Characterization of a New Plasmid-mediated Extended-spectrum β-Lactamase from *Serratia marcescens*

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A new extended spectrum β-lactamase was detected in *Serratia marcescens* 42039 that was isolated from urine of patients with complicated urinary tract infection in Japan. This strain produced three different β-lactamase types (TEM-1, a cephalosporinase, and a new β-lactamase: CKH-1). The TEM-1 and CKH-1 encoding genes were conjugated from *S. marcescens* 42039 to *Escherichia coli* K-12 at frequencies of 10^{-5} to 10^{-6}. The MICs of β-lactams against the transconjugant were: ampicillin >1600, piperacillin 800, cephalothin 1600, ceftazidime 6.25, cefotaxime 100, and ceftriaxone 200 μg/ml. The CKH-1 enzyme was purified to more than 90% by ion-exchange chromatography. The molecular weight of purified CKH-1 was 30 K dalton and the isoelectric point was 8.2. Relative Vmax/*Km* values (cephaloridine=100) of penicillin G, cephalothin, and oxyiminocephalosporins such as cefuroxime, ceftriaxone, and cefotaxime, were 256, 226, 116, 87, and 49, respectively. The I_{50} values of tazobactam, BRL-42715, and clavulanic acid against CKH-1 enzyme were 0.0011, 0.0002, and 0.097 μM, respectively. The enzymatic activity of CKH-1 was not inhibited by EDTA and anti-TEM-1 serum. These findings indicate that CKH-1 is a member of the groups of class A β-lactamases. This is the first report of a plasmid-mediated oxyiminocephalosporin hydrolyzing broad-spectrum β-lactamase from clinical isolates of *S. marcescens*.

Extended-spectrum β-lactamases are plasmid-mediated enzymes which confer resistance to oxyimino-β-lactams such as cefotaxime, ceftazidime, and aztreonam, that were designed to be effective against strains producing known plasmid-determined β-lactamases. Extended-spectrum β-lactamases, first recognized in Europe, have become increasingly prevalent there and are being reported all around the world[1~5]. In Japan however, only three studies on extended β-lactamases from clinical isolates have been reported[6~8]. These enzymes are different in type from those found in Europe and the United States. Their β-lactamases in Japan were metallo-enzymes belonging to AMBLER class B[9] or plasmid-mediated cephalosporinase included in class C[6]. We have identified an extended-spectrum β-lactamase from clinical isolates of *Serratia marcescens* which belongs to class A[10].

S. marcescens isolates usually produce a chromosomal class C β-lactamase with predominant cephalosporinase activity[11]. This enzyme is generally expressed inducibly but may become stably derepressed *via* mutation. Plasmid-mediated β-lactamases, particularly the TEM-1 enzyme, also occur frequently in *S. marcescens* strains[12], causing resistance to penicillins and older cephalosporins, but not to penems, carbapenems, or newer cephalosporins. Recently, several strains of *S. marcescens* were reported to exhibit resistance to carbapenems as well[7,13]. On the other hand, there have been no reports of plasmid-mediated oxyiminocephalosporin hydrolyzing β-lactamase (class 2e[3]) from *S. marcescens*.

We evaluated the possible resistance mechanisms to oxyiminocephalosporins of clinical isolates of *S. marcescens*, and identified a β-lactamase which hydrolyzes oxyiminocephalosporins such as cefuroxime, cefotaxime, and cefmenoxime as well as cephaloridine. The biochemical properties of this enzyme were different from those of chromosomal cephalosporinases from *S. marcescens*[14]. We confirmed that this enzyme was plasmid mediated and was similar to those of oxyiminocephalosporinase type I[15].

Materials and Methods

Bacterial Strain, and Plasmid

All the *S. marcescens* clinicaly isolates used in this study, including 42039, were isolated from patients in Tokyo Clinical Research Center, Japan. *S. marcescens* 42039, which encodes CKH-1 β-lactamase, was isolated from a complicated urinary tract infection in 1986. This

Table 1. Bacterial strains used in this study.

