Brief Report

Relatively high rate of cefotaxime- and ceftriaxone-non-susceptible isolates among group B streptococci with reduced penicillin susceptibility (PRGBS) in Japan

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Running title: High rate of CTX- and CRO-non-susceptibility among PRGBS isolates

Synopsis

Objectives: Our previous report identified group B *Streptococcus* (GBS) clinical isolates with reduced penicillin susceptibility (PRGBS) that were non-susceptible to cefotaxime; however, the rate of cefotaxime- and ceftriaxone-non-susceptible ones among the PRGBS has never been reported. Therefore, we firstly measured the MICs of 22 antibacterial drugs/compounds for the 74 PRGBS isolates collected in Japan, and secondly, determined the rate of cefotaxime- and ceftriaxone-non-susceptible ones among them.

Methods: We used 74 clinical PRGBS isolates, which were previously collected in Japan and have confirmed to harbour relevant amino acid substitutions in the PBP2X. We also used 80 penicillin-susceptible GBS (PSGBS) clinical isolates as the control. The MICs of 22 antibacterial drugs/compounds for all the 154 GBS isolates were determined via microdilution and/or agar dilution methods, as CLSI recommended.

Results: The rates of non-susceptibility/resistance to ampicillin-, cefotaxime-, ceftriaxone-, and levofloxacin- for the 80 PSGBS were 0%, 0%, 0%, and 30%, respectively, but were 14% (P = 0.0003), 28% (P <0.0001), 36% (P <0.0001), and 93% (P <0.0001) for the 74 PRGBS isolates, respectively. No PRGBS isolates were

identified to be non-susceptible to meropenem, doripenem, vancomycin, quinupristin/dalfopristin, daptomycin or linezolid.

Conclusions: We found that cefotaxime- and ceftriaxone-non-susceptible PRGBS isolates occur at a relatively high rate in Japan. Importantly, this finding suggests that the range of drugs likely to be effective in treating PRGBS infections may be limited compared to those available for penicillin-susceptible GBS infections; therefore, clinicians should be careful in considering drug choice and their efficacy for PRGBS infections.

Introduction

Group B *Streptococcus* (GBS, *Streptococcus agalactiae*) is the leading cause of neonatal sepsis and meningitis, and can also cause invasive diseases in elderly people, pregnant women, and people suffering from underlying medical diseases, such as diabetes.[1,2] Since all the clinical GBS isolates to date have been regarded to be susceptible to β-lactams, including penicillin G, these drugs are currently used for the first-line treatment and prevention of GBS infections.[1-3] However, we previously reported the emergence of GBS with reduced penicillin-susceptibility (PRGBS) and characterised molecularly, [4,5] and clinical PRGBS isolates have since been identified in Japan,[6,7] Canada,[8,9] the United States[10] and Mozambique.[11] These isolates have been shown to acquire reduced susceptibility to penicillin G, oxacillin, ceftizoxime, ceftibuten[12] and cefoxitin[13] via point mutations that cause amino acid substitutions, especially Val405Ala and/or Gln557Glu, that are located adjacent to conserved active-site motifs in the transpeptidase domain of the PBP2X.[5] In addition to reduced penicillin susceptibility, PRGBS clinical isolates are also often non-susceptible to macrolides and fluoroquinolones,[14] and nosocomial spread caused by MDR-PRGBS has already been reported.[15] Thus, limited treatments are available for MDR-PRGBS infections.

A previous report showed that some PRGBS isolates are non-susceptible to cefotaxime.[5] In the clinical setting, cefotaxime and ceftriaxone are often prescribed to treat meningitis because they exhibit good penetration into cerebrospinal fluid; however, to the best of our knowledge, the rates of cefotaxime- and ceftriaxone-non-susceptibility among PRGBS isolates have never been reported. Therefore, the present study aimed firstly to establish the MICs of 22 antibacterial drugs/compounds (including cefotaxime and ceftriaxone) for 74 PRGBS isolates collected from various regions in Japan, and secondly, to determine the rates of cefotaxime- and ceftriaxone-non-susceptibility among these isolates.

