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Correlation of Serotypes and Genotypes of Macrolide-Resistant *Streptococcus agalactiae*

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Despite the necessity for studies of group B streptococci (GBS), due to the increase in serious adult infections, the emergence of new serotypes, and the increased resistance to macrolide antibiotics, such studies have been limited in Korea. The primary purpose of the present study was to determine the frequency trends of GBS serotypes, including serotypes VI, VII, and VIII. The final objective was to elucidate the relationship between the genotypes and serotypes of macrolide-resistant GBS isolates from a Korean population. Among 446 isolates of *Streptococcus agalactiae,* isolated between January 1990 and December 2002 in Korea, the frequency of serotypes were III (36.5%), Ib (22.0%), V (21.1%), Ia (9.6%), VI (4.3%), II (1.8%), VIII (1.3%), IV (1.1%), and VII (0.9%). The resistance rates to erythromycin, by serotype, were 85% (V), 23% (III), 21% (VI), 3% (Ib), and 2% (Ia). Of 135 erythromycin-resistant *S. agalactiae*, *erm*B was detected in 105 isolates, *mef*A in 20 isolates, and *erm*TR in seven isolates; most type V isolates harbored the *erm*B gene, Ib type isolates had an equal distribution of resistance genes, type III isolates accounted for 70% of all isolates carrying *mef*A genes, and one fourth of type VI isolates had *mef*A genes.

Key Words: Group B streptococci, *Streptococcus agalactiae*, serotyping, erythromycin, macrolides, antibiotic resistance, genotype

INTRODUCTION

Streptococcus agalactiae (group B streptococci, GBS) is still susceptible to penicillin but has shown an increasing resistance to erythromycin and clindamycin during the last decade in many parts of the world.[1-5] In Korea, resistance rates to erythromycin and clindamycin among isolates of GBS have been increasing.[6] As determined in our previous studies,[7,8] the rates of erythromycin and clindamycin resistance among GBS isolates have increased from 0% in 1990-1995 to 41% in 2002, and 0% in 1990-1993 to 48% in 2002, respectively. Over the past decade, several investigators have reported that an increasing proportion of GBS disease is due to serotype V organisms.[4,5,9,10] In addition, several reports documented an association between macrolide resistance and GBS serotype V.[5,9,11,12] Strains of serotypes IV, VI, VII, and VIII have rarely been isolated from patients in the United States and Europe.[13] However, the emergence of new serotypes has been reported in Japan.[14] In our previous study,[12] it was determined that the proportion of non-typeable (NT) serotypes was increasing among GBS. The aims of this study were to investigate the distribution of GBS serotypes, including new types by year of isolation, and to elucidate the correlation between serotypes and genotypes of macrolide-resistant *S. agalactiae* in a Korean population.

MATERIALS AND METHODS

The GBS isolates in this study were collected between January 1990 and December 2002 at Wonju Christian Hospital, a university hospital in South Korea. Pure isolates of 138 GBS (2001-2002) and 24 non-typeable GBS (1990-2000) were tested for serotyping. The results of antimicrobial susceptibilities, phenotypes, and genotypes of erythromycin-resistant GBS isolates were adopted

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from our previous data.[7,8,12] Serotypes Ia, Ib, and II-V were determined by use of Hemolytic Streptococcus Group B Typing Sera (Denka Seiken Co., Ltd., Tokyo, Japan), and serotypes VI-VIII were serotyped with coagglutination (ESSUM Group B Streptococcus Serotyping Test; Bacterum AB, Umeå, Sweden).

RESULTS

The overall serotype frequencies of the 446 GBS isolates were III (36.5%), Ib (22.0%), V (21.1%), Ia (9.6%), VI (4.3%), II (1.8%), VIII (1.3%), IV (1.1%), and VII (0.9%). In our hospital between 1990 and 1995, Ib was the most common serotype, followed by serotypes III and Ia; III was by far the most common serotype from 1996 to 2000. Serotype V was not detected before 1996; it was the most common serotype from 2001 to 2002. Serotype VI did not exist before 1997. Since then, its frequency ranged from 2.5% to 9.1%. In this hospital, Serotypes II, IV, VII, and VIII were rarely isolated (Table 1).

The overall resistance rates to erythromycin, clindamycin, and tetracycline were 30%, 35%, and 96%, respectively (Table 2).

Among the 135 erythromycin-resistant strains in this study, *erm*B was detected in 105 strains (77.8%); *mef*A in 20 strains (14.8%); and *erm*TR in

Table 1. Isolation Frequency of Serotypes of *S. agalactiae* by Year

Year	Serotypes										Total
	Ia	Ib	II	III	IV	V	VI	VII	VIII	NT*	
1990	1	3	0	2	0	0	0	0	0	0	6
1991	0	3	1	1	0	0	0	0	0	0	5
1992	1	8	0	3	0	0	0	0	0	0	12
1993	0	0	1	2	0	0	0	0	0	0	3
1994	1	6	0	5	0	0	0	0	1	0	13
1995	1	4	0	2	0	0	0	0	0	0	7
1996	4	17	2	22	0	3	0	1	0	1	50
1997	7	15	0	15	0	9	2	0	0	0	48
1998	2	9	1	20	0	5	1	0	0	2	40
1999	6	8	0	26	3	10	2	1	1	1	58
2000	5	8	0	22	1	19	6	2	2	1	66
2001	7	12	0	28	1	32	4	0	0	1	85
2002	8	5	3	15	0	16	4	0	2	0	53
Total	43	98	8	163	5	94	19	4	6	6	446

*NT, not-typeable.

