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In vitro Activities of Oral Cephem and Telithromycin Against Clinical Isolates of Major Respiratory Pathogens in Japan

The in vitro antibacterial activities of oral cephem antibiotics and ketolide telithromycin against major respiratory pathogens possessing β-lactam-resistant mutations (within the *pbp* gene) and/or macrolide-resistant genes (*erm* and *mef*) were examined in clinical isolates collected at 66 institutes in all over the Japan between 2002 and 2003. Telithromycin showed the strongest antibacterial activity against methicillin-susceptible *Staphylococcus aureus* strains with and without macrolide-resistant genes, such as *ermA* or *ermC* gene. All the cephem antibiotics showed potent antibacterial activity against *Streptococcus pyogenes*, with minimum inhibitory concentrations (MICs) of 0.015 mg/L or lower. Cefdinir had a much higher MIC_{90} against genotypic penicillin-resistant *Streptococcus pneumoniae* (gPRSP) than cefditoren and cefcapene (8 mg/L cefdinir vs. 1 mg/L cefditoren and cefcapene). The majority of gPRSP harbored either *ermB* or *mefA*, and the antibacterial activity of telithromycin against these strains was decreased however some susceptibility was still sustained. Cefditoren exerted the strongest antibacterial activity against β-lactamase-negative ampicillin-resistant *Haemophilus influenzae*, with an MIC_{90} of 0.5 mg/L. These results underline the importance of checking the susceptibility and selecting an appropriate antibiotic against target pathogens.

Key Words : *cefditoren; telithromycin; Microbial Sensitivity Tests; Minimum Inhibitory Concentration; beta-Lactams*

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INTRODUCTION

Methicillin-susceptible *Staphylococcus aureus* (MSSA), *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Haemophilus influenzae* are major causative pathogens of respiratory infectious disease and established causes of pneumonia, otitis media and paranasal sinusitis. In Japan and other Asian countries, *S. pneumoniae* has been rapidly developing β-lactam resistance by amino-acid substitutions in penicillin-binding proteins (PBPs). Isolates of these new strains also frequently show resistance to macrolides conferred by macrolide-resistant genes (1-3). Macrolide resistance in *S. pneumoniae* is conferred by 23S ribosome methylation by ErmB and/or drug efflux systems by MefA (4). Similarly, encounters with *ermA*-, *ermB*-, *ermC*- or *mefA*-positive MSSA and *S. pyogenes* have been growing more common in clinical settings. Clinicians in Japan have also been isolating new strains of *H. influenzae*, β-lactamase-negative ampicillin resistant strains (BLNAR), with increasing frequency (1, 5). Taking all of these developments together, it seems certain that respiratory pathogens are acquiring β-lactam and macrolide resistance. This makes it all the more important to examine the antibacterial activity against multidrug-resistant bacteria isolated from patients with respiratory tract infections (2, 5-9).

In this study we examine the in vitro activities of 7 antibiotics, oral cephems (cefditoren, cefcapene and cefdinir), macrolide (erythromycin), ketolide (telithromycin) and controls (benzylpenicillin and ampicillin) against clinical isolates of four major respiratory pathogens (MSSA, *S. pyogenes*, *S. pneumoniae,* and *H. influenzae*) collected in Japan between 2002 and 2003.

MATERIALS AND METHODS

Clinical isolates

MSSA, *S. pyogenes*, *S. pneumoniae* and *H. influenzae*, which were isolated from the respiratory tract and collected at 66 institutions (20 university hospitals; 34 local central hospitals; 4 clinics; 8 general practitioners) in all over the Japan between 2002 and 2003, were used in this study. Forty-three isolates of MSSA and 49 isolates of *S. pyogenes* strains were randomly selected. *S. pneumoniae* was classified in three categories, based on the mutations of the *pbp* genes associated with β-lactam resistance. As a result, the following isolates were studied: 34 isolates of genotypic penicillin-susceptible *S. pneumoniae* (gPSSP) with no mutations in the *pbp1a*, *2x* or *2b* genes; 47 isolates of genotypic penicillin-intermediate-resistant *S.*

pneumoniae (gPISP) with mutations in two of three *pbp* genes (*1a* and *2x*, *1a* and *2b* or *2x* and *2b*) and 68 isolates of genotypic penicillin-resistant *S. pneumoniae* (gPRSP) with mutations in all three *pbp* genes, as described by Ubukata et al. (9). The *H. influenzae* isolates were classified into two variants. The first, BLNAR, had M377I, S385T, L389F and N526K mutations of the *ftsI* gene encoding PBP3 without carrying the TEM-1 gene encoding β-lactamase. The second, β-lactamase-negative ampicillin-susceptible *H. influenzae* (BLNAS) had no mutations of the *ftsI* gene and did not carry the TEM-1 gene. Twenty-five BLNAS isolates and 24 BLNAR isolates were selected for inclusion in this study.