Strain or plasmid	Relevant properties	Source or reference
Strains		
Escherichia coli K-12 C-600	Rifampicin-resistant	S. MITSUHASHI
E. coli CKT-10	Transconjugant, TEM-1 and CKH-1 ß-lactamase producer	This study
E. coli CKT-11	Transconjugant, TEM-1 and CKH-1 ß-lactamase producer	This study
Serratia marcescens 200L	TEM-1 and chromosomal cephalosporinase ß-lactamase producer	J.D. WILLIAMS
S. marcescens 42039	Clinical isolate, chromosomal cephalosporinase, TEM-1 and CKH-1 ß-lactamases producer	This study
Klebsiella pneumoniae CTX-1	CTX-1 ß-lactamase producer	N. A. KUCK(27)
Plasmid		
pCKH-1	CKH-1 encode	This study

organism was identified by VITEK System (ASM III, Vitek System Inc. U.S.A.). Table 1 shows the strains and plasmid used for the analysis of CKH-1.

Antibiotics

Commercially available ampicillin, piperacillin, cephalothin, ceftazidime, cefotaxime, ceftriaxone, cefuroxime, ceftizoxime, cefmetazole, latamoxef, aztreonam, imipenem/cilastatin, gentamicin, kanamycin, rifampicin, minocycline, cloxacillin, and nitrocefin were used. Clavulanic acid, sulbactam, BRL-42715, and tazobactam were synthesized in our laboratories.

Susceptibility Testing

The activity of antibiotics was determined by the agar dilution method. Hundred-fold dilutions of overnight cultures in Mueller-Hinton broth (Difco Laboratories, Detroit. Mich.) were inoculated with a multipoint replicating apparatus onto Mueller-Hinton agar plates containing serial two-fold dilutions of antibiotic. MICs were read after incubation at 37°C for 18 hours.

Plasmid Content and Conjugation Assays

Transfer of resistance into *E. coli* K-12 C-600 was attempted by liquid mating-out assay at 37°C. Transconjugants were selected on Mueller-Hinton agar plates containing rifampicin (200 μg/ml) and cefotaxime (200 μg/ml). Plasmid DNA was obtained by alkaline treatment and analyzed by 0.8% agarose gel electrophoresis[16]. Plasmid molecular weight was determined by comparison with the fragment of a *Hind*III digest of bacteriophage lambda DNA (Takara, Japan).

β-Lactamase Extraction and Purification

An overnight culture was grown in Mueller-Hinton broth (Difco), diluted 100-fold into 5 liters of the same broth and incubated at 37°C with shaking for 4 hours. Cells were harvested by centrifugation at 15,000 × *g* for 15 minutes at 37°C, washed once with 50 mM phosphate buffer (pH 7.0), suspended in the same buffer at 200 times their original density, disrupted by sonication, and the cellular debris were removed by centrifugation (15,000 × *g*, 15 minutes, 4°C). The crude extracts were centrifuged for 30 minutes at 15,000 × *g* at 4°C, and streptomycin (2% w/v) was added to the supernatant which was dialyzed overnight against distilled water, and a 40 to 80% ammonium sulfate precipitate was dialyzed against 10 mM phosphate buffer (pH 6.0). The dialysate was applied to a carboxymethyl Sephadex C-50 column, and the column was equilibrated in 10 mM phosphate buffer (pH 6.0). The enzyme was eluted with a linear gradient of NaCl (0 to 1 M) in the same buffer.

Molecular Weight Measurement

Molecular weight of CKH-1 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Purified enzyme and marker proteins (Pharmacia, Uppsala, Sweden) were treated with 1% sodium dodecyl sulfate - 3% β-mercaptoethanol at 100°C for 2 minutes and subjected to electrophoresis in a 16% polyacrylamide gel with a current of 30 mA for 4 hours at room temperature.

Isoelectric Focusing

Crude β-lactamase extracts and purified β-lactamase were subjected to isoelectric focusing on pH 3.5 to pH 9.5 polyacrylamide gels (Pharmacia) for 1 hour at 15 W of constant power on flatted apparatus (KS-8300; Malisor, Japan) at 10°C. The β-lactamases were visualized by overlaying the gel with nitrocefin solution (0.125%) in 100 mM phosphate buffer (pH 7.0)[17].

