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Evaluation of antimicrobial susceptibility for β-lactams using the Etest method against clinical isolates from 100 medical centers in Japan (2006)

Yoshikazu Ishii*, Kazuhiro Tateda, Keizo Yamaguchi, the JARS[1]

Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Faculty of Medicine, Tokyo 1438540, Japan

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Abstract

This antimicrobial resistance surveillance study was performed in 100 medical centers. Susceptibility testing (Etest; AB BIODISK, Solna, Sweden) of 9152 strains including *Escherichia coli* (991 strains), *Klebsiella* spp. (1000 strains), *Enterobacter* spp. (971 strains), *Citrobacter* spp. (803 strains), indole-positive *Proteae* spp. (834 strains), *Serratia* spp. (902 strains), *Acinetobacter* spp. (874 strains), *Pseudomonas aeruginosa* (992 strains), oxacillin-susceptible *Staphylococcus aureus* (984 strains), and coagulase-negative staphylococci (CoNS; 801 strains) was performed with 7 β-lactams (cefepime, cefpirome, ceftazidime, cefoperazone/sulbactam, imipenem and piperacillin for Gram-negative bacteria, or oxacillin for Gram-positive bacteria). No strain resistance to these β-lactams (except for ceftazidime) was found in oxacillin-susceptible *S. aureus* and CoNS. Of the *E. coli* clinical isolates, 17.1% were resistant to piperacillin, whereas 2.9% or less (cefpirome = 2.9%) were resistant to other β-lactam agents. *Klebsiella* spp. strains were more susceptible to imipenem (99.9%), cefepime (99.2%), ceftazidime (98.6%), and cefpirome (98.3%). Isolates of *Enterobacter* spp., *Citrobacter* spp., indole-positive *Proteae*, and *Serratia* spp. were susceptible to imipenem, cefepime, and cefpirome as well. *Acinetobacter* spp. strains were least resistant to cefoperazone/sulbactam (0.7% resistance), imipenem (2.6%), cefepime (6.6%), and ceftazidime (7.7%) compared with other β-lactam antibiotics tested. Isolates of *P. aeruginosa* were more susceptible to ceftazidime (8.7% resistance), cefoperazone/sulbactam (9.8%), and cefepime (8.9%) than piperacillin (11.9%), cefpirome (16.2%), and imipenem (12.4%). The percentage of imipenem-resistant *P. aeruginosa* was approximately 13% in clinical isolates in Japan. The proportion of strains resistant to β-lactam antimicrobials has been decreasing compared with data from 2004, suggesting that reduced consumption of β-lactams has reflected the decreased rates of resistant bacterial isolates in Japan.

Keywords: β-Lactams; Etest; Susceptibility; Drug resistance

1. Introduction

β-Lactam antimicrobial agents have been widely used in clinical practice for more than 60 years. β-Lactamases have become the major resistance mechanism toward these agents in Gram-negative bacteria (Jacoby and Munoz-Price, 2005). Another prominent resistance mechanism in Gram-negative bacteria has been the decrease of antimicrobial concentrations to inhibit bacterial cell wall biosynthesis enzymes, for example, target enzymes (Alekshun and Levy, 2007).

Previously, extended-spectrum β-lactamase (ESBL)–producing organisms were reported in numerous countries worldwide (Canton and Coque, 2006). These ESBLs can hydrolyze penicillins and cephalosporins including oxyimino-cephalosporins. CTX-M–type ESBL enzymes prefer to hydrolyze cefotaxime as its major substrate (Ishii et al., 2007). These enzyme-producing isolates are found not only in clinical specimens (Canton and Coque, 2006; Ishii et al., 2005b) but also in animals and the environment in Japan (Ahmed et al., 2004; Kojima et al., 2005).

Plasmid-borne class B β-lactamases, metallo-β-lactamases (MBLs), are classified into 5 main molecular groups: IMP-, VIM-, SPM-, GIM-, and SIM-type enzymes (Walsh

* Corresponding author. Tel.: +81-3-3762-4151x2396; fax: +81-3-5493-5415.
E-mail address: yoishii@med.toho-u.ac.jp (Y. Ishii).
[1] The Japan Antimicrobial Resistance Surveillance Participants Group.

