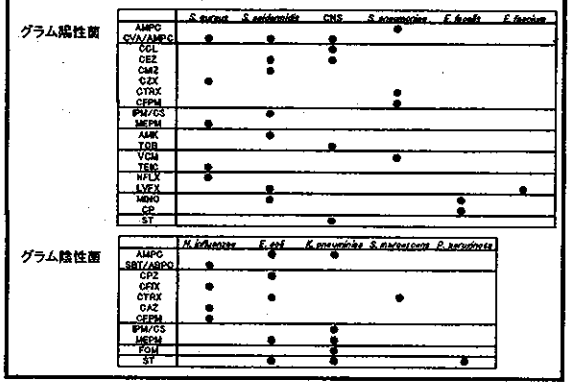
図1. 再評価の必要がある薬剤感受性結果の一覧



厚生労働科学研究費補助金（新興・再興感染症研究事業）
分担研究報告書

集中治療室（ICU）の院内感染率の比較検討

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研究要旨：　アメリカのNNISは関連するディバイス装着日数を分母にした感染率を提唱しているが、本来、除くべき感染後のディバイス装着日数まで分母にカウントされており、疫学的観点から、算出された値の精度が疑問視される。本研究では、JANISのICU部門の研究班（19施設）から収集した患者データを用いて、NNISが提唱している感染率を含めて、分母の設定が異なる4種類の院内感染率を比較検討した。ICU入室数あたりとICU在室日数あたりの感染率はほぼ一致した傾向をみとめ、ICU入室数あたりの感染率でも施設間の比較に耐え得ると考えられた。ディバイス装着日数あたりの感染率は感染後のディバイス装着日数を含めた場合（NNISが提唱している感染率）と感染後のディバイス装着日数を除いた場合とで有意差を認めず、NNISが提唱している感染率でも一般的使用に耐え得ると考えられた。

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A．研究目的

院内感染サーベイランスは院内感染対策の中心的役割を果たしている。ICU部門はJANIS専用の入力支援ソフトを開発して、標準化された患者データを収集できる体制を整備した。今後、収集した患者データを集計・解析するプログラムを確立して、システム化する必要がある。

院内感染率は全体および各施設の院内感染の発生状況を表わす基本的指標である。アメリカのNational Nosocomial Infection Surveillance（NNIS）は関連するディバイス装着日数を分母にした感染率を提唱しており[1]、世界各国でひろく用いられている。集計・解析のプロセスが簡略化され、実践的である一方、本来、除くべき感染後のディバイス装着日数まで分母にカウントされており、疫学的観点から、算出された値の精度が疑問視される。本研究では、NNISが提唱している感染率を含めて、分母の設定が異なる4種類の院内感染率を比較検討した。

B．研究方法

2002年6月～2003年12月、JANISのICU部門の研究班（19施設）から収集した患者データのうち、他院ICU転出者を除いて、16歳以上、ICU在室24時間以上1000時間未満、APACHE IIスコアの情報が得られた者は11221名である。院内感染の定義はICU入室2日目以降の感染である[1,2]。そこで、ICU在室2日以上かつICU入室2日目までに感染を確認されていない10339名を対象にして、ICU入室からICU退室まで追跡した。

全体および各施設の感染部位別（肺炎、尿路感染、敗血症）の院内感染率とディバイス装着率を算出した。ディバイス装着率の定義は「ICU在室中の全ディバイス装着日数／ICU在室日数」である[1]。感染と関連するディバイスの対応は肺炎と人工呼吸器、尿路感染と尿道カテーテル、敗血症と

中心静脈カテーテルである。

院内感染率は分母の設定が異なる以下の4種類を算出した。なお、NNISが提唱している感染率は③(1)に相当する。

① ICU入室数あたり
全感染者／ICU入室数

② ICU在室日数あたり
全感染者／感染日以前のICU在室日数

③ ディバイス装着日数あたり
(1) 全ディバイス装着日数あたり
ディバイス装着日の翌日以降に感染した者／ICU在室中の全ディバイス装着日数

(2) 感染前ディバイス装着日数あたり
ディバイス装着日の翌日以降に感染した者／ICU在室中の感染日以前のディバイス装着日数

統計学的解析はSAS version 8.2を使用した。

C．研究結果

• 感染者数

肺炎の感染者は、肺炎よりまえに肺炎以外の感染を確認されていない10314名の3.6%、375名であり、そのうち、336名（90%）が人工呼吸器装着日の翌日以降に感染した。10314名の人工呼吸器装着率は46.9%である。

尿路感染の感染者は、尿路感染よりまえに尿路感染以外の感染を確認されていない10325名の0.4%、43名であり、そのうち、40名（93%）が尿道カテーテル装着日の翌日以降に感染した。10325名の尿道カテーテル装着率は92.2%である。

敗血症の感染者は、敗血症よりまえに敗血症以外の感染を確認されていない10317名の0.6%、64名であり、そのうち、59名（92%）が中心静脈カテーテル装着日の翌日以降に感染した。10317名の中心静脈カテーテル装着率は75.7%である。

• ICU入室数あたりとICU在室日数あたりの感染率の比較

表1にICU入室数あたりとICU在室日数あたりの感染率を示した。

肺炎に関して、図1に施設別の値のプロットを示した。ICU入室数あたりとICU在室日数あたりの感染率はほぼ一致した傾向をみとめた。

• 全ディバイス装着日数あたりと感染前ディバイス装着日数あたりの感染率の比較

表2に全ディバイス装着日数あたりと感染前ディバイス装着日数あたりの感染率を示した。感染前ディバイス装着日数あたりの感染率を基準にして全ディバイス装着日数あたりの感染率の95%信頼区間を求めると、肺炎が117.0～164.6、尿路感染が2.6～14.4、敗血症が7.4～23.2であり、感染前ディバイス装着日数をまたぎ、有意差を認めない。

肺炎に関して、図2に施設別の値のプロットを示した。全ディバイス装着日数あたりと感染前ディバイス装着日数あたりの感染率はほぼ一致した傾向をみとめた。感染前ディバイス装着日数あたりの感染率を基準にして全ディバイス装着日数あたりの感染率の95%信頼区間を求めると、感染前ディバイス装着日数をまたぎ、有意差を認めない。ただ、感染率の高い施設とディバイス装着率の低い施設とで両者の開きが大きい傾向をみとめた。

D．考察

本研究では、NNISが提唱している感染率を含めて、分母の設定が異なる4種類の院内感染率を算出した。これまでICU患者を対象にして複数の院内感染率を比較検討した報告は見られない。