β-Lactamase Assays

Assays of β-lactamase activity were performed by UV spectrophotometry with freshly prepared antibiotic solutions in 50 mM phosphate buffer (pH 7.0). The assays were run at 30°C, and the following wavelengths were used; 232 nm for piperacillin, 233 nm for penicillin G, 235 nm for ampicillin, 257 nm for ceftizoxime and ceftriaxone, 260 nm for cephaloridine, 262 nm for cephalothin and cefuroxime, 264 nm for cefotaxime, 265 nm for ceftazidime, 270 nm for cefmenoxime, 273 nm

for cefoperazone, 274 nm for cefamandole, 275 nm for cefmetazole and latamoxef, 276 nm for cefotiam, 299 nm for imipenem and 318 nm for aztreonam. The values of *Km* and Vmax were derived by linear regression analysis of Lineweaver-Burk plots of initial velocity data that were obtained at 18 different substrates at the concentrations of 10 to 100 μM for the cephalosporins and imipenem/cilastatin and 10 to 200 μM for penicillins and aztreonam. The *Km* was expressed in micromolar concentrations, and Vmax and Vmax/*Km* were expressed as the relative ratio to that of cephaloridine (Vmax, Vmax/*Km*=100).

Inhibition of β-Lactamase Activity

At the various concentrations of inhibitors (up to 0.1 mM), tazobactam, sulbactam, clavulanic acid, and BRL-42715 were preincubated for 5 minutes at 30°C before testing the cephalothin hydrolysis rate for CKH-1 or ampicillin hydrolysis rate for TEM-1. *Ki* values were determined by Dixon plot. Inhibition by EDTA, Fe^{2+} and Mg^{2+} (final concentration, 100 μM) or anti-TEM-1 serum (5 Prime—3 Prime, Inc., U.S.A.)[18] was tested by preincubation with enzyme for 5 minutes or 1 hour at 30°C or 37°C before testing the cephalotin or ampicillin hydrolysis rate.

Results

Antibiotic Susceptibility

The MICs of β-lactams showed that *S. marcescens* 42039 was highly resistant to piperacillin and cephalosporins including third generation cephems (Table 2). The susceptibility of *S. marcescens* 42039 was partially restored by the addition of tazobactam. MICs of β-lactams against *E. coli* CKT-10 harboring plasmid pCKH-1 showed that the resistance to oxyiminocephalosporins, ceftazidime, cefotaxime, ceftriaxone, cefuroxime, and aztreonam was caused by acquisition of plasmid pCKH-1. Against this strain, MICs of cefotaxime and ceftriaxone were markedly high compared with ceftazidime and aztreonam. The MICs of cefmetazole, latamoxef, and imipenem were lower. The MICs of most β-lactams tested were lower in the presence of tazobactam, and the MIC of β-lactams was decreased markedly against *E. coli* CKT-10. Both *S. marcescens* 42039 and *E. coli* CKT-10 remained fully susceptible to imipenem.

Transfer of Cephalosporin Resistance

Cephalosporin-resistant transconjugants were obtained at frequencies of 10^{-5} to 10^{-6}. All transconjugants had two β-lactamases with pIs 5.4 (TEM-1) and 8.2 (CKH-1). No transconjugants with a pI 8.2 β-lactamase were obtained.

Identification of Plasmid DNA

Plasmid DNA was purified from donor and transconjugant and analyzed by agarose gel electrophoresis. Donor and transconjugant had the same size of plasmid, pCKH-1. The pCKH-1 plasmid was estimated to be about 100 kbps and the pTEM-1 plasmid was estimated to be about 45 kbps.

Table 2. Activity of antibiotics against *S. marcescens* 42039 and *E. coli* strains.

Antibiotic	MIC(μg/ml)					
	S. marcescens 42039		*E. coli* CKT-10		*E. coli* K-12 C600	
	alone	+Tazobactam	alone	+Tazobactam	alone	+Tazobactam
Ampicillin	>1600	1600	>1600	6.25	6.25	6.25
Piperacillin	>1600	100	800	3.13	3.13	3.13
Cephalothin	>1600	>1600	1600	6.25	12.5	6.25
Ceftazidime	50	6.25	6.25	0.78	0.39	0.39
Cefotaxime	>1600	50	100	≤0.10	≤0.10	≤0.10
Ceftriaxone	>1600	200	200	≤0.10	≤0.10	≤0.10
Cefuroxime	>1600	>1600	1600	12.5	12.5	6.25
Ceftizoxime	100	25	0.78	≤0.10	≤0.10	≤0.10
Cefmetazole	800	800	3.13	1.56	1.56	0.78
Latamoxef	400	400	0.78	0.39	0.20	0.20
Aztreonam	800	50	25	0.20	≤0.10	≤0.10
Imipenem/cilastatin	3.13	1.56	0.20	0.20	0.10	0.10
Gentamycin	50		0.78		0.78	
Kanamycin	100		3.13		3.13	
Ciprofloxacin	3.13		0.025		0.025	
Riphampicin	12.5		>200		>200	
Minocycline	12.5		3.13		3.13	

