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Endemic and Epidemic Occurrences of Metallo-Beta-Lactamases in Japanese Medical Centers (1998-2002): Report from the SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Background: Metallo-ß-lactamases (MßL) were initially characterized in Japan, usually of the IMP-type and found in *P. aeruginosa* (PSA), *Acinetobacter* spp. (ACB) or *S. marcescens* (SM). The number of MßL types has increased worldwide, but geographic dissemination in Japan has appeared limited. This study compares baseline levels of MßL-resistance (R) from two, 22 center studies (1996-97) to the longitudinal sample (3 sites) of Japanese isolates from the SENTRY Program (1998-2002).
Methods: All MICs were determined by reference NCCLS methods. 84/456 (18.4%) PSA, 3/88 (3.4%) ACB, and 6/258 (2.3%) Enterobacteriaceae (Enterobacters and SM) with R to both monitored carbapenems (CARB; MIC, ≥ 8 mg/L) were screened for MßL by disk approximation (EDTA and 2MPA inhibitors), CARB hydrolysis by enzyme extracts and selected PCR primers for all known MßLs. All MßL-positive strains (10) were sequenced to determine type. Clonality in each center was determined by automated ribotyping and PFGE, where needed.
Results: The CARB-R rates in PSA (15.5 to 28.0%) appears to be increasing over the monitored interval (1998-2002), but varied by medical center location. Among CARB-R isolates, 2.2% were attributed to MßL strains (1.1% of all PSA tested). MßL identification showed: 5 PSA (3 IMP-1; 2 IMP-2), 4 SM (1 IMP-1; 2 IMP-1 + OXA-1, and 1 IMP-11). The sequence of the new MßL most closely resembles IMP-8 and has a complex integron (integrase-MßL-aacA4-ORF1-QAC/SUL). Also a single ACB had an IMP-1. 8 of 10 MßLs occurred between 2000 and 2002; 4 in 2002 (ACB, PSA, 2 SM). BRL42715, an AMP-C inhibitor confirmed AMP-C-mediated R in 87.3% of PSA and OMP changes were also discovered by membrane preparations. Prior (1997-98; 22 sites) results showed CARB-R at 22.4 - 25.6% and 0.5 - 0.9% MßLs (IMP-1) overall.
Conclusions: MßL-producing strains from several species persist in Japan, but represent a minority of all CARB-R isolates (1998-2002). Epidemic (SM 196-3 ribotype in 2002) or endemic dissemination was observed; and some novel ß-lactamase combinations and MßL-types were discovered (IMP-11). MßL rates appear generally stable in Japan. Continued global surveillance for these R mechanisms appears to be a prudent practice due to the mobility of the genetic determinants (plasmids or integrons) and the emergence of novel enzyme types, especially in SM and other non-PSA species.

INTRODUCTION

Metallo-ß-lactamases responsible for resistances to carbapenem antimicrobials have been consistently described in Japan since 1991 and the complete group of carbapenemases have been of grave concern to effective infection chemotherapy. The number of metallo-ß-lactamases has recently expanded to include SPM-1 from Latin America, GIM-1 from Germany and the first reported case from the United States, VIM-7. These latter reports have emerged from the structured surveillance effort of the SENTRY Antimicrobial Surveillance Program, initiated in 2002 using antimicrobial resistance phenotype indicators and various screening tests.

Several investigators have documented the emergence and spread of different types of metallo-ß-lactamases (bla_{IMP}, bla_{VIM}, bla_{SPM}) in various geographic areas. Specifically in Japan, IMP-1 has persisted in dominant numbers on class 1 integrons usually found in *Pseudomonas aeruginosa, Serratia marcescens, P. putida/fluorescens, Alcaligenes xylosoxidans, Acinetobacter baumannii* and *Klebsiella pneumoniae*. VIM-2 has also been observed in Japan as well as gene carriage on *intI3*. In this report, we examine the metallo-ß-lactamases contributing to the increasing carbapenem resistance rates in three Japanese medical centers contributing to the SENTRY Program between 1998 and 2002. These findings were compared to results from two earlier trials published by the Japan Antimicrobial Resistance Study Group (JARS Group) et al.