ICUの在室が長期化すると感染リスクが増加するという考えから、person-dayの概

念を用いた院内感染率が推奨されている[1]。しかし、本研究の結果から、ICU入室数あたりとICU在室日数あたりの感染率はほぼ一致した傾向をみとめ、ICU入室数あたりの感染率でも施設間比較に耐え得ると考えられた。ICU在室日数は平均5.2（±5.3）日であり、10日を越える割合は1割以下である。ICUの場合、観察期間の長さが限定されるため、観察期間の影響を受けにくいと考えられた。

ICU入室数あたりの感染率やICU在室日数あたりの感染率を用いる場合、感染のリスク要因は考慮されない。本研究の対象は国立病院を中心にしており、比較的均質であるが、施設間較差が大きくなれば、感染のリスク要因を調整した感染率の使用が望ましいと考えられる[3]。ディバイス装着はextrinsic factor(感染の外部リスク要因)であり、ディバイス装着を調整した感染率がひろく用いられている[1,3]。本研究の結果から、ディバイス装着日数あたりの感染率は感染後のディバイス装着日数を含めた場合（NNISが提唱している感染率）と感染後のディバイス装着日数を除いた場合とで有意差を認めず、NNISが提唱している感染率でも一般的使用に耐え得ると考えられた。ただ、感染率の高い施設とディバイス装着率の低い施設とで両者の開きが大きい傾向をみとめており、注意が必要である。また、感染を確認した日とディバイスを外した日が一致している患者が肺炎で9.2%（31/336）、尿路感染で25.0%（10/40）、敗血症で8.5%（5/59）みられ、相当数の患者が感染を理由にディバイスを外したと推察される。全ディバイス装着日数にしめる感染後のディバイス装着日数の割合は人工呼吸器で9.6%、尿道カテーテルで0.4%、中心静脈カテーテルで1.5%であり、これらの数値が増加すれば、感染後のディバイス装着日数を含めた場合の誤差が大きくなるかもしれない。

JANISにおいても、今後、収集した患者データを集計・解析するプログラムを確立して、システム化する必要がある。NNISが提唱している感染率はたしかに限界もあるが、収集すべきデータ項目が簡素化され、集計・解析のプロセスが簡略化され、実践的であり、疫学的観点からも許容できる値を算出できる。JANISのシステムにNNISが提唱している感染率を計算する機能を組み入れ、集計・解析を自動化することも、十分、検討の余地があると考えられた。

E．参考文献

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表1：入室数あたりとICU在室日数あたりの感染率の比較

	ICU入室数	ICU在室日数	感染	院内感染率	
				/100入室数	/1000在室日数
肺炎	10314	47776	375	3.64	7.85
尿路感染	10325	50794	43	0.42	0.85
敗血症	10317	50181	64	0.62	1.28

表2：全ディバイス装着日数あたりと感染前ディバイス装着日数あたりの感染率の比較

	ディバイス装着日数		感染	院内感染率 /10000装着日数	
	全体	感染前		全日数	感染前日数
肺炎	23861	21561	336	140.8	155.8
尿路感染	47054	46872	40	8.5	8.5
敗血症	38476	37903	59	15.3	15.6