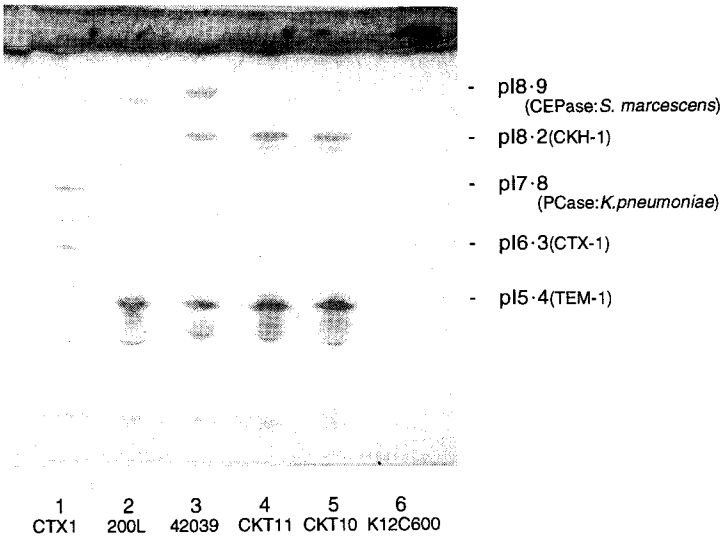
Tazobactam concentration is 10 μg/ml.

Isoelectric Focusing and CKH-1 β-Lactamase Activity

Isoelectric focusing revealed that *S. marcescens* 42039 had β-lactamase activity at pIs 5.4, 8.2 and 8.9. *E. coli* CKT-10 and CKT-11 had β-lactamase activity at pIs 5.4 and 8.2 (Fig. 1). Therefore, *S. marcescens* 42039 possesses three β-lactamases, one is the chromosomal cephalosporinase (pI 8.9) and the other two correspond to the transferred extended-spectrum β-lactamases CKH-1 (pI 8.2) and TEM-1 (pI 5.4).

Kinetic parameters of the CKH-1 β-lactamase and TEM-1 obtained from a culture of *E. coli* CKT-10 were subsequently determined (Table 3). The TEM-1 (pI 5.4) and CKH-1 (pI 8.2) enzymes purified from *E. coli* CKT-10 differed markedly in their hydrolytic properties. The former enzyme (which was not purified to homogeneity) showed a pattern similar to that of the TEM-1 enzyme of *Enterobacteriaceae*, having a relative Vmax value for penicillin G that was 223% of that of cephaloridine[2]. On the other hand, the Vmax/*Km* value of the CKH-1 (pI 8.2; CKH-1) was high for cefuroxime,

Fig. 1. Isoelectric focusing gel electrophoretic patterns of each β-lactamase.



The β-lactamases were extracted from *K. pneumoniae* CTX1 (CTX-1, lane 1), *S. marcescens* 200L (TEM-1 & CEPase, lane 2), *S. marcescens* 42039 (Donor, TEM-1, CKH-1 & CEPase, lane 3), *E. coli* CKT-11 (transconjugant, TEM-1 & CKH-1, lane 4), *E. coli* CKT-10 (transconjugant, TEM-1 & CKH-1, lane 5) and *E. coli* K-12 C-600 (Recipient, lane 6).

Table 3. Kinetic parameters of CKH-1 β-lactamase.

	Km(μM)			Vmax*			Vmax/*Km**		
Substrate	CKH-1	FEC-1**	TEM-1	CKH-1	FEC-1**	TEM-1	CKH-1	FEC-1**	TEM-1
Cephaloridine	119	152	282	100	100	100	100	100	100
Cephalothin	197	134	109	373	198	14	226	225	36
Cefamandole	101	122	174	124	125	33	146	156	53
Cefotiam	36	38	36	22	43	1	73	170	10
Cefoperazone	1.2	2.8	111	2	2.6	31	166	139	78
Cefuroxime	27	27	53	26	32	1	116	179	5
Ceftizoxime	21	821	631	1	12	4	7	2.3	2
Cefotaxime	35	61	35	15	23	1	49	59	7
Cefmenoxime	50	84	ND	24	61	<1	57	110	ND
Ceftazidime	61	393	ND	1	0.13	<1	1	0.05	ND
Ceftriaxone	23	27	25	17	14	1	87	80	8
Cefmetazole	ND			<1			ND		
Latamoxef	ND			<2			ND		
Aztreonam	ND		7.4	<4		5	ND		180
Penicillin G	10		54	22		223	256		1172
Ampicillin	12	30	92	9	17	304	97	89	928
Piperacillin	17		73	10		168	67		647
Imipenem/cilastatin	134		24	2		1	2		16

