Original Article

High isolation rate and multidrug resistance tendency of penicillin-susceptible group B *Streptococcus* with reduced ceftibuten susceptibility in Japan

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Abstract

Group B *Streptococcus* (GBS) clinical isolates with reduced penicillin susceptibility (PRGBS) have emerged through acquisition of amino acid substitutions in penicillin-binding protein 2X (PBP2X). Moreover, we also reported the emergence of penicillin-susceptible GBS clinical isolates with reduced ceftibuten susceptibility (CTB^r PSGBS) due to amino acid substitutions in PBPs. However, whether or not these amino acid substitutions are responsible for the reduced ceftibuten susceptibility (RCTBS) profile remains unclear. Furthermore, the rate of CTB^r PSGBS isolation and their multidrug resistance tendency remain uncertain. Therefore, we collected 377 clinical GBS isolates from multiple regions in Japan between August 2013 and August 2015. These isolates were characterised by determining MICs and sequencing the *pbp2x* gene. The isolation rate of CTB^r PSGBS was 7.2% (27/377). CTB^r PSGBS isolate harbor two types of amino acid substitutions in PBP2X [(T394A type) and (I377V, G398A, Q412L, and H438H type)]. The relevance of the amino acid substitutions found to the RCTBS was confirmed with allelic exchange techniques. Allelic exchange recombinant clones acquired two types of amino acid substitutions in PBP2X showed RCTBS. Furthermore, total ratio of resistance and non-susceptibility to both macrolides and fluoroquinolones in CTB^r PSGBS was 51.9% (14/27). The isolation rate of CTB^r PSGBS is

non-negligibly high and the CTB^{r} PSGBS tends to exhibit resistance and non-susceptible profile to both macrolides and fluoroquinolones.

Keywords group B *Streptococcus* · reduced ceftibuten · susceptibility, · penicillin-binding protein 2X · multidrug resistance

Introduction

Group B *Streptococcus* (*Streptococcus agalactiae*, GBS) is one of the most important causes of serious neonatal infections, such as sepsis and meningitis, and is an important pathogen in elderly people suffering from various medical disorders [1-5]. Invasive infections caused by GBS in neonates are associated with high mortality; surviving neonates often suffer from severe neurological sequelae such as mental retardation and visual and/or auditory disabilities [6-8]. Currently, almost all GBS clinical isolates are considered to be susceptible to β-lactam antibiotics, making them the first-line drugs for the treatment and prevention of GBS infections [4, 8]. However, since our first report in 2008 [9], GBS isolates with reduced penicillin susceptibility (PRGBS) have been identified in Japan [10, 11], Canada [12, 13], and the United States [14]. PRGBS have emerged through the acquisition of amino acid substitutions in penicillin-binding protein 2X (PBP2X), such as V405A and/or Q557E, and have also shown a tendency for resistance to macrolides and non-susceptibility to fluoroquinolones [15, 16]. Interestingly, there has also been a report of penicillin-susceptible group B streptococcus (PSGBS) clinical isolates exhibiting no growth inhibition zone around a ceftibuten (CTB) disk (CTB^r PSGBS) [17]. These isolates were shown to share a T394A or/and G429S substitution in PBP2X and a T567I substitution in PBP2B or/and T154A in

PBP1A [17]. However, the report did not elucidate whether the amino acid substitutions in the PBPs were responsible for reduced CTB susceptibility (RCTBS), and six clinical CTB^r PSGBS isolates in that report were recovered from one hospital. Moreover, the isolation rate of PSGBS with reduced ceftibuten susceptibility (CTB^r PSGBS) and whether or not CTB^r PSGBS isolates exhibit a multidrug resistance tendency remains unknown worldwide.

In this study, we examined the isolation rate of PRGBS and CTB^r PSGBS among clinical GBS isolates from multiple regions in Japan between August 2013 and August 2015. Moreover, we analyzed the multidrug resistance tendency of CTB^r PSGBS and the relationship between RCTBS and amino acid substitutions in PBP2X.