Table 2. Rates of Antimicrobial Resistance of *S. agalactiae* by Serotypes

Antimicrobials	No.(%) of resistance isolates of serotype										
	Ia [43]†	Ib [98]	II [8]	III [163]	IV [5]	V [94]	VI [19]	VII [4]	VIII [6]	NT* [6]	Total [446]
Erythromycin	1 (2)	3 (3)	1 (13)	38 (23)	3 (60)	80 (85)	4 (21)	0 (0)	3 (50)	2 (33)	135 (30)
Clindamycin	6 (14)	11 (11)	1 (13)	47 (29)	3 (60)	80 (85)	1 (5)	0 (0)	4 (67)	3 (50)	156 (35)

*NT, not-typeable.
†The numbers in brackets mean the total No. of GBS isolates by serotype.
‡All GBS isolates in this study were susceptible to penicillin, ceftriaxone, and vancomycin.

Table 3. Resistance Phenotypes and Genotypes of Erythromycin of *S. agalactiae* According to Serotypes

Phenotypes and genotypes	No. of serotypes										Total (%)
	Ia	Ib	II	III	IV	V	VI	VII	VIII	NT	
ERY-I/R *erm*B	0	1	0	20	3	77	1	0	3	0	105 (23.5)
*erm*TR	0	1	1	3	0	1	0	0	0	1	7 (1.6)
*mef*A	1	1	0	14	0	0	3	0	0	1	20 (4.5)
ND	0	0	0	1	0	2	0	0	0	0	3 (0.7)
ERY-S, CLI-S	36	86	7	105	2	14	15	4	2	3	274 (61.4)
ERY-S, CLI-I/R	6	9	0	20	0	0	0	0	1	1	37 (8.3)
Total	43	98	8	163	5	94	19	4	6	6	446 (100)

Abbreviations: NT, not-typeable; ERY-I/R, erythromycin intermediate or resistant; ND, not detected; ERY-S, erythromycin susceptible; CLI-S, clindamycin susceptible; CLI-I/R, clindamycin intermediate or resistant.

seven strains (5.2%). The most prevalent serotypes of erythromycin-resistant GBS isolates were V (n=80, 59.3%) and III (n=38, 28.1%) (Table 3).

DISCUSSION

A recent study in Japan showed that serotypes VIII (36%) and VI (25%) were the most common serotypes in healthy pregnant women.[14] Matsuura et al.[15] also reported that 28.6% to 35.3% of GBS isolated from the sputum of elderly patients were serotype VIII. In throat isolates from school children, Okuyama et al.[16] reported that Ia was dominant from 1980 to 1988, III from 1989 to 1990, and VI from 1991 to 1992. In Korea, Lee et al.[6] reported that serotypes VI and VIII were not detected from 1979 to 1992, but from 1993 to 1996, the proportions of VI and VIII serotypes were 3% (6/200) and 2% (4/200), respectively. Serotype VI and VIII strains were known to be less virulent. However, Ekelund et al.[17] recently reported that serotype VIII constituted 6% of all of the invasive GBS infections in Denmark. The differences in serotype distribution among various populations also may reflect differences in pathogenesis among the serotypes. Therefore, monitoring the serotype distribution of all nine serotypes is important for complete surveillance of GBS infections and for vaccine formulation.

The resistance rates to erythromycin found for our common serotypes (> 10 isolates) were (in decreasing order): 85% (V), 23% (III), 21% (VI), 3% (Ib), and 2% (Ia). These results were consistent with most other reports,[11,18] although some researchers[1,19] reported that serotype Ib had a lower rate of susceptibility to erythromycin than did serotype Ia, III, or V isolates. The resistance rates to clindamycin of our serotype Ia, Ib, and III isolates were higher than those of erythromycin while the other serotypes, with the exception of serotype VI, had nearly equal susceptible rates to erythromycin and clindamycin. Our results show that resistance to clindamycin is more common than resistance to erythromycin, and similar results were reported in Taiwan[1] and New Zealand.[20] The distribution of MLS_B (macrolide-lincosamide-streptogramin B) resistance genes and the isolation frequency of serotypes of GBS may be major factors contributing to differences in erythromycin and clindamycin resistance in different countries. Malbruny et al.[20] recently reported that a new LSA (lincosamide-streptogramin A) phenotype was noted in erythromycin-susceptible, clindamycin-resistant *S. agalactiae* isolates from New Zealand and that III (13/19) and I (5/19) were main the serotypes of GBS with an LSA phenotype. Further studies are needed to characterize our erythromycin-susceptible, clindamycin-resistant GBS isolates.

The distribution of MLS_B resistance genes of GBS is influenced by geographical variation and serotypes and also changes with time. Among the erythromycin-resistant isolates in this study, most serotype V isolates harbored the *erm*B gene, serotype Ib isolates had an equal distribution of resistance genes, and serotype III isolates accounted for 70% of GBS carrying *mef*A genes. Of new serotypes with erythromycin-resistant genes, 25% of type VI isolates had *mef*A genes, 100% of type VIII isolates had *erm*B genes, and type VII isolates did not carry any resistance genes.

In conclusion, the high resistance rates to erythromycin and clindamycin among our GBS isolates were primarily due to a continued emergence of serotype V GBS with the *erm*B gene. Serotype VI isolates showed an increasing frequency trend in our hospital. Further studies are required to observe any change in the trends of serotype distribution and antimicrobial susceptibilities.

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