative gram gram-positive cocci were detected as group B streptococci by using The BBL™ Streptocard™ Acid Latex Test (Becton Dickinson, USA). In this study for capsular serotyping, *Streptococcus agalactiae* Type Ia, Ib, II, III, IV, V, VI, VII monospecific antibodies and *S. aureus* ATCC 25923 standard strains were used.

Capsular serotyping

Specific GBS capsule antigens reacted with serotype monospecific antibodies (through the carrier protein *S. aureus* protein A) and capsular serotypes were detected by a visible agglutination.

Preparation of protein A by co-agglutination method

Initially, *S. aureus* Cowan strain was cultivated on sheep blood agar and cultured at 37°C for 24 hours. The growing colony was cultivated in tryptic soy broth (Difco Laboratories, Detroit, Mich) and cultured at 37°C for 18 hours. After the incubation, tryptic soy broth media were centrifuged at 3000 rpm for one minute and the pellet was washed in phosphate-buffered saline (PBS) with 0.5% formaldehyde. Then the sediment was mixed and kept at room temperature for three hours. The mixture was centrifuged at 3000 rpm for one minute. The sediment was washed with 0.5% formaldehyde. PBS containing 0.1% NaN^3 was added onto the pellets. Final mixture was incubated at 80°C water bath for 10 minutes (12).

Acquisition of serotype-specific antigens with Hot application- HCL Method

GBS strains were inoculated on sheep blood agar and incubated at 37°C for 24 hours. The colonies were inoculated in 40 ml of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich) and incubated at 37°C for 18-24 hours. After the centrifugation (3000 rpm for one minute) the supernatant was discarded. Additionally, 0.35 ml 0.2 N HCl was added to the sediment, and mixed. This mixture was incubated at a 52°C water bath for two hours and mixed 15 minutes intervals. After cooling, it was centrifuged at 3000 rpm for one minute and fragmented antigen molecules including protein and polysaccharide became a soluble form. The supernatant was used (pH=7.2) for the study (12).

Preparation of co-agglutination marker

In the first step, one ml of S. aureus protein A and 140 ml serotype-specific antiserum were added and kept at room temperature for 10 minutes. The Staphylococcus suspension containing monospecific antibody, was washed twice with PBS. The pellet was suspended in 1 ml PBS containing 0.01% NaN3. This final mixture was stored at 4 °C until used (12).

Co-agglutination test

One drop of GBS study the antigen (HCl extract) and one drop of monospecific GBS serotypes reagent as a co-agglutination marker were mixed on the slide. The agglutination was observed and the presence of agglutination was considered to be a positive reaction for used serotype.

RESULTS

Among 100 GBS isolates, 50 were colonizing and 50 were infecting isolates. The distribution of GBS strains according to sample type and the patients' mean age were stated in Table 1, and Table 2. The results of co-agglutination test showing the agglutination reaction of specific antisera and reference serotype antigens for GBS capsular serotyping were stated in Table 3.

Table 1. Distribution of colonized GBS strains according to sample type and the mean age of the patients

Sample	Number of samples	Mean Age
Genital swab	37	32
Throat swab	13	9

Table 2. Distribution of the causative GBS strains according to sample type and the mean age of the patients

Sample	Number of samples	Mean Age
Urine	33	35
Blood	7	50
Wound swap	4	50
Sterile body Fluids	4	71
Respiratory samples	2	88

Table 3. The results of agglutination GBS serotype specific antisera with reference serotype antigens.

Specific Antisera	Reference serotype antigens										
	Ia	Ib	Ic	II	III	IV	V	VII	VIII	R	X
Ia	+	+	-	-	-	-	-	-	-	-	-
Ib	-	+	-	-	-	-	-	-	-	-	-
Ic	-	-	+	-	-	-	-	-	-	-	-
II	-	-	-	+	-	-	-	-	-	+	+
III	-	-	-	-	+	-	-	-	-	±	-
IV	-	-	-	-	-	+	-	-	-	-	-
V	-	-	-	-	-	-	+	-	-	-	-
VII	-	-	-	-	-	-	-	+	-	-	-
VIII	-	-	-	-	-	-	-	-	+	-	-
R	-	-	-	-	-	-	-	-	-	+	±
X	-	-	-	-	-	-	-	-	-	-	+

Abbreviations:(+): A positive agglutination reaction for 15-45 seconds. (-): Negative agglutination for 0-60 seconds (±): Weak agglutination reaction for 45 to 60 seconds

Colonized strain distribution values for serotype Ia, serotype II, serotype III, serotype Ic, serotype R, serotype V, serotype IV, serotype II/R, serotype III/R, and non-serotyping (NT) stains were 12 (24%), 11 (22%), 7 (14%), 3 (6%), 3 (6%) , 2 (4%),1 (2%) , 2 (4%), 1 (2%), 8 (16%) respectively.