Materials and methods

Clinical isolates

This study analysed 74 clinical PRGBS isolates that were previously collected from various locations in Japan, and characterized (Table S1).[5,6,16] The penicillin G MIC for these isolates was shown to be >0.12 mg/L, and since the CLSI stipulates that the penicillin G susceptibility breakpoint is any level ≤0.12 mg/L,[3] these isolates were classed as penicillin-non-susceptible. The isolates were previously shown to harbour *pbp2x* mutations that caused several amino acid substitutions in the PBP2X protein. The oxacillin, ceftizoxime, and ceftibuten MIC values for all the 74 isolates were high; therefore, the isolates were confirmed as PRGBS.

The study also used 80 previously reported penicillin-susceptible GBS (PSGBS) clinical isolates as the controls (Table S1).[16] All the 154 (PRGBS and PSGBS) isolates tested were confirmed as GBS by the formation of β-haemolytic colonies on sheep blood agar plates and specific agglutination with anti-Lancefield B antigen serum using the Lancefield antigen examination kit (PASTOREX strep, BIO-RAD).

MIC determination

Microdilution or agar dilution methods were used to determine the MICs of 22 antibacterial drugs/compounds, comprising ampicillin, cefazolin, cefotaxime,

ceftriaxone, imipenem, meropenem, doripenem, biapenem, tebipenem, faropenem, vancomycin, teicoplanin, gentamicin, arbekacin, levofloxacin, quinupristin /dalfopristin, daptomycin, tigecycline, linezolid, novobiocin, apigenin and auranofin for all 154 GBS isolates, as recommended by the CLSI.[3] Isolate susceptibility/resistance was determined according to the ampicillin, cefotaxime, ceftriaxone, doripenem, meropenem, vancomycin, levofloxacin, daptomycin, linezolid and quinupristin /dalfopristin breakpoints for the *Streptococcus* spp. β-hemolytic Group stipulated by the CLSI.[3] *S. pneumoniae* ATCC49619 was used as the control of antimicrobial susceptibility testing.

Statistical analysis

Data were analysed by performing Chi-square (χ^2) tests with GraphPad Prism 7 software (GraphPad software, Inc., San Diego, California). A P value ≤ 0.05 was considered to indicate a statistically significant difference.

Results

Table 1 shows the MIC ranges, and the MIC_{50}, and MIC_{90} values of the 22 analysed antibacterial drugs/compounds for the analysed 74 PRGBS and 80 PSGBS isolates. Notably, the ampicillin, cefazolin, cefotaxime and ceftriaxone MIC ranges and MIC_{50}, and MIC_{90} values were higher for the PRGBS than the PSGBS isolates. Furthermore, while no ampicillin-, cefotaxime-, nor ceftriaxone-non-susceptible PSGBS isolates were identified, the rate of ampicillin-, cefotaxime-, and ceftriaxone-non-susceptibility among the PRGBS isolates was found to be 14% (P = 0.0003), 28% (P <0.0001), and 36% (P <0.0001), respectively (Table 2). The carbapenem and penem MIC_{50} and MIC_{90} values were slightly higher for the PRGBS than the PSGBS isolates, but were relatively low for both isolate types. Moreover, no meropenem- and/or doripenem-non-susceptible isolates were identified. The glycopeptide MICs for both the PRGBS and PSGBS isolates were also relatively low, and all isolates were found to be susceptible to vancomycin.

We previously reported that PRGBS clinical isolates tend to be non-susceptible not only to penicillin G, but also to macrolides and fluoroquinolones.[14] In this study, although 24 PSGBS isolates (24/80, 30%) were resistant to levofloxacin, 69 PRGBS isolates (69/74, 93%) were resistant to levofloxacin (Table 2) (χ^2 test, P <0.0001). The

MICs of the relatively new drugs quinupristin /dalfopristin, daptomycin, tigecycline and linezolid for both the PRGBS and PSGBS isolates were low, and no PRGBS isolates that were non-susceptible to quinupristin /dalfopristin, daptomycin, or linezolid was identified. Although arbekacin is an aminoglycoside as an anti-MRSA drug approved in Japan, the gentamicin and arbekacin MICs for both PRGBS and PSGBS were relatively high. The MIC ranges of the other anti-MRSA drug, novobiocin, were 0.5–4 and 0.5–16 for the PRGBS and PSGBS isolates, respectively. Notably, although apigenin was previously reported to be an anti-quinolone-resistance antibiotic,[17] the apigenin MICs for both the PRGBS and PSGBS isolates were high. Conversely, the MICs of auranofin, which is an organogold FDA-approved antirheumatic drug that has been reported to have bactericidal activity against Gram-positive bacteria,[18,19] were relatively low for both the PRGBS and PSGBS isolates.