Materials and methods

Clinical isolates

In this study, 377 clinical GBS isolates were collected from multiple regions in Japan between August 2013 and August 2015 by the Miroku Medical Laboratory, a private clinical microbial testing laboratory. These clinical GBS isolates were recovered from various specimens: sputum, urine, vaginal specimens and several others (Supplemental Table S1). The age distribution of these patients is shown in Supplemental Table S2. All the 377 isolates

were confirmed as GBS by the presence of β-hemolytic colonies on sheep blood agar (Nissui, Tokyo, Japan), and specific agglutination with anti-Lancefield B antigen serum using the Lancefield antigen examination kit (Prolex streptococcal grouping kit, Iwaki, Tokyo, Japan). GBS with non-hemolytic activity were confirmed as GBS by specific agglutination with anti-Lancefield B antigen serum and anti-GBS serotype-specific serum (Denka Seiken, Tokyo, Japan).

Determination of MICs

The MICs of penicillin G (PEN), ampicillin, oxacillin, cefaclor, cefazolin, ceftizoxime, ceftibuten (CTB), cefepime, meropenem, vancomycin, erythromycin, clindamycin and levofloxacin were determined for the 377 isolates using the agar dilution method with Muller-Hinton agar supplemented with 5% defibrinated sheep blood, as recommended by the CLSI [18]. Streptococcus pneumoniae strain ATCC 49619 was used as the quality control strain.

PCR and Sequence analysis

Sequence analysis of the *pbp2x* gene was performed for the 52 clinical isolates showing RCTBS (CTB MIC ≥128 μg/ml). PCR amplification, including primers used, and sequencing were performed as previously described [9].

Allelic exchange experiments

To generate GBS strain 2603 V/R (ATCC BAA-611; GenBank accession number NC004116) allelic exchange recombinants containing the chromosomally encoded *pbp2x* gene of NUBL-8805 or NUBL-12951, pG+host6Δamp-8805 and pG+host6Δamp-12951 were constructed by cloning a fragment encompassing the chromosomal region from position 295821 to 297819 of strain 2603 V/R (corresponding to the *pbp2x* gene) from either strain into the thermosensitive plasmid pG+host6Δamp backbone. Details of pG+host6Δamp are as previously described [9]. Both pG+host6Δamp-8805 and pG+host6Δamp-12951 were introduced into *Escherichia coli* DH10B for amplification, and the GBS ATCC strain 2603 V/R were transformed with purified plasmids, and the transformants were selected on Todd-Hewitt agar (THA) containing 0.5 μg/ml erythromycin at 30°C. Successful GBS clones harboring recombinant plasmids were incubated in Todd-Hewitt broth (THB) containing 5 μg/ml of erythromycin at 37°C for 16 h, and then were cultivated for 3 days in THB at 30°C without erythromycin selection to facilitate vector excision [19]. The allele exchanged *S. agalactiae* recombinant clones were selected on THA containing 64 μg/ml of CTB, and susceptibility to erythromycin was confirmed using THA containing 5 μg/ml of erythromycin. These *S. agalactiae* recombinant clones were confirmed as GBS using a Lancefield antigen

examination kit and the absence of additional changes in the *pbp2x* gene was confirmed by sequencing the *pbp2x* gene. These recombinant clones were subjected to MIC measurements.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed as previously described [20], with minor modifications. DNA was digested with the ApaI restriction enzyme (Nippon Genes, Tokyo, Japan) and subjected to electrophoresis using the CHEF-DRIII power module (Bio-Rad) with the following program: switch time of 1–18 s for 23 h with a 120° angle at a temperature of 14°C and a voltage gradient of 6 V/cm. The lambda ladder PFGE marker kit (New England BioLabs) was used as a DNA size marker. Gel images were further analyzed using FPQuest software v.5 (Bio-Rad Laboratories). PFGE cluster analyses were performed using the Unweighted Pair Group Method with Arithmetic means (UPGMA).

Isolation rate and statistical analysis

The isolation rate of CTB^r PSGBS and PRGBS from August 2013 to August 2015 was calculated using the MIC data of the clinical isolates in this study. The isolation rate of CTB^r PSGBS from January 2012 to July 2013 was determined by re-analyzing the raw data of a previous study [21]. Concerning isolates in the both durations, CTB^r PSGBS and PRGBS were judged using the same clarification (PRGBS, PEN MIC $\geq$0.25 μg/ml and CTB MIC

≥128 μg/ml; CTB^r PSGBS, PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA).

Ethical statement

As this study pertains to the characterization of bacterial isolates only, and as we did not obtain or use clinical or personal information, this type of study does not require examination or approval of an ethical committee according to the related guidelines of the Japanese government.

