

## SPREAD OF METALLO- $\beta$ -LACTAMASES: SCREENING REVEALS THE PRESENCE OF A $bla_{SPM-1}$ GENE IN HOSPITAL SEWAGE IN SOUTHERN BRAZIL

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Submitted: November 09, 2007; Returned to authors for corrections: March 15, 2008; Approved: February 15, 2009.

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### ABSTRACT

Of 396 *Pseudomonas aeruginosa* strains isolated from hospital sewage, the  $bla_{SPM-1}$  gene was confirmed in nine. This is the first report of environmental *P. aeruginosa* strains carrying the  $bla_{SPM-1}$  gene in Brazil. The carbapenem resistance, already disseminated among clinical isolates, has been detected among environmental isolates.

**Key words:** Metallo- $\beta$ -Lactamases, hospital sewage, *P. aeruginosa*, bacterial resistance

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Metallo- $\beta$ -lactamases (MBLs) are emerging worldwide as a source of acquired carbapenem resistance in Gram-negative bacteria, especially *Pseudomonas aeruginosa*, *Acinetobacter* sp. and Enterobacteriaceae. A particular concern is that acquired MBL genes are located on integron structures that reside on mobile genetic elements such as plasmids or transposons (17), thus enabling widespread dissemination. The emergence of these enzymes drastically compromises effective treatments of infections by these microorganisms, since MBLs are capable of hydrolyzing most  $\beta$ -lactams, including carbapenems, and are not susceptible to inhibitors.

During the last few years, several MBL-producing bacteria have been reported in Brazil (4,6,7,10,12,15). These isolates were found almost exclusively in hospital settings. Recently, a *Pseudomonas pseudoalcaligenes* VIM-2 strain was isolated outside hospital boundaries, from hospital sewage. This finding suggests that the ongoing spread of the  $bla_{VIM-2}$  is occurring simultaneously in several dimensions, since it can now be found in different environments and in several bacterial species (13). Another study revealed the presence of  $bla_{VIM-2}$  in two unrelated *P. aeruginosa* strains from aquatic environments (14).

The release of antibiotic-resistant bacteria into the community is a particular concern, since they might proliferate in soil and surface water, persist and spread in different

environments, and transfer antibiotic-resistance genes among different species (3). However, the role and the presence of outside hospital niches acting as a reservoir for bacteria that carry acquired MBLs genes is still poorly established, and there is a need for further evaluation. Because the  $bla_{SPM-1}$  MBL gene is the most prevalent in Brazil, its presence was evaluated in *P. aeruginosa* isolates from hospital sewage and surface-water samples, in order to obtain epidemiological data on the spread and dissemination of this gene in environmental samples in southern Brazil. This is the first report of environmental *P. aeruginosa* strains carrying a  $bla_{SPM-1}$  like gene.

Sewage samples were collected from the Hospital São Vicente de Paulo (HSVP), located in Passo Fundo, Rio Grande do Sul (RS), Brazil, and the Hospital Divina Providência (HDP), located in Porto Alegre, RS, Brazil. Samples were also collected from waterbodies upstream and downstream of the hospitals' sewage discharge outlets (Table 1). Samples of 1 L of surface water or hospital sewage were collected in sterile bottles and stored at 4°C until processing. Aliquots of 100 mL were filtered on membranes of mixed esters of 0.45  $\mu$ m porosity. The membranes were then transferred to the selective asparagine broth. The appearance of green fluorescence under ultraviolet light was considered a positive result. Aliquots of 100  $\mu$ l from the positive tubes were transferred to acetamide broth and subsequent

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**Table 1.** Description of sampling points, and numbers of isolates recovered from the different points of Hospital São Vicente de Paulo (HSVP) and Hospital Divina Providência (HDP).