ディバイスは肺炎ならば人工呼吸器、尿路感染ならば尿道カテーテル、敗血症ならば中心静脈カテーテルを表わす。

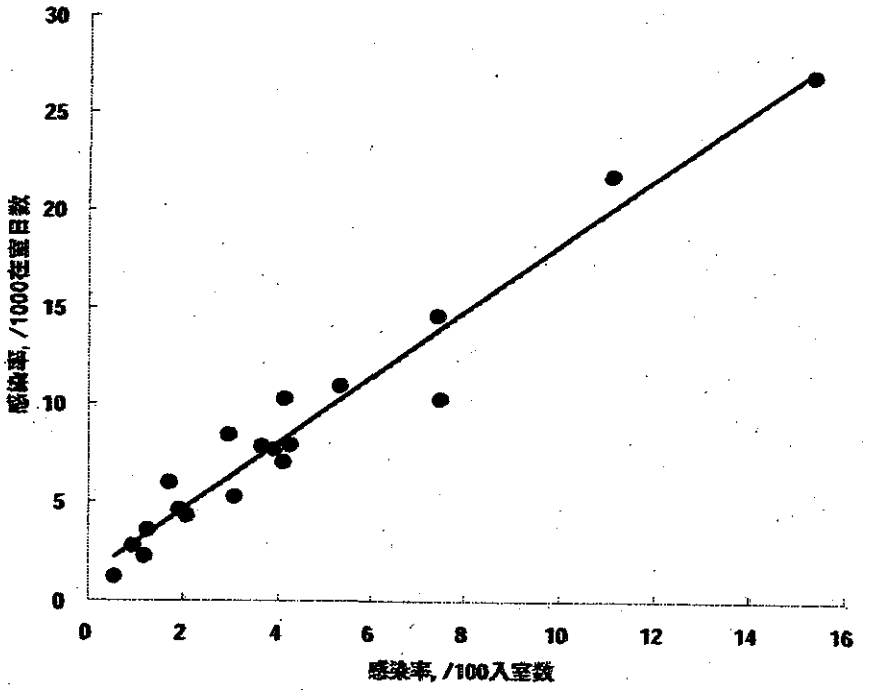

図1：入室数あたりとICU在室日数あたりの肺炎の感染率（19施設）

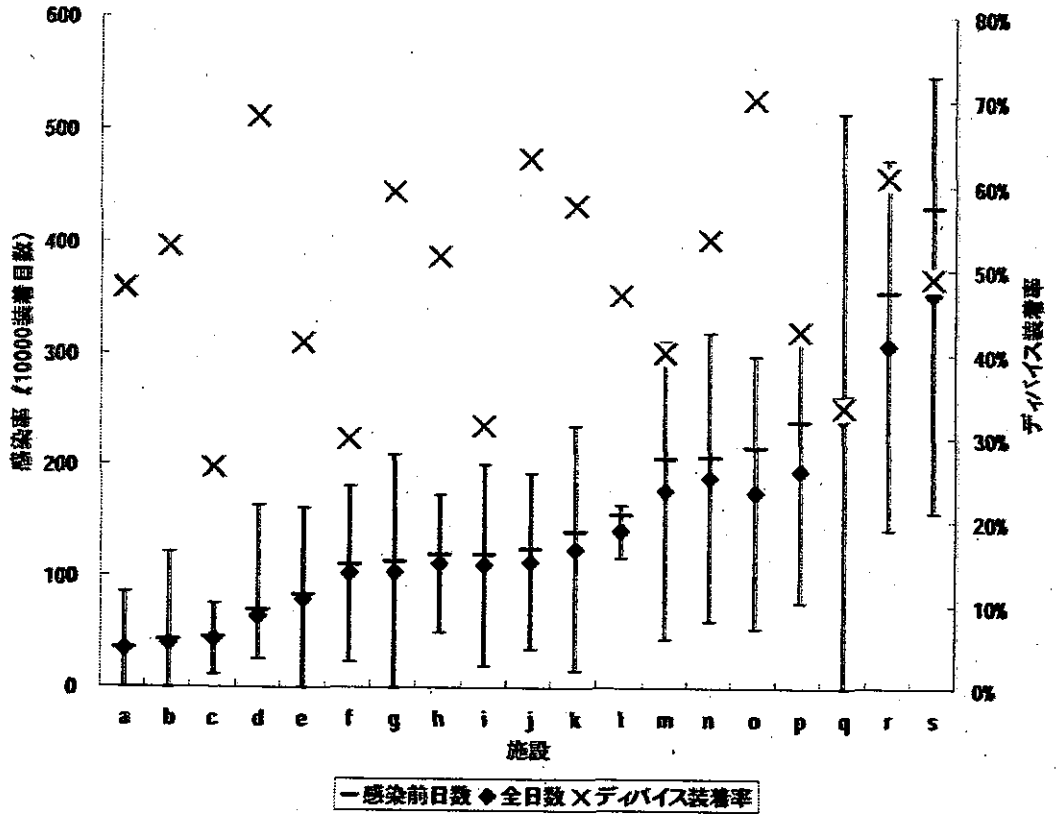

図２：全ディバイス装着日数あたりと感染前ディバイス装着日数あたりの肺炎の感染率
ディバイスは肺炎ならば人工呼吸器、尿路感染ならば尿道カテーテル、
敗血症ならば中心静脈カテーテルを表わす。
グラフの上下のバーは感染前ディバイス装着日数あたりの感染率を基準にして
全ディバイス装着日数あたりの感染率の95％信頼区間を表わす。

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G.知的所有権の取得など

1.特許許可

2.実用新案登録

3.その他

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Metallo-β-Lactamase-Producing Gram-Negative Bacilli: Laboratory-Based Surveillance in Cooperation with 13 Clinical Laboratories in the Kinki Region of Japan

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A total of 19,753 strains of gram-negative rods collected during two 6-month periods (October 2000 to March 2001 and November 2001 to April 2002) from 13 clinical laboratories in the Kinki region of Japan were investigated for the production of metallo-β-lactamases (MBLs). MBLs were detected in 96 (0.5%) of the 19,753 isolates by the broth microdilution method, the 2-mercaptopropionic acid inhibition test, and PCR and DNA sequencing analyses. MBL-positive isolates were detected in 9 of 13 laboratories, with the rate of detection ranging between 0 and 2.6% for each laboratory. Forty-four of 1,429 (3.1%) *Serratia marcescens*, 22 of 6,198 (0.4%) *Pseudomonas aeruginosa*, 21 of 1,108 (1.9%) *Acinetobacter* spp., 4 of 544 (0.7%) *Citrobacter freundii*, 3 of 127 (2.4%) *Providencia rettgeri*, 1 of 434 (0.2%) *Morganella morganii*, and 1 of 1,483 (0.1%) *Enterobacter cloacae* isolates were positive for MBLs. Of these 96 MBL-positive strains, 87 (90.6%), 7 (7.3%), and 2 (2.1%) isolates carried the genes for IMP-1-group MBLs, IMP-2-group MBLs, and VIM-2-group MBLs, respectively. The class 1 integrase gene, *intI1*, was detected in all MBL-positive strains, and the *aac (6′)-Ib* gene was detected in 37 (38.5%) isolates. Strains with identical PCR fingerprint profiles in a random amplified polymorphic DNA pattern analysis were isolated successively from five separate hospitals, suggesting the nosocomial spread of the organism in each hospital. In conclusion, many species of MBL-positive gram-negative rods are distributed widely in different hospitals in the Kinki region of Japan. The present findings should be considered during the development of policies and strategies to prevent the emergence and further spread of MBL-producing bacteria.

Metallo-β-lactamases (MBLs) are enzymes belonging to Ambler's class B that can hydrolyze a wide variety of β-lactams, including penicillins, cephems, and carbapenems (14, 30, 42). The acquisition by gram-negative rods of MBLs, which are often encoded by mobile genetic elements such as cassettes inserted into integrons, confers a multidrug resistance profile against many clinically important β-lactams as well as other antimicrobial agents (1). This fact raises a significant problem with respect to antimicrobial chemotherapy (38). Plasmid-mediated MBLs are categorized into three major molecular types: they are IMP-type, VIM-type, and SPM-type enzymes (14, 21, 30, 32, 39, 42). Among them, IMP-1-type MBLs have been identified in various gram-negative bacilli belonging to the family *Enterobacteriaceae* and in several non-glucose-fermenters, such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. (13, 18, 19, 20, 36–38, 43). Furthermore, in Japan, several variants of the IMP-1 type, including IMP-3 from *Shigella flexneri* (15), IMP-6 from *Serratia marcescens* (47), IMP-10 from *P. aeruginosa* and *Alcaligenes xylosoxidans* (16), and IMP-11 (EMBL/GenBank accession no. AB074437) from *P. aeruginosa* and *Acinetobacter baumannii*, have been characterized recently. VIM-type MBLs, including VIM-1 and VIM-2 from *P. aeruginosa* isolates in Italy and France, respectively (21, 32), were first described in 1999. Outbreaks of VIM-type MBL-positive strains have also been reported in Italy and Greece (8, 40). SPM-1, a member of the third group of plasmid-mediated MBLs, was recently detected in *P. aeruginosa* isolates in Brazil, and SPM-1 producers appear to be widely disseminated in Brazil (11).