Discussion

This study revealed that the rates of cefotaxime- and ceftriaxone-non-susceptibility among the 74 analysed PRGBS (penicillin G MIC >0.12 mg/L) isolates were relatively high, at 28% and 36%, respectively. Importantly, this finding indicates that not all PRGBS are cefotaxime- and ceftriaxone-non-susceptible, and demonstrates that an isolate that is identified as being non-susceptible to penicillin in the clinical setting may yet be susceptible to cefotaxime and/or ceftriaxone. Nevertheless, as we previously reported, PRGBS isolates tend to be also non-susceptible to macrolides and fluoroquinolones,[14] this finding suggests that the range of drugs that are likely to be effective treatments for PRGBS (as opposed to penicillin-susceptible GBS) infections in Japan is limited, highlighting the need for careful drug selection for the GBS infections in the clinical setting. Moreover, PRGBS were reported to comprise 14.7% and 6.6% of GBS infections in Japan between 2012–2013 and 2013–2015, respectively,[16] indicating that the incidence of PRGBS in Japan is high. The CLSI asserts that routine drug (especially penicillin) susceptibility testing is not necessary for GBS infections in the clinical setting because PRGBS isolates are extremely rare[3]; however, this is clearly not the case in Japan. In fact, the results of the present study suggest that this recommendation by the CLSI is not applicable to the clinical setting in Japan, and that the

159 necessity of routine testing for β-lactam-non-susceptible GBS infections should be
160 reconsidered.

161 Importantly, this study also revealed that all the PRGBS isolates tested retained
162 susceptibility to meropenem, doripenem, vancomycin, quinupristin/dalfopristin,
163 daptomycin and linezolid, suggesting that these drugs are likely effective in treating
164 PRGBS infections. Similarly, the MICs of imipenem, biapenem, tebipenem, faropenem,
165 teicoplanin, tigecycline, novobiocin and auranofin for the PRGBS isolates were
166 relatively low; thus, although further (particularly clinical) research is needed, these
167 drugs/compounds are promising potential therapeutic agents to treat PRGBS infections.

168 Notably, PRGBS detection is often problematic at clinical laboratories, since
169 penicillin MICs (0.25–1 mg/L) for PRGBS isolates are usually close to the breakpoint
170 level (≤ 0.12 mg/L) stipulated by the CLSI. Furthermore, most routine drug
171 susceptibility testing at clinical laboratories does not measure oxacillin, ceftizoxime,
172 ceftibuten, or cefoxitin MICs, all of which are generally high among the PRGBS
173 isolates.[12,13] The findings of the present study strongly support the need for improved
174 detection protocols of PRGBS at clinical laboratories, and thus the introduction of the
175 disk diffusion method[12] and selective agar plate[20] that were previously developed by our
176 research group. Although clinical studies concerning the clinical effectiveness of drugs

with the elevated MICs for PRGBS against PRGBS infections are urgent, accurate PRGBS detection, and subsequent drug-susceptibility testing for specific PRGBS isolates are essential to facilitate the selection of appropriate drugs that are likely to be effective against PRGBS infections in the clinical setting.

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Transparency declaration

The authors have no conflicts of interest to declare. The manuscript was edited by Editage, a language-editing company.

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Table 1. MIC range, and MIC_{50} and MIC_{90} values of 22 antibacterial drugs/compounds for the analysed 74 PRGBS and 80 PSGBS isolates

