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Molecular characterization and antimicrobial resistance of *Streptococcus agalactiae* isolated from pregnant women in Japan, 2017–2021

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ABSTRACT

Objectives: This study aimed to elucidate the molecular characteristics and antimicrobial resistance of *Streptococcus agalactiae* (group B streptococcus, GBS) colonizing pregnant women in Japan.
Methods: GBS isolates obtained from screening of pregnant women from 2017 to 2021 were analyzed for capsular serotype, sequence type (ST), and antimicrobial susceptibility. For levofloxacin-resistant isolates, mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA, gyrB,* and *parC* genes were analyzed.
Results: Seventy-six GBS isolates were recovered from 1090 women (isolation rate: 7.0%). Of the 76 isolates, serotype III (31.6%) was the most prevalent, followed by V (19.7%), Ia (17.1%), and Ib (10.5%). Among the 22 STs identified, capsular serotype III/ST335-clonal complex (CC) 19 lineage was dominant (13.2%), followed by Ia/ST23, III/ST17, and V/ST1. Levofloxacin resistance was detected in 15.8% (n=12) of all the isolates, with serotype Ib being the most common. Most levofloxacin resistant isolates belonged to serotype Ib/CC10 or serotype V/CC19, with double mutations in the QRDRs, Ser81Leu in GyrA and Ser79Phe in ParC.
Conclusions: The present study indicates the prevalence of the serotype III/ST335 (CC19) lineage, and the spread of serotype Ib/CC10 and serotype V/CC19 lineages, which are responsible for levofloxacin resistance in colonizing GBS in pregnant women in Japan.

Introduction

Colonization by *Streptococcus agalactiae* (group B streptococcus, GBS) in pregnant women is an important risk factor for newborn diseases due to vertical transmission during childbirth. Since 2002, the Centers for Disease Control and Prevention has published guidelines for the prevention of perinatal GBS disease, recommending routine culture-based GBS screening for all pregnant women (Verani et al. 2010). In Japan, the GBS screening based on the national guidelines has been recommended for all pregnant women since 2008 (Japan Society of Obstetrics and Gynecology. 2020). Recently, an increasing trend has been concern for GBS with reduced susceptibility to penicillin, and resistance to macrolide and fluoroquinolone (Seki et al. 2015). Although epidemiological profiles of GBS responsible for invasive infections have been described in Japan (Morozumi et al. 2016), characteristics of the colonizing strains in pregnant women are poorly documented. In this study, we investigated the molecular features and antimicrobial resistance of GBS isolates from pregnant women in Hokkaido, the main northern island of Japan.

Methods

From October 2017 to December 2021, 76 non-duplicate GBS isolates were obtained from 1090 pregnant women screened for GBS in a single hospital. GBS was identified by MALDI-TOF mass spectrometry using the MALDI Biotyper (BRUKER). Capsular serotype was assigned by multiplex PCR (Imperi et al. 2010). Sequence type (ST) was determined based on the multilocus sequence typing (MLST) scheme (Jones et al. 2003). Minimum inhibitory concentrations (MICs) of 11 antimicrobials were determined by the broth microdilution method using Dry Plate (Eiken Chemical, Japan), and susceptibility was inter-

Abbreviations: GBS, group B streptococcus; ST, sequence type; QRDRs, quinolone resistance-determining regions; MLST, multilocus sequence typing; MICs, minimum inhibitory concentrations; CC, clonal complex.

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Table 1
Distribution of capsular serotypes and clonal complexes (CCs)/sequence types (STs) for 76 GBS isolates.