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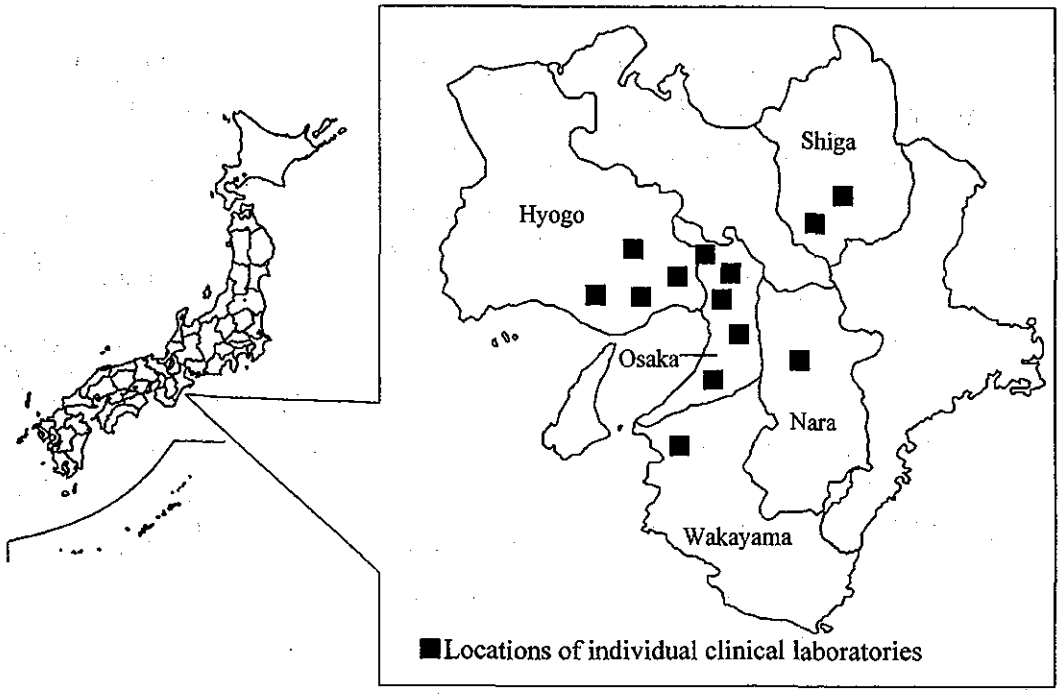

FIG. 1. Locations of the 13 institutions involved in this study of the Kinki region of Japan.

Previously reported survey data from the Kinki region of Japan revealed that 0.7% of isolates produced IMP-1-group MBLs (44). The prevalence of IMP-1-group MBLs among gram-negative rods has also been investigated (19, 36); however, the prevalence of the new plasmid-mediated MBLs, such as the IMP-2 group (33) and the VIM-2 type (32), in Japan remains unclear.

For the present study, to assess the prevalence and types of MBL-positive bacteria in the Kinki region of Japan, we investigated almost 20,000 isolates collected from 12 general hospitals and one commercial laboratory.

MATERIALS AND METHODS

Bacterial isolates. This laboratory-based surveillance study was conducted with the cooperation of 13 institutions (12 hospital clinical laboratories and one commercial laboratory) in the Kinki region, which is located in western Japan (Fig. 1), with the assistance of the National Institute of Infectious Diseases of Japan. Between October 2000 and March 2001 (first study period) and November 2001 and April 2002 (second study period), a total of 19,753 isolates of gram-negative bacilli, including *P. aeruginosa* (6,198 isolates), *Acinetobacter* spp. (1,108 isolates), *Escherichia coli* (4,347 isolates), *Klebsiella pneumoniae* (2,354 isolates), *S. marcescens* (1,429 isolates), *Enterobacter cloacae* (1,483 isolates), *Citrobacter freundii* (544 isolates), *Klebsiella oxytoca* (627 isolates), *Enterobacter aerogenes* (454 isolates), *Proteus mirabilis* (470 isolates), *Morganella morganii* (434 isolates), *Proteus vulgaris* (178 isolates), and *Providencia rettgeri* (127 isolates), were isolated from various clinical specimens and then tested. A single isolate was selected from each patient and identified by use of a MicroScan Neg Combo 5J panel (Dade Behring, Tokyo, Japan). Moreover, *Acinetobacter* isolates were identified by use of an ID TEST NF-18 panel (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). For *Acinetobacter* spp. other than *A. baumannii*, PCR amplification of the 16S rRNA gene was performed, with genomic DNA as the template, according to a previously published protocol (34), and the amplicons were sequenced. The sequence data were submitted to the DNA Data Bank of Japan (DDBJ) database to check the identity or similarity of each sequence against the database by use of the FASTA program (http://www.ddbj.nig.ac.jp/search/Welcome-e.html).

First screening for MBL production. MIC criteria for the first screening of MBL producers were >16 μg of ceftazidime/ml for *Acinetobacter* spp. and >16 μg of both ceftazidime and cefoperazone-sulbactam/ml for gram-negative organisms other than *Acinetobacter* spp. The production of MBLs was assessed with a 2-mercaptopropionic acid inhibition (2-MPA) test as described previously (2, 37). Test strains were cultured, adjusted to a 0.5 McFarland standard, diluted with 0.85% saline, and inoculated onto Mueller-Hinton agar plates according to the protocol recommended by the NCCLS (27). Two Senci-Disks (Becton Dickinson Co., Ltd., Tokyo, Japan) containing 30 μg of ceftazidime, 10 μg of imipenem, and 30 μg of cefepime were placed at a distance of 50 mm from each other on the plate, and one blank disk was placed near one of the Senci-Disks at a distance of 20 mm. Two to 3 μl of 2-MPA was added to the blank disk. After an overnight incubation at 35°C, if an expansion of the growth inhibition zone around either the ceftazidime, imipenem, or cefepime disk was observed, the strain was interpreted as being positive for MBL.

Susceptibility testing for antimicrobial agents. The MICs of antimicrobial agents for isolates that tested positive in the 2-MPA test were subjected to antimicrobial susceptibility testing by the broth microdilution method with dry plates (Biken Chemical Co., Ltd., Tokyo, Japan), which conformed to NCCLS guidelines (26, 28). The following antimicrobial agents and concentrations were used: piperacillin (2 to 128 μg/ml), piperacillin-tazobactam (1-4 to 128-4 μg/ml), ceftazidime (1 to 128 μg/ml), cefepime (1 to 128 μg/ml), cefoperazone-sulbactam (1-0.5 to 128-64 μg/ml), aztreonam (1 to 128 μg/ml), cefmetazole (1 to 128 μg/ml), moxalactam (1 to 128 μg/ml), meropenem (0.25 to 32 μg/ml), imipenem (0.25 to 32 μg/ml), gentamicin (1 to 8 μg/ml), amikacin (4 to 32 μg/ml), minocycline (4 to 8 μg/ml), levofloxacin (2 to 4 μg/ml), trimethoprim-sulfamethoxazole (9.5-0.5 to 38-2 μg/ml), and chloramphenicol (8 to 16 μg/ml). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as reference strains for quality control of the tests (26).

