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Molecular Characteristics of Group B Streptococci Isolated from Adults with Invasive Infections in Japan

Miyuki Morozumi,[a] Takeaki Wajima,[b] Misako Takata,[a] Satoshi Iwata,[a] Kimiko Ubukata[a]

Department of Infectious Diseases, Keio University School of Medicine, Tokyo, Japan[a]; Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan[b]

***Streptococcus agalactiae* (group B streptococcus) isolates ($n = 443$) obtained from Japanese adults with invasive infections between April 2010 and March 2013 were analyzed for capsular serotype, multilocus sequence type (ST), antibiotic susceptibility, and resistance genes. Among these cases, bacteremia without primary focus was the most common variety of infection (49.9%), followed by cellulitis (12.9%) and pneumonia (9.0%). Concerning patient age (18 to 59, 60 to 69, 70 to 79, 80 to 89, and 90 years old or older), the incidence of pneumonia increased in patients in their 70s and 80s ($P < 0.001$), while younger patients (18 to 59 and 60 to 69 years old) were more likely to have abscesses ($P < 0.05$). The mortality rate was 10.2% for all ages. The most common capsular serotype was Ib (39.5%), followed by V (16.0%), III (13.8%), VI (9.5%), and Ia (8.6%). The main ST of serotype Ib strains was ST10, which belonged to clonal complex 10 (88.0%). The predominant clonal complexes of serotypes V and III, respectively, were 1 (78.9%) and 19 (75.4%). Among these isolates, 9 strains (2.0%) were identified as group B streptococci with reduced penicillin susceptibility, reflecting amino acid substitutions in penicillin-binding protein 2X (PBP2X). In addition, 19.2% of all strains possessed *mef*(A/E), *erm*(A), or *erm*(B) genes, which mediate macrolide resistance, while 40.2% of strains were resistant to quinolones resulting from amino acid substitutions in GyrA and ParC. Our data argue strongly for the continuous surveillance of microbial characteristics and judicious antibiotic use in clinical practice.**

Streptococcus agalactiae or Lancefield group B streptococcus (GBS), a leading cause of neonatal infections with high mortality and morbidity rates, also occurs as an invasive infection in elderly persons, causing sepsis, cellulitis, and, less frequently, meningitis (1, 2).

Invasive GBS (iGBS) infections are increasing, particularly in elderly persons with underlying diseases, such as diabetes, cardiovascular disease, and cancer (3, 4). The most common clinical manifestations of iGBS in adults are bacteremia without focus, soft tissue infection, and pneumonia, which are in contrast to neonatal infection, where the main clinical manifestations are meningitis and sepsis (3, 5). In the United States, the incidence of iGBS disease in adults was found to have doubled, from 3.6 cases per 100,000 population in 1990 to 7.3 cases per 100,000 population in 2007 (4). As iGBS infection is increasing among elderly persons worldwide, especially in developed countries, the prevention and treatment of these infections are growing in importance (2, 4, 6, 7).

In 2008, GBS with reduced penicillin (PEN) susceptibility (penicillin-resistant GBS [PRGBS]) was first reported in Japan; these strains, isolated from the sputum samples of elderly patients from 1995 to 2005 (8), showed a limited number of amino acid substitutions adjacent to conserved motifs of Ser-Ser-Asn (SSN) and Lys-Ser-Gly (KSG) in penicillin-binding protein 2X (PBP2X). Subsequently, PRGBS isolates were found to possess various PBPs (PBP1A, PBP2A, PBP2B, and PBP2X) among isolates from adult patients in Canada (9, 10) and the United States (11).

In the present study, we examined the genetic diversity of iGBS isolates from adult patients in Japan from April 2010 to March 2013. The iGBS isolates, collected through a surveillance program, were investigated by identifying the capsular serotype using real-time PCR, by multilocus sequence typing (MLST), and by making antimicrobial susceptibility determinations.

MATERIALS AND METHODS

Strains. GBS strains isolated from adult patients over 18 years old with invasive GBS infections, such as bacteremia without known focus, cellulitis, pneumonia, arthritis, and meningitis, were collected from 341 general hospitals with clinical microbiology laboratories participating in the surveillance of invasive pneumococcal and streptococcal infections. These hospitals were located throughout Japan.