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et al., 2005). These MBLs destroy most β-lactam antimicrobials including the carbapenems. IMP-1, the predominant MBL in Japan, has been found in clinical pathogens such as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Acinetobacter baumannii*, *Achromobacter xylosoxidans*, *Serratia marcescens*, *Enterobacter cloacae*, *Citrobacter youngae*, *Klebsiella pneumoniae*, or *Shigella flexneri* (Walsh et al., 2005). MBL-producing *P. aeruginosa* and *Providencia rettgeri* isolates were detected in our previously reported surveillance program in 2002 (Ishii et al., 2002, 2005a, 2006; Kimura et al., 2005a; Shiroto et al., 2005). On the other hand, nosocomial infection caused by IMP-1–producing *K. pneumoniae* has occurred in general hospital in Japan (Fukigai et al., 2007). The isolation frequencies of MBL-producing *P. aeruginosa* in 2002 and 2004 were 1.9% and 2.3%, respectively (Ishii et al., 2005a, 2006).

A surveillance program by the Japan Antimicrobial Resistance Study Group was carried out from 1997 to 2004 (Ishii et al., 2002, 2005b, 2006; Lewis et al., 1999; Yamaguchi et al., 1999). The present study was designed to provide up-to-date β-lactam antimicrobial susceptibility for clinical isolates including *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., indole-positive *Proteus* spp. (*Proteus vulgaris*, *Providencia* spp., and *Morganella morganii*), *Serratia* spp., *Acinetobacter* spp., *P. aeruginosa*, oxacillin-susceptible *Staphylococcus aureus* (MSSA), and oxacillin-susceptible coagulase-negative staphylococci (CoNS) in Japan. One hundred hospitals participated in this surveillance program during 2006. Participating centers represented all geographic regions in Japan. In the present study, we compare the incidences of β-lactam–resistant bacteria and the consumption of β-lactam antimicrobials in Japan.

2. Materials and methods

2.1. Bacterial isolates

The collection and subsequent testing of clinical isolates by the 100 participant centers began in July and was concluded in September 2006. Each participant center had an average of 632 beds. Fifty-five and 32 participating centers use MicroScan WalkAway system (Dade Behring, Tokyo, Japan) and Vitek system (bioMérieux, Tokyo, Japan) to identify the organisms, respectively. Twelve centers used other systems such as the BD Phoenix system (Becton Dickinson, Tokyo, Japan), Raisus system (Nissui Pharmaceutical, Tokyo, Japan) or API sires (bioMérieux), Enterotube system (Becton Dickinson), and so on. Each laboratory was instructed to construct a collection of consecutive bacterial strains of up to 10 nonduplicate patient isolates for each designated species groups (10 total) as stated in a prevalence format. These 10 organism groups were *E. coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., indole-positive *Proteus* spp., *Serratia* spp., *Acinetobacter* spp., *P. aeruginosa*, MSSA (oxacillin MIC, ≤2 μg/mL), and oxacillin-susceptible CoNS (MIC, ≤0.25 μg/mL). The overall collection of bacterial strains from the 100 centers totaled 9152 strains including 991 *E. coli*, 1000 *Klebsiella* spp., 971 *Enterobacter* spp., 803 *Citrobacter* spp., 834 indole-positive *Proteus* spp., 902 *Serratia* spp., 874 *Acinetobacter* spp., 992 *P. aeruginosa*, 984 MSSA, and 801 oxacillin-susceptible CoNS.

The specimens from which the strains in this study were isolated are listed on Table 1. Although compliance was complete, 1 *S. aureus* strain was omitted from the analysis because the documented oxacillin-resistant criteria was redefined by the Clinical and Laboratory Standards Institute (CLSI, 2006) during the protocol period. Also, 6 proteae isolates were omitted from analysis because these strains were identified as *Proteus mirabilis*, an indole-negative proteae by the BD Phoenix system in the coordinating laboratory (Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Tokyo, Japan).