HSVP			HDP		
Sample point	Number of isolates	Description	Sample point	Number	Description of isolates
A	0	Passo Fundo River near its source, 11,5 Km from HSVP	G	56	Hospital sewage
B	53	Passo Fundo River 800 m upstream of hospital sewage discharge	H	50	Hospital sewage
C	18	Passo Fundo River 600 m downstream of hospital sewage discharge	I	44	Gruta Streamlet
D	42	Hospital sewage	J	31	Cascata Arroyo downstream of hospital sewage discharge
E	44	Hospital sewage	K	17	Cascata Arroyo upstream of hospital sewage discharge
F	41	Hospital sewage			
<b>Total</b>	<b>198</b>		<b>Total</b>	<b>198</b>	

isolation in acetamide agar, for selection of the characteristic colonies. Biochemical tests and amplification of 16S rDNA were used to identify all *P. aeruginosa* strains (16). Susceptibility was determined by the disk-diffusion method according to CLSI guidelines (5). The isolates were screened for MBL production using the 2-mercaptopyruvic acid double-disk potentiation method and the imipenem-EDTA double-disk synergy test (1, 19). The MBL Etest (AB Biodisk, Solna, Sweden) was used as a further test of MBL production. The MBL Etest was only done with the isolates that showed reduced susceptibility to imipenem and/or meropenem. The presence of the bla<sub>SPM-1</sub> gene was determined by polymerase chain reaction (PCR) with the following pair of primers: SPMF (5'-TCG GAT CAT GTC GAC TTG CC-3') and SPMR (5'-CCT TCG CTT CAG ATC CTC GT-3'). *P. aeruginosa* SPM-1 producer was used as a positive control in PCR amplification reactions. The PCR fragments were confirmed by sequencing.

A total of 198 *P. aeruginosa* strains were recovered from HSVP (Table 1). Among these, 78 isolates showed reduced susceptibility to imipenem and/or meropenem, and were submitted to the MBL Etest, where 7.8% (n= 6) of the isolates showed positive results. From HDP, 198 isolates were recovered (Table 1). Eleven isolates showed reduced susceptibility to imipenem and/or meropenem, and were also submitted to the MBL Etest. Of these, 27.3% (n=3) showed positive results, including H9, H11 and H12 strains. All isolates were susceptible to polymyxin B. The H8, H9, H11, H12 and H13 strains from HDP were resistant to all antimicrobials tested, except for piperacillin-tazobactam, aztreonam and polymyxin B. The bla<sub>SPM-1</sub> gene was detected by PCR amplification among four strains from the HSVP (strains

D30, F3, F7 and F20). Five isolates (strains H8, H9, H11, H12 and H13) from Point H hospital sewage were confirmed with the 344 bp fragment of bla<sub>SPM-1</sub> gene in HDP. Sequence analysis of the fragments showed 100% identity with the bla<sub>SPM-1</sub> gene of *P. aeruginosa* (accession number DQ145284). The accession number for sequences of the PCR fragments from H8, H9, H11, H12 and H13 strains, are FJ197850, FJ197851, FJ197852, FJ197853 and FJ197854, respectively. Great variability was seen in the resistance profile among the different isolates from hospital sewage and surface water. The *P. aeruginosa* isolates from HSVP showed imipenem and/or meropenem resistance in samples taken at all points, including Passo Fundo River points B and C. Point C isolates averaged 55.5% imipenem and/or meropenem resistance, but no multiresistant strains were detected (Table 2). The high percentage of carbapenem resistance observed in a strain from Point C suggests that selection has occurred in this environment; it possibly originated from the HSVP activities, since this point is located downstream from the HSVP sewage discharge. Point B averaged 22.6% carbapenem resistance. Although this point is located upstream from the HSVP sewage outlet, it seems that this area receives hospital sewage and also domestic effluents. This type of resistance may indicate the degree of selection exerted by the indiscriminate use of antibiotics in a community setting. The domestic use of antimicrobials has expanded, and may be exercising enough selection pressure to permit the appearance of resistance profiles, therefore confirming the problem of resistance in the community (2).

It is generally assumed that antibiotic use has a significant impact on bacterial resistance rates. Lepper *et al.* (2002) demonstrated that the consumption of imipenem was correlated

**Table 2.** Susceptibility results from *P. aeruginosa* isolates from Hospital São Vicente de Paulo (HSVP) and Hospital Divina Providência (HDP).

