의학석사 학위논문

Genotype Characterization of Group B *Streptococcus* Isolated from Infants with Invasive Diseases in South Korea

소아 침습성 질환에서 분리된 그룹 B 사슬알균의 유전자형 분석

2016년 2월

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A thesis of the Master's degree

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강 현 미

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Genotype Characterization of Group B *Streptococcus* Isolated from Infants with Invasive Diseases in South Korea

by
Hyun Mi Kang

A thesis submitted to the Department of Medicine
in partial fulfillment of the requirements for the
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Approved by Thesis Committee:

Professor _____ Chairman

Professor _____ Vice chairman

Professor _____

Abstract

Genotype Characterization of Group B *Streptococcus* Isolated from Infants with Invasive Diseases in South Korea

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Background: Group B *Streptococcus* (GBS) is one of the leading causes of invasive bacterial diseases in infants, and the disease risk is highest during the first three months of age. The purpose of this study was to investigate the genotypic diversity of GBS isolated from infants with invasive diseases in South Korea, and to observe the prevalence of the highly virulent clone.

Method: Isolates from infants with invasive GBS diseases were collected prospectively from infants with invasive GBS diseases admitted at 4 referral hospitals from 1995 to 2015, in South Korea. Capsular serotype was determined by sequencing analysis, and multilocus sequence typing (MLST) was carried out. All GBS isolates were tested for the presence of the gene encoding the hypervirulent surface adhesin (*hvgA*) by PCR amplification.

Antibiotic susceptibility testing was done by gradient diffusion E-test, and *ermB* and *mefA* genes were detected using PCR amplification.

Results: Among the 98 GBS isolates collected, 16.3% of the isolates were early-onset disease (EOD), 69.4% were late-onset disease (LOD), and 14.3% were late late-onset disease (LLOD). Fourteen STs were found; ST1 (20.4%), ST17 (19.4%) and ST19 (18.4%) were the most prevalent. eBURST analysis revealed 2 clonal complexes (CCs) and 6 singletons. Serotype III (n=50, 51.0%) was the most prevalent, followed by V (n=18, 18.4%), Ia (n=15, 15.3%), and Ib (n=13, 13.3%). Isolates of the same ST usually expressed one dominant serotype capsule. The dominant serotype capsule expressed by ST1 was serotype V, ST17 and ST19 was serotype III, and ST23 was serotype Ia. The *hvgA* gene was detected in 19.4% (n=19) of the isolates; all ST17 and serotype III (P<0.001). A significant temporal trend of the isolates in serotype III was observed; as ST17 increased (P=0.001) in proportion, ST19 decreased (P=0.009). ST1 was a significant pathogen in EOD (43.8%) compared to LOD (16.2%) and LLOD (14.3%) (P=0.039), and was less inclined to cause meningitis (P=0.005) compared with other STs. Erythromycin resistance was significantly associated with ST1 than other STs (P<0.001), with 85% (n=17/20) of the isolates resistant. In contrast, only 7.1% (n=1/14) of ST23 were resistant to erythromycin.

Conclusion: Genotype analysis helps in understanding the specific traits of strains that cause invasive diseases in infants. In particular, ST1 is an important strain causing EOD with a high rate of macrolide resistance, and manifests as diseases other than meningitis. The hypervirulent ST17 is

prevalent in South Korea, and a trend in the increase in its proportion is seen together with the decrease in ST19.

Keywords: Group B Streptococcus, invasive infection, serotype, multilocus sequence type, *hvgA*

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Contents

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List of Abbreviations

CSF, cerebrospinal fluid

CC, clonal complex

EOD, early-onset disease

GBS, group B Streptococcus

hvgA, hypervirulent GBS adhesion

IAP, intrapartum antibiotic prophylaxis

LLOD, late late-onset disease

LOD, late-onset disease

MIC, minimum inhibitory concentration

MLST, multilocus sequence typing

ST, sequence type

1. Introduction

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS), is one of the leading causes of invasive bacterial diseases in infants, and the disease risk is highest during the first three months of age. Because early-onset disease (EOD) (age 0 to 72 hours) results from the transmission of GBS during or before delivery from a colonized mother, universal screening and intrapartum antibiotic prophylaxis (IAP) (1-3) has been extremely effective in reducing the incidence of EOD (4). Meanwhile, the incidence of late-onset disease (LOD) (age 3-89 days), which is usually acquired from the mother or from the environment, remained unchanged (5, 6).

In South Korea, the low rate of GBS colonizers in pregnant woman ranging from 2% to 6% (7-10) and the low prevalence of neonatal GBS sepsis (11) have been barriers to the universal adoption of IAP throughout the country. However, the most recent study on GBS carriage rate in pregnant women revealed that the colonization rate has increased to 10% (12). Also, in 2011-2013, a multicenter study of 25 hospitals throughout the country revealed that GBS was responsible for 44% of invasive bacterial infections in patients below 3 months old (13), heightening the awareness of the importance of GBS.

The polysaccharide capsule is an important virulence factor in causing invasive infections, and GBS are classified into ten capsular polysaccharide types, serotypes Ia, Ib, II-IX. Worldwide five serotypes (Ia, Ib, II, II, and V) account for up to 96% of all neonatal invasive GBS infections (4, 14, 15). Among the 5 common serotypes, serotype III strains are especially important,

as they are responsible for a large proportion of EOD and majority of LOD in infants worldwide.