2.2. Antimicrobial susceptibility testing

Susceptibility testing of each isolate was determined by using Etest (AB BIODISK, Solna, Sweden) following the protocol described previously (Ishii et al., 2002, 2005a, 2006; Lewis et al., 1999; Yamaguchi et al., 1999). Bacteria were cultured on a 90-mm-diameter Mueller–Hinton agar (Becton Dickinson) for 16 h at 35 °C. Isolated colonies were suspended in sterile saline to obtain a turbidity of 0.5 McFarland. Each cell suspension was spread on a 135-mm-diameter Mueller–Hinton agar plate (Becton Dickinson) with a cotton swab, and the Etest strips were placed on the plates according to the manufacturer's instructions. The following strips were used: oxacillin (for Gram-positive bacteria), piperacillin (for Gram-negative bacteria), ceftazidime, cefepime, cefpirome, cefoperazone/sulbactam, and imipenem. Results were recorded after 16 to 20 h of incubation at 35 °C except for *S. aureus* and CoNS for which incubation was extended to 24 h. MIC values were interpreted as the point of intersection of the inhibition ellipse with the Etest strips edge. All clinical laboratories used the same lot of Etest strips, Mueller–Hinton agar plates, and reference strains. Clinical and Laboratory Standards Institute (2007) does not have criteria (susceptible, intermediate, or resistant) for either cefpirome or cefoperazone/sulbactam. For comparison only, the same values for cefepime (CLSI, 2007) were used as criteria for cefpirome, and the value for cefoperazone alone were used as criteria for cefoperazone/sulbactam. All 100 hospitals provided their results to the Department of Microbiology and Infectious Diseases, Toho University School of Medicine, for analysis. If uncertain data were found in the provided results, including identification and susceptibility testing, all tests were repeated. Identification

Table 1
Specimens used in this study

	S. aureus	CoNS	*E. coli*	*Klebsiella* spp.	*C. freundii*	*Enterobacter* spp.	Indole-positive *Proteae*	*Serratia* spp.	Acinetobacter spp.	*P. aeruginosa*
Urinary tract	57	108	547	252	274	157	375	148	75	220
Urine	27	65	373	156	182	95	233	92	42	116
Urinary catheter	12	10	117	74	71	40	123	51	23	88
Others	18	33	57	22	21	22	19	5	10	16
Pulmonary tract	392	96	106	411	112	418	85	487	542	449
Sputum	158	24	63	282	68	246	54	295	306	294
BALF	9	1	5	19	2	7	–	6	9	5
Intratracheal sputum	26	1	16	42	11	55	20	105	92	84
Pharyngeal mucus	95	24	12	47	18	67	3	44	82	28
Others	104	46	10	21	13	43	8	37	53	38
Gastrointestinal tract	24	13	98	122	246	118	139	35	28	62
Gastric or duodenal secretion	0	2	6	16	9	12	5	5	7	
Feces	20	6	59	63	179	67	112	19	13	383
Others	4	5	33	43	58	39	22	11	8	21
Blood and fluids	83	223	104	95	33	77	23	56	68	81
Blood	55	182	87	75	24	50	14	42	57	27
Spinal fluid	1	7	–	1	1	–	–	1	3	1
Others	27	34	17	19	8	27	9	13	8	13
Others	428	361	136	120	138	201	212	176	161	220
Drain fluid (thoracic cavity abdominal cavity)	15	10	26	24	25	37	32	19	18	29
Ophthalmic secretion	29	57	1	1	3	1	7	8	7	6
Ear secretion	95	53	3	7	4	23	10	20	23	36
Abscess	167	98	71	57	72	69	94	73	57	90
Skin or decubitus	83	55	14	9	17	17	50	26	23	27
Unspecified	39	88	21	22	17	54	19	30	33	32

BALF = bronchoalveolar lavage fluid.

and determination of MIC values was performed using the BD Phoenix system.

2.3. Quality control

For quality control (QC) of the Etest strips, the following reference strains were used: *E. coli* ATCC25922, *S. aureus* ATCC29213, and *P. aeruginosa* ATCC27853 (CLSI, 2006). CLSI does not have MIC QC ranges regarding cefpirome and cefoperazone/sulbactam. In this study, a range was determined near (±1 doubling dilution) the median MIC for cefepime and cefoperazone/sulbactam, as what was done in our previous reports (Ishii et al., 2006). The laboratories were required to test a set of all QC organisms in a replicate manner.