Clonal complex (CC) (no. of isolates)	Sequence type (ST)	Total no. (%)	Number of isolates (% in each ST) Capsuler serotype							
			Ia	Ib	II	III	IV	V	VI	VIII
CC1 (23)	ST1	12 (15.8)	0	0	1 (8.3)	1 (8.3)	0	7 (58.3)	2 (16.7)	1 (8.3)
	ST196	4 (5.3)	0	0	0	0	4 (100)	0	0	0
	ST676	4 (5.3)	0	0	4 (100)	0	0	0	0	0
	ST3	2 (2.6)	0	2 (100)	0	0	0	0	0	0
	ST2	1 (1.3)	0	0	1 (100)	0	0	0	0	0
CC19 (20)	ST335	10 (13.2)	0	0	0	10 (100)	0	0	0	0
	ST19	6 (7.9)	0	0	3 (50.0)	0	0	3 (50.0)	0	0
	ST27	2 (2.6)	0	0	0	2 (100)	0	0	0	0
	ST131	1 (1.3)	0	0	0	0	0	1 (100)	0	0
	ST324	1 (1.3)	0	0	0	0	0	1 (100)	0	0
CC23 (14)	ST23	9 (11.8)	8 (88.9)	0	0	0	0	1 (11.1)	0	0
	ST144	3 (3.9)	3 (100)	0	0	0	0	0	0	0
	ST88	1 (1.3)	1 (100)	0	0	0	0	0	0	0
	ST199	1 (1.3)	1 (100)	0	0	0	0	0	0	0
CC17 (7)	ST17	7 (9.2)	0	0	0	7 (100)	0	0	0	0
CC10 (6)	ST10	3 (3.9)	0	3 (100)	0	0	0	0	0	0
	ST1410	2 (2.6)	0	2 (100)	0	0	0	0	0	0
	ST752	1 (1.3)	0	1 (100)	0	0	0	0	0	0
CC452 (3)	ST452	3 (3.9)	0	0	0	0	3 (100)	0	0	0
CC26 (1)	ST26	1 (1.3)	0	0	0	0	0	1 (100)	0	0
CC529 (1)	ST529	1 (1.3)	0	0	0	1 (100)	0	0	0	0
CC1524 (1)	ST1524	1 (1.3)	0	0	0	0	0	1 (100)	0	0
Total no. (%)		76 (100)	13 (17.1)	8 (10.5)	6 (7.9)	24 (31.6)	7 (9.2)	15 (19.7)	2 (2.6)	1 (1.3)

Table 2
Antimicrobial susceptibility of 76 GBS isolates belonging to different capsular serotypes recovered from pregnant women.

Antimicrobial agent[a]	Percentage of intermediate resistance (I) and resistance (R), and (I/R) in each capsuler serotype								
	Ia (n=13) I+R (I/R)	Ib (n=8) I+R (I/R)	II (n=6) I+R (I/R)	III (n=24) I+R (I/R)	IV (n=7) I+R (I/R)	V (n=15) I+R (I/R)	VI (n=2) I+R (I/R)	VIII (n=1) I+R (I/R)	Total (n=76) I+R (I/R)
Erythromycin	23.1 (0/23.1)	37.5 (0/37.5)	33.3 (0/33.3)	41.6 (8.3/33.3)	42.9 (0/42.9)	60.0 (6.7/53.3)	50.0 (0/50.0)	0	40.7 (3.9/36.8)
Azithromycin	23.1 (0/23.1)	50.0 (12.5/37.5)	33.3 (0/33.3)	70.8 (0/70.8)	42.9 (0/42.9)	60.0 (0/60.0)	50.0 (0/50.0)	0	51.3 (1.3/50.0)
Clarithromycin	30.8 (0/30.8)	37.5 (0/37.5)	33.3 (0/33.3)	66.7 (25.0/41.7)	42.9 (0/42.9)	60.0 (0/60.0)	50.0 (0/50.0)	0	50.0 (7.9/42.1)
Clindamycin	15.4 (7.7/7.7)	50.0 (12.5/37.5)	0	37.5 (0/37.5)	42.9 (0/42.9)	40.0 (0/40.0)	50.0 (0/50.0)	0	34.2 (3.9/30.3)
Levofloxacin	0	87.5 (0/87.5)	0	4.2 (0/4.2)	0	26.7 (0/26.7)	0	0	15.8 (0/15.8)

[a] All isolates were susceptible to penicillin, ampicillin, imipenem, meropenem, cefotaxime, and vancomycin (data not shown).

preted according to the breakpoints indicated by the Clinical and Laboratory Standards Institute guidelines 2018. For 12 GBS isolates showing levofloxacin resistance (MIC; ≥ 8 μg/mL), mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA, gyrB*, and *parC* genes were analyzed. Statistical analyses were performed using IBM SPSS software version 25.