MATERIALS AND METHODS

Organisms tested. Among 456 *P. aeruginosa* isolates tested from participating Japanese medical centers (1998 - 2002), a total of 104 carbapenem-resistant strains were available for further testing. Each isolate was forwarded from the hospital, had its identity confirmed by the monitor (Adelaide, Australia), and tested against 25 - 35 additional antimicrobial agents. These strains were selected for their resistance to carbapenems (MIC, ≥ 8 mg/L for imipenem and/or meropenem). Similarly, any Enterobacteriaceae that was non-susceptible to the tested carbapenems (four of 69 *S. marcescens*; two of 189 *Enterobacter* spp.) were subjected to further tests, as were three of 88 *Acinetobacter* spp. isolates.

MATERIALS AND METHODS (Cont.)

Additional tests. All qualifying strains were retested by reference MIC methods [NCCLS, 2003 and 2004] to confirm the resistant antibiogram of the isolates (JMI Laboratories, North Liberty, IA, USA). Reproducible isolates (113 strains) were tested by the agar dilution method for imipenem with and without BRL42715, a novel ß-lactamase inhibitor active against Bush group 1-producing organisms. A reduction in the MIC of imipenem in the presence of 4 or 8 mg/L of this inhibitor compared to the imipenem MIC alone was suggestive of elevated expression of an Amp C enzyme.

Organisms resistant to the two tested carbapenems and ceftazidime were screened for the presence of a metallo-ß-lactamase using a disk approximation method that utilized two chelators (EDTA and 2-MPA) and four ß-lactam substrates (aztreonam, ceftazidime, imipenem, meropenem). Enhanced zones of inhibition between two or more substrates and inhibitors was considered presumptive evidence of a metallo-ß-lactamase that was further confirmed by the Etest (AB BIODISK, Solna, Sweden) metallo-ß-lactamase test, and an assay for imipenem or meropenem hydrolysis performed by BCARE (Bristol, UK). A total of 11 strains were subjected to hydrolysis studies, 10 confirmed to possess a carbapenem-hydrolyzing enzyme.

PCR primer sets were selected to screen for previously recognized metallo-ß-lactamase types as described earlier. Enzyme type was confirmed by gene sequence analyses using methods published before, and the sequences (both strands) were compared to previously described GenBank accessions (example AB074437 for IMP-11). An OXA-1 enzyme was also detected in a *S. marcescens* cluster of isolates.

Carbapenem-resistant isolates (nine strains of *P. aeruginosa*) having no evidence of high Amp C expression or metallo-ß-lactamase production, were studied for alterations in the outer membrane proteins (OMP). The electrophoretic patterns were compared to control, carbapenem-susceptible strains from the SENTRY Program (Japan). Any change in the membrane preparations in the intensity or position of the bands as compared to controls was considered as positive for OMP alteration, especially the deletion of the D2 protein.

Possible clonal occurrences of carbapenem-resistant strains were studied by two epidemiologic typing methods, automated ribotyping (RiboPrinter, Qualicon, Wilmington, DE) and pulsed-field gel electrophoresis (PFGE; BioRad Laboratories, Hercules, CA) as described earlier. Two epidemic/endemic clones were recognized.