3. Results

3.1. Quality assurance

The validity of generated data was assured by employing appropriate QC and quality assurance measures. Values obtained for the challenge set of strains resulted in 28 of 1073 values falling out of the appropriate susceptibility category (2.6%). Of these 2.6%, 0.2% ($n = 2$ strains) were very major (false-susceptible) errors and 0.4% ($n = 4$ strains) were major (false-resistant) errors. Overall, this equates to 97.4% of MIC categoric results being accurate.

3.2. Activity against staphylococci

Because the CLSI (2006, 2007) recommends that oxacillin-resistant staphylococci be considered as resistant to all β-lactams, only oxacillin-susceptible strains were collected in this study. Of all the tested staphylococci, 984 isolates of *S. aureus* and 801 isolates of oxacillin-susceptible CoNS strains were susceptible to cefepime, cefpirome, cefoperazone/sulbactam, and imipenem (Table 2). However, 22 *S. aureus* (0.7%) and 23 oxacillin-susceptible CoNS (1.1%) were resistant to ceftazidime. The rank order of activity for all the tested agents using MIC_{90} values was imipenem (0.032 μg/mL) > oxacillin > cefpirome > cefoperazone/sulbactam > cefepime > ceftazidime (12–16 μg/mL).

3.3. Activity against E. coli and Klebsiella spp.

A total of 991 *E. coli* and 1000 *Klebsiella* spp. isolates were tested. Generally, all agents tested, except piperacillin (17.1% resistant), were highly active against *E. coli* and *Klebsiella* spp. (Table 2). No imipenem-resistant strains of *E. coli* or *Klebsiella* spp. were observed in this study.

3.4. Activity against other Enterobacteriaceae

Enterobacter spp. and *Citrobacter freundii* showed lower rates of susceptibility to piperacillin (77.6–83.4%), ceftazidime (79.5–83.0%), and cefoperazone/sulbactam

Table 2
Antimicrobial activity of 7 tested β-lactams against clinical isolates (2006)