Results

Measurement of MIC

Three hundred and seventy-six of the 377 collected clinical isolates were confirmed as GBS by the formation of β-hemolytic colonies on sheep blood agar plates and specific agglutination with anti-Lancefield B antigen serum. One clinical isolate showed no β-hemolytic activity, but was confirmed as GBS by specific agglutination with anti-Lancefield B antigen serum and anti-serotype III serum. The CTB MIC for 52 of the 377 clinical isolates (13.8%) was ≥128 μg/ml (Fig. 1). Moreover, we confirmed that 25 isolates were PRGBS (PEN MIC ≥0.25 μg/ml and CTB MIC ≥128 μg/ml; Supplemental Table S3), and that 27 isolates were CTB^r PSGBS (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml;

Table 1). A number of CTB^r PSGBS isolates also showed resistance to erythromycin (15/27; MIC ≥1 μg/ml) and non-susceptibility to levofloxacin (26/27) (MIC ≥4 μg/ml). Additionally, the resistance and non-susceptibility ratio for both macrolides and fluoroquinolones in PSGBS, CTB^r PSGBS, and PRGBS were 14.7% (48/325), 51.9% (14/27) and 76.0% (19/25), respectively (Table 2; P value ≤0.0001 by chi-square test). The vancomycin MIC for all GBS clinical isolates was equal or lower than 2 μg/ml.

Sequencing the *pbp2x* gene from PRGBS and CTB^r PSGBS

We performed sequencing analysis of the *pbp2x* genes of 52 clinical isolates with a CTB MIC ≥128 μg/ml. All 25 PRGBS clinical isolates showed amino acid substitutions, including V405A and/or Q557E (amino acid substitutions often found among the PRGBS isolates) in PBP2X (supplemental table S3). Moreover, seven of the 27 CTB^r PSGBS isolates also showed amino acid substitutions, including V405A and/or Q557E, in PBP2X (Table 3). The other 20 CTB^r PSGBS isolates also demonstrated amino acid substitutions in PBP2X, which could be divided into two substitution types: the T394A type and the I377V, G398A, Q412L, H438Y type (Table 3).

Allelic exchange with CTB^r PSGBS-derived *pbp2x* genes

To examine the contribution of the two altered *pbp2x* gene types found in CTB^r PSGBS to

158 the reduction in CTB susceptibility, we constructed recombinants of strain 2603 V/R
159 containing the *pbp2x* genes derived from CTB^r PSGBS clinical isolates NUBL-8805 and
160 NUBL-12951 using allelic exchange techniques. Both NUBL-8805 and NUBL-12951
161 isolates contain a T394A, and I377V, G398A, Q412L and H438Y, in PBP2X, respectively.
162 These isolates were selected because they showed the least number of alterations in their
163 *pbp2x* genes. The MICs of the recombinants were comparable to those of the parental clinical
164 isolates (Table 4). These results confirm that both types of altered *pbp2x* genes are major
165 determinants of RCTBS in GBS.

166 **PFGE analysis**

167 We performed PFGE with the 25 PRGBS isolates and the 27 clinical CTB^r PSGBS isolates
168 (Fig. 2). Overall, although each of the PRGBS and CTB^r PSGBS isolates showed relatively
169 similar band patterns in clusters, most PRGBS and CTB^r PSGBS isolates seemed to belong to
170 different clusters. The 25 PRGBS 27 CTB^r PSGBS isolates did not exhibit the same
171 pulsotype, suggesting that the 25 PRGBS and 27 CTB^r PSGBS isolates were not clonal.

172 **Isolation rate of PRGBS and CTB^r PSGBS**

173 The isolation rate of PRGBS and CTB^r PSGBS among clinical isolates was 6.6% (25/377)
174 and 7.2% (27/377) between August 2013 and August 2015, respectively. In addition, the