Multilocus sequence typing (MLST) is also used to characterize GBS, which involves sequencing the seven housekeeping genes (16). Because of the higher discriminatory power of MLST, diverse lineages within serotypes have been distinguished providing information on GBS colonization dynamics; the extent of changes over time and differences in clonality in different areas. Assessing clonality is also useful for understanding the characteristics and virulence potentials of the clones, as well as identifying specific clones that are more easily acquired or are difficult to eradicate which are significant in the prevention of neonatal GBD infections (16-18).

Through serotype and MLST analysis, ST17 of serotype III were found to be responsible for a large majority of neonatal invasive diseases, and became known as “highly virulent clones” (17-19). These strains express a surface-anchored protein, known as the hypervirulent GBS adhesion *(hvgA)*. This anchor protein enables them to adhere to epithelial and endothelial cells, thus promoting persistent intestinal colonization, translocation across the intestinal barrier, and the crossing of the blood-brain barrier (20).

The primary aim of this study was to investigate the genotypic diversity of GBS and to observe the prevalence of highly virulent clones that cause invasive diseases in infants. The secondary objectives were to explore the phenotypic characteristics, specifically the antibiotic resistance profiles and clinical manifestations of the genotypes.

2. Materials and Methods

2.1 Study Design

This study was conducted at four tertiary hospitals in South Korea, with cases from Seoul National University Hospital collected from April, 1995 to July, 2015. Cases from Seoul National University Bundang Hospital and Chonbuk National University Hospital were included starting January 2003, and Busan Paik Hospital from January, 2006.

A case of invasive GBS infection was defined as an infant with GBS isolated from a normally sterile site including the blood, cerebrospinal fluid (CSF), and synovial fluid. Invasive GBS infection was categorized depending on the onset of infection: within 72 hours from birth as early-onset disease (EOD), from 72 hours to 89 days as late-onset disease (LOD), and beyond 90 days after birth as late late-onset disease (LLOD). The demographic information on the cases included in this study were collected from the medical records, including details on birth history, any underlying conditions, clinical manifestations, onset of infection, image findings, treatment methods and drugs, and outcome.

The institutional review board of Seoul National University Hospital approved this study. Informed consent was waived.

2.2 Bacterial Isolates and Antimicrobial Susceptibility Testing

GBS isolates from invasive infections from children below 1 year old were collected prospectively. These isolates were stored at -70°C. GBS

identification was initially performed using automated microbiology systems, included Vitek-1 (bioMerieux, Marcy L'Etoile, France) and MicroScan Walk-Away (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Subculture of the stored GBS isolates were carried out on 5% sheep blood agar plates showing Gram-positive, β-hemolysis on sheep blood agar, and CAMP test-positive (2).

The minimum inhibitory concentrations (MICs) were determined by gradient diffusion E-test for penicillin, erythromycin, and clindamycin; and double disk synergy test (D-test) was performed to determine inducible resistance of erythromycin and clindamycin. The following MICs were used to define susceptibility, according to the 2014 Clinical and Laboratory Standards Institute guidelines (CLSI, 2014): for penicillin, MIC ≤0.12 μg/mL, and for both erythromycin and clindamycin, MIC ≤0.25 μg/mL. Resistance in erythromycin and clindamycin was defined as a MIC ≥1 μg/mL.

2.3 DNA Extraction

Chromosomal DNA was extracted from the GBS isolates that were cultured overnight, grown on 5% sheep blood agar using a DNA extraction kit (DNeasy Kit; Qiagen GmbH) according to the instructions of the manufacturer.

2.4 Capsular Serotyping and Multilocus Sequence Typing

The capsular polysaccharide (CPS) type-specific regions of the cps locus were amplified by polymerase chain reaction (PCR). The PCR products were then sequenced and capsular polysaccharide types were determined for serotypes Ia, Ib, and II through VII as described previously (21).

Internal fragments of the seven housekeeping genes (*adhP*, *atr*, *glck*, *glnA*, *pheS*, *sdhA*, and *tkt*) were PCR-amplified using previously described oligonucleotide primers by Jones et al. (16). The amplification products were then sequenced and then submitted to the GBS MLST database (http://pubmlst.org/sagalactiae/) for designation at each of the 7 loci. Each isolate was designated by a seven-integer number, known as its allelic profile, and entered into the GBS MLST database for assignment of the sequence type (ST). Sequence types that shared 6 identical alleles of the 7 loci were clustered into a clonal complex (CC) using eBURST (version 3; http://eburst.mlst.net).

2.5 *HvgA* gene detection

All 98 GBS isolates underwent PCR amplification of the 210 base pair genetic region encoding the S10 domain of the surface protein to detect the presence of surface protein *hvgA* (17). The amplified products were analyzed by pulsed-field gel electrophoresis to detect the presence of *hvgA*.

2.6 Detection of macrolide-resistant genes

On all the isolates non-susceptible to erythromycin, PCR amplification of

the macrolide resistance genes *ermA, ermB* and *mefA* was performed. Primers for these three genes were designed based on previously published sequences (22, 23). The amplified products were then analyzed by pulsed-field gel electrophoresis to detect the presence *ermA, ermB* and *mefA*.