Detection of drug-resistance genes by PCR

Mutations in the *pbp1a*, *2x* and *2b* genes and the presence of the *mefA* and *ermB* genes in *S. pneumoniae* were detected by a penicillin-resistant *S. pneumoniae* detection kit (Wakunaga Pharmaceutical, Osaka, Japan). Mutations in the *ftsI* gene and the presence of the TEM-1 gene in *H. influenzae* were detected using an *H. influenzae* gene detection kit (Wakunaga Pharmaceutical). The presence of the *ermA* and *ermC* genes in MSSA, and the presence of the *mefA* and *ermB* genes in *S. pyogenes*, were detected by conventional PCR methods (6, 10).

Antibiotic susceptibility test

Cefditoren and ampicillin were supplied by Meiji Seika Kaisha, Ltd., Tokyo, Japan. Cefdinir was purchased from Kemprotec Ltd., Middlesborough, U.K. Erythromycin and benzylpenicillin was purchased from Sigma-Aldrich Japan. Cefcapene was synthesized in our laboratory. Telithromycin was purified from the commercial product (Aventis Pharma Japan, Tokyo, Japan). Minimum inhibitory concentrations (MICs) were determined by the agar dilution method with 2-fold serial dilutions of antibiotics. The following Mueller-Hinton agar (MHA, Becton, Dickinson and Company, Sparks, MD, U.S.A.) preparations were used for the drug susceptibility tests: MHA alone for MSSA; MHA supplemented with 5% defibrinated equine blood for *S. pneumoniae*; MHA supplemented with 5% defibrinated equine blood heated until chocolate for *H. influenzae*, and MHA supplemented with 5% defibrinated ovine blood for *S. pyogenes*. Each isolate was inoculated at 10^4 CFU/spot on each agar plate. The MIC was determined as the minimum drug concentration showing no bacterial growth after 18-20 hr of incubation at 35℃. *S. aureus* ATCC29213, *S. pneumoniae* ATCC49619, *H. influenzae* ATCC49247 and ATCC49766 were used to control the accuracy of the MIC determinations. *S. pneumoniae* ATCC49619 was used as a control in the measurement of the susceptibility of *S. pyogenes* isolates. As for the breakpoint values of antibiotics for pneumoniae, we referred to the section of pneumoniae in the journal issued by the Sensitivity Determination Committee for Antibiotics, Japanese Society of Chemotherapy, except for benzylpenicillin that was not provided this journal. Judging from the breakpoint value, we calculated the resistance or susceptibility rate of each antibitotic against pathogens.

RESULTS

Antibacterial activity against MSSA

Of the 43 MSSA isolates used in this study, 10 isolates (23.3 %) and 3 isolates (7.0%) carried either *ermA* or *ermC*, respectively, while none of the strains carried both genes. Telithromycin showed the most potent antibacterial activity against MSSA, followed by cefdinir, cefditoren, and cefcapene in descending order (Table 1). The antibacterial activity of erythromycin against MSSA was markedly attenuated by the presence of the *erm* genes, whereas the MIC_{90} of telithromycin was only 0.06 mg/L, even in the presence of the *erm* genes, with no cross-resistance to erythromycin.

Table 1. Antibacterial activity of oral cephems, erythromycin and telithromycin against methicillin-susceptible *Staphylococcus aureus* (MSSA)

Macrolide-resistant gene	No. of strains	Drug	Minimum inhibitory concentration (MIC) (mg/L)			Breakpoint (mg/L)*	Resistance-rate (%)
			Range	MIC_{50}	MIC_{90}		
ermA or *ermC*	13	Cefditoren	1	1	1	1	0
		Cefcapene	1-2	1	2	0.5	100
		Cefdinir	0.25-0.5	0.5	0.5	1	0
		Erythromycin	1->128	8	>128	0.5	100
		Telithromycin	0.03-0.13	0.06	0.13	2	0
None	30	Cefditoren	0.5-1	1	1	1	0
		Cefcapene	1.-2	1	2	0.5	100
		Cefdinir	0.25-1	0.5	0.5	1	0
		Erythromycin	0.13-32	0.25	0.25	0.5	3.3
		Telithromycin	0.015-0.13	0.06	0.06	2	0

*, Breakpoint value of each antibiotic for pneumoniae was cited from the journal issued by that was defined by the Sensitivity Determination Committee for Antibiotics, Japanese Society of Chemotherapy.