Between April 2010 and March 2013, our laboratory received 443 GBS isolates, each accompanied by an anonymous questionnaire survey form that was completed by the attending physician. Infections with invasive GBS were defined as cases in which GBS was isolated from normally sterile clinical samples, such as blood, spinal fluid, or joint fluid, or from pus obtained from a closed space. Isolates identified as GBS at each facility were sent to our laboratory. After receiving an isolate, we reidentified GBS according to the Manual of Clinical Microbiology (12).

Capsular typing. Capsular types of GBS were identified by real-time PCR as described in previous reports (13). One colony on each blood agar plate (Nippon Becton Dickinson, Tokyo, Japan) was picked up and placed in 50 μl of lysis solution containing 2 U of mutanolysin (Sigma-Aldrich, MO, USA). The lytic reaction was carried out for 10 min at 37°C and 10 min at 60°C, followed by 5 min at 94°C. Lysate was added to each of the tubes containing PCR mixtures for the following: (i) the *dltS* gene, which

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Address correspondence to Kimiko Ubukata, ubukatak@keio.jp.
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TABLE 1 Correlations between age groups and clinical manifestations

Clinical manifestation	No. (%)[a] of cases by age range (yr)					Total no. (%) of cases	*P* value
	18–59	60–69	70–79	80–89	≥90		
STSS[b]	1	1				2 (0.5)	
Necrotizing fasciitis	2	3				5 (1.1)	
Meningitis	3 (17.6)	4 (23.5)	8 (47.1)	1 (5.9)	1 (5.9)	17 (3.8)	0.276
Bacteremia without primary focus	50 (22.6)	38 (17.2)	61 (27.6)	57 (25.8)	15 (6.8)	221 (49.9)	0.722
Endocarditis	4 (23.5)	7 (41.2)	4 (23.5)	1 (5.9)	1 (5.9)	17 (3.8)	0.133
Pneumonia	2 (5.0)	1 (2.5)	12 (30.0)	20 (50.0)	5 (12.5)	40 (9.0)	<0.001
Arthritis	3 (21.4)	1 (7.1)	4 (28.6)	6 (42.9)		14 (3.2)	0.341
Osteomyelitis/spondylitis	4 (30.8)	4 (30.8)	4 (30.8)	1 (7.7)		13 (2.9)	0.439
Cholangitis/peritonitis	1	1	4	2	1	9 (2.0)	
Cellulitis	12 (21.1)	9 (15.8)	17 (29.8)	11 (19.3)	8 (14.0)	57 (12.9)	0.270
Abscess, noncutaneous	9 (34.6)	10 (38.5)	4 (15.4)	3 (11.5)		26 (5.9)	0.015
Others[c]	10 (45.5)	5 (22.7)	5 (22.7)	1 (4.5)	1 (4.5)	22 (5.0)	0.054
Total no.	101	84	123	103	32	443	

[a] Percentages are for each subtotal. Blank spaces indicate no cases.
[b] Streptococcal toxic shock syndrome.
[c] Others mostly consisted of erysipelas, lymphangitis, intrauterine infection, and discitis.

encodes a histidine kinase specific to GBS; (ii) capsular serotypes Ia and Ib; (iii) capsular serotype II; (iv) capsular serotypes III and IV; (v) capsular serotypes V and VI; and (vi) capsular serotypes VII and VIII (13, 14).

The total volume (30 μl) of the PCR mixture included 20 pmol of each primer, 25 pmol of each probe, 2× Multiplex Powermix (Bio-Rad, Hercules, CA), and DNase- and RNase-free distilled water. DNA amplification was carried out for 40 cycles as follows: 95°C for 10 s, 50°C for 30 s, and 72°C for 20 s.

MLST analysis. Primer sets corresponding to 7 housekeeping genes (*adhP*, *atr*, *glcK*, *glnA*, *pheS*, *sdhA*, and *tkt*) used for MLST analysis were constructed with reference to the MLST website (http://pubmlst.org/sagalactiae/). MLST was applied to the sequence for these 7 genes according to previously described methods (15), with alleles and sequence type (ST) assignments determined using the *S. agalactiae* MLST database. Alleles and STs that were not previously posted were entered into the *S. agalactiae* MLST database. The relationships of each ST were analyzed by eBURST version 3.1 (http://eburst.mlst.net/v3/mlst_datasets/).