PCR amplification and DNA sequencing. Isolates that tested positive in the 2-MPA test were then assessed for their MBL type by PCR and DNA sequencing. PCRs were performed as described previously (35). PCR primers for the amplification of each MBL gene were constructed as described in previous reports for bla_{IMP-1} (35), bla_{IMP-2} (33), and bla_{VIM-2} (32). Primers for the amplification of integrase genes (*intI1*, *intI2*, and *intI3*) (31, 37) and the aminogly-

TABLE 1. Primers for PCR and sequencing of MBL genes

Target	Use	Primer name	Primer sequence (5′ to 3′)	Position[a]	Product length (bp)	Reference
IMP-1	Amplification	IMP1L	CTACCGCAGCAGAGTCTTTG	47–66	587	35
		IMP2R	AACCAGTTTTGCCTTACCAT	633–614		
	Sequencing	IMP1-SQ-F	ACCGCAGCAGAGTCTTTGCC	49–68	587	37
		IMP1-SQ-R	ACAACCAGTTTTGCCTTACC	635–616		
IMP-2	Amplification	IMP2L	GTGTATGCTTCCTTTGTAGC	23–42	174	33
		IMP2R	CAATCAGATAGGCGTCAGTGT	196–176		
	Sequencing	IMP2-SQ-F	GTTTTATGTGTATGCTTCC	16–34	678	37
		IMP2-SQ-R	AGCCTGTTCCCATGTAC	693–677		
VIM	Amplification	VIMB	ATGGTGTTTGGTCGCATATC	152–171	510	32
		VIMF	TGGGCCATTCAGCCAGATC	661–643		
	Sequencing	VIM2-SQ-F	ATGTTCAAACTTTTGAGTAAG	1–21	801	37
		VIM2-SQ-R	CTACTCAACGACTGAGCG	801–784		

[a] Position number 1 for every MBL gene corresponds to the first base of the start codon.

coside resistance gene [*aac (6′)-Ib*] (35) were described previously. The PCR and DNA sequencing primers used are listed in Table 1.

PCR products were sequenced by the dideoxynucleotide chain termination method (45) in an automated DNA sequencer (ABI 3100; Perkin-Elmer Applied Biosystems, Foster City, Calif.). Similarity searches against sequence databases were performed with an updated version of the FASTA program available from the Center for Information Biology and DNA Data of Japan for Biotechnology Information server of the National Institute of Genetics of Japan (http://www.ddbj.nig.ac.jp/).

RAPD pattern analysis. The isolates that were confirmed to be positive for the MBL gene by PCR and chromosomal DNA typing were analyzed by random amplified polymorphic DNA (RAPD) analysis to generate a DNA fingerprint (29). The RAPD primers were ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) for *Enterobacteriaceae* other than *S. marcescens* (41), HLWL-74 (5′-CGTCTATGCA-3′) and 1254 (5′-AACCCACGCC-3′) for *S. marcescens* (12), 272 (5′-AGCGGGCCAA-3′) for *P. aeruginosa* (6), and A5 (5′-GCCGGGGCCT-3′) for *Acinetobacter* spp. (31).

RESULTS

Prevalence of MBL-positive isolates. The prevalence of isolates that produced MBLs is shown in Table 2. Seven hundred fifty-seven isolates (3.8%) fulfilled the MIC criteria for the production of MBLs. Of these 757 isolates, 96 (12.7%) were positive in the 2-MPA test. Of these 96 positive isolates, only 1 *E. cloacae* isolate appeared to have a weak and ambiguous growth inhibition zone (data not shown) in the 2-MPA test. All 96 isolates that tested positive in the 2-MPA test were positive for at least one MBL gene by PCR and DNA sequencing. The numbers of MBL-positive isolates with an MBL gene were 44 (3.1%) for *S. marcescens*, 22 (0.4%) for *P. aeruginosa*, 21 (1.9%) for *Acinetobacter* spp., 4 (0.7%) for *C. freundii*, 3 (2.4%) for *Providencia rettgeri*, 1 (0.2%) for *M. morganii*, and 1 (0.1%) for *E. cloacae*. Of 21 isolates of *Acinetobacter* spp., 18 were identified as *A. baumannii*, and the remaining three strains were *A. johnsonii*, *A. junii*, and *A. calcoaceticus* according to 16S rRNA sequencing analysis and their biochemical properties.

The results of the MBL assessments in each laboratory are shown in Table 3. These 13 laboratories included 5 laboratories in university hospitals, 7 laboratories in general hospitals, and 1 commercial laboratory. MBL-positive isolates were detected in 9 of 13 laboratories; the overall rate of detection was 0.5% and ranged from 0 to 2.6% in each laboratory.

Genetic characterization of MBL-producing isolates. Some characteristics and selected clinical associations of MBL-producing isolates are shown in Table 4. All MBL-producing iso-

TABLE 2. Prevalence of metallo-β-lactamase-producing isolates

Organism	No. of isolates collected			No. of isolates fulfilling MIC criteria			No. (%) of MBL-producing isolates[c]		
	2000[a]	2001[b]	Total	2000[a]	2001[b]	Total	2000[a]	2001[b]	Total
Pseudomonas aeruginosa	2,645	3,553	6,198	141	141	282	8 (0.3)	14 (0.4)	22 (0.4)
Escherichia coli	1,334	3,013	4,347	3	8	11	0 (0)	0 (0)	0 (0)
Klebsiella pneumoniae	867	1,487	2,354	1	2	3	0 (0)	0 (0)	0 (0)
Serratia marcescens	615	814	1,429	101	55	156	26 (4.2)	18 (2.2)	44 (3.1)
Enterobacter cloacae	565	918	1,483	81	90	171	0 (0)	1 (0.1)	1 (0.1)
Acinetobacter spp.	388	720	1,108	20	14	34	13 (3.4)	8 (1.1)	21 (1.9)
Citrobacter freundii	234	310	544	23	37	60	0 (0)	4 (1.3)	4 (0.7)
Klebsiella oxytoca	227	400	627	0	0	0	0 (0)	0 (0)	0 (0)
Enterobacter aerogenes	194	260	454	11	12	23	0 (0)	0 (0)	0 (0)
Proteus mirabilis	176	294	470	1	4	5	0 (0)	0 (0)	0 (0)
Morganella morganii	166	268	434	4	2	6	1 (0.6)	0 (0)	1 (0.2)
Proteus vulgaris	97	81	178	0	0	0	0 (0)	0 (0)	0 (0)
Providencia rettgeri	45	82	127	5	1	6	3 (6.7)	0 (0)	3 (2.4)
Total	7,553	12,200	19,753	391	366	757	51 (0.7)	45 (0.4)	96 (0.5)

[a] First study period, October 2000 to March 2001.
[b] Second study period, November 2001 to April 2002.
[c] Percentages were calculated as follows: no. of MBL-producing isolates/no. of isolates collected × 100%.