RESULTS

- Carbapenem resistance was highest (18.4%) among the 456 *P. aeruginosa*, nearly doubling in rate from 1998 to 2002. The carbapenem-resistance rates for the three other monitored organisms ranged from 1.6% (*Enterobacter* spp.) to 7.2% (*S. marcescens*).
- Three non-carbapenem, broad-spectrum ß-lactams (cefepime, ceftazidime, piperacillin/tazobactam) were also tested by reference methods against the entire collection of 802 strains (data not shown). The occurrence of resistant level MIC results for these antimicrobials was: cefepime (7.4% at > 16 mg/L); ceftazidime (15.1% at > 16 mg/L); and piperacillin/tazobactam (11.3% at > 64 mg/L). These rates compare to an imipenem resistance rate of 11.6%.
- At the individual medical centers, the carbapenem resistance rates were modestly to significantly increased among *P. aeruginosa* isolates over the study interval. The collective imipenem-resistant rates were 19.3% (range, 3.8 - 38.5%) in 1998 and 38.0% (range, 20.9 - 62.1%) in 2002. In the last year of the study, the strains that were carbapenem-resistant represented increases of 4.8, 23.6 and 35.2% in the three monitored sites over the five years.
- Strains combining resistances to carbapenems and ceftazidime were tested and 11 strains (10 confirmed) were discovered with enhanced zones of inhibition between a carbapenem or ceftazidime and either EDTA or 2-MPA.
- The 10 metallo-ß-lactamases were detected across all three medical centers (one to six per site), in all years monitored, and represented four enzymes types or combinations (Table 2). Among the *P. aeruginosa*, three IMP-1- and two IMP-2-producing isolates were detected from two medical centers. In the *S. marcescens* strains, three variations of metallo-ß-lactamases were observed, one cluster (ribotype 196-3) having IMP-1 and OXA-1 enzymes isolated in a single patient (site 206).

RESULTS

Table 1. Trends in carbapenem resistance among three monitored Japanese medical centers (SENTRY Antimicrobial Surveillance Program, 1998 - 2002).

Organism (no. tested)	% carbapenem resistance by year:[a] 1998	1999	2000	2001	2002	% all years
Acinetobacter spp. (88)	5.9	5.9	0.0	0.0	4.8	3.4
P. aeruginosa (456)	19.3	20.5	24.0	40.5	38.0	28.6
Enterobacter spp. (189)	0.0	0.0	0.0	9.1	1.9	1.6
S. marcescens (69)	10.0	9.5	0.0	0.0	8.3	7.2

a. MIC for imipenem at ≥ 8 mg/L.

Table 2. List of metallo-ß-lactamase producing strains from Japan identified in the SENTRY Antimicrobial Surveillance Program (1998-2002).

Organism/ strain no.	Medical center	Ribotype	Imipenem hydrolysis rate [k_{cat}/K_m ($\mu M^{-1}s^{-1}$)]	Metallo-ß-lactamase (year of isolation)
P. aeruginosa				
5344	205	197-4	ND[a]	IMP-2(2000)
5353	205	197-4	0.079	IMP-1(2000)
1675	206	219-4	ND	IMP-1(2001)
1677	206	197-2	ND	IMP-2(2001)
3100	206	ND	ND	IMP-1(2002)
A. baumannii				
3466	207	ND	ND	IMP-1(2002)
S. marcescens				
825	206	196-2	0.018	IMP-1(1998)
1297	205	511-8	0.387	IMP-11(1999)
3105	206	196-3[b]	0.150	IMP-1+OXA-1(2002)
3106	206	196-3[b]	0.059	IMP-1+OXA-1(2002)

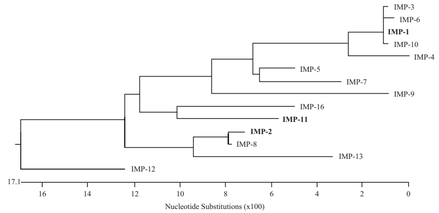
a. ND = not determined, PCR results preceded hydrolysis studies.
b. Cluster with the gene cassettes on a complex class 1 integron (gene context pending).

Table 3. Carbapenem resistance mechanisms associated with elevated imipenem and meropenem MIC values by each year of the study and isolated species.