Organism (no. tested)	Antimicrobial agent	MIC (μg/mL)		MIC (μg/mL)			Category (%)	
		50%	90%	Range			S	R
S. aureus (984)	Oxacillin	0.38	0.5	0.023	–	2	100.0	0.0
	Ceftazidime	12	16	0.125	–	48	24.7	0.7
	Cefepime	3	4	0.032	–	8	100.0	0.0
	Cefpirome	1	1.5	0.032	–	4	100.0	0.0
	CP–SB	2	3	0.032	–	6	100.0	0.0
	Imipenem	0.032	0.032	<0.016	–	2	100.0	0.0
Coagulase-negative staphylococci (801)	Oxacillin	0.19	0.25	<0.016	–	0.25	100.0	0.0
	Ceftazidime	6	12	0.125	–	>256	85.5	1.1
	Cefepime	1	2	0.064	–	>256	99.8	0.2
	Cefpirome	0.38	0.75	0.023	–	>256	99.6	0.2
	CP–SB	1	2	0.064	–	>256	99.6	0.2
	Imipenem	0.023	0.032	<0.016	–	>256	99.8	0.2
E. coli (991)	Piperacillin	2	>256	0.023	–	>256	72.9	17.1
	Ceftazidime	0.125	0.75	0.023	–	>256	97.2	2.4
	Cefepime	0.032	0.125	<0.016	–	>256	97.4	1.4
	Cefpirome	0.047	0.125	<0.016	–	>256	95.5	2.9
	CP–SB	0.25	2	<0.016	–	>256	98.7	0.4
	Imipenem	0.25	0.38	<0.016	–	4	100.0	0.0
Klebsiella spp. (1000)	Piperacillin	6	48	0.125	–	>256	86.5	8.5
	Ceftazidime	0.125	0.5	<0.016	–	>256	98.6	1.0
	Cefepime	0.047	0.125	<0.016	–	96	99.2	0.4
	Cefpirome	0.047	0.125	<0.016	–	>256	98.3	1.0
	CP–SB	0.25	2	<0.016	–	>256	96.4	3.0
	Imipenem	0.25	0.38	0.023	–	6	99.9	0.0
C. freundii (803)	Piperacillin	2	>256	0.032	–	>256	77.6	17.9
	Ceftazidime	0.5	>256	0.047	–	>256	79.5	18.2
	Cefepime	0.032	1.5	<0.016	–	>256	98.9	0.6
	Cefpirome	0.064	3	<0.016	–	>256	96.1	2.2
	CP–SB	0.5	16	0.023	–	>256	90.4	2.9
	Imipenem	0.5	1	0.032	–	4	100.0	0.0
Enterobacter spp. (971)	Piperacillin	2	128	0.047	–	>256	83.4	10.5
	Ceftazidime	0.25	96	0.023	–	>256	83.0	13.7
	Cefepime	0.047	1	<0.016	–	>256	98.4	0.6
	Cefpirome	0.064	2	<0.016	–	>256	96.5	1.5
	CP–SB	0.38	16	<0.016	–	>256	91.6	3.5
	Imipenem	0.5	1	<0.064	–	12	99.4	0.0
Indole-positive *Proteae* (834)	Piperacillin	0.5	6	0.047	–	>256	92.9	4.9
	Ceftazidime	0.094	1	0.016	–	>256	95.4	2.6
	Cefepime	0.032	0.125	<0.016	–	>256	99.4	0.2
	Cefpirome	0.064	0.38	<0.016	–	>256	98.6	0.8
	CP–SB	1	3	0.032	–	>256	98.9	0.5
	Imipenem	1.5	3	0.047	–	>256	98.3	0.6
Serratia spp. (902)	Piperacillin	2	48	0.064	–	>256	83.3	6.9
	Ceftazidime	0.19	1	0.023	–	>256	96.7	2.5
	Cefepime	0.064	0.75	<0.016	–	>256	97.9	0.9
	Cefpirome	0.064	0.5	<0.016	–	>256	97.8	1.2
	CP–SB	1	16	0.032	–	>256	92.7	4.9
	Imipenem	0.5	1	<0.016	–	>256	99.4	0.6
Acinetobacter spp. (874)	Piperacillin	12	64	0.032	–	>256	91.8	7.8
	Ceftazidime	4	16	0.032	–	>256	88.1	7.7
	Cefepime	2	12	0.023	–	>256	88.2	6.6
	Cefpirome	2	16	0.023	–	>256	88.9	8.5
	CP–SB	2	4	0.125	–	>256	97.6	0.7
	Imipenem	0.38	1	0.032	–	>256	95.3	2.6
P. aeruginosa (992)	Piperacillin	4	>256	0.25	–	>256	87.4	11.9
	Ceftazidime	2	16	0.19	–	>256	87.0	8.7
	Cefepime	3	24	0.19	–	>256	79.3	8.9
	Cefpirome	4	64	0.125	–	>256	70.7	16.2
	CP–SB	4	48	0.25	–	>256	80.5	9.8
	Imipenem	1.5	16	0.094	–	>256	74.8	12.4

CP–SB = cefoperazone/sulbactam (2:1); S = susceptible; R = resistance.

(90.4–91.6%) compared with the other tested β-lactams (Table 2). Susceptibility rates for cefepime (98.4–98.9%) and imipenem (99.4–100%) were superior to cefpirome (96.1–96.5%). For the indole–positive *Proteus* spp., susceptibility rates of piperacillin (92.9%) and ceftazidime (95.4%) were lower than for the other β-lactam antibiotics. *Serratia* spp. also showed lower rates of susceptibility to piperacillin (83.3%) and cefoperazone/sulbactam (92.7%) compared with the other tested β-lactams (96.7–99.4%).

3.5. Activity against nonfermentative Gram-negative bacilli

For *Acinetobacter* spp., cefoperazone/sulbactam was the most active antimicrobial (combination) (97.6% susceptible), followed by imipenem (95.3%), cefpirome (88.9%), cefepime (88.2%), and ceftazidime (88.1%). Piperacillin (91.8%) showed the lowest susceptibility rate when compared with the other tested β-lactams (Table 2).