TABLE 3. Isolation frequencies of metallo-β-lactamase-producing isolates in 13 laboratories

Laboratory code (type)	No. of isolates collected			No. (%) of MBL-producing isolates[c]		
	2000[a]	2001[b]	Total	2000[a]	2001[b]	Total
A (university hospital)	1,293	1,571	2,864	7 (0.5)	3 (0.2)	10 (0.4)
B (university hospital)	951	1,281	2,232	5 (0.5)	11 (0.9)	16 (0.7)
C (university hospital)	1,139	1,003	2,142	31 (2.7)	24 (2.4)	55 (2.6)
D (university hospital)	965	873	1,838	0 (0)	1 (0.1)	1 (0.1)
E (university hospital)	883	728	1,611	0 (0)	0 (0)	0 (0)
F (general hospital)	482	627	1,109	0 (0)	2 (0.3)	2 (0.2)
G (general hospital)	463	453	916	0 (0)	0 (0)	0 (0)
H (general hospital)	347	421	768	1 (0.3)	2 (0.5)	3 (0.4)
I (general hospital)	304	280	584	1 (0.3)	0 (0)	1 (0.2)
J (general hospital)	255	251	506	4 (1.5)	2 (0.8)	6 (1.2)
K (general hospital)	212	293	505	2 (0.9)	0 (0)	2 (0.4)
L (general hospital)	259	152	411	0 (0)	0 (0)	0 (0)
M (commercial laboratory)	ND[d]	4267	4267	ND[d]	0 (0)	0 (0)

[a] First study period, October 2000 to March 2001.
[b] Second study period, November 2001 to April 2002.
[c] Percentages are no. of MBL-producing isolates/no. of isolates collected × 100.
[d] ND, not determined.

lates were isolated from inpatients with bacterial infections. With respect to the MBL genotypes, 87 (90.6%) isolates carried genes encoding IMP-1-group MBLs, including IMP-1, IMP-3, IMP-6, and IMP-10. Similarly, seven (7.3%) isolates carried genes for IMP-2-group MBLs, such as IMP-2, IMP-8, and IMP-11. Genes encoding VIM-2-group MBLs, such as VIM-2, VIM-3, and VIM-6, were carried by two (2.1%) isolates. Genes for IMP-1-group MBLs were detected in 21 isolates of *P. aeruginosa* at hospitals B, C, and H; 14 isolates of *Acinetobacter* spp. at hospitals A, C, F, H, and J; 44 isolates of *S. marcescens* at hospitals A and C; 4 isolates of *C. freundii* at hospital C; 3 isolates of *Providencia rettgeri* at hospital C; and 1 isolate of *M. morganii* at hospital C. Genes for IMP-2-group MBLs were detected in seven isolates of *Acinetobacter* spp. at hospitals J and K. Genes for VIM-2-group MBLs were detected in one isolate of *P. aeruginosa* at hospital I and one isolate of *E. cloacae* at hospital D. The integrase gene (identified as *intI1*) was detected in all 96 MBL-producing isolates. The *aac (6')-Ib* gene was detected in 37 (38.5%) MBL-positive isolates.

Of 96 MBL-positive isolates, 68 (70.8%), 8 (8.3%), 7 (7.3%), 4 (4.1%), 3 (3.1%), 2 (2.1%), 2 (2.1%), and 2 (2.1%) were recovered from urine, sputum, throats, pus, drains, blood, tracheal tubes, and other samples, respectively. The majority of *A. baumannii* isolates were recovered from respiratory tract specimens, and bacterial species belonging to the family *Enterobacteriaceae* and *P. aeruginosa* were recovered from urine.

RAPD typing with the 272 primer of 22 *P. aeruginosa* isolates from four hospitals identified 11 distinct types. Two or more isolates with the same banding patterns were observed for two of the four hospitals. Of 21 *A. baumannii* isolates from six hospitals, 8 isolates (same ward) from hospital A were found to belong to the same clonal lineage, and 5 isolates (two wards) from hospital J belonged to another clonal lineage. Of 44 *S. marcescens* isolates from hospitals A and C, 2 isolates from hospital A had the same pattern, and two distinct patterns were observed for 42 isolates recovered from hospital C. Forty-one of the isolates from hospital C shared the same pattern, and they had been isolated from eight different wards. At hospital C, the two isolates of *Providencia rettgeri* had the same pattern, and four isolates of *C. freundii* also shared the same pattern. Genetically related isolates, such as those of *P. aeruginosa* in hospital B, *A. baumannii* in hospitals A and J, and *S. marcescens*, *Providencia rettgeri*, and *C. freundii* in hospital C, were isolated from the same ward, suggesting a nosocomial spread of these organisms.

Susceptibility of MBL-producing isolates. The results of susceptibility tests are shown in Table 5. The susceptibilities of the 96 MBL-positive isolates to several antimicrobial agents varied. For the MBL-producing bacterial species belonging to the family *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp., the MICs at which 50% of the isolates were inhibited (MIC_{50}s) of imipenem were 32, 16, and 16, respectively, and the MIC_{90}s of the same agent were >32, 32, and 32 μg/ml, respectively. For species belonging to the family *Enterobacteriaceae*, piperacillin-tazobactam, aztreonam, gentamicin, amikacin, and levofloxacin had relatively high activities. *P. aeruginosa* isolates had similar or lower susceptibilities to non-β-lactam agents than bacterial species belonging to the family *Enterobacteriaceae* or *Acinetobacter* spp. Piperacillin-tazobactam and cefoperazone-sulbactam appeared to have the most potent activities against MBL-producing *Acinetobacter* spp. The MICs for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were within the NCCLS control ranges.