2.7 Statistical analyses

Categorical variables were compared by using Chi-square test or Fisher's exact test, and continuous variables were compared by Mann-Whitney U test. The R2 was used to assess the strength of the relationship and the F-test was used to assess the fit of the regression model of the ST trend. Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) software (ver. 21.0; SPSS Inc., U.S.). All tests were 2-tailed and were considered statistically significant when the *P* value was less than 0.05.

3. Results

3.1 Demographic and Clinical Characteristics

During 1995 to 2015, a total of 98 invasive GBS isolates were collected from patients less than 1 years of age admitted at Seoul National University Hospital (n=38), Seoul National University Bundang Hospital (n=18), Chonbuk National University Hospital (n=31), and Busan Paik Hospital (n=11). Sixteen (16.3%) cases were EOD, 68 (69.4%) cases were LOD, and 14 (14.3%) cases were LLOD. Fifteen (15.3%) cases were infections in preterm infants, who had a median gestational age of 30+1 weeks. Overall, the median age at onset was 21 days (IQR 10-57 days), and bacteremia (n=48, 49.0%) was the most common diagnosis, followed meningitis (n=42, 42.9%). All 3 cases of pneumonia occurred as early-onset diseases (Table 1).

Table 1. Clinical characteristics of patients with invasive Group B *Streptococcus* isolated from infants less than 1 year old from South Korea according to disease category

	No. of cases (%)				*P*[1]
	Total (N=98)	Early-onset (N=16)	Late-onset (N=68)	Late late-onset (N=14)	
Birth history					
Term infants	83 (84.7)	15 (93.8)	56 (82.4)	12 (85.7)	0.519
Preterm infants	15 (15.3)	1 (6.2)	12 (17.6)	2 (14.3)	
Median gestational age					
Term infants, weeks+days	39+6	39+5	40+0	40+0	*NA*
Preterm infants, weeks+days	30+1	28+0	31+4	25+0	
Median age at onset, days (IQR)	21 (10-57)	1 (0-1)	21 (13-44)	139 (109-365)	*NA*
Clinical diagnoses					
Bacteremia	48 (49.0)	9 (56.3)	31 (45.5)	8 (57.1)	0.599
Meningitis	42 (42.9)	4 (25)	32 (47.1)	6 (42.9)	0.276
Osteomyelitis	5 (5.1)	-	5 (7.4)	-	0.313
Bacteremic pneumonia	3 (3.0)	3 (18.8)	-	-	<0.001

[1]Pearson's chi-square test

Abbreviations: NA not applicable; IQR interquartile range

3.2 Serotype Distribution and Multilocus Sequence Types

Six capsular serotypes were identified in 98 GBS isolates. Serotype III (n=50, 51.0%) was the most prevalent, followed by V (n=18, 18.4%), Ia (n=15, 15.3%), and Ib (n=13, 13.3%). There was one isolate of serotype II and VI.

Among the 98 invasive GBS isolates, a total of 14 STs were found. The most prevalent type was ST1 (n=20, 20.4%), followed by ST17 (n=19, 19.4%), ST19 (n=18, 18.4%), and ST23 (n=14, 14.3%).

Isolates of the same ST usually expressed one dominant serotype capsule, showing a relationship between STs and serotypes. The dominant serotype capsule expressed by ST1 was serotype V, ST17 and ST19 was serotype III, and ST23 was serotype Ia (Table 2).

Table 2. The relationship between sequence type and capsular serotype of the 98 invasive Group B *Streptococcus* isolates

Sequence type (ST)	No. of isolates	No. of serotypes	Capsular serotype
ST1	20	3	III (1), V (18), VI (1)
ST3	1	1	Ib (1)
ST8	4	1	Ib (4)
ST10	4	2	Ib (3), II (1)
ST12	1	1	Ib (1)
ST17	19	1	III (19)
ST19	18	1	III (18)
ST23	14	1	Ia (14)
ST24	1	1	Ia (1)
ST27	2	1	III (2)
ST112	2	1	III (2)
ST335	7	1	III (7)
ST529	1	1	III (1)
ST654	4	1	Ib (4)

Through eBURST analysis, 2 clonal complexes, CC19 (n=29, 29.6%) and CC10 (n=12, 12.2%), and six singletons were identified (Fig. 1).

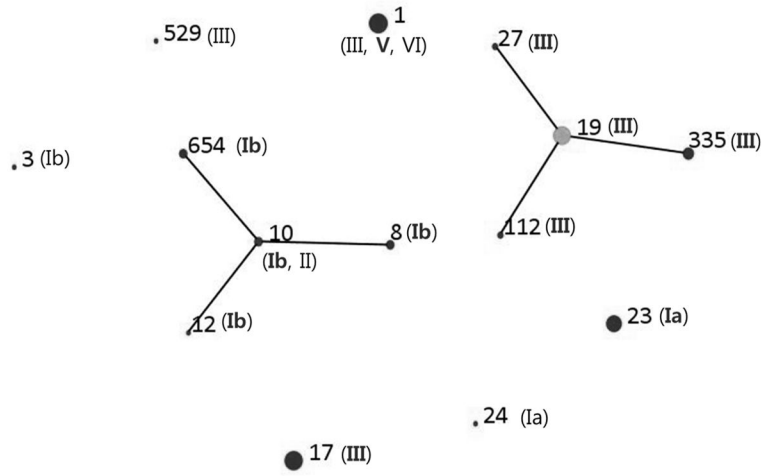

Figure 1. eBURST analysis of the 98 invasive Group B *Streptococcus* isolates. Two clonal complexes and 6 singletons were found. ST19 (large grey circle) is predicted to be the primary founder of the CC19, and ST10 (small grey circle) of CC10. Numbers indicate ST (Serotype). The size of each of the circles correlates with the number of isolates of that particular ST.