Table 2. Antibacterial activity of oral cephems, erythromycin and telithromycin against *Streptococcus pyogenes*

Macrolide-resistant gene	No. of strains	Drug	MIC (mg/L)			Breakpoint (mg/L)*	Resistance -rate (%)
			Range	MIC_{50}	MIC_{90}		
ermB	6	Cefditoren	0.008-0.015	–	–	1	0
		Cefcapene	0.008-0.015	–	–	0.5	0
		Cefdinir	0.008-0.015	–	–	1	0
		Erythromycin	1->128	–	–	0.5	100
		Telithromycin	0.06-64	–	–	2	66.7
mefA	17	Cefditoren	0.008-0.015	0.008	0.015	1	0
		Cefcapene	0.008-0.015	0.008	0.015	0.5	0
		Cefdinir	0.008-0.015	0.008	0.015	1	0
		Erythromycin	8-32	16	32	0.5	100
		Telithromycin	0.5-2	1	1	2	0
None	26	Cefditoren	0.008-0.015	0.008	0.015	1	0
		Cefcapene	0.008-0.015	0.008	0.015	0.5	0
		Cefdinir	0.008-0.015	0.008	0.015	1	0
		Erythromycin	0.03-0.25	0.06	0.13	0.5	0
		Telithromycin	0.015-0.06	0.015	0.03	2	0

*, Breakpoint value of each antibiotic for pneumoniae was cited from the journal issued by that was defined by the Sensitivity Determination Committee for Antibiotics, Japanese Society of Chemotherapy.

Antibacterial activity against *S. pyogenes*

As shown in Table 2, 6 isolates (12.2%) and 17 isolates (34.7%) of the *S. pyogenes* strains carried the *ermB* and *mefA* genes, respectively. The MIC of the cephem antibiotics was no more than 0.015 mg/L against all the isolates of *S. pyogenes* tested. The MIC_{90} of telithromycin was increased when the isolates carried either *ermB* or *mefA*. Especially high resistance against telithromycin (MIC=64 mg/L) was observed in 4 of 6 isolates carrying the *ermB* gene.

Antibacterial activity against *S. pneumoniae*

Subdivision of the gPSSP, gPISP, and gPRSP strains based on the presence of the *ermB* and *mefA* genes revealed that *S. pneumoniae* carried either *ermB* or *mefA* at a high frequency (Table 3). The β-lactams showed potent antibacterial activity against gPSSP, and cefditoren showed the strongest antibacterial activity against gPSSP, gPISP, and gPRSP of all 3 β-lactam antibiotics. The presence of the *ermB* and *mefA* genes within *S. pneumoniae* strains did not affect the activity the β-lactams. Telithromycin and erythromycin showed no attenuation in antibacterial activity even in the presence of a mutated *pbp* gene, while telithromycin exerted the strongest antibacterial activities against all three of the *S. pneumoniae* strains. However, the antibacterial activity of telithromycin was weakened in the presence of either the *ermB* or *mefA* gene.

Antibacterial activity against *H. influenzae*

Cefditoren and cefcapene showed stronger antibacterial activity than cefdinir, telithromycin, and erythromycin against BLNAS (Table 4). The antibacterial activities of the oral cephems were weaker against BLNAR than against BLNAS, whereas the MIC_{90} of cefditoren was 0.5 mg/L, the most potent level measured among all the antibiotics tested in this study. Telithromycin showed an equal MIC_{90} against BLNAR and BLNAS, and the same result was obtained for erythromycin.

DISCUSSION

The incidence of drug-resistant pathogens differs greatly between countries, presumably in accordance with differences in the dosage and usage of antibiotics. In this study we focused on the antibacterial activities of three different types of antibiotics (cephem, macrolide and ketolide) against recently isolated pathogens from respiratory tract infections carrying various drug-resistance genes in the Japanese population. The different frequencies of drug-resistant pathogens between Japan and neighboring Asian countries have also been considered.