	HSVP sample points (n° isolates / %)					HDP sample points (n° isolates / %)				
	B	C	D	E	F	G	H	I	J	K
<i>Susceptibility to all antimicrobials tested</i>	41/77.3	8/44.4	34/80.9	29/65.9	4/9.7	37/66	40/80	41/93.2	28/90.3	15/88.2
<i>Resistance to IPM and/or MER</i>	12/22.6	10/55.5	6/14.3	15/34	30/73.2	1/1.8	9/18	1/2.3	0/0	1/5.9
<i>Resistance to at least four antimicrobial classes</i>	0/0	0/0	2/4.8	0/0	32/78	0/0	6/12	0/0	0/0	0/0

IPM = Imipenem, MER = Meropenem.

with  $\beta$ -lactam resistance in *P. aeruginosa*, in a hospital setting. This behavior must have also occurred in the community setting of the present study. The percentage of carbapenem resistance from HDP was much lower than HSVP, and the number of multiresistant strains was also lower (Table 2). This difference may be a function of the smaller size of the HDP, which accepts approximately 9,000 internments per year; the HSVP accepts around 28,000 internments. Recently, clinical isolates of *P. aeruginosa* producing SPM-1 were reported in the São Lucas Hospital (SLH) and the Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil (19). It is important to note that the five isolates with positive SPM-1 results found in the present study (strains H8, H9, H11, H12 and H13) were collected from locations that are geographically distant from the health institutions mentioned by Zavascki *et al.* (2006), and that there was no physical link between SLH, HCPA and HDP. Nevertheless, the presence of the *bla*<sub>SPM-1</sub> gene in the five strains found in the present study suggests that genes carrying antibiotic resistance associated with the clinical setting might be continuously contaminating the community environment through wastewater discharge. High rates of carbapenem resistance were found in *P. aeruginosa* strains from this study, especially in HSVP isolates (Table 2). Many isolates showed a positive MBL screening test, but did not have the *bla*<sub>SPM-1</sub> gene. Because they were previously detected in clinical isolates of *P. aeruginosa* in Porto Alegre, RS, the genes *bla*<sub>IMP-1</sub> and *bla*<sub>VIM-1</sub> were also investigated by PCR; however, neither of these was detected in the present study (7,11). Therefore, these isolates may be carrying another type or another allele variant of the MBL gene, or may even show another mechanism of resistance to the carbapenems.

It is noteworthy that poor sanitation and proper care with hospital effluents is not observed, and these effluents are often discharged completely untreated into waterbodies. These waterbodies receiving effluents containing strongly selected bacteria can constitute an important route of transfer of multiresistance between hospital (high-selection compartments) and the community.

This study is the first report of an environmental *P. aeruginosa* strain carrying the *bla*<sub>SPM-1</sub> gene in Brazil. It now

seems clear that this type of carbapenem resistance has already crossed the hospital boundaries. Reducing the release of bacteria or genetic elements from the clinical setting into the community is becoming a critical issue, to avoid the buildup of environmental reservoirs of antibiotic resistance. Not only is the case of transfer of genetic resistance elements in the environment a reminder for proper hygiene, but it is also illustrates the importance of reducing the use of antimicrobial agents so as decrease the level of antimicrobial resistance among bacteria. There is general agreement that the pool of resistance genes in the environment is amplified by the use of antimicrobial agents (8). Minimizing the use of antimicrobial agents will reduce the risk of spread of resistance factors in the environment, securing the continuous benefit of antimicrobial drugs.

#### ACKNOWLEDGMENTS

Our thanks to Dr. Ana Cristina Gales (*Laboratório Alerta and Laboratório Especial de Microbiologia Clínica, Division of Infectious Diseases, Universidade Federal de São Paulo*) who kindly provided the *Pseudomonas aeruginosa* used as the positive control in this study. We are also grateful to CAPES-PROF for financial support.

#### RESUMO

##### **Disseminação de Metallo- $\beta$ -Lactamases: Triagem revela a presença do gene *bla*<sub>SPM-1</sub> em efluente hospitalar no Sul do Brasil**

Ao todo, 396 isolados de *Pseudomonas aeruginosa* foram estudados. O gene *bla*<sub>SPM-1</sub> foi encontrado em nove isolados de efluente hospitalar. Este estudo é o primeiro relato de isolados ambientais de *P. aeruginosa* com o gene *bla*<sub>SPM-1</sub> no Brasil. A resistência aos carbapenêmicos, amplamente disseminada entre isolados clínicos, já é detectada em isolados ambientais.

**Palavras-chave:** Metallo- $\beta$ -Lactamases, efluente hospitalar, *P. aeruginosa*, resistência bacteriana

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