Study year	Organism (no. tested)	Resistance mechanism (no./%)[a]
1998	*A. baumannii* (1)	Amp C (1/100.0)
	P. aeruginosa (15)	Amp C (15/100.0)
	S. marcescens (1)	MßL (1/100.0)[b]
1999	*A. baumannii* (1)	Amp C (1/100.0)
	P. aeruginosa (20)	Amp C (18/90.0)
		OMP (2/10.0)
	S. marcescens (1)	MßL (1/100.0)[c]
2000	*P. aeruginosa* (22)	Amp C (19/86.4)
		OMP (1/4.5)
		MßL (2/9.1)[a]
2001	*E. aerogenes* (1)	Amp C (1/100.0)
	P. aeruginosa (16)	Amp C (13/81.3)
		OMP (1/6.2)
		MßL (2/12.5)[d]
2002	*A. baumannii* (1)	MßL (1/100.0)[b]
	E. cloacae (1)	Amp C (1/100.0)
	P. aeruginosa (31)	Amp C (25/80.6)
		OMP (5/16.1)
		MßL (1/3.3)[b]
	S. marcescens (2)	MßL (2/100.0)[e]

a. Amp C = hyper-expression of Amp C enzyme; MßL = metallo-ß-lactamase; OMP = outer membrane protein alteration compared to site-specific controls.
b. IMP-1.
c. IMP-11.
d. IMP-1 and IMP-2.
e. IMP-1 and OXA-1-type combination in each strain (same ribotype, 196-3 [see Table 2]) from the same patient.

Figure 1. Phylogenetic chart displaying the relationship between members of the IMP family of MßL enzymes. Bolded enzyme types were discovered in this survey.



- The phylogenetic relationships of the three encountered IMP enzymes are shown in Figure 1. The bla_{IMP-11} differs by 22 and 30 positions (nucleotide changes) when compared to bla_{IMP-2} and bla_{IMP-1}, respectively.
- The occurrence of metallo-ß-lactamases among the entire collection of Japanese strains was 10 in 802 isolates (1.1%; Tables 1 and 2). This compares favorably with two earlier multi-center Japanese investigations (22 sites) that observed metallo-ß-lactamase rates of 0.5 - 0.9%. However, among the tested carbapenem-resistant strains, 10.8% of these organisms had a metallo-ß-lactamase. The medical center with the highest carbapenem resistance rate was also the location of the largest number (six) of metallo-ß-lactamase-producing strains (site 206).
- The use of OMP preparations and an Amp C inhibitor suggested several mechanisms were responsible for the resistances to the carbapenems and other ß-lactam agents. The dominant resistance mechanism was elevated Amp C expression with or without OMP changes (103 of 113 screens; 91.2%), see Table 3. Overall, OMP alterations (D2 protein) as the only detected resistance mechanism occurred more rarely in *P. aeruginosa* strains (8.7%).

CONCLUSIONS

- Gram-negative bacilli with bla_{IMP} have a long history in Japan and recently the bla_{VIM-2} has been described usually on class 1 integrons that also possess *aacA4* gene cassettes. Our results, however, failed to detect VIM-2 in Japan, but did validate the IMP-type enzymes in *P. aeruginosa* (five occurrences), *S. marcescens* (four occurrences) and *A. baumannii* (one occurrence).
- The first documented case of bla_{IMP-11} was also discovered from a patient isolate in 1999, and a unique bla_{IMP-1} + bla_{OXA-1} combination was detected in a cluster of *S. marcescens* cases in 2002.
- All monitored sites experienced increasing carbapenem resistance throughout the study (1998 - 2002) and each center contributed at least one metallo-ß-lactamase case.
- The screening methods used and double disk or Etest (AB BIODISK) confirmation tests were sensitive and specific for detecting metallo-ß-lactamases of the IMP-type in Japan. The lowest level of resistance was noted for the "fourth-generation" cephalosporin, cefepime with only 7.4% resistance (MIC, > 16 µg/ml) compared to resistance rates of 11.3 and 15.1% for piperacillin/tazobactam and ceftazidime, respectively. Clearly the incidence of metallo-ß-lactamases in Japan has modified the rank order of ß-lactams for empiric therapy reducing the role of parenteral carbapenem agents and ceftazidime.
- Because of the rapid emergence of metallo-ß-lactamase types and dissemination geographically or between species, focused resistance surveillance initiatives should be expanded to include local monitoring of multi-drug-resistant Gram-negative bacilli.

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