4. Discussion

All centers participating in this surveillance were not small-sized hospitals (average number of beds = 632), so results reflect large hospital data. Imipenem maintained antimicrobial activity against Gram-positive and Gram-negative bacteria except for some indole-positive *Proteus* spp., *Acinetobacter* spp., and *P. aeruginosa* (Table 2) compared with previous studies (Ishii et al., 2002, 2005a, 2006; Lewis et al., 1999; Yamaguchi et al., 1999). Against *Acinetobacter* spp., the combination of cefoperazone and sulbactam had the most potent antimicrobial effect. Sulbactam has been recognized as one of the effective agents against carbapenem-resistant *A. baumannii* (Go et al., 1994). In 2004, the resistance rates of *Acinetobacter* spp. to cefepime or cefpirome were 7.6% and 11.6%, respectively (Ishii et al., 2006). The present surveillance data show that the resistance rates for cefepime and cefpirome were only 7.0% and 8.6%, respectively. Multidrug-resistant (MDR) *Acinetobacter* spp. isolates have become a problem in Europe (Paterson, 2006). Fortunately, these reported data suggested that expanded-spectrum cephalosporin-resistant *Acinetobacter* spp. are not increasing in Japan.

ESBL-producing Enterobacteriaceae are well known as cephalosporin-resistant strains (Ahmed et al., 2004). In this study, 6.2% (61 strains) of *E. coli* and 4.2% (42 strains) of *K. pneumoniae* showed a MIC value of 2 μg/mL or more for ceftazidime, which suggests that they are ESBL producers according to the CLSI (2006). Among *Klebsiella* spp., 26 *K. pneumoniae* and 16 *Klebsiella oxytoca* isolates were possible ESBL producers according to this screening test. These *Klebsiella* spp. isolates were collected in 33 hospitals, and the *E. coli* were isolated from 40 hospitals. ESBL producers were confirmed by the CLSI disk with clavulanate test (2007). Because some *K. oxytoca* strains produce K1 enzyme, which behaves like an ESBL and in which it was impossible to separate the K1 β-lactamase from an ESBL by a phenotypic

Table 3
Annual changing of resistant percentages of 7 β-lactams against clinical isolates (1997–2006)

Organism	Antimicrobial agents	Year (%)					
		1997	1998	2000	2002	2004	2006
S. aureus	Oxacillin	0	0	0	0	0	0
	Ceftazidime	6.4	0.5	2	2.4	2.2	0.7
	Cefepime	0	0	0	0	0	0
	Cefpirome	0	0.5	0	0	0	0
	CP–SB	0	0	0	0	0	0
	Imipenem	0	0	0	0	0	0
CoNS	Oxacillin	0	0	0	0	0	0
	Ceftazidime	15.4	1.6	2.1	3.9	2.9	1.1
	Cefepime	0	0	0	0	0.7	0.2
	Cefpirome	0	0	0	0	0.6	0.2
	CP–SB	0	0	0	0	0.5	0.2
	Imipenem	0	0	0	0	0.7	0.2
E. coli	Piperacillin	14.6	12.6	11.9	10.8	16.5	17.1
	Ceftazidime	0.5	0	1	0.5	1	2.4
	Cefepime	0.5	0	0.5	0.7	0.9	1.4
	Cefpirome	0.5	0	1	1.3	1.5	2.9
	CP–SB	0.5	0	0.5	1	0.7	0.4
	Imipenem	0.5	0	0	0	0	0
Klebsiella spp.	Piperacillin	7.2	9.6	7.2	7.4	11.2	8.5
	Ceftazidime	1.8	0.9	0.2	1	1.1	1
	Cefepime	0	0	0.2	0.2	0.8	0.4
	Cefpirome	1.4	0.5	0.7	0.3	1.4	1
	CP–SB	2.7	1.4	1.5	2.5	3.9	3
	Imipenem	0	0	0	0	0.2	0
C. freundii	Piperacillin	26.1	22.6	18.4	18.7	19.2	17.9
	Ceftazidime	25	22.1	19.5	19.7	16.7	18.2
	Cefepime	0	0	0.6	0.6	1.6	0.6
	Cefpirome	1.1	2.1	1.7	1.6	2	2.2
	CP–SB	8.3	6.3	5.8	2	5.9	2.9
	Imipenem	0	0	0	0.2	0.1	0
Enterobacter spp.	Piperacillin	18.5	25.1	18	15	14.5	10.5
	Ceftazidime	20.5	24.2	22.8	20.2	16.8	13.7
	Cefepime	1	0.5	1.3	2.1	1.7	0.6
	Cefpirome	3.9	4.3	5.3	3.7	3.4	1.5
	CP–SB	15.1	10.6	8.5	5.9	7.1	3.5
	Imipenem	0.5	0.5	0	0.5	0.1	0
Indole-positive *Proteae*	Piperacillin	8.7	8	6.3	5.5	6	4.9
	Ceftazidime	0.5	3	2.6	4.5	2	2.6
	Cefepime	0.5	0.5	0	1.4	1.2	0.2
	Cefpirome	3.1	0.5	0	0.6	1.9	0.8
	CP–SB	1.5	0	1.7	2.4	0.7	0.5
	Imipenem	1	5	0.9	3.3	0.7	0.6
Serratia spp.	Piperacillin	25	22.3	15	9.8	10.1	6.9
	Ceftazidime	9.5	6.8	8	7.1	3.7	2.5
	Cefepime	5	5.8	6.5	5.3	3.2	0.9
	Cefpirome	8.5	6.3	7.8	4.7	3.2	1.2
	CP–SB	23.5	16	14.2	10.9	6.1	4.9
	Imipenem	4.5	4.4	4.5	3.6	1.5	0.6
Acinetobacter spp.	Piperacillin	31.2	30.2	5.9	9.3	13.3	7.8
	Ceftazidime	8	4	4.5	5.8	6	7.7
	Cefepime	5	8	5.1	7.6	7	6.6
	Cefpirome	12.1	15	5.4	11.6	8.6	8.5
	CP–SB	0.5	0.5	0.3	1.5	0.8	0.7
	Imipenem	2.5	6.5	3.1	5	3.2	2.6
P. aeruginosa	Piperacillin	20.1	18.5	15.7	15	15.5	11.9
	Ceftazidime	11.4	8.7	10.8	12.3	9.9	8.7
	Cefepime	9.1	9.1	12.5	12.6	11.2	8.9
	Cefpirome	27.9	27.2	26	22.6	19.1	16.2
	CP–SB	13.7	11.5	13.2	12.5	14.9	9.8
	Imipenem	22.4	24.9	20.3	30.8	19.3	12.4