		AMP	CFZ	CTX	CRO	IPM	MEM	DOR	BIPM	TBPM	FRPM	VAN	TEC	GEN	ABK	LVX	Q/D	DAP	TGC	LZD	NB	API	AUR
PRGBS	MIC range	0.06-	0.5-	0.03-	≦0.015-	0.015-	0.06-	0.015-	0.03-	≦0.007-	0.03-	0.25-	0.06-	32-	64-	0.5-	0.06-	0.25-	0.06-	0.5-	0.5-	>256-	1-
(*n*=74)	(mg/L)	0.5	4	1	1	0.06	0.25	0.12	0.5	0.03	0.12	0.5	0.25	>256	256	256	1	1	0.25	2	4	>256	4
	MIC_{50} (mg/L)	0.25	1	0.25	0.5	0.03	0.12	0.06	0.06	0.03	0.06	0.5	0.25	128	128	64	0.25	0.5	0.12	2	2	>256	2
	MIC_{90} (mg/L)	0.5	2	1	1	0.06	0.25	0.12	0.12	0.03	0.12	0.5	0.25	256	256	128	0.5	1	0.25	2	4	>256	4
PSGBS	MIC range	0.03-	0.06-	0.03-	0.03-	≦0.007-	0.03-	0.015-	0.015-	≦0.007-	0.015-	0.5-	≦0.03-	32-	64-	0.5-	0.06-	0.25-	0.06-	0.5-	0.5-	256-	1-
(*n*=80)	(mg/L)	0.25	1	0.5	0.25	0.03	0.25	0.06	0.12	0.06	0.12	1	0.25	256	512	128	1	2	0.5	2	16	>256	4
	MIC_{50} (mg/L)	0.12	0.25	0.06	0.06	0.015	0.06	0.03	0.03	0.015	0.03	0.5	0.25	128	256	1	0.25	1	0.12	2	4	>256	2
	MIC_{90} (mg/L)	0.25	0.25	0.06	0.12	0.03	0.06	0.03	0.06	0.015	0.06	1	0.25	128	256	64	0.5	1	0.25	2	4	>256	4

PRGBS (PEN MIC >0.12 mg/L), PSGBS (PEN MIC ≤0.12 mg/L)

Abbreviations: PRGBS, group B streptococcus with reduced penicillin susceptibility; PSGBS, penicillin susceptible group B streptococcus; PEN, penicillin G; AMP, ampicillin; CFZ, cefazolin; CTX, cefotaxime; CRO ceftriaxone; IPM, imipenem; MEM, meropenem; DOR, doripenem; BIPM, biapenem; TBPM, tebipenem; FRPM, faropenem; VAN, vancomycin; TEC, teicoplanin; GEN, gentamicin; ABK, arbekacin; LVX, levofloxacin; Q/D, quinupristin/dalfopristin; DAP, daptomycin; TGC, tigecycline; LZD, linezolid; NB, novobiocin; API, apigenin; AUR, auranofin.

Table 2. Rate of drug non-susceptibility/resistance among the analysed 74 PRGBS and 80 PSGBS isolates.

	Identified non-susceptible/resistant isolates, *n* (%)									
Drug	AMP	CTX	CRO	MEM	DOR	VAN	LVX	Q/D	DAP	LZD
PRGBS (*n* = 74) (PEN MIC >0.12 mg/L)	11 (14)†	21 (28)††	27 (36)†††	0 (0)	0 (0)	0 (0)	69 (93)††††	0 (0)	0 (0)	0 (0)
PSGBS (*n* = 80) (PEN MIC ≤0.12 mg/L)	0 (0)†	0 (0)††	0 (0)†††	0 (0)	0 (0)	0 (0)	24 (30)††††	0 (0)	1 (1)	0 (0)

$^{\dagger}P = 0.0003$, $^{\dagger\dagger}P < 0.0001$, $^{\dagger\dagger\dagger}P < 0.0001$, $^{\dagger\dagger\dagger\dagger}P < 0.0001$. according to a χ^2 test.

Abbreviations: PRGBS, group B streptococcus with reduced penicillin susceptibility; PSGBS, penicillin susceptible group B streptococcus; PEN, penicillin G; AMP, ampicillin; CTX, cefotaxime; CRO ceftriaxone; MEM, meropenem; DOR, doripenem; VAN, vancomycin; LVX, levofloxacin; Q/D, quinupristin/dalfopristin; DAP, daptomycin; LZD, linezolid.

Table S1. Specimen types and isolation periods for the analysed 74 PRGBS and 80 PSGBS clinical isolates

	PRGBS	PSGBS
Specimen type		
Sputum	61	49
TTA	3	1
Urea	3	9
Blood	2	0
PHA	2	3
Pus	1	7
Decubitus ulcer	1	1
CS	1	0
Nasal cavity	0	1
Skin	0	1
Vaginal discharge	0	8
Year of isolation		
1995	1	0
1997	6	0
1998	2	0
2001	0	3
2002	0	3
2003	3	9
2004	5	12
2005	7	0
2006	2	4
2007	0	3
2008	2	2
2011	1	0
2012	25	44
2013	20	0

Abbreviations: PRGBS, group B streptococcus with reduced penicillin susceptibility; PSGBS, penicillin-susceptible group B streptococcus; TTA, transtracheal aspirate; CS, conjunctival sac discharge; PHA, pharyngeal swab.