In EOD, the most prevalent STs were ST1 (n=7/16, 43.8%) and ST19 (n=4/16, 25%); however, in LOD and LLOD, ST17 (n=14/68, 20.6% in LOD and n=4/14, 28.6% in LLOD) and ST19 (n=11/68, 16.2% in LOD and n=3/14, 21.4% in LLOD) were predominant. ST1 was a significant pathogen in EOD (43.8%) compared to LOD (16.2%) and LLOD (14.3%) (P=0.039). Table 3 shows the distribution of each ST/CC in relation to the disease onset. Although all the STs and CCs manifested as both meningitis and non-meningitis, ST1 rarely manifested as meningitis (P=0.005) (Table 4).

Table 3. The distribution of sequence type and clonal complexes in relation to disease onset

CC/ST	No. of cases [%]			*P*[1]
	EOD N=16	LOD N=68	LLOD N=14	
CC19[2]	5 (31.3)	19 (27.9)	5 (35.7)	0.834
CC10[3]	-	12 (17.6)	1 (7.1)	0.133
ST1	7 (43.8)	11 (16.2)	2 (14.3)	0.039
ST17	1 (6.3)	14 (20.6)	4 (28.6)	0.275
ST23	2 (12.5)	10 (14.7)	2 (14.3)	0.975
ST3	-	1 (1.5)	-	0.8
ST24	1 (6.3)	-	-	0.075
ST529	-	1 (1.5)	-	0.8

Abbreviations: EOD early-onset disease; LOD late-onset disease; LLOD late late-onset disease;

[1] Pearson's chi-square test showing the significance of the ST in relation to EOD, LOD, and LLOD

[2] CC19 includes ST19, ST335, and ST 112

[3] CC10 includes ST8, ST10, ST12, and ST 654

Table 4. Distribution of each sequence type and clonal complexes in relation to the clinical diagnoses

CC/ST	No. of cases (%)			*P*-value[1]
	Total (N=98)	Meningitis	Non-meningitis	
CC19[2]	29	16 (55.2)	13 (44.8)	*0.110*
CC10[3]	13	5 (38.5)	8 (61.5)	*0.731*
ST1	20	3 (15)	17 (85)	*0.005*
ST17	19	10 (52.6)	9 (47.4)	*0.338*
ST23	14	7 (50)	7 (50)	*0.560*
ST3	1	1	0	*0.246*
ST24	1	0	1	*0.384*
ST529	1	0	1	*0.384*

Abbreviations: CC clonal complex; ST sequence type
[1]Pearson's chi-square test
[2]CC19 includes ST19, ST112, and ST 335
[3]CC10 includes ST8, ST10, ST12 and ST 654

3.3 Hypervirulent GBS clone

The *hvgA* gene encoding the surface anchored adhesin was detected in 19.4% (n=19) of the 98 invasive GBS isolates. All *hvgA* gene carrying isolates were ST17, serotype III ($P<0.001$), and no acquisition was found by non-ST17 strains. Only one isolates carrying this virulence factor was EOD (n=1), the remaining were LOD and LLOD. The isolates carrying the *hvgA* gene manifested as meningitis (n=10, 52.6%) and bacteremia (n=9, 47.4%).

3.4 Analysis of Serotype III

Of the 50 isolates expressing the serotype III capsule, the highly virulent clone, ST17 (n=19 of 50, 37.3%), was found to be the most common ST,

followed by ST19 (n=18 of 50, 35.3%) and ST335 (n=7 of 50, 13.7%).

Before 1997, ST17 (n=2, 100%) was the only ST expressing a serotype III capsule to cause invasive diseases in infants. With the emergence of ST19 (n=2, 100%) in 1998-2000, ST17 became undetected. Beginning 2004-2006, ST17 reemerged (n=1 of 7, 14.3%) and began increasing in proportion as ST19 (n=4 of 7, 57.4%) began to decrease. A significant temporal trend of the isolates in serotype III was observed; as ST17 increased (*P*=0.001), ST19 decreased (*P*=0.009) (Fig. 2).