CP–SB = cefoperazone/sulbactam (2:1).

test, all *K. oxytoca* isolates were omitted from the confirmatory testing. Thirteen *E. coli* strains (1.3%) and 4 *K. pneumoniae* strains (0.4%) were confirmed as ESBL producers. These values are the same as our previous report. Moreover, 2 *C. freundii* isolates (0.2%) were confirmed as ESBL producers by the CLSI disk with clavulanate test using Mueller–Hinton agar plates in the presence of 300 μg/mL of 3-aminophenyl boronic acid (final concentration) as a specific inhibitor of class C β-lactamases (Yagi et al., 2005). This result suggested that it is important to survey ESBL producers, not only *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*, but also the other Enterobacteriaceae.

Twenty-five strains of *P. aeruginosa* (2.5%) were confirmed as MBL producers in this surveillance program by using imipenem and ceftazidime disk in the presence/absence of dipicolinic acid (Kimura et al., 2005b). Of the *P. aeruginosa*, 1.9% and 2.3% produced MBL in 2002 and 2004, respectively (Ishii et al., 2005a and 2006). The present data suggest that MBL-producing *P. aeruginosa* are increasing in Japan. On the other hand, imipenem-resistant *P. aeruginosa* were present in 18.6% (185 isolates) of the isolates in this study. So, this result suggests that class B β-lactamases are not the main mechanism for carbapenem resistance in *P. aeruginosa*. In 2006, MBL producers among the tested Enterobacteriaceae and *Acinetobacter* spp. were present in 0.2% (13 isolates) and 0.2% (2 isolates) by phenotypic testing. The isolation frequency of Enterobacteriaceae producing an MBL (2004) has the same value (0.2%: 12/5596 isolates). On the other hand, MBL producers of *Acinetobacter* spp. decreased from 1.2% (2004) to 0.2%. In 2004, MBL-producing *Acinetobacter* spp. (11 isolates) were isolated from only 5 hospitals.