Antibiotic susceptibility and identification of resistance genes. Susceptibility testing of GBS strains was performed using an agar dilution method conforming to the standards of the Clinical and Laboratory Standards Institute (www.clsi.org). *Streptococcus agalactiae* ATCC 12403 and *Streptococcus pneumoniae* ATCC 49619 were used as reference strains in the antibiotic susceptibility test.

Oral antimicrobial agents employed in this study were PEN, ampicillin (AMP), amoxicillin (AMX), clarithromycin (CLR), clindamycin (CLI), and levofloxacin (LVX). Parenteral agents were cefotaxime (CTX), panipenem (PAM), meropenem (MEM), and vancomycin (VAN). The antimicrobial agents were obtained from their respective manufacturers.

Macrolide (ML) resistance genes, specifically *erm*(A), *erm*(B), and *mef*(A/E), were identified by PCR (16). Four genes (*gyrA*, *gyrB*, *parC*, and *parE*) related to quinolone (QL) resistance were analyzed according to previously described methods (17). The *pbp2x* gene encoding the PBP2X enzyme, which mediates septum formation during cell wall synthesis, was also sequenced using previously reported primers (8).

Statistical analysis. We assessed the statistical significance of differences by age group and specific infectious disease, ML or QL resistance, and capsular serotype. We performed χ^2 tests or Fisher exact tests using Ekuseru-Toukei 2012 software for statistics (Social Survey Research Information, Tokyo, Japan).

RESULTS

Relationships between age groups and clinical manifestations. The 443 adult cases of invasive GBS infection that were collected between April 2010 and March 2013 are summarized in Table 1 according to age group (18 to 59, 60 to 69, 70 to 79, 80 to 89, and 90 years old or older) and clinical manifestations. Among the patients, 232 (52.4%) were males, 198 (44.7%) were females, and the sex of 13 was unknown.

The mean and median ages of the cases were 70.4 and 73.0 years, respectively. The mean age at onset of GBS infection is different from those at the onset of infections with other beta-hemolytic streptococci, such as *Streptococcus pyogenes* (group A streptococcus [GAS]; mean, 61 years) and *Streptococcus dysgalactiae* subspecies *equisimilis* (mean, 75 years) (18).

GBS caused a variety of invasive infections. Bacteremia without primary focus was predominant (49.9%), followed by cellulitis (12.9%), pneumonia (9.0%), endocarditis (3.8%), and meningitis (3.8%). Streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis were infrequent. The incidence of pneumonia was particularly high among persons in their 70s and 80s ($P < 0.001$), while younger patients (18 to 59 years and those in their 60s) were more likely to have abscesses ($P < 0.05$).

Overall mortality at 28 days was 10.2% (45/443); for subjects 18 to 59 years old ($n = 2$), it was 2.0%; for those in their 60s ($n = 5$), it was 6.0%; for those in their 70s ($n = 16$), it was 13.0%; for those in their 80s ($n = 15$), it was 14.6%; and for those 90 years old or older ($n = 7$), it was 21.9%. The mortality rate was higher among patients who were 70 years old or older ($P < 0.001$). The male-to-female ratio of subjects who died due to infection was 2:1.

Comorbid conditions, including cases with multiple underlying diseases, were present in 83.5% of patients, particularly diabetes (30.2%), cancer (24.6%), liver or renal dysfunction (23.3%), and cardiac diseases (15.8%). Various other comorbidities were present in 28.0% of patients.

Clinical infections in fatal cases were primarily bacteremia without primary focus in 55.6% (25/45) of cases and pneumonia in 24.4% (11/45; $P = 0.001$) of cases.