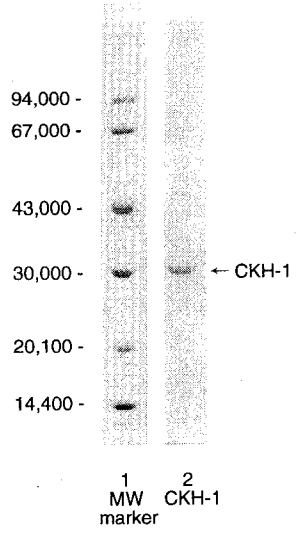
* Value relative to cephaloridine hydrolysis = 100. ND: not detected.
** FEC-1 is reported by MATSUMOTO[19].

cefotaxime, cefmenoxime, and ceftriaxone ranging from 49 to 116, as well as for less stable cephems such as cephaloridine, cephalothin, cefamandole, and cefotiam. The Vmax/*Km* value against cefoperazone was also greater than that of cephaloridine, despite of having a lower Vmax value than ceftizoxime and ceftazidime. The enzyme did not extensively hydrolyze cephamycins (cefmetazole and latamoxef). This substrate profile resembles that of FEC-1 from *E. coli* isolated in the fecal flora of dog[19], chromosomal enzyme from *Proteus vulgaris*[20], *Proteus penneri*[21], *Klebsiella oxytoca*[22], and *Bacteroides* spp.[23] and could thus be classified into the oxyiminocephalosporinase type I[15] and class 2e.

Purification of β-Lactamase

By ion-exchange chromatography, the pI 5.4 and pI 8.2 β-lactamases which were produced by *E. coli* CKT-10 were separated at the 100 mM and 360 mM NaCl concentration, respectively. The pI 8.2 enzyme was more than 90% homogeneous (Fig. 2) even by column chromatography. SDS-PAGE indicated that its molecular weight was 30,000 dalton.

Fig. 2. SDS-polyacrylamide gel electrophoresis of CKH-1 β-lactamase from *E. coli* CKT-10.



Inhibition of CKH-1 and TEM-1 Enzyme

Table 4 shows the *Ki* value of CKH-1 and TEM-1. The enzyme activities of CKH-1 and TEM-1 were markedly inhibited by tazobactam and BRL-42715. Sulbactam and clavulanic acid inhibited the activities of CKH-1 and TEM-1 less than BRL-42715 and tazobactam. The activity of CKH-1 was not inhibited by anti-TEM-1 serum and 100 μM of EDTA, Fe^{2+}, and Mg^{2+}. The pI 5.4 enzyme activity disappeared with anti-TEM-1 serum preincubated for 1 hour, but this enzyme was not inhibited by preincubation with 100 μM EDTA for 5 minutes. Therefore, the pI 5.4 enzyme was considered to be the TEM-1 enzyme.

Discussion

S. marcescens isolates typically have a chromosomal β-lactamase of the class C type[11]. *S. marcescens* 42039, isolated from the urinary tract infected patient, encoded three β-lactamases: a chromosomal cephalosporinase with a pI of 8.9 (the same pI as cephalosporinase from *S. marcescens*[14]), TEM-1 with a pI of 5.4 and an activity inhibited by an anti-TEM-1 serum, and a new β-lactamase, CKH-1, with a pI of 8.2.