Causative agents distribution values for serotype II, serotype Ia, serotype III, serotype R, serotypes IV and serotype VIII, II / R, III / R, and NT stains were 15 (30%), 13 (26%), 10 (20%), 2 (4%), 1 (2%), 1 (2%), 3 (6%), 2 (4%) and 2 (4%) respectively.

DISCUSSION

GBS isolates can be classified according to the surface protein antigens and capsular polysaccharide antigens. Ten GBS serotypes (Ia, Ib, II–IX) have been identified till now according to the classification of the GBS capsular polysaccharide antigen. Serotypes III, V, and Ia have been described as the main serotypes responsible for infections caused in newborns and adults in various studies, however this distribution can alter in different countries (13-19).

Terakubo et al. collected GBS isolates from 1404 pregnant out-patient women, and they investigated serotypes of 187 GBS isolates by using hemolytic streptococcus-typing immune sera. The common serotypes were VIII (32%), VI (27%), III (10%), and Ia (8%), respectively (14).

Tsolia et al. collected rectal and vaginal swabs from 1014 parturient or pregnant women in Athens, Greece and found that the most prevalent serotypes among colonized isolates were II, III, Ia, Ib and V with 26.9%, 22.4%, 19%, 12%, and 9% respectively (15).

In a study in Italy, among invasive infectious isolates, the serotype distribution was as follows: Type Ia (29%), III (22.6%), II (19.4%), V (12.9%), Ib (6.5%), VII (3.2%), NT (3.2%). In Italy study, serotype distribution was reported as Tip V (25 %), III (16.7 %), II (11.1 %), Ia (13.9 %), Ib (5.5 %), NT (19.4%) in non-invasive infectious isolates and Type III (33.3%), II (12.5%), Ia (8.3%), Ib (4.2%), IV (4.2%) in colonizing isolates (16).

In a study conducted in Denmark at 2019, it was found that in female carriage isolates the common serotypes were IX (21 %), serotype III (19 %), and serotype II (14.9 %), and the resistance against erythromycin and clindamycin was 21% and 26 %, respectively. Including invasive GBS strains (female/male) cultured from cerebrospinal fluid or blood, the predominant serotypes were serotype V , III, Ia, II and NT isolates that constituted 23.5 %, 21.8 %, 16.8 %, 11.4 % and 9.4 % respectively. Erythromycin and clindamycin resistance rates were 23 and 15 %, respectively in invasive GBS isolates. Serotype IX was not detected in an instance of early-onset illness patients. This study indicated that early-onset disease development was caused by GBS transmission from mother to newborn except for serotype IX (19).

In Turkey, Topkaya et al. investigated the serotype dispersion and the prevalence of GBS species isolated from rectal and vaginal swabs of 1026 pregnant women. They reported that 9.74% of women were positive for GBS and serotype Type Ia (29%), serotype II (25%), and III (15%) were the common serotypes (17). In a different study published by the same authors, 46 GBS strains were isolated from mothers' specimens and eight from newborns. Mother and newborn colonization incidences were detected as 9.2% and 1.6%, respectively. Type Ia, Type II, and Type III serotypes were the most prevalent in GBS isolates from mothers and newborns, and the vertical transmission rate was 15.2% (18).

In our study, we found that common serotypes were serotype Ia (24%), serotype II (22%), serotype III (14%), respectively in colonized GBS strains and serotype II (30%), serotype Ia (26%), serotype III (20%) in invasive GBS strains. Our data showed that in İstanbul, serotype II, serotype Ia, and serotype III are common serotypes but multicenter studies are needed to investigate the dominant serotypes of GBS isolates in our country.

On the other hand, recent studies showed that detecting the common surface proteins of GBS isolates would have a potential role in the development of vaccines. The C protein, which consists of α and β proteins, is the first surface protein found in GBS as a result of research. The major surface-localised proteins of GBS were α-C-protein, β-C-protein, the R-proteins (R1, R3, R4), and the α-like proteins (Alp2 and Alp3) which may be R1 variants, the ε protein (Alp1). In a study in The United States of America, 289 invasive and 2660 colonizing GBS strains were investigated. The most frequent surface protein distribution pattern in different GBS serotypes was found as alpha in type Ia; alpha plus beta in type Ib, alpha and R4 in type II; R4 in type III, and co-expression

of R1 plus R4 in isolates of type V. The most common (33.3%) expression profile was R1 plus R4 among the 72 non-typable colonizing strains. They concluded that the GBS surface proteins and the prevalent serotypes were dispersed evenly in colonizing and invasive strains and trypsin-resistant, alpha, and alpha-like proteins, R1 and R4 were the most common proteins (13). Pregnant women can use the GBS vaccine as a preventative measure to avoid neonatal and newborn GBS illness. The World Health Organisation (WHO), in 2015, mentioned that GBS vaccine development for maternal immunization in pregnancy in low-income and middle-income is a priority (20). Our study and literature data indicated that we need further studies detecting the common capsular polysaccharide antigens and surface proteins of GBS isolates; the molecular-based studies will contribute to GBS vaccine development and the management of GBS infections.