Correlation between capsular serotype and clinical manifestations. The correlations between capsular serotype and clinical manifestations are shown in Table 2. The most common capsular serotype was Ib (39.5%), followed by V (16.0%), III (13.8%), VI

TABLE 2 Correlations between capsular serotypes and clinical manifestations

Clinical manifestation	Serotype (no. [%])[a]								Total no. (%)	*P* value
	Ia	Ib	II	III	V	VI	VIII	Others[b]		
STSS[c]				2					2 (0.5)	
Necrotizing fasciitis	2		1			1	1		5 (1.1)	
Meningitis	2 (11.8)	9 (52.9)		3 (17.7)	1 (5.9)	1 (5.9)	1 (5.9)		17 (3.8)	0.740
Bacteremia without primary focus	16 (7.2)	98 (44.3)	14 (6.3)	27 (12.2)	37 (16.7)	21 (9.5)	4 (1.8)	4 (1.8)	221 (49.9)	0.158
Endocarditis		9 (53.0)		4 (23.5)	2 (11.8)		1 (5.9)	1 (5.9)	17 (3.8)	0.212
Pneumonia	4 (10.0)	15 (37.5)	4 (10.0)	5 (12.5)	3 (7.5)	7 (17.5)	2 (5.0)		40 (9.0)	0.504
Arthritis	1 (7.1)	3 (21.4)	2 (14.3)	3 (21.4)	4 (28.6)		1 (7.1)		14 (3.2)	0.522
Osteomyelitis/spondylitis	1 (7.7)	3 (23.1)	2 (15.4)	3 (23.1)	2 (15.4)	1 (7.7)		1 (7.7)	13 (2.9)	0.385
Cholangitis/peritonitis	3	2		2	1		1		9 (2.0)	
Cellulitis	4 (7.0)	23 (40.4)	2 (3.5)	8 (14.0)	12 (21.1)	4 (7.0)	4 (7.0)		57 (12.9)	0.584
Abscess, noncutaneous	4 (15.4)	9 (34.6)	4 (15.4)		6 (23.1)	3 (11.5)			26 (5.9)	0.177
Others[d]	1 (4.5)	4 (18.2)	4 (18.2)	4 (18.2)	3 (13.6)	4 (18.2)	2 (9.1)		22 (5.0)	0.142
Total no. (%)	38 (8.6)	175 (39.5)	33 (7.4)	61 (13.8)	71 (16.0)	42 (9.5)	17 (3.8)	6 (1.4)	443	

[a] Percentages are for each subtotal. Blank spaces indicate no cases.
[b] Others include serotypes IV ($n = 4$), VII ($n = 1$), and nontypeable ($n = 1$).
[c] Streptococcal toxic shock syndrome.
[d] Others mostly consisted of erysipelas, lymphangitis, intrauterine infection, and discitis.

(9.5%), Ia (8.6%), II (7.4%), VIII (3.8%), IV (0.9%), and VII (0.2%). Only 1 strain was not typeable.

Capsular serotypes did not correlate significantly with clinical manifestations. Mortality was highest in patients with type VI (16.7%, 7/42), followed by type Ib (11.4%, 20/175), III (9.8%, 6/61), II (9.1%, 3/33), Ia (7.9%, 3/38), V (7.0%, 5/71), VIII (5.9%, 1/17), IV (0%, 0/4), and VII (0%, 0/1).

Capsular serotype, clonal complex, and ST. Associations between capsular serotype, ST, and clonal complex (CC) were analyzed for all GBS strains by MLST using the GBS website.

As shown in Table 3, strains were classified into 8 CCs, which included 25 STs. CC1 and CC10 were most prevalent and accounted for 35.4% ($n = 157$) and 39.5% ($n = 175$) of isolates, respectively, followed by CC19 (10.8%), CC23 (9.3%), and CC26 (2.7%). Correlations between serotype and CC were evident, such as Ia with CC23, Ib with CC10, III with CC19, V with CC1 and CC26, and II, VI, and VIII with CC1. The CC17, ST17 strains, which are known to be highly virulent, numbered only 7 (1.6%).

The results of eBURST analysis are shown in Fig. S1 in the supplemental material. Among these, CC10 including 9 STs was most prevalent, followed by CC1, CC19 including 4 STs, and CC23 including 5 STs. The most prevalent STs were ST1 and ST10, which accounted for 34.8% ($n = 154$) and 35.7% ($n = 158$) of isolates, respectively, followed by ST335 (6.1%), ST23 (6.1%), and ST19 (3.6%). No significant correlation was found between ST, CC, and mortality rate in patients with iGBS infection.

