

Detection of *Pseudomonas aeruginosa* producing metallo- β -lactamase VIM-2 in a central hospital from Portugal

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Pseudomonas aeruginosa remains one of the most important pathogens in the nosocomial setting [1]. *P. aeruginosa* exhibits intrinsic resistance to several antimicrobial agents. The antipseudomonal β -lactams represent a major weapon against *Pseudomonas* infections, either for monotherapy or for combination therapy, for which β -lactams almost invariably represent one of the components. Therefore, resistance to these agents constitutes a major challenge for anti-*Pseudomonas* chemotherapy. Several mechanisms can contribute to β -lactam resistance in *P. aeruginosa*, including β -lactamase production, outer membrane impermeability and active efflux mediated by RND-type efflux systems [1]. During the last decade, the metallo- β -lactamases (MBLs) have emerged as new threatening mechanisms of broad-spectrum β -lactam resistance in *P. aeruginosa*. In fact, these enzymes can efficiently degrade virtually all antipseudomonal β -lactams (except aztreonam), while they are not susceptible to therapeutic β -lactamase inhibitors [2]. Based on amino acid sequence homology, these MBLs have been classified into four major types: IMP, VIM, SPM

and GIM. Clinical isolates harbouring the MBLs IMP and VIM have been increasingly reported worldwide, mostly in European and Asian countries [2]. This increase in occurrence, types and rate of dissemination of MBLs makes early detection very critical. The benefits of such treatment include the timely implementation of strict infection control practices, as well as clinical guidance.

The aim of this study was to determine the presence of these enzymes in imipenem-resistant *P. aeruginosa* isolates collected at the Centro Hospitalar of Coimbra (CHC), during a two-year period (April 2003 to April 2005) and to ascertain their clonal relationship. CHC is a cluster formed by a central hospital and several specialised units located at distances of less than 8 km, namely, Hospital dos Covões (central hospital), Instituto Maternal (maternity hospital) and Hospital Pediátrico (paediatric hospital). The Microbiology Laboratory also analysed samples from another hospital (Hospital de Pombal) located 30 km away.

Bacterial identification and minimal inhibitory concentrations (MIC) were performed with the MicroScan Walk-Away (DadeBehring) system according to the instructions of the manufacturer and API32GN or API PSE systems (bioMérieux). All intermediate strains were considered as nonsusceptible strains. The MICs of β -lactams were also determined by E-test in isolates that presented the enzyme VIM-2. The results were interpreted on the basis of CLSI recommended breakpoints [3]. The screening of MBLs was done by the double combined disk test [4]. Polymerase chain reaction (PCR) analyses for the detection of MBLs genes (bla_{IMP} , bla_{VIM} , bla_{GIM} and bla_{SPM-1}) were carried out for all strains in which the screening test gave positive results [5]. The amplicons were directly sequenced on both DNA strands on an ABI PRISM 377 automated sequencer. The nucleotide and deduced amino acid sequences were analysed with software available online (<http://blast.ncbi.nlm.nih.gov/>

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Blast.cgi). VIM-2-producing isolates were typed by random amplified polymorphic DNA (RAPD) with primer 272 [5]. Samples with the same banding pattern were assigned a letter indicating a single RAPD type. Those with no more than one major band or three minor band differences were thought to be related and great differences in the banding patterns were considered to be a different pattern [5].

On the basis of resistance phenotype (resistance or intermediate susceptibility to imipenem), 134 isolates were selected and studied for MBL production. The susceptibility of these *P. aeruginosa* isolates relative to β -lactams antibiotics was: ceftazidime, 56.7%; piperacillin plus tazobactam, 47.4%, and aztreonam, 37.3%. Meropenem still demonstrated some activity (20.3%). Among these imipenem-resistant isolates, 3.7% were characterised by multidrug resistance, including all of the tested drugs. In our study, carbapenem resistance rates did not correlate with the presence of VIM-2, as 80.6% of the strains were resistant to imipenem without MBL production. This demonstrates that other resistance mechanisms were involved, such as permeability mutations via the loss of porins or the up-regulation of efflux systems [1].

The combined disk method was positive in 40 imipenem-resistant isolates, but by PCR, the presence of an amplicon of 815 pb in size (detection of *bla*_{VIM}) was observed in only 26 isolates. No amplification was observed with *bla*_{IMP}, *bla*_{SPM} and *bla*_{GIM} primers. DNA sequencing of the amplicons confirmed that these 26 isolates harboured *bla*_{VIM-2}. MBL-producing gram-negative organisms have now been reported in many geographic regions [2]. The MBL found in our hospital is VIM-2, which is scattered in several European countries, namely, France, Italy and Greece [2]. The *bla*_{VIM}-positive isolates were detected during the time of the study and appeared to be widely distributed in the central hospital, and even outside of it. *bla*_{VIM}-positive *P. aeruginosa* represented 19.4% of the carbapenem-resistant *P. aeruginosa* isolates. These numbers are lower than those reported for MBL producers from a SENTRY Antimicrobial Surveillance Programme [6], but exceed other reports from Italy [7]. Among these isolates, VIM-2 producers' MICs were determined by E-test and the results are presented in Table 1. Resistance to all of the tested drugs was observed in 15.4% of these isolates. Furthermore, the majority of the multidrug-resistant isolates