175 isolation rate of CTB^r PSGBS among clinical isolates was 9.5% (29/306) between January
176 2012 and July 2013 (Table 5).
177
178 **Discussion**
179 To the best of our knowledge, this study is the first one to determine the isolation rate of
180 CTB^r PSGBS (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml) among the clinical isolates.
181 The isolation rates between January 2012 and July 2013 and between August 2013 and
182 August 2015 were 9.5% (29/306) and 7.2% (27/377), respectively. These values are
183 non-negligibly high and suggest that CTB^r PSGBS clinical isolates are not rare at present in
184 Japan. Because the same data collection methods were used in past [21] and present studies,
185 these results suggest the steady spread of CTB^r PSGBS in Japan since 2012. CTB^r PSGBS
186 seems susceptible to penicillin and has apparent reduced cefaclor susceptibility; although
187 CTB is hardly used against gram-positive bacterial infections, cefaclor was previously one of
188 the most widely used oral cephalosporins for empirical treatment of acute upper respiratory
189 infections in Japan. Therefore, when using some cephalosporins, e.g. cefaclor, against CTB^r
190 PSGBS infections, it is important to pay attention to the drug susceptibility of CTB^r PSGBS.
191 In addition, using two allelic exchange recombinants, we demonstrated that two types of

amino acid substitutions (the T394A type and the I377V, G398A, Q412L, H438Y type) in PBP2X confer RCTBS. Although the T394A type and I377V, G398A, Q412L, H438Y type amino acid substitutions in PBP2X have been previously reported [17, 22], allelic exchange recombinants of both types had not been previously generated. Therefore, to the best of our knowledge, this is the first report of allelic exchange recombinants with amino acid substitutions in PBP2X without having the PRGBS-characteristic amino acid substitutions V405A and/or Q557E. The PEN MIC for the recombinant 2603 V/R strain (NUBL-12951 PBP2X) generated in this study containing the I377V, G398A, Q412L, and H438Y type amino acid substitutions increased from 0.03 to 0.12 μg/ml (Table 4). This indicates that in some cases, amino acid substitutions in PBP2X other than Q557E and V405A can also elevate the PEN MIC up to just near the breakpoint of PEN non-susceptible (≥0.25 μg/ml).

Typically, the PEN MICs for clinical isolates harboring the PRGBS-characteristic amino acid substitutions V405A and/or Q557E in PBP2X are equal to or above the PEN breakpoint (≥0.25 μg/ml). However, in the current study, 7 of 27 CTB^r PSGBS harbored the PRGBS-characteristic amino acid substitutions V405A and/or Q557E in PBP2X, although the PEN MICs for these CTB^r PSGBS were 0.12 μg/ml, which is below the PEN breakpoint (≥0.25 μg/ml). Another group also reported that the PEN MIC for one clinical isolate,

7507-03, harboring Q557E in PBP2X was 0.12 μg/ml [23]. These results suggest that the amino acid substitutions V405A and/or Q557E in PBP2X often found among the PRGBS isolates usually confer reduced PEN susceptibility (PEN MIC ≥0.12 μg/ml) and do not necessarily confer PEN non-susceptibility (PEN MIC ≥0.25 μg/ml).

There are a few limitations of our study. The first is that the isolation rate of CTB^r PSGBS is dependent on the isolation site and the age of the patients, as CTB^r PSGBS is often isolated from respiratory specimens of the elderly. The second limitation of this study is that because the clinical information pertaining to the isolates in this study is limited, isolates from this study may reflect colonization, rather than infection.

This study is the first one to demonstrate a resistance and non-susceptible tendency to both macrolides and fluoroquinolones among CTB^r PSGBS. Although CTB^r PSGBS are susceptible to PEN, because they have a tendency of resistance and non-susceptible to macrolides and fluoroquinolones, as well as reduced susceptibility to several cephalosporins (e.g. cefaclor and CTB), the drug choices for treating CTB^r PSGBS infections are limited in patients allergic to penicillin. Therefore, as the isolation rate of CTB^r PSGBS is high and the CTB^r PSGBS isolates also tend to have a resistance and non-susceptible profile against both macrolides and fluoroquinolones, rapid and precise detection of CTB^r PSGBS is necessary in

clinical settings, and the multidrug resistance tendency of CTB^r PSGBS should be taken into consideration when treating CTB^r PSGBS infections.

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Compliance with ethical standards

Conflict of interest

None of the authors has any conflicts of interest. The manuscript was edited by Editage, a language editing company.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent is not applicable, as this article does not contain any studies with human participants.

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Figure legends

Fig. 1 Distribution of 377 clinical GBS isolates according to ceftibuten MIC. The CTB MIC was measured for 377 clinical GBS isolates. Values above the bars indicate the number of clinical isolates. CTB, ceftibuten.