DISCUSSION

We investigated the distribution and prevalence of MBL-producing gram-negative rods with the cooperation of 12 clinical laboratories at large-scale general hospitals and one commercial clinical laboratory in the Kinki region of Japan. Such isolates were identified at a rate of 0.5%. In a previous laboratory-based surveillance conducted in 1998 and 2000, MBL-producing isolates were found only in specimens collected by a commercial laboratory (44). However, MBL producers were isolated from 9 of 13 laboratories in the present multi-institutional surveillance study, and the prevalence of MBL-positive isolates ranged from 0 to 2.6%, suggesting that there is a continuous proliferation of MBL producers in Japan. The ma-

TABLE 4. Characteristics of MBL-producing strains and selected clinical data

Species (no. of strains)	RAPD type	MBL type[a] (No. of strains)	No. of *aac(6')-Ib*-a positive strains	Hospital	Ward[b] (no. of strains)	Antibiogram[c] (no. of strains)
P. aeruginosa (22)	a	IMP-1 (5)	4	B	ICU (1), NICU (1), pediatrics 7E (3)	GM, MINO, ST, CP (4), GM, ST, CP (1)
	b	IMP-1 (6)	6	B	Internal medicine 11E (2), internal medicine 13E (1), internal medicine 8E (1), pediatrics 7E (2)	GM, MINO, ST, CP (6)
	c	IMP-1 (1)	0	B	Internal medicine 11E (1)	GM, MINO, LVFX, ST, CP (1)
	d	IMP-1 (1)	1	B	Urology 8W (1)	GM, MINO, ST, CP (1)
	e	IMP-1 (1)	0	B	Urology 8W (1)	MINO, ST, CP (1)
	f	IMP-1 (2)	2	B	Urology 8W (2)	GM, AMK, MINO, LVFX, ST, CP (1), GM, MINO, LVFX, ST, CP (1)
	g	IMP-1 (1)	0	C	Emergency 1S (1)	GM, MINO, LVFX, ST, CP (1)
	h	IMP-1 (1)	0	C	Emergency 1S (1)	GM, AMK, MINO, LVFX, ST, CP (1)
	i	IMP-1 (1)	0	C	Plastic surgery 4C (1)	GM, MINO, LVFX, ST, CP (1)
	j	IMP-1 (2)	2	H	Urology 5W (1), internal medicine MICU (1)	GM, MINO, LVFX, ST, CP (2)
	k	VIM-2 (1)	1	I	Internal medicine 6E (1)	GM, MINO, LVFX, ST, CP (1)
A. baumannii (18)	A	IMP-1 (8)	8	A	Internal medicine 11SNS (8)	GM, ST, CP (8)
	B	IMP-1 (1)	1	C	Otolaryngology 7E (1)	GM, CP (1)
	C	IMP-1 (1)	1	F	Cardiac surgery ICU (1)	ST, CP (1)
	D	IMP-1 (1)	1	F	Brain surgery SCU (1)	ST, CP (1)
	E	IMP-1 (1)	1	H	Internal medicine 7E (1)	CP (1)
	F1	IMP-2 (5)	0	J	Surgery W6 (3), brain surgery W7 (2)	ST, CP (5)
	F2	IMP-2 (1)	0	K	Internal medicine 5A (1)	GM, ST, CP (1)
A. junii (1)		IMP-1 (1)	1	C	Otolaryngology 7E (1)	GM, ST (1)
A. calcoaceticus (1)		IMP-1 (1)	0	J	Internal medicine ICU (1)	AMK, ST (1)
A. johnsonii (1)		IMP-2 (1)	0	K	Internal medicine 5A (1)	ST (1)
S. marcescens (44)	1	IMP-1 (2)	0	A	Neurology 7SNS (1), urology 6SNS (1)	MINO, LVFX, ST, CP (1) MINO, LVFX, CP (1)
	2	IMP-1 (1)	0	C	Urology 7F (1)	MINO, ST, CP (1)
	3	IMP-1 (41)	0	C	Emergency 1S (14), internal medicine CCU (13), urology 7F (5), brain surgery 6F (2), cardiac surgery 4S (2), internal medicine 3S (2), plastic surgery 5C (2), surgery 4F (1)	CP (7), AMK (1), MINO (1), MINO, CP (1)
Providencia rettgeri (3)	1	IMP-1 (2)	2	C	Urology 7F (2)	ST, CP (2)
	2	IMP-1 (1)	1	C	Internal medicine 8J (1)	MINO, LVFX, ST (1)
Citrobacter freundii (4)	1	IMP-1 (4)	4	C	Internal medicine CCU (3), internal medicine 3S (1)	CP (1)
Morganella morganii (1)		IMP-1 (1)	1	C	Urology 7F (1)	MINO, LVFX, ST (1)
Enterobacter cloacae (1)		VIM-2 (1)	0	D	Pediatrics E6 (1)	MINO, ST, CP (1)

[a] MBL types: IMP-1, IMP-1-group MBLs, including IMP-1, IMP-3, IMP-6, and IMP-10; IMP-2, IMP-2-group MBLs, including IMP-2, IMP-8, and IMP-11; VIM-2, VIM-2 MBLs.
[b] ICU, intensive care unit; NICU, neonatal intensive care unit; MICU, medical intensive care unit; SCU, surgical care unit; CCU, coronary care unit.
[c] Antibiogram: AMK, amikacin (64 μg/ml); GM, gentamicin (16 μg/ml); MINO, minocycline (16 μg/ml); LVFX, levofloxacin (8 μg/ml); ST, sulfamethoxazole-trimethoprim (4 μg/ml); CP, chloramphenicol (32 μg/ml).

jority of MBL producers detected in the present study were *S. marcescens*, *P. aeruginosa*, or *Acinetobacter* spp., which was similar to the results of previous studies (33, 37). Moreover, in the present study, several strains of *Providencia rettgeri* and *M. morganii* that produce MBLs were found, suggesting that plasmid-mediated horizontal transfer of the MBL genes is so far likely to occur continuously among gram-negative bacilli, as reported previously (14, 35). This finding gives us an alert on the further dissemination of MBL genes among various gram-negative bacilli. In the present study, the predominant type of MBL in Japan was found to be the IMP-1 group, but MBLs belonging to the IMP-2 group and the VIM-2 group were also detected. MBL genes encoding bla_{IMP-2} and bla_{VIM-2} have been reported in Italy (21, 33), France (32), Korea (22), Taiwan (45, 46), Portugal (7), and Greece (25). Recently, a genetic classification of the MBLs detected in Japan was reported (37), but the frequency of isolation of MBL producers from clinical specimens was not described. The present study provides the

TABLE 5. Susceptibility of MBL-producing isolates to various antimicrobial agents