MDR *P. aeruginosa* is a serious problem in the world (Paterson, 2006). These MDR organisms are resistant to carbapenems, fluoroquinolones, and aminoglycosides. We determined additional antimicrobial susceptibility testing results for imipenem-resistant *P. aeruginosa* by using the BD Phoenix system. Nineteen isolates showed resistance to amikacin and 119 isolates to levofloxacin (data not shown). Eighteen amikacin-resistant isolates were also resistant to levofloxacin, so the incidence of MDR *P. aeruginosa* was 1.7% (17 strains). This present data suggest, however, that the isolation frequency of MDR *P. aeruginosa* was not increasing compared with the 2004 results.

All tested Gram-negative organisms with the exception of *E. coli* and *Acinetobacter* spp. improved their susceptibilities for β-lactams (Table 3). For example, the percentages of imipenem-resistant isolates of *Serratia* spp. were 4.5% (1997), 4.4% (1998), 4.5% (2000), 3.6% (2002), 1.5% (2004), and 0.6% (2006) (Ishii et al., 2002, 2005a, 2006; Lewis et al., 1999; Yamaguchi et al., 1999). Fig. 1 illustrates the consumption of β-lactams in Japan from 1997 to 2006 (IMS-Japan K.K., Tokyo, Japan, agreed to present this data). These data indicate that the consumption of β-lactam exception of penicillins has been decreasing year by year. Also, these results suggest that regulation of antimicrobial usage and dosing can improve antimicrobial susceptibility patterns for Japanese clinical isolates.

In conclusion, the susceptibility of *P. aeruginosa* to almost β-lactam antimicrobial agents has improved compared with previous reported years. Overall, cefepime is maintaining its in vitro activity against Gram-positive and Gram-negative bacteria. It is very important to continue

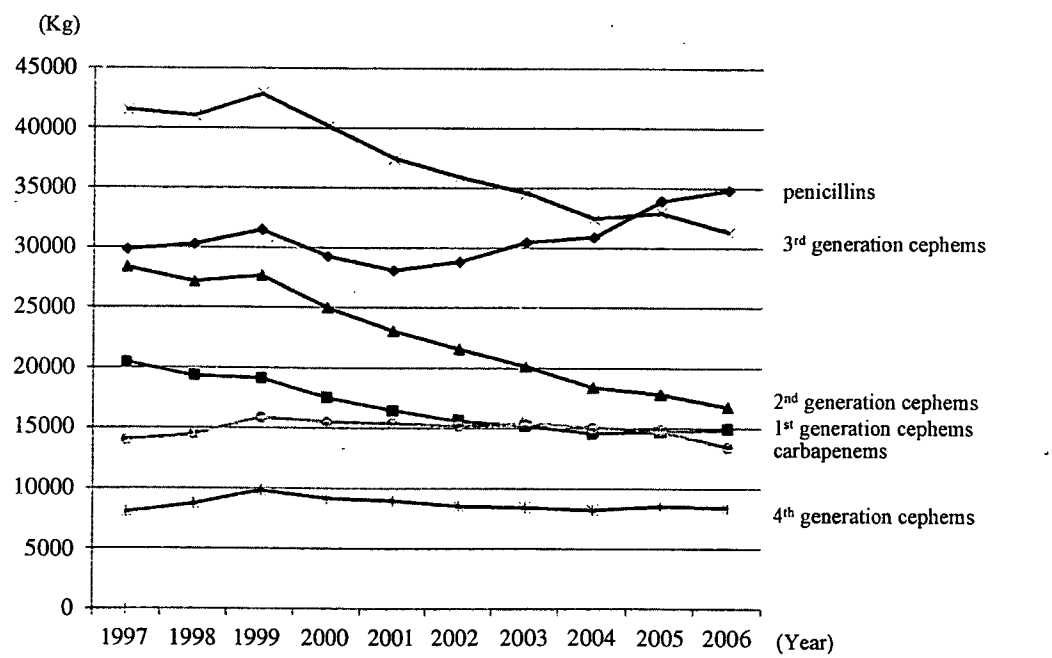

Fig. 1. The consumption of β-lactam antibiotics in Japan during 1997 to 2006 (Copyright 2007 IMS Japan. All rights reserved. Source, IMS JPM. Reprinted with permission).

surveillance for the MDR bacteria because of limited treatment options that could lead to optimal outcomes.

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