Fig. 2 PFGE of 25 PRGBS isolates (PEN MIC ≥0.25 μg/ml and CTB MIC ≥128 μg/ml) and 27 clinical CTB^r PSGBS isolates (PSGBS with reduced CTB susceptibility; PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml). * indicates CTB^r PSGBS (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml). CTB, ceftibuten; PEN, penicillin G.

Table 1

Table 1 Origin and MICs of clinical CTB^r PSGBS isolates

Strain, isolate	Specimen	Isolation location	Sex	Age	MIC (μg/ml)												
					PEN	AMP	OXA	CEC	CFZ	ZOX	CTB	FEP	MEM	VAN	ERY	CLI	LVX
2603 V/R	-	-	-	-	0.06	0.03	0.25	1	0.12	0.12	32	0.06	0.12	0.5	0.12	0.06	0.5
NUBL-12951	Urine	Tokyo	Unknown	Unknown	0.12	0.5	2	4	1	4	256	0.5	0.06	0.12	0.25	0.03	32
NUBL-15885	Throat swab	Chiba	F	94	0.12	0.5	1	8	0.5	2	＞256	0.5	0.25	0.5	0.12	0.06	32
NUBL-16354	Throat swab	Tokyo	F	73	0.12	0.5	2	8	1	2	256	0.25	0.12	0.5	0.12	0.06	128
NUBL-10588	Sputum	Miyagi	M	81	0.06	0.25	0.5	4	0.25	1	128	0.25	0.06	0.5	0.25	0.12	64
NUBL-8792	Urine	Kanagawa	F	90	0.03	0.06	0.5	4	0.12	1	128	0.12	0.03	1	＞128	＞128	128
NUBL-8805	Sputum	Nagano	F	87	0.06	0.12	0.5	8	0.25	2	256	0.12	0.06	1	＞128	＞128	128
NUBL-8814	Sputum	Tokyo	M	85	0.06	0.12	0.5	8	0.25	2	256	0.12	0.06	1	0.12	0.25	8
NUBL-9802	Sputum	Chiba	F	90	0.06	0.06	0.5	8	0.25	1	256	0.12	0.06	0.5	0.12	0.06	128
NUBL-10571	Sputum	Aomori	M	85	0.06	0.12	0.5	8	0.25	0.25	256	0.25	0.03	0.5	0.12	0.06	128
NUBL-10587	Sputum	Fukushima	M	74	0.06	0.12	0.25	4	0.25	1	128	0.12	0.03	1	＞128	＞128	128
NUBL-11464	Sputum	Tokyo	F	90	0.06	0.12	0.25	4	0.25	1	128	0.12	0.06	1	＞128	＞128	32
NUBL-11937	Sputum	Tochigi	F	Unknown	0.03	0.12	0.25	4	0.12	1	128	0.06	0.03	0.5	0.12	0.06	8
NUBL-11966	Throat swab	Tokyo	M	77	0.03	0.12	0.25	4	0.12	2	128	0.12	0.03	0.5	＞128	＞128	64
NUBL-11982	Sputum	Chiba	M	77	0.06	0.12	0.5	4	0.12	1	256	0.12	0.03	0.5	＞128	＞128	128

NUBL-12930	Sputum	Nagano	M	68	0.06	0.25	0.25	4	0.12	0.25	128	0.12	0.03	0.5	＞128	＞128	8
NUBL-13594	Throat swab	Tokyo	M	86	0.03	0.12	0.5	8	0.12	1	256	0.12	0.06	0.5	＞128	128	16
NUBL-13598	Sputum	Chiba	F	79	0.03	0.12	0.5	8	0.12	1	256	0.12	0.06	0.5	＞128	＞128	128
NUBL-13604	Wound	Shizuoka	F	29	0.06	0.12	0.5	8	0.12	4	>256	0.5	0.12	0.5	4	0.06	0.5
NUBL-15884	Sputum	Chiba	M	76	0.06	0.06	0.25	4	0.25	1	256	0.12	0.06	0.5	＞128	＞128	64
NUBL-16368	Sputum	Tokyo	M	66	0.03	0.06	0.25	8	0.06	1	128	0.12	0.03	0.5	＞128	＞128	64
NUBL-12950	Sputum	Tokyo	M	60	0.12	0.06	2	0.5	1	2	256	0.25	0.06	0.5	4	0.03	128
NUBL-12912	Sputum	Tokyo	M	91	0.12	0.06	2	4	1	4	>256	0.25	0.12	0.5	0.12	0.06	64
NUBL-11942	Sputum	Chiba	M	64	0.12	0.06	2	8	1	2	256	0.25	0.12	0.5	2	0.06	128
NUBL-11448	Throat swab	Saitama	F	77	0.12	0.06	2	1	1	4	>256	0.5	0.06	0.5	0.25	0.06	128
NUBL-11965	Sputum	Chiba	M	85	0.12	0.12	2	8	1	8	>256	0.5	0.25	0.25	2	0.06	64
NUBL-11959	Throat swab	Gunmma	M	59	0.12	0.25	2	16	0.25	16	>256	0.5	0.12	0.5	0.12	0.06	64
NUBL-10593	Sputum	Fukushima	M	63	0.12	0.50	2	8	1	16	>256	0.5	0.12	0.5	0.12	0.06	64