The CKH-1 enzyme is an extended-spectrum cephalosporin hydrolyzing β-lactamase susceptible to inhibition by tazobactam, sulbactam, and clavulanic acid. Extended-spectrum β-lactamases susceptible to clavulanic acid are mainly derivatives from plasmid-mediated TEM and SHV enzymes[4] and have been extensively land in *Enterobacteriaceae*. TEM derivatives had an acidic pI, but CKH-1 had an alkaline pI. The CKH-1 was different from TEM derivatives in pI. The pIs of SHV derivatives are alkaline. However, SHV-2, 3, 4 and 5 producing bacteria are resistant to ceftazidime and cefotaxime[24]. *E. coli* CKT-10 harboring CKH-1 was highly resistant to cefotaxime and ceftriaxone but not to ceftazidime. SHV-5 hydrolyzed ampicillin and piperacillin more than cephalothin[25], however, CKH-1 hydrolyzed cephalothin more than ampicillin. These observations suggest that

Table 4. *Ki* value of β-lactamase inhibitors for CHK-1 and TEM-1 β-lactamase.

Inhibitor	*Ki* (μM)	
	CKH-1(pI8.2)*	TEM-1(pI5.4)**
Tazobactam	0.0011	0.015
Sulbactam	0.2696	1.034
Clavulanic acid	0.0970	0.159
BRL-42715	0.0002	0.005

Substrate: *; cephalothin, **; ampicillin.
Culuture was preincubated for 5 minutes.

CKH-1 and SHV-derivatives are different types of β-lactamases. CMY-1, CMY-2 and MIR-1, alkaline pI β-lactamase producing bacteria, were resistant to cefotaxime and cephamycins such as cefoxitin and cefmetazole. The susceptibilities of cefoxitin and cefmetazole were not completely recovered by clavulanic acid or tazobactam[26]. *E. coli* CKT-10 was susceptible to cefmetazole. The hydrolyzing characteristics of CKH-1 were similar to those of chromosomally encoded β-lactamases (class 2e[3]) from *P. vulgaris*[20], *P. penneri*[21], *K. oxytoca*[22], and *Bacteroides* spp.[23]. The CKH-1 and FEC-1[19] enzymes were plasmid-mediated. The CKH-1 enzyme has characteristics very similar to those from FEC-1, concerning pI, substrate profile and inhibitor profile. They differed in origin; CKH-1 was a clinical isolate of *S. marcescens* and FEC-1 was a laboratory isolate of *E. coli*, and their molecular weights, the former is 30,000 and the later is 48,000.

S. marcescens 42039 produced three different β-lactamases and was resistant to broad spectrum cephalosporins. CKH-1 represents the first report of plasmid-mediated oxyiminocephalosporin hydrolyzing broad-spectrum β-lactamase (class 2e) from clinical isolates of *S. marcescens*.

Based on these data, care should be executed in therapy by cephalosporins alone, because *S. marcescens*, which produces three types of β-lactamases such as cephalosporinase, TEM-1 and CKH-1, is resistant to all β-lactams except carbapenems. The CKH-1 gene was also transferable to *E. coli* at high frequency. Therefore, CKH-1 may be a cause for failure of therapy of gram-negative bacterial infections by extended-spectrum cephalosporin alone.