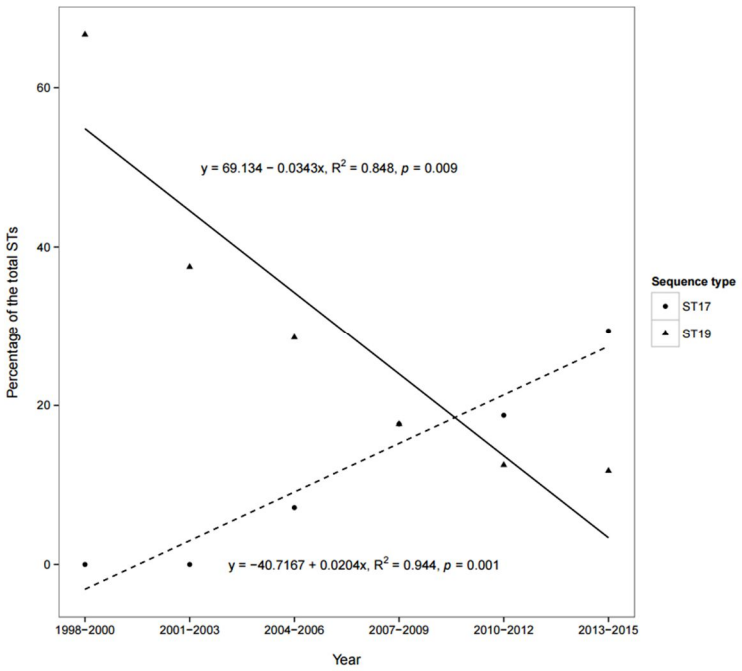

Figure 2. Temporal changes in the trend of the dominant sequence type (ST) of serotype III. The trend shows an increase in the proportion of ST17 (dashed line) and a decrease in ST19 (solid line) as the predominant strain of serotype III causing invasive diseases in infants.

3.5 Antimicrobial Susceptibility and Macrolide Resistance Genes

Of the 98 invasive GBS isolates, all were susceptible to penicillin. Resistance to erythromycin and clindamycin were seen in 41.8% (n=41) and 46.9% (n= 46), respectively. Of the isolates resistant to erythromycin, 19.5% (n=8/41) carried the *ermA* gene, 58.5% (n=24/41) carried the *ermB* gene, 17.1% (n=7/41) carried both *ermA* and *ermB*, and 4.9% (n=2/41) carried the *mefA* gene. There were no strains that carried both *ermB* and *mefA* genes. The highest rate of erythromycin resistance was seen in ST1, with 85% (n=17/20) of the isolates resistant ($P<0.001$), all carrying the *ermB* gene. In contrast, only 7.1% (n=1/14) of ST23 were resistant to erythromycin ($P=0.003$) (Table 5). The *ermA* gene was significantly responsible for macrolide resistance in ST335 than in other STs ($P<0.001$), with all ST335 macrolide resistant isolates carrying *ermA* (n=6). No temporal trend showing changes in the proportion of macrolide resistant isolates was observed.

Table 5. Relationship between sequence type and distribution of Macrolide Resistance Genes in Erythromycin Resistant Strains

	No. of isolates (%)			
	ermA(+)	*ermB*(+)	*ermA*(+) *ermB* (+)	*mefA*(+)
ST1 (n=17)	0	11 (64.7)	6 (35.3)	0
ST17 (n=9)	1 (11.1)	8 (88.9)	0	0
ST335 (n=6)	6 (100)	0	0	0
ST19 (n=4)	1 (25)	0	1 (25)	2 (50)
ST3 (n=1)	0	1 (100)	0	0
ST8 (n=1)	0	1 (100)	0	0
ST10 (n=1)	0	1 (100)	0	0
ST12 (n=1)	0	1 (100)	0	0
ST23 (n=1)	0	1 (100)	0	0
Total (n=41)	8 (19.5)	24 (58.5)	7 (17.1)	2 (4.9)

Abbreviations: ST sequence type

4. Discussion

The purpose of this study was to investigate the genotypic diversity of GBS isolated from infants with invasive diseases in South Korea, and to examine the prevalence of the highly virulent clone, ST17. Among the 98 GBS isolates collected, fourteen STs were found, with ST1, ST17 and ST19 most prevalent. *HvgA* gene was positive in 19.4% of the isolates, and all were ST17 strains in serotype III. A temporal trend showing an increase in the proportion of the highly virulent clone, ST17, was observed together with the decrease in ST19 as the predominant ST of serotype III.

Overall, the predominant capsular serotypes causing invasive GBS infections in South Korea were serotype III (51.0%), V (18.4%), Ia (15.3%), and Ib (13.3%), which accounted for 98% of the cases. Serotype III accounted for more than 50% of the infections, similar to the worldwide trend, however, serotype II was uncommon relative to other regions that reported up to 6.2% in prevalence (4). The most recent GBS colonization study done on 2624 pregnant women in South Korea (2006-2008) showed that the major serotypes were types III (43.8%), V (20.3%), Ia (12.1%) and Ib (9.5%),(12) portraying the serious impact of maternal GBS colonization on invasive GBS infections.

One of the major STs in this study was ST1. The importance of ST1 was highlighted by the fact that it was a significant pathogen responsible for EOD compared to LOD and LLOD (P=0.011). Other than ST1, the predominant STs (ST19, ST17, and ST23) manifested as meningitis in ≥50% of the cases. In contrast, ST1 was significantly shown to present as diseases other than

meningitis (*P*=0.005). This may be due to the lack or absence of virulence factors have been reported to contribute to the pathogenesis of GBS meningitis, such as streptococcal fibronectin binding protein A (24), β-hemolysin/cytolysin toxin (25), lipoteichoic acid (26), pili (27), *hvgA* (20), serine-rich repeat glycoproteins (28), and the alpha C protein (29). Although this study was unable to distinguish the relationship between a particular virulence factor causing meningitis and STs, it shows that not all STs have neurotropism.