Results and Discussion

The prevalence of GBS colonization among the pregnant women was 7.0% (n=76/1090). The most prevalent serotype was III (31.6%), followed by V (19.7%), Ia (17.1%), and Ib (10.5%); collectively, they accounted for 79% of the isolates (Table 1). A systematic review reported that the estimated rate of maternal GBS colonization is 18% worldwide, with regional variation (11–35%) and a lower prevalence in Eastern Asia (10–12%); the dominant serotypes were I (Ia and Ib) and III (Russell et al. 2017). Our results also support the low frequency of GBS colonization in Eastern Asia; nevertheless, the relatively higher prevalence of serotype V was noted. Serotype V was the most frequent among isolates from pregnant women in a Japanese study (2007-2010) (Ueno et al. 2012), and also reported as the second most common type among invasive GBS in Argentina (Arias et al. 2022). Accordingly, further monitoring may be required for the trend of this serotype.

MLST analysis revealed the presence of 22 STs grouped into nine clonal complexes (CCs), among which ST1 (15.8%) was the most common, followed by ST335 (CC19) (13.2%), ST23 (CC23), and ST17 (CC17) (Table 1). The most common serotype/ST combinations were III/ST335 (n=10), Ia/ST23 (n=8), III/ST17 (n=7), and V/ST1 (n=7). In only ST1 (CC1), the isolates were assigned to various serotypes representing wide genetic diversity. The similar diversity in CC1 has also been observed in Italy (Piccinelli et al. 2015), Sri Lanka (Sapugahawatte et al. 2022), and other regions of Japan (Morozumi et al. 2016). The serotype III/ CC19 clone was previously described as the most common in pregnant women in China (Wang et al. 2015) and the second most prevalent in invasive infections in Japan (Morozumi et al. 2016). ST335 was first identified in Japan from invasive infections in infants and was the second most common type among CC19 following ST19 (Morozumi et al. 2014). The dominance of ST335 in GBS in this study suggests the prevalence of ST335 as a colonizing GBS strain in pregnant women in Japan.

All GBS isolates in the present study were susceptible to β-lactams (Table 2), consistent with previous studies (Hays et al. 2016, Wang et al. 2015). In contrast, a high resistance rate to levofloxacin (35.7–42.0%) has been documented in Asia-Pacific countries (Wang et al. 2015, Morozumi et al. 2016), whereas the resistance rates were low in European countries (1.4–1.5%) (Piccinelli et al. 2015, Hays et al. 2016). In the present study, 12 isolates (15.8%) showed resistance to levofloxacin, with one or double amino acid mutations in the QRDR of the examined genes (Supplementary Table 1). Most isolates had double mutations: Ser81Leu in GyrA and Ser79Phe in ParC, as

described in previous studies (Piccinelli et al. 2015, Hays et al. 2016, Morozumi et al. 2016). In the present study, the rate of resistance to levofloxacin was significantly higher in serotype Ib (n=7/8, 87.5%; p=0.003) than in other serotypes, and most of the levofloxacin-resistant isolates (75.0%) belonged to Ib/CC10 or V/CC19. According to the published literature, levofloxacin-resistant GBS belonged to III/CC19, V/CC19, and Ib/CC10 in France (Hays et al. 2016), Ib/CC19 in Italy (Piccinelli et al. 2015), and Ib/CC10 in Japan (Morozumi et al. 2016). The findings suggest that the particular lineages, Ib/CC19 or CC10, and V/CC19 are distributed worldwide as levofloxacin-resistant GBS.

In conclusion, the present study revealed the genetic characteristics and antimicrobial resistance traits of GBS colonizing isolates from pregnant women in northern Japan. Further surveillance may be required regarding the prevalence of CC19 GBS, with particular reference to ST335, and the potential spread of the serotype Ib/CC10 and serotype V/CC19 lineages with levofloxacin resistance.

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Ethical statement

In this study, no human participants were directly involved. Hence, clearance of human ethics is not required. We used isolates routinely cultured from clinical specimens from hospitals and clinics.

Conflict of interest

The authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2022.07.002.

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