In *S. aureus*, ribosome methylation and the presence of drug efflux systems have been shown to confer resistance against macrolide antibiotics (4). The isolates that exhibited low susceptibility to erythromycin without the *erm* gene in this study may have overexpressed multidrug efflux systems such as MsrA (11). Interestingly, all MSSA strains examined in this study were susceptible to telithromycin, even when they were positive for the presence of the *erm* gene. The expression of the *erm* gene can be inducible or constitutive, and MSSA is resistant to telithromycin when the expression is constitutive (12). Thus, we know that the isolates used in this study might harbor the inducible type of gene expression. The rate of MSSA resistance to telithromycin in Asia, 20%, is higher than the rates in Europe and the U.S.A. (13).

β-lactam resistance has not yet been reported in *S. pyogenes*,

Table 3. Antibacterial activity of β-lactams, erythromycin and telithromycin against *Streptococcus pneumoniae*

Organism macrolide-resistant gene	No. of strains	Drug	Minimum inhibitory concentration (MIC) (mg/L)			Breakpoint (mg/L)*	Resistance -rate (%)
			Range	MIC_{50}	MIC_{90}		
gPSSP	7	Cefditoren	0.008-0.03	–	–	1	0
ermB		Cefcapene	0.008-0.06	–	–	0.5	0
		Cefdinir	0.03-0.13	–	–	1	0
		Erythromycin	>128	–	–	0.5	100
		Telithromycin	0.03-1	–	–	2	0
		Benzylpenicillin	0.004-0.03	–	–	–	–
gPSSP	3	Cefditoren	0.008-0.03	–	–	1	0
mefA		Cefcapene	0.008-0.06	–	–	0.5	0
		Cefdinir	0.03-0.06	–	–	1	0
		Erythromycin	4-8	–	–	0.5	100
		Telithromycin	0.06-0.13	–	–	2	0
		Benzylpenicillin	0.004-0.015	–	–	–	–
gPSSP	24	Cefditoren	0.004-0.03	0.015	0.03	1	0
None		Cefcapene	0.004-0.13	0.008	0.06	0.5	0
		Cefdinir	0.03-0.13	0.03	0.13	1	0
		Erythromycin	0.06-0.25	0.13	0.13	0.5	0
		Telithromycin	0.008-0.06	0.008	0.015	2	0
		Benzylpenicillin	0.004-0.015	0.015	0.015	–	–
gPISP†	22	Cefditoren	0.03-0.25	0.13	0.13	1	0
ermB		Cefcapene	0.03-0.5	0.13	0.25	0.5	0
		Cefdinir	0.03-0.5	0.13	0.5	1	0
		Erythromycin	128->128	>128	>128	0.5	100
		Telithromycin	0.03-1	0.13	0.5	2	0
		Benzylpenicillin	0.015-0.25	0.03	0.25	–	–
gPISP†	8	Cefditoren	0.13-1	–	–	1	0
mefA		Cefcapene	0.13-1	–	–	0.5	25
		Cefdinir	0.25-2	–	–	1	25
		Erythromycin	1->128	–	–	0.5	100
		Telithromycin	0.06-1	–	–	2	0
		Benzylpenicillin	0.06-0.25	–	–	–	–
gPISP	17	Cefditoren	0.03-0.5	0.25	0.5	1	0
None		Cefcapene	0.03-2	0.5	0.5	0.5	11.8
		Cefdinir	0.06-2	2	2	1	64.7
		Erythromycin	0.06-1	0.13	0.13	0.5	5.9
		Telithromycin	0.008-0.13	0.015	0.03	2	0
		Benzylpenicillin	0.03-0.5	0.25	0.5	–	–
gPRSP†	22	Cefditoren	0.25-1	0.5	1	1	0
ermB		Cefcapene	0.25-1	0.5	1	0.5	45.5
		Cefdinir	1-8	4	8	1	95.5
		Erythromycin	128->128	>128	>128	0.5	100
		Telithromycin	0.03-1	0.06	0.13	2	0
		Benzylpenicillin	0.5-4	1	2	–	–
gPRSP†	38	Cefditoren	0.13-2	0.5	1	1	7.9
mefA		Cefcapene	0.13-4	0.5	1	0.5	34.2
		Cefdinir	0.5-8	4	8	1	92.1
		Erythromycin	1->128	4	8	0.5	100
		Telithromycin	0.06-1	0.13	0.25	2	0
		Benzylpenicillin	0.5-4	1	2	–	–
gPRSP	8	Cefditoren	0.25-1	–	–	1	0
None		Cefcapene	0.5-1	–	–	0.5	25
		Cefdinir	2-8	–	–	1	100
		Erythromycin	0.06-0.13	–	–	0.5	0
		Telithromycin	0.015-0.03	–	–	2	0
		Benzylpenicillin	0.5-1	–	–	–	–

gPSSP, genotypic susceptible *S. pneumoniae*; gPISP, genotypic penicillin-intermediate-resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. *, Breakpoint value of each antibiotic for pneumoniae was cited from the journal issued by that was defined by the Sensitivity Determination Committee for Antibiotics, Japanese Society of Chemotherapy; †, Including one strain with both *ermB* and *mefA* genes.