A relationship between capsular serotypes and STs was observed in the invasive GBS isolates. In particular, ST23 was the dominant ST in serotype Ia, CC10 in serotype Ib, ST19 and ST17 in serotype III, and ST1 in serotype V. Two STs in this study was seen in more than one serotype, ST1 and ST10. As found in other studies, ST1 is a dominant strain of serotype V (30). However, in this present study, ST1 was also found among isolates with capsular serotype III and VI. Capsular switching is a possible mechanism for this phenomenon, which has been reported in other studies (31, 32).

Serotype III GBS are known to cause more invasive diseases than other serotypes. Activation of the alternative complement pathways is inhibited by the presence of terminal sialic acid residues on the type III polysaccharide, which then disables phagocytosis of GBS (33, 34). There are two main lineages of serotype III; ST19 and ST17. Different regions have a more predominant clone; ST19 in Canada (35) and the USA (32); ST17 in Beijing (36), Italy (37), Portugal (38), and Spain (39). In Korea, ST17 and ST19 are

both dominant strains of serotype III.

Hypervirulent ST17 clones carry the *hvgA* gene which encodes a surface adhesion protein that mediates GBS colonization and meningeal crossing (20). In France, of the 651 clinical GBS isolates, this hypervirulent clone accounted for >80% of neonatal meningitis (20). In a study of Swiss invasive GBS, there was a highly significant association between this clone and neonatal infections (P=0.006) (40). This gene was known to be carried only by ST17, belonging to capsular serotype III (17, 41). However, after the *hvgA* gene was detected in serotype IV GBS isolates, whole genome sequencing revealed capsular switching to occur (15). Since then, there have been reports of this phenomenon (31), which is important to surveillance in the GBS population because capsular switching ultimately means that GBS has the ability to switch targets that are used to make vaccines. Another mechanism for the transfer of the *hvgA* gene is acquisition of the genome by conjugal transfer (31, 42). All 98 invasive GBS isolates included in this study underwent PCR analysis for the *hvgA* gene. However, this virulence factor was detected only in ST17 strains. Thus, none of the isolates underwent capsular switching, and *hvgA* gene transfer has not yet occurred in the GBS isolated from infants with invasive diseases in South Korea.

A high rate of erythromycin resistance was seen in the GBS isolates of this study, and through genotype analyses, specific clones were found to have significant associations with macrolide resistance. In particular, ST1 strains were significantly resistant compared to other STs (P<0.001). In contrast, most ST23 strains were susceptible to erythromycin (P=0.003). The

determination of the specific clones that are less likely to be eradicated through IAP is important in preventing neonatal GBD disease (32). Although South Korea currently has not adopted the CDC guidelines for IAP, the clearance of ST1 strains colonized in pregnant woman via antibiotics may be difficult and other preventive measures have to be considered.

A limitation of this study was that the GBS isolates included in this study were not from a nationwide surveillance. However, this study includes the largest number of invasive GBS isolates from 4 tertiary hospitals located in remote regions throughout South Korea. Considering that all the isolates collected were from invasive diseases in children, data on serotype distribution, genotype diversity, and antibiotic susceptibility patterns from these isolates are of value.

4.1 Conclusions

In summary, 14 STs were found; ST1, ST19, and ST17 were the predominant STs causing invasive GBS infections in South Korea. In particular, ST1 is an important strain causing EOD with a high rate of macrolide resistance, and manifests as diseases other than meningitis. The hypervirulent ST17 is prevalent in South Korea, and the *hvgA* gene was found only in hypervirulent ST17 strains, showing no evidence of capsular switching or gene transfer. A temporal trend in the isolates expressing a serotype III capsule was observed; an increase in ST17 was seen together with the decrease in ST19. In conclusion, genotype analysis helps in understanding the specific traits of strains that cause invasive diseases in infants.

6. References

1. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 1996 May 31;45(RR-7):1-24

2. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 2010 Nov 19;59(RR-10):1-36

3. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 2002 Aug 16;51(RR-11):1-22

4. Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet (London, England). 2012 Feb 11;379(9815):547-56

5. Schrag SJ, Zell ER, Lynfield R, Roome A, Arnold KE, Craig AS, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. The New England journal of medicine.

2002 Jul 25;347(4):233-9

6. Moore MR, Schrag SJ, Schuchat A. Effects of intrapartum antimicrobial prophylaxis for prevention of group-B-streptococcal disease on the incidence and ecology of early-onset neonatal sepsis. The Lancet Infectious diseases. 2003 Apr;3(4):201-13

7. Uh Y, Jang I, Yoon K, Lee C, Kwon J, Kim M. Colonization rates and serotypes of group B streptococci isolated from pregnant women in a Korean tertiary hospital. European Journal of Clinical Microbiology and Infectious Diseases. 1997;16(10):753-6

8. Choi KU, Koh SK, Lee JY, Park JH, Hwang SO, Lee BI, et al. Clinical significance of group B streptococcal infection in pregnant women. Korean Journal of Obstetrics and Gynecology. 2002;45(5):811-5

9. Kim MW, Jang HO, Chang DY, Cho JR, Kim YA, Choi HM, et al. Group B streptococcal colonization rate in Korean pregnant women. Korean Journal of Obstetrics and Gynecology. 2006;49(2):337-44

10. Kim TH, Park SE, Kim KH. A study of group B streptococcal infection in pregnant women, by LIM broth media. Korean Journal of Pediatrics. 2004;47(10):1072-5