Antimicrobial susceptibility and macrolide, quinolone, and penicillin resistance. The MIC, MIC_{50}, and MIC_{90} values of 10 antimicrobial agents, including 3 penicillins (PEN, AMP, AMX), CTX, 2 carbapenems (MEM and PAM), VAN, CLR, CLI, and LVX, for iGBS are shown in Table S1 in the supplemental material. The MIC_{90} values of CLR, CLI, and LVX were high at 16 μg/ml, 8 μg/ml, and ≥64 μg/ml, respectively. Nine strains showed reduced susceptibility to PEN (0.125 to 0.5 μg/ml), AMP (0.25 μg/ml), and CTX (0.125 to 1 μg/ml).

Table 4 shows correlations between ML and QL susceptibility, resistance genes, and capsular serotype. ML-resistant strains possessing the *erm*(B) gene, mediating high ML resistance; the *erm*(A)

TABLE 3 Relationships between clonal complex and capsular serotype in 443 invasive GBS isolates

Serotype	Clonal complex (no.)[a]									Total no.
	CC1	CC7	CC10	CC17	CC19	CC22	CC23	CC26	Singleton	
Ia	1		5				32			38
Ib	7	1	166						1	175
II	29		3			1				33
III	1		1	7	46		6			61
IV	3						1			4
V	56				2		1	12		71
VI	42									42
VII	1									1
VIII	17									17
NT[b]							1			1
Total no. (%)	157 (35.4)	1 (0.2)	175 (39.5)	7 (1.6)	48 (10.8)	1 (0.2)	41 (9.3)	12 (2.7)	1 (0.2)	443

[a] Blank spaces indicate no cases.
[b] NT, nontypeable.

TABLE 4 Relationships between macrolide and quinolone resistance and capsular serotype

Serotype	Isolates (no.)	No. (%)[a] with ML resistance			Total no. (%)	No. (%)[a] with QL resistance (*gyrA* + *parC*)[b]
		mef(A/E)	*erm*(A)	*erm*(B)		
Ia	38	3 (7.9)		2 (5.3)	5 (13.2)	3 (7.9)
Ib	175	5 (2.9)	5 (2.9)	15 (8.6)	25 (14.3)	162 (92.6)
II	33		2 (6.1)	4 (12.1)	6 (18.2)	1 (3.0)
III	61		15 (24.6)	15 (24.5)	30 (49.2)	9 (14.8)
IV	4					
V	71		2 (2.8)	14 (19.7)	16 (22.5)	1 (1.4)
VI	42	1 (2.4)		1 (2.4)	2 (4.8)	2 (4.8)
VII	1					
VIII	17			1 (5.9)	1 (5.9)	
NT[c]	1					
Total no. (%)	443	9 (2.0)	24 (5.4)	52 (11.7)	85 (19.2)	178 (40.2)

[a] Percentages are for each subtotal.
[b] Amino acid substitutions include Ser81Leu or Glu85Lys in *gyrA* and Ser79Phe, Ser79Tyr, or Asp83Gly in *parC*.
[c] NT, nontypeable.

gene, mediating inducible high ML resistance; and the *mef*(A/E) gene, mediating intermediate ML resistance (2 μg/ml to 16 μg/ml), were included in 11.7%, 5.4%, and 2.0% of isolates, respectively, totaling 19.2% of all strains. ML resistance was prevalent in serotype III (49.2%) and serotype V (22.5%).

On the other hand, QL-resistant strains showed a variety of amino acid substitutions such as Ser81Leu or Glu85Lys in GyrA and Ser79Phe, Ser79Tyr, or Asp83Gly in ParC. Substitutions were particularly prevalent in serotype Ib (92.6%).

CC1 and CC19 were dominant in ML-resistant strains, and CC10 was dominant in QL-resistant strains (data not shown here).