Table 4. Antibacterial activity of β-lactams, erythromycin and telithromycin against *Haemophilus influenzae*

Organism	No. of strains	Drug	Minimum inhibitory concentration (MIC) (mg/L)			Breakpoint (mg/L)*	Resistance -rate (%)
			Range	MIC_{50}	MIC_{90}		
BLNAS	25	Cefditoren	0.015-0.03	0.015	0.03	1	0
		Cefcapene	0.015-0.03	0.015	0.03	0.5	0
		Cefdinir	0.25-1	0.5	1	1	0
		Erythromycin	2-8	4	8	0.5	100
		Telithromycin	1-4	2	4	2	24
		Ampicillin	0.25-0.5	0.25	0.25	0.5	0
BLNAR	24	Cefditoren	0.03-0.5	0.25	0.5	1	0
		Cefcapene	0.25-8	4	8	0.5	91.7
		Cefdinir	2-32	8	16	1	100
		Erythromycin	2-16	8	8	0.5	100
		Telithromycin	1-8	2	4	2	50
		Ampicillin	1-64	2	8	0.5	100

BLNAS, β-lactamase-negative ampicillin-susceptible *H. influenzae*; BLNAR, β-lactamase-negative ampicillin-resistant *H. influenzae*.
*, Breakpoint value of each antibiotic for pneumoniae was cited from the journal issued by that was defined by the Sensitivity Determination Committee for Antibiotics, Japanese Society of Chemotherapy.

but macrolide-resistant strains of this pathogen have been frequently isolated (6-8, 13, 14). Some of the *S. pyogenes* isolates carrying *ermB* have become highly resistant to telithromycin (MIC >32 mg/L) (6-8). In our study, telithromycin and erythromycin showed reduced antibacterial activity against *S. pyogenes* isolates carrying either *ermB* or *mefA*. The incidence of telithromycin-resistant *S. pyogenes* (MIC ≥4 mg/L) differs from region to region, presumably due to the different usage of antibiotics. For example, the incidence in Japan stands at less than 1%, while the incidences in Korea and Europe are generally above 10% (13).

As previously reported, we categorized *S. pneumoniae* into gPRSP or gPISP based on mutations in the *pbp* gene (9). *ermB* and *mefA* have been found to be present at high frequencies in recent Japanese isolates of PISP and PRSP (2, 3). Our results also revealed many gPRSP and gPISP isolates carrying either *ermB* or *mefA*. In Korea and Hong Kong, PRSP accounts for ≥60% of clinical isolates of *S. pneumoniae*, and 50% or more of these isolates carry either *ermB* or *mefA* (3). It thus comes as no surprise that the MIC of telithromycin has already risen to 4 mg/L against newly emerging strains of *S. pneumoniae* in Taiwan (15) and to 2-8 mg/L against strains in other countries (7).

Just as Japan is witnessing a rising prevalence of BLNAR and falling prevalence of β-lactamase-producing strains, the very opposite trends are being observed in Korea (1, 3). It might be reasonable to assume that penicillin is more frequently prescribed in Korea than in Japan. Accordingly, the oral cephems such as cefditoren, an agent stable against β-lactamase in *H. influenzae*, may be useful and effective in both of these countries. Although macrolide-resistant genes such as *erm* or *mef* have not yet been found in *H. influenzae*, the antibacterial activity of telithromycin and erythromycin was lower than that of cefditoren against the *H. influenzae* isolates analyzed in the present study. Peric et al. demonstrated that more than 98% of *H. influenzae* strains have a macrolide efflux mechanism (16). Thus, the *H. influenzae* isolates investigated in this study might also express efflux pumps, such as AcrAB (17).

In conclusion, the variable antibacterial activity against the clinical pathogens in the presence of drug-resistant genes demonstrated in this study suggests that the inappropriate prescription of antibiotic agents may lead to clinical failure. It will be important to choose suitable antibiotics and to make the most of the ability of the prescribed agents in order to prevent the further emergence of drug-resistant pathogens.

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