CTB^{r} PSGBS, penicillin-susceptible group B *Streptococcus* with reduced ceftibuten susceptibility (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml); PEN, penicillin G; AMP, ampicillin; OXA, oxacillin; CEC, cefaclor; CFZ, cefazolin; ZOX, ceftizoxime; CTB, ceftibuten; FEP, cefepime; MEM, meropenem; VAN, vancomycin; ERY, erythromycin; CLI, clindamycin; LVX, levofloxacin

Table 2

Table 2 Rate of resistance/non-susceptibility to both macrolides and fluoroquinolones among PSGBS, CTB^r PSGBS, and PRGBS isolates

Category	PSGBS	CTB^r PSGBS	PRGBS
Resistance/non-susceptibility to both macrolides and fluoroquinolones	48 (14.7%)	14 (51.9%)	18 (72.0%)
Non-resistance/non-susceptibility to both macrolides and fluoroquinolones	277	13	7

$P \leq 0.0001$ by chi-square test.

Abbreviations: PSGBS, penicillin susceptible group B *Streptococcus*; CTB^r PSGBS, penicillin susceptible group B *Streptococcus* with reduced ceftibuten susceptibility; PRGBS, group B streptococci with reduced penicillin susceptibility. Resistance/non-susceptibility indicates resistance to macrolides and non-susceptibility to fluoroquinolones.

Table 3 Amino acid substitutions in PBP2X of clinical CTB^r PSGBS isolates

Strain, isolate	PBP2X												
	377	394	398	400	405	433	412	437	438	510	557	575	648
2603 V/R	I	T	G	A	V	R	Q	L	H	V	Q	N	G
NUBL-12951	V		A				L		Y				
NUBL-15885	V		A				L		Y				
NUBL-16354	V		A				L	P	Y			D	
NUBL-10588		A										D	
NUBL-8792		A											
NUBL-8805		A											
NUBL-8814		A											
NUBL-9802		A											
NUBL-10571		A											
NUBL-10587		A											
NUBL-11464		A											
NUBL-11937		A											
NUBL-11966		A											
NUBL-11982		A											
NUBL-12930		A											
NUBL-13594		A											
NUBL-13598		A											
NUBL-13604		A											
NUBL-15884		A											
NUBL-16368		A											
NUBL-12950				V							E		
NUBL-12912				V							E		
NUBL-11942				V							E		
NUBL-11448				V							E		
NUBL-11965	V		A		A	D							
NUBL-11959	V				A	D			Y	I			A
NUBL-10593	V				A	D			Y	I			A

CTB^r PSGBS, penicillin-susceptible group B *Streptococcus* with reduced ceftibuten susceptibility (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml); PBP2X, penicillin-binding protein 2X

Table 4

Table 4 MICs of nine β-lactams for GBS ATCC strain 2603V/R, clinical isolates and 2603V/R recombinants with amino acid substitutions in PBP2X

Strain, isolate	MIC (μg/ml)								
	PEN	AMP	OXA	CEC	CFZ	ZOX	CTB	FEP	MEM
2603 V/R	0.03	0.06	0.25	1	0.12	0.25	32	0.06	0.03
NUBL-8805	0.06	0.12	0.5	8	0.12	1	128	0.12	0.06
2603 V/R (NUBL-8805 PBP2X)	0.03	0.12	0.25	8	0.12	1	256	0.06	0.03
NUBL-12951	0.12	0.5	2	8	0.5	1	>256	0.12	0.12
2603 V/R (NUBL-12951 PBP2X)	0.12	0.5	2	8	0.5	2	>256	0.25	0.12

2603 V/R (NUBL-8805 PBP2X) and 2603 V/R (NUBL-12951 PBP2X) signify 2603V/R recombinants with amino acid substitutions in PBP2X derived from NUBL-8805 and NUBL-12951, respectively.