Antimicrobial agent[a]	MIC (μg/ml) for organism								
	Enterobacteriaceae (n = 53)			*P. aeruginosa* (n = 22)			*Acinetobacter* spp. (n = 21)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Ceftazidime	16–>128	>128	>128	64–>128	128	>128	128–>128	>128	>128
Piperacillin	8–>128	16	>128	4–>128	16	>128	8–>128	32	128
Piperacillin-tazobactam[a]	≤1–>128	8	16	2–>128	8	>128	≤1–32	≤1	4
Cefepime	2–>128	64	128	32–>128	64	>128	64–>128	128	128
Cefoperazone-sulbactam	32–>128	>128	>128	64–>128	64	>128	≤1–16	2	4
Aztreonam	≤1–64	16	32	2–>128	8	32	4–32	16	32
Cefmetazole	>128–>128	>128	>128	>128–>128	>128	>128	64–>128	>128	>128
Latamoxef	64–>128	>128	>128	>128–>128	>128	>128	>128–>128	>128	>128
Meropenem	1–>32	>32	>32	16–>32	32	>32	8–>32	32	>32
Imipenem	1–>32	32	>32	1–>32	16	32	8–>32	16	32
Gentamicin	≤1–4	≤1	2	2–>8	>8	>8	≤1–>8	8	>8
Amikacin	≤4–>32	16	32	≤4–>32	16	32	≤4–>32	8	16
Minocycline	≤4–>8	≤4	>4	>8–>8	>8	>8	≤4–≤4	≤4	≤4
Levofloxacin	≤2–>4	≤2	4	≤2–>4	4	>4	≤2–4	≤2	≤2
Sulfamethoxazole-trimethoprim	≤0.5–>2	≤0.5	>2	>2–>2	>2	>2	≤0.5–>2	>2	>2
Chloramphenicol	≤8–>16	16	>16	>16–>16	>16	>16	≤8–>16	>16	>16

[a] Tazobactam was tested at a fixed concentration of 4 μg/ml.

first reported data on the prevalence of bacteria carrying the genes for MBLs, including IMP-1, IMP-2, and VIM-2 MBLs, in the western portion of Japan.

A total of 96 MBL-positive isolates were typed by RAPD analysis to determine the stabilities of the strain genotypes. The RAPD typing results are summarized in Table 4. The 16 isolates of *P. aeruginosa* from hospital B yielded seven different RAPD patterns and originated from six different wards. Eight *A. baumannii* isolates from hospital A had the same RAPD pattern and were from the same ward. The five *A. baumannii* isolates carrying the genes for IMP-2-group MBLs, isolated from two wards of hospital J, appeared to be of the same clonal lineage. This is the first report of nosocomial spread of *A. baumannii* isolates carrying genes for IMP-2-group MBLs in Japan. Of the 41 *S. marcescens* isolates from hospital C, 13 isolates from the internal medicine coronary care unit had the same RAPD pattern and were isolated within a 5-month period, suggesting that there was probable nosocomial spread within the same ward. Thus, the same or closely related isolates were identified repeatedly by PCR fingerprinting by RAPD analysis from five hospitals, suggesting the nosocomial spread of these organisms in each hospital. Furthermore, long-term cross-transmission of plasmids that carry MBL genes among different bacterial strains and species could result in the current complicated features of MBL producers, especially in hospital B.

The 2-MPA test, which is a simple test that was first described by Arakawa et al. (2), is a useful method for the routine laboratory detection of MBLs (45). Moreover, in the present study, all isolates that tested positive in the 2-MPA test were subsequently confirmed to be positive for the MBL gene by PCR. However, the growth inhibition zones of bla_{VIM-2}-positive *E. cloacae* isolates were weak and ambiguous, possibly due to the excessive production of AmpC and/or a change in membrane permeability. The production of some extended-spectrum β-lactamases as well as the excessive production of the chromosomal AmpC cephalosporinase could be responsible for the characteristics of these strains that were previously reported for *E. cloacae* (2, 7). In such cases, imipenem and meropenem disks would be better than ceftazidime disks for the detection of MBL production because imipenem and meropenem are essentially not hydrolyzed by extended-spectrum β-lactamases and class C cephalosporinases.

With respect to antimicrobial susceptibilities, various β-lactam antimicrobial agents such as ureidopenicillin, cephalosporins, cephamycins, and carbapenems had high MICs for most MBL-positive isolates, whereas monobactam and piperacillin typically had low MICs for MBL producers. Low MICs of cefepime, meropenem, and imipenem were observed for several isolates, even though MBLs can hydrolyze these agents. The production of MBLs in these isolates could be cryptic or suppressed in strains showing low-level carbapenem resistance (14). It is also possible that IMP-3 and IMP-6 MBLs, which have low-level hydrolytic activities against these agents (15, 47), are produced in such isolates. The increased ability of active efflux systems and decreased outer membrane permeabilities have been reported to contribute to β-lactam resistance in *P. aeruginosa* (23, 24). Therefore, the low-level MICs of piperacillin, cefepime, and carbapenems for some isolates may be due to higher permeability coefficients or less efficient efflux pumps in the bacterial membranes in addition to the molecular mechanisms described above.

The MICs of monobactam and piperacillin for MBL producers were relatively low compared to those of oximinocephalosporins, cephamycins, and carbapenems (35, 36); however, this finding does not necessarily reflect their clinical efficacy against MBL producers because most gram-negative rods have the intrinsic ability to produce chromosomal AmpC cephalosporinases, which can hydrolyze monobactam and piperacillin (17). Although the administration of high doses of aztreonam or tazobactam-piperacillin was reported to be useful for the reduction of MBL-producing strains in rats suffering from experimental pneumonia (3), it is possible that the induction of intrinsic chromosomal AmpC production in MBL producers may promote the emergence of multiple-β-lactam-resistant gram-negative rods in clinical settings.

In the present study, MICs of tazobactam-piperacillin and cefoperazone-sulbactam were generally low for MBL-positive *Acinetobacter* isolates. Strains producing IMP-1 or VIM-2 usually show high-level resistance to oximinocephalosporins and cephamycins, but the MIC of piperacillin for these strains is usually lower than those of oximinocephalosporins and cephamycins (9, 30, 32). Because the activities of MBLs are not reduced significantly by β-lactamase inhibitors, such as sulbactam and tazobactam (5), the observations for *Acinetobacter* isolates suggested that the phenotypes related to these combination drugs may depend mainly on the intrinsic production of AmpC cephalosporinase (4, 10) as well as the low-level production of MBLs and alterations in membrane permeability. Thus, the low MIC levels of tazobactam-piperacillin and cefoperazone-sulbactam for MBL-producing *Acinetobacter* isolates could be an intrinsic feature of this bacterial genus.

In conclusion, plasmid-mediated MBL-producing gram-negative rods were first described approximately 13 years ago in Japan, and in the present study, such isolates were found to have disseminated to many hospitals in the Kinki region of Japan. It is conceivable that several isolates have spread nosocomially among a number of hospitals. The results of the present study should be considered when health care facilities develop policies and strategic practices to prevent and address the emergence and spread of MBL-producing gram-negative microorganisms in clinical environments.

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