Amino acid substitution of gPRGBS. Table 5 shows information concerning PRGBS, including amino acid substitutions in PBP2X encoded by the *pbp2x* gene (gPRGBS), capsular serotype, ST, and MIC of 5 antibiotics. Serotypes of gPRGBS included III ($n = 6$), Ia ($n = 2$), and Ib ($n = 1$). Pneumonia ($n = 3$) was prevalent in patients with gPRGBS and was followed by bacteremia without primary focus ($n = 2$), arthritis ($n = 1$), osteomyelitis ($n = 1$), spondylitis ($n = 1$), and cellulitis ($n = 1$).

All 9 gPRGBS strains possessed a few amino acid substitutions that were adjacent to conserved motifs of Ser402-Ser403-Asn404 (SSN) and/or Lys552-Ser553-Gly554 (KSG) in PBP2X.

As described above, gPRGBS was found in 2.0% ($n = 9$) of isolates in this study. These strains showed a few amino acid substitutions in PBP2X.

Notably, four strains had the amino acid substitution Val405Ala, which was adjacent to conserved motifs of SSN, and showed an MIC for CTX of ≥0.5 μg/ml. The amino acid substitution Gln557Glu was involved in this resistance. These strains had reduced penicillin susceptibility as well as resistance to CLR and LVX.

DISCUSSION

GBS is an important pathogen that causes invasive infections in neonates and adults. The proportion of GBS infections in nonpregnant adults that involve the elderly is increasing worldwide (2, 4, 6, 19). The incidence of invasive disease is approximately 7 cases per 100,000 nonpregnant adults, with the highest incidence among adults 65 years of age and older (20 to 25 cases per 100,000) (3).

In the present Japanese study of iGBS in adults, elderly persons (≥70 years old) accounted for more than half of all iGBS cases, and 83.5% of patients had some underlying disease. As in previous reports (3), diabetes (30.2%) and malignant neoplasia (24.6%) were the most common underlying diseases associated with invasive GBS infection in adults. Among clinical forms of infection,

TABLE 5 Capsular serotype, sequence type, MICs of 5 agents, and amino acid substitutions in PBP2X in gPRGBS

Strain no.	Site of isolation	Serotype	ST	MIC (μg/ml)					Amino acid substitutions in PBP2X									
				PEN	AMP	CTX	CLR	LVX	Lys372	Ile377	Gly398	Val405	Gln412	Gly429	His438	Asp478	Glu513	Gln557
KK0132[a]	Blood	III	1	0.063	0.125	0.063	0.125	64										
KK1419	Pleural effusion	Ia	10	0.25	0.25	1	>64	32			Ala	Ala						Glu
KK1949	Blood	III	10	0.25	0.25	1	>64	32			Ala	Ala						Glu
KK2145	Blood	Ia	1	0.25	0.25	1	>64	32	Glu		Ala	Ala		Asp				-
KK1244	Blood	Ib	358	0.5	0.25	0.5	>64	>64			Ala	Ala				Ala	Gln	Glu
KK1746	Joint	III	24	0.125	0.25	0.25	0.125	32		Val	Ala		Leu		Tyr			
KK1756	Closed pus	III	24	0.125	0.25	0.25	>64	32		Val	Ala		Leu		Tyr			
KK1863	Joint	III	24	0.125	0.25	0.25	>64	32		Val	Ala		Leu		Tyr			
KK842	Blood	III	464	0.125	0.25	0.125	>64	32		Val	Ala		Leu		Tyr			
KK872	Blood	III	464	0.125	0.25	0.125	0.125	32		Val	Ala		Leu		Tyr			

[a] This strain is a β-lactam-susceptible strain.

bacteremia without primary focus was predominant, followed by cellulitis and pneumonia, as in previous reports (4, 6, 20, 21). The mortality rate was high in old patients ($P = 0.002$) and in those with bacteremia without primary focus or pneumonia ($P = 0.001$). In our study, mortality was high in patients with serotypes VI and Ib, which is unlike other reports where serotypes Ia and II showed increased mortality (7).