PBP2X, penicillin-binding protein 2X; PEN, penicillin G; AMP, ampicillin; OXA, oxacillin; CEC, cefaclor; CFZ, cefazolin; ZOX, ceftizoxime; CTB, ceftibuten; FEP, cefepime; MEM, meropenem.

Table 5 Isolation rate of clinical PRGBS and CTB^r PSGBS

Category	March 2005 to February 2006	January 2012 to July 2013	August 2013 to August 2015
PRGBS	10/442 (2.3%)	45/306 (14.7%)	25/377 (6.6%)
CTB^r PSGBS	Unknown*	29/306 (9.5%)	27/377 (7.2%)

PRGBS, Group B streptococci with reduced penicillin susceptibility (PEN MIC ≥0.25 μg/ml and CTB MIC ≥128 μg/ml); CTB^r PSGBS, penicillin-susceptible group B *Streptococcus* with reduced ceftibuten susceptibility (PEN MIC ≤0.12 μg/ml and CTB MIC ≥128 μg/ml); PEN, penicillin G; CTB, ceftibuten

*In this study, CTB MICs were not determined.

Figure 1

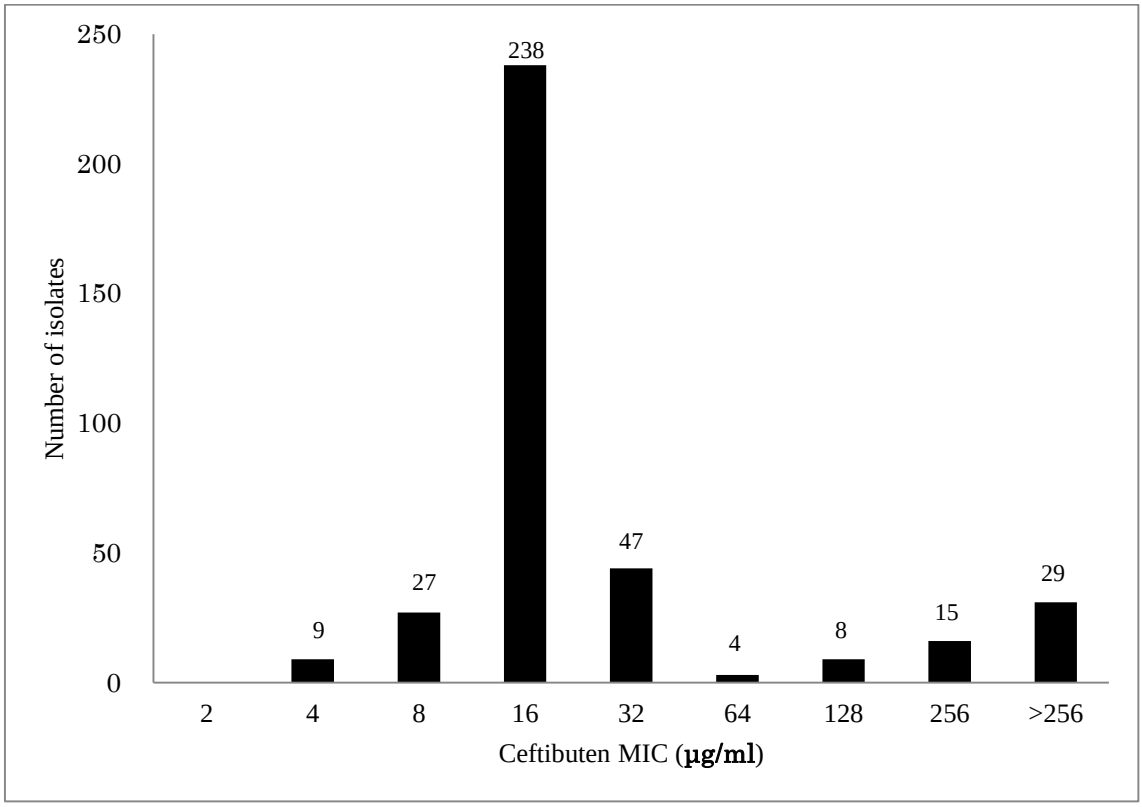

Fig. 1

Figure 2

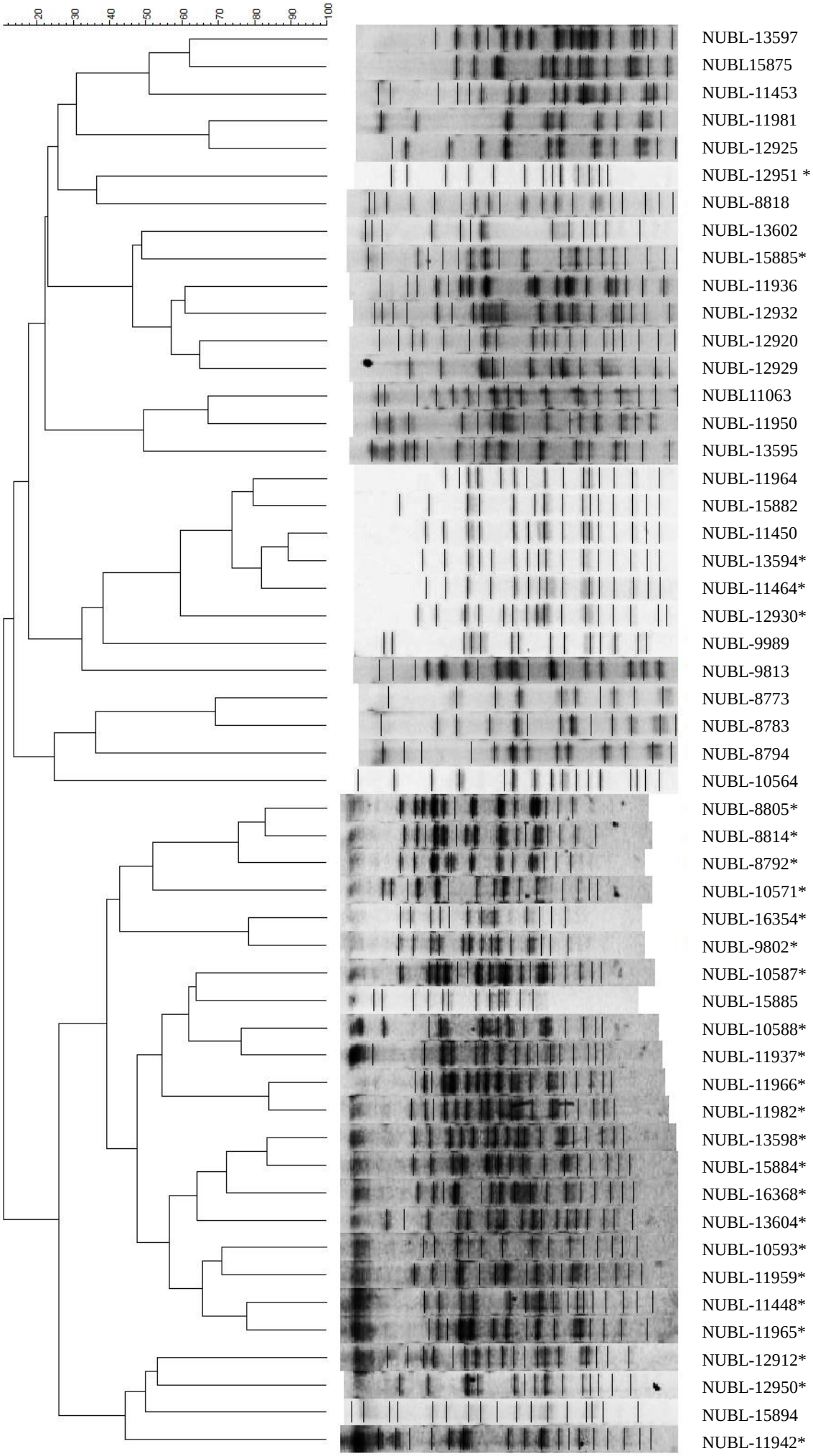

Fig. 2