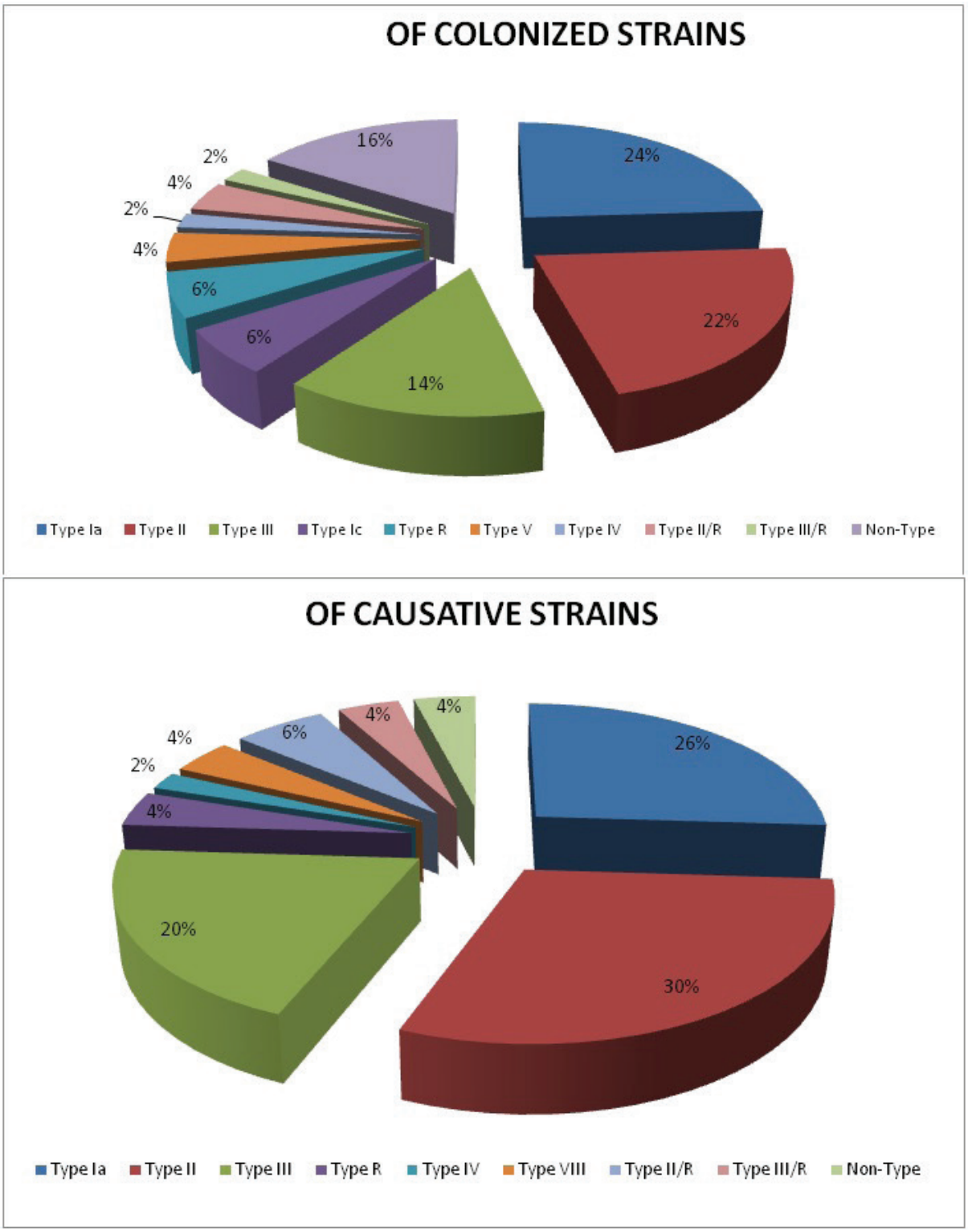

Figure 1. Serotype percentages of colonized and causative GBS isolates.

Limitations:

This research has a few constraints. The research has been studied in a single center and has a comparably small sample size. Another limitation is the absence of the use of molecular-based tests.

CONCLUSION

Serotype Ia, serotype II, and serotype III were the most prevalent serotypes in our clinical isolates of İstanbul. The multicenter studies investigating the dominant serotypes of GBS isolates and the common surface in our country are essential in order to reduce maternal colonization and prevent transmission to neonates. Additionally, using next-generation sequencing techniques for understanding molecular epidemiology and virulence of GBS will contribute to GBS vaccine development and the management of GBS infections.

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Author contributions: Design of the study; ÇA, FB - Supervision; ÇA, TD - Data collection &/or processing; ÇA, İHE - Performed data analysis; ÇA, İHE - Literature search; Ç.A, TD- Written by; TD, ÇA - Critical review;FB, İHE, ÇA, TD

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