11. Kim KA, Shin SM, Choi JH. A Nationwide Survey on the Causative Organisms of Neonatal Sepsis in Korea. Korean J Pediatr. 2002 01;45(1):55-63

12. Hong J-S, Choi CW, Park K-U, Kim SN, Lee HJ, Lee HR, et al. Genital group B streptococcus carrier rate and serotype distribution in Korean pregnant women: implications for group B streptococcal disease in Korean

neonates. Journal of perinatal medicine. 2010;38(4):373-7

13. Lee J-H, Cho HK, Kim K-H, Kim CH, Kim DS, Kim KN, et al. Etiology of invasive bacterial infections in immunocompetent children in Korea (1996-2005): a retrospective multicenter study. Journal of Korean medical science. 2011;26(2):174-83

14. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. Jama. 2008 May 7;299(17):2056-65

15. Bellais S, Six A, Fouet A, Longo M, Dmytruk N, Glaser P, et al. Capsular switching in group B Streptococcus CC17 hypervirulent clone: a future challenge for polysaccharide vaccine development. The Journal of infectious diseases. 2012 Dec 1;206(11):1745-52

16. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, et al. Multilocus sequence typing system for group B streptococcus. Journal of clinical microbiology. 2003 Jun;41(6):2530-6

17. Lamy MC, Dramsi S, Billoet A, Reglier-Poupet H, Tazi A, Raymond J, et al. Rapid detection of the "highly virulent" group B Streptococcus ST-17 clone. Microbes and infection / Institut Pasteur. 2006 Jun;8(7):1714-22

18. Davies HD, Jones N, Whittam TS, Elsayed S, Bisharat N, Baker CJ. Multilocus sequence typing of serotype III group B streptococcus and correlation with pathogenic potential. The Journal of infectious diseases. 2004 Mar 15;189(6):1097-102

19. Musser JM, Mattingly SJ, Quentin R, Goudeau A, Selander RK. Identification of a high-virulence clone of type III Streptococcus agalactiae

(group B Streptococcus) causing invasive neonatal disease. Proceedings of the National Academy of Sciences of the United States of America. 1989 Jun;86(12):4731-5

20. Tazi A, Disson O, Bellais S, Bouaboud A, Dmytruk N, Dramsi S, et al. The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates. The Journal of experimental medicine. 2010 Oct 25;207(11):2313-22

21. Kong F, Gowan S, Martin D, James G, Gilbert GL. Serotype identification of group B streptococci by PCR and sequencing. Journal of clinical microbiology. 2002 Jan;40(1):216-26

22. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. Antimicrobial agents and chemotherapy. 1996 Nov;40(11):2562-6

23. Morosini MI, Canton R, Loza E, del Campo R, Almaraz F, Baquero F. Streptococcus pyogenes isolates with characterized macrolide resistance mechanisms in Spain: in vitro activities of telithromycin and cethromycin. The Journal of antimicrobial chemotherapy. 2003 Jul;52(1):50-5

24. Mu R, Kim BJ, Paco C, Del Rosario Y, Courtney HS, Doran KS. Identification of a group B streptococcal fibronectin binding protein, SfbA, that contributes to invasion of brain endothelium and development of meningitis. Infection and immunity. 2014 Jun;82(6):2276-86

25. Doran KS, Liu GY, Nizet V. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. The Journal of

clinical investigation. 2003 Sep;112(5):736-44

26. Doran KS, Engelson EJ, Khosravi A, Maisey HC, Fedtke I, Equils O, et al. Blood-brain barrier invasion by group B Streptococcus depends upon proper cell-surface anchoring of lipoteichoic acid. The Journal of clinical investigation. 2005 Sep;115(9):2499-507

27. Banerjee A, Kim BJ, Carmona EM, Cutting AS, Gurney MA, Carlos C, et al. Bacterial Pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration. Nature communications. 2011;2:462

28. Seo HS, Mu R, Kim BJ, Doran KS, Sullam PM. Binding of glycoprotein Srr1 of Streptococcus agalactiae to fibrinogen promotes attachment to brain endothelium and the development of meningitis. PLoS pathogens. 2012;8(10):e1002947

29. Chang YC, Wang Z, Flax LA, Xu D, Esko JD, Nizet V, et al. Glycosaminoglycan binding facilitates entry of a bacterial pathogen into central nervous systems. PLoS pathogens. 2011 Jun;7(6):e1002082

30. Imperi M, Gherardi G, Berardi A, Baldassarri L, Pataracchia M, Dicuonzo G, et al. Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011 Dec;17(12):1834-9

31. Teatero S, McGeer A, Low DE, Li A, Demczuk W, Martin I, et al. Characterization of invasive group B streptococcus strains from the greater Toronto area, Canada. Journal of clinical microbiology. 2014

May;52(5):1441-7

32. Manning SD, Lewis MA, Springman AC, Lehotzky E, Whittam TS, Davies HD. Genotypic diversity and serotype distribution of group B streptococcus isolated from women before and after delivery. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008 Jun 15;46(12):1829-37

33. Edwards MS, Kasper DL, Jennings HJ, Baker CJ, Nicholson-Weller A. Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. Journal of immunology (Baltimore, Md : 1950). 1982 Mar;128(3):1278-83

34. Marques MB, Kasper DL, Pangburn MK, Wessels MR. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. Infection and immunity. 1992 Oct;60(10):3986-93

35. Manning SD, Springman AC, Lehotzky E, Lewis MA, Whittam TS, Davies HD. Multilocus sequence types associated with neonatal group B streptococcal sepsis and meningitis in Canada. Journal of clinical microbiology. 2009 Apr;47(4):1143-8

36. Wang P, Tong JJ, Ma XH, Song FL, Fan L, Guo CM, et al. Serotypes, antibiotic susceptibilities, and multi-locus sequence type profiles of Streptococcus agalactiae isolates circulating in Beijing, China. PloS one. 2015;10(3):e0120035

37. Gherardi G, Imperi M, Baldassarri L, Pataracchia M, Alfarone G, Recchia S, et al. Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy.