In the present study, capsular serotypes Ib (39.5%), V (16.0%), and III (13.8%) accounted for the majority of GBS infections in adults. This serotype distribution was similar to that reported in adult patients in Taiwan (22) but was slightly different from reports from France (23), the United States (4), the United Kingdom (2), and Iceland (19). Among our serotype Ib strains, 92.6% showed QL resistance; these strains belonged to CC10. QL-resistant GBS was first isolated in Japan in 2003 (24), and isolates from other countries were reported in subsequent years (4, 25–27). In most countries, QL resistance remains uncommon (0.9% to 4.8%); however, in Korea, such resistance is frequent (32.7%), and most isolates belong to CC10 (71.4%) (28), which is similar to our results. In Italy, most QL-resistant GBS isolates were serotype Ib and belonged to CC19 (26).

Our study detected resistance to MLs in 19.2% of isolates, which is more often than that in the United Kingdom (2) (9%) or in Iceland (8.3%) (19) but less than that in Taiwan (44.0%) (22), France (35.3%) (23), or the United States (40.0%) (4). We found this resistance to be almost exclusively among serotypes III and V as in reports from other countries (4, 23, 25).

In our CC analysis, almost all CC10 strains were serotype Ib, while CC1 strains were distributed across various serotypes (II, V, VI, and VIII). Although relationships between serotypes and CCs have been reported in various countries (29–33), the prevalence was different among the studied population.

Among adults, ST17, which is known to include highly virulent strains, accounted for only 7 (1.6%) of all of the strains, which is in contrast to neonatal infection where ST17 has been predominant (29.3%) (5); ST17 is the main ST that causes neonatal meningitis. These ST17 strains possess hypervirulent GBS adhesin (HvgA), which enhances their ability to invade the neonatal central nervous system (34). Since the incidence of meningitis was lower in adults, ST17 strains were few. However, capsular switching from serotype III to IV within CC17 has been reported recently (35, 36). These *hvgA*-positive strains were obtained from adult patients. Almost all serotype IV strains were detected in invasive isolates from adults, and these have been reported to be increasing (29, 37). Capsular switching may interfere with current efforts to develop GBS vaccines since serotype IV is not included (38). No *hvgA*-positive serotype IV strain was observed in our study, while all ST17 strains were *hvgA*-positive.

Importantly, 9 gPRGBS isolates have presently been detected among those submitted from invasive GBS infection in various regions in Japan. PRGBS was first isolated from the sputum samples of elderly patients in 1995 (8); these isolates showed a few amino acid substitutions adjacent to the conserved amino acid motifs of SSN and KSG in PBP2X. In particular, substitutions of Val405Ala and/or Gln557Glu of PBP2X correlated with increases in the MIC, indicating a reduced PEN susceptibility. Elsewhere, PRGBS possessing substitutions of PBPs (PBP1A, PBP2A, PBP2B, and PBP2X) were isolated from adult patients in Canada (9, 10) and the United States (11).

In Canada, PRGBS strains were isolated from patients with invasive infection in 2007 and again in 2009. One strain, which belonged to serotype II and ST2, was isolated after prolonged therapy with PEN. These strains possessed a few amino acid substitutions in PBP1A, PBP2A, PBP2B, and PBP2X but not Val405Ala or Gln557Glu in PBP2X. PRGBS with substitutions in PBPs may have been favored by the acquisition of drug selection pressure; these substitutions have contributed to reduced susceptibility. In a 2003 U.S. study, 4 PRGBS strains were isolated from invasive GBS infections; all of these strains were serotype III and ST19 with a Gln557Glu mutation in PBP2X (11).

Another important trend is the identification of PRGBS strains with resistance to multiple drugs, such as ML plus QL (multidrug-resistant PRGBS [MDR-PRGBS]). In Japan, PRGBS and MDR-PRGBS are increasing among adults, not including pregnant women (39). Although these resistant strains have not yet been confirmed in neonatal infections, such an event would represent a serious threat.

Since invasive GBS infection in elderly persons with underlying conditions has become an important problem, considerable effort has been made to develop an effective vaccine against GBS infection (40). Over 20 years, polysaccharide vaccines, conjugate vaccines, and protein-based vaccines have been developed to prevent neonatal GBS infection (11, 38). Despite all of these initiatives, GBS infection remains a major problem both in neonates and adults.

Continuous epidemiologic surveillance and proper clinical antibiotic use are necessary to provide better prevention of GBS infection in elderly people.

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