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References

1) BUSH, K.: Characterization of β-lactamases. Antimicrob. Agents Chemother. 33: 259~263, 1989
2) BUSH, K.: Classification of β-lactamases: Group 1, 2a, 2b, and 2b'. Antimicrob. Agents Chemother. 33: 264~270, 1989
3) BUSH, K.: Classification of β-lactamases: Group 2c, 2d, 2e, 3, and 4. Antimicrob. Agents Chemother. 33: 271~276, 1989
4) JACOBY, G. & A. A. MEDEIROS: More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. 35: 1697~1704, 1991
5) PHILIPPON, A.; R. LABIA & G. JACOBY: Extended-spectrum β-lactamases. Antimicrob. Agents Chemother. 33: 1131~1136, 1989
6) HORII, T.; Y. ARAKAWA, M. OHTA, S. ICHIYAMA, R. WACHAROTAYANKUN & N. KATO: Plasmid-mediated ampC-type β-lacamase isolated from *Klebsiella pneumoniae* confers resistance to broad-spectrum β-lactams, including moxalactam. Antimicrob. Agents Chemother. 37: 984~990, 1993
7) OSANO, E.; Y. ARAKAWA, R. WACHAROTAYANKUN, M. OHTA, T. HORII, H. ITO, F. YOSHIMURA & N. KATO: Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob. Agents Chemother. 38: 71~78, 1994
8) WATANABE, M.; S. IYOBE, M. INOUE & S. MITSUHASHI: Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 35: 147~151, 1991
9) AMBLER, R. P.: The structure of β-lactamases. Philos. Trans. R. Soc. London Ser. B 289: 321~331, 1980
10) AMBLER, R. P. & F. W. COULSON, (J.-M. FRERE, J.-M. GHUYSEN, B. JORIS, M. FORSMAN, R. C. LEVESQUE, G. TIRABY, S. G. WALEY): A standard numbering scheme for the class A β-lactamases. Biochem. J. 276: 269~272, 1991
11) RICHMOND, M. H. & R. B. SYKES: The β-lactamases of gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. 9: 31~88, 1973
12) ROY, C.; A. FOX, C. SEGURA, M. TIRADO, C. FOSTER & R. REIG: Plasmid-determined β-lactamases identified in a group of 204 ampicillin-resistant *Enterobacteriaceae*. J. Antimicrob. Chemother. 12: 507~510, 1983
13) YANG, Y.; P. WU & D. M. LIVERMORE: Biochemical characterization of a β-lactamase that hydrolyzes penems and carbapenems from two *Serratia marcescens* isolates. Antimicrob. Agents Chemother. 34: 755~758, 1990
14) TAJIMA, M.; S. MASUYOSHI, M. INOUE, Y. TAKENOUCHI, S. SUGAWARA & S. MITSUHASHI: Purification and properties of β-lactamases from *Serratia marcescens*. J. General Microbiology 126: 179~184, 1981
15) FUJII, T.; K. SATO, K. MIYATA, M. INOUE & S. MITSUHASHI: Biochemical properties of β-lactamase produced by *Legionella gormanii*. Antimicrob. Agents Chemother. 29: 925~926, 1986
16) KADO, C. I. & S. T. LIU: Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145: 1365~1373, 1981
17) MATTHEW, M.; A. M. HARRIS, M. J. MARSHALL & G. W. ROSS: The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. General Microbiology 88: 169~178, 1975
18) SUTCLIFFE, J. G.: Nucleotide sequence of ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. U.S.A. 75: 3737~3741, 1978
19) MATSUMOTO, Y.; F. IKEDA, T. KAMIMURA, Y. YOKOTA & Y. MINE: Novel plasmid-mediated β-lactamase from *Escherichia coli* that inactivates oxyimino-cephalosporins. Antimicrob. Agents Chemother. 32: 1243~1246, 1988
20) MATSUBARA, M.; M. INOUE & S. MITSUHASHI: Purification and some properties of a cephalosporinase from *Proteus vulgaris*. Antimicrob. Agents Chemother. 19: 185~187, 1981
21) GRACE, M. E.; F. J. GREGORY, P. P. HUNG & K. P. FU: Purification and properties of a β-lactamase from *Proteus penneri*. J. Antibiotics 39: 938~942, 1986
22) LABIA, R.; A. MORAND, M. GUIONIE, M. HEITZ & J. S. PITTON: *Klebsiella oxytoca* β-lactamase: an analysis of their action on third-generation cephlosporins. Pathol. Biol. 34: 611~615, 1986
23) ELEY, A. & D. GREENWOOD: β-Lactamase of type culture

strains of the *Bacteroides fragilis* group and of strains that hydrolyse cefoxitin, latamoxef and imipenem. J. Med. Microbiol. 21: 49~57, 1986

24) LABIA, R.; A. THABAUT, A. MORAND, K. TIWARI, D. SIROT & J. SIROT: The kinetics of CAZ-5, a novel SHV-related plasmid-mediated β-lactamase with enhanced hydrolytic activity against ceftazidime. Drugs Exptl. Clin. Res. 15: 535~540, 1989

25) GUTMANN, L.; B. FERRE, F. W. GOLDSTEIN, N. RIZK, E. PINTO-CHUSTER, J. F. ACAR & E. COLLATZ: SHV-5, a novel SHV-type β-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. Antimicrob. Agents Chemother. 33: 951~956, 1989

26) BAUERNFEIND, A.; Y. CHONG & S. SCHWEIGHART: Extended broad spectrum β-lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. Infection 17: 316~321, 1989

27) KUCK, N. A.; N. V. JACOBUS, P. J. PETERSEN, W. J. WEISS & R. T. TESTA: Comparative *in vitro* and *in vivo* activities of piperacillin combined with the β-lactamase inhibitors tazobactam, clavulanic acid, and sulbactam. Antimicrob. Agents Chemother. 33: 1964~1969, 1989