Journal of clinical microbiology. 2007 Sep;45(9):2909-16

38. Martins ER, Melo-Cristino J, Ramirez M. Dominance of serotype Ia among group B Streptococci causing invasive infections in nonpregnant adults in Portugal. Journal of clinical microbiology. 2012 Apr;50(4):1219-27

39. Martins ER, Andreu A, Correia P, Juncosa T, Bosch J, Ramirez M, et al. Group B streptococci causing neonatal infections in barcelona are a stable clonal population: 18-year surveillance. Journal of clinical microbiology. 2011 Aug;49(8):2911-8

40. Luan SL, Granlund M, Sellin M, Lagergard T, Spratt BG, Norgren M. Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching. Journal of clinical microbiology. 2005 Aug;43(8):3727-33

41. Sorensen UB, Poulsen K, Ghezzo C, Margarit I, Kilian M. Emergence and global dissemination of host-specific Streptococcus agalactiae clones. mBio. 2010;1(3)

42. Brochet M, Rusniok C, Couve E, Dramsi S, Poyart C, Trieu-Cuot P, et al. Shaping a bacterial genome by large chromosomal replacements, the evolutionary history of Streptococcus agalactiae. Proceedings of the National Academy of Sciences of the United States of America. 2008 Oct 14;105(41):15961-6

국 문 초 록

B군 사슬알균은 영아 침습성 세균감염의 주요 원인균으로 생후 3개월 미만인 소아에서 높은 발생률을 보인다. 본 연구의 목적은 영아 침습성 세균 감염증에서 분리된 B군 사슬알균의 유전형 분석과 독성이 강한 클론(highly virulent clone)의 유병률을 확인하고자 하였다.

1995년부터 2015년사이에 서울대학교 어린이병원, 분당서울대학교병원, 전북대학교병원, 부산백병원 소아청소년과에 B군 사슬알균의 침습성 감염증으로 입원한 1세 미만 영아들의 무균성 체액에서 분리된 군주를 전향적으로 수집하여 염기서열 분석으로 B군 사슬알균의 피막 혈청형과 염기서열 타입(Sequence type, ST)을 분석하였다.

총 98개의 B군 사슬알균이 수집되었으며, 임상적 진단은 발현시기에 따라, 조발형 16.3%, 지발형 69.4%, 그리고 지지발형 14.3%이었다. 염기서열 분석에서 15개의 염기서열 타입 (Sequence type, ST)이 발견되었고, 그 중 ST1 (20.4%), ST17 (19.4%), 그리고 ST19 (18.4%) 이 가장 흔한 유형이었다. eBURST 분석을 통하여 2개의 군집 군 (Clonal complex, CC)과 6개의 싱글톤이 발견되었다. 혈청형의 분포는 III형(n=50, 51%)이 가장 흔하였고, 다음으로 V형(n=18, 18.4%), Ia형(n=15, 15.3%), Ib형(n=13, 13.3%) 순

으로 나타났다. ST의 종류와 혈청형간에 연관성이 있었는데, ST1은 주로 혈청형 V가 포함되었으며, ST17과 ST19는 혈청형 III, ST23는 혈청형 Ia가 포함되었다. *HvgA* 유전자는 전체 균 중 19개에서 검출되었고(19.4%), 모두 ST17이면서 혈청형 III인 특징을 보였다. 혈청형 III 중 ST17은 연구 기간의 초반에는 드물게 분리되었으나 최근에 분리율이 증가하는 경향이었으며(P=0.001), ST19는 최근에 분리율이 감소하는 시간적 추이가 관찰되었다(P=0.009). 다른 감염 시기에 비해 ST1은 조발형 감염증(43.8%)의 흔한 원인균이었고(P=0.039), 다른 ST에 비하여 수막염보다는 균혈증으로 진단된 경우가 많았다(P=0.005). ST1이 다른 타입에 비해 에리스로마이신 내성률이 높은 반면(n=17/20, P<0.001), ST23는 상대적으로 에리스로마이신 내성률이 낮았다(n=1/14, P=0.003).

본 연구를 통하여 침습성 감염증을 일으키는 B군 사슬알균의 ST별로 중요한 특성을 확인할 수 있었다. 특히, ST1은 조발형의 중요한 원인으로 높은 에리스로마이신 내성률을 보였고, 수막염보다는 균혈증의 주요 원인임을 알 수 있었다. 또한, 국내 영아에서 진단된 침습성 B군 사슬알균 감염증의 원인으로 독성이 강한 클론의 유병률이 최근에 증가하는 추세임을 알 수 있었다.

주요어: B군 사슬알균, 침습성 감염, 혈청형, 염기서열 타입, *hvgA*

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