Table 1 Minimal inhibitory concentrations (MICs) of β -lactams of VIM-2-producing *Pseudomonas aeruginosa* and respective random amplified polymorphic DNA (RAPD) types

Isolate no.	MIC (mg/L)					Ward/hospital	RAPD type
	IP	MP	CAZ	AZT	PIP		
17735/03	>32	32	24	3	16	Neurosurgery/Hosp. Covões	A
17872/03	>32	>32	24	3	8	Orthotraumatology/Hosp. Covões	A
14593/03	>32	32	>256	16	>256	Reanimation/Hosp. Covões	A
17000/03	>32	>32	32	3	24	Reanimation/Hosp. Covões	A
19210/03	>32	>32	24	3	24	Reanimation/Hosp. Covões	A
1891/04	>32	>32	2	3	24	Reanimation/Hosp. Covões	A
12321/04	>32	>32	32	3	16	Reanimation/Hosp. Covões	A
18441/03	>32	6	16	2	16	Medicine/Hosp. Covões	A
712/04	>32	>32	24	3	16	Medicine/Hosp. Covões	A
1825/04	>32	>32	32	256	32	Medicine/Hosp. Covões	A
3647/04	>32	>32	32	2	16	Medicine/Hosp. Covões	A
4584/04	>32	>32	32	2	24	Medicine/Hosp. Covões	A
6741/04	>32	>32	24	16	24	Medicine/Hosp. Covões	A
1801/04	>32	4	32	2	16	Pneumology/Hosp. Covões	A
14349/04	>32	3	16	2	12	Pneumology/Hosp. Covões	A
21800/04	>32	>32	32	4	16	Pneumology/Hosp. Covões	A
429/05	>32	>32	32	6	32	Pneumology/Hosp. Covões	A
17053/04	>32	8	32	1,5	32	Reanimation/Hosp. Covões	A1
541/05	>32	>32	16	3	32	Pneumology/Hosp. Covões	A1
15153/04	>32	>32	32	>256	>256	Medicine/Hosp. Pombal	B
18769/04	>32	>32	24	64	>256	Medicine/Hosp. Pombal	B
5783/03	>32	3	24	1	48	Infectious/Hosp. Covões	C
11874/04	>32	>32	>256	96	>256	Infectious/Hosp. Covões	D
17083/03	>32	>32	64	3	48	Infectious/Hosp. Covões	D
7147/03	>32	4	2,5	3	4	Medicine/Hosp. Paediatric	E
19168/03	>32	>32	64	2	16	UIC/Hosp. Paediatric	F

IP=imipenem; MP=meropenem; CAZ=ceftazidime; AZT=aztreonam; PIP=piperacillin

were producers of VIM-2, which agrees with other studies carried out in Italy that described 76% VIM-2-producing *P. aeruginosa* clinical isolates with resistant phenotype [8].

In this work, *P. aeruginosa* isolates that produce VIM-2 were detected in six different wards of Hospital dos Covões and in another two geographically distinct hospitals (Hospital Pediátrico and Hospital de Pombal). Six genotypically different strains were identified (Table 1). Pattern A comprised 17 isolates (65.4%) that were disseminated in five wards of the central hospital. Subpattern A1 was constituted of two isolates closely related to pattern A, suggesting that a small mutation occurred in isolate A, creating the former pattern. These results demonstrated that we had the same strain harbouring VIM-2 disseminated in various wards of the central hospital. This reality was different from another study carried out in Greece where the VIM-2 isolates were unrelated [9]. Pattern B represented two isolates of Hospital de Pombal, a geographically distinct hospital. In the Infectious ward, two samples of *P. aeruginosa* with identical patterns classified as D and another isolate classified as pattern C were isolated. This diversity suggested the spread of the *bla*_{VIM-2} gene among genetically distinct *P. aeruginosa* strains [9, 10]. In this ward, clone A was not found, suggesting that the prevention measures and control of the spread of pathogen are more effective in this ward. The remaining two isolates were identified as patterns E and F, respectively, and were recovered from the Medicine ward and the Unit of Intensive Care of Paediatric Hospital, another geographically distinct hospital.

In our study, MBL-positive strains had a common multidrug-resistant phenotype that not only included carbapenems, but also last-generation cephalosporins, aminoglycosides and fluoroquinolones. The possibility that spreading transferable MBL genes among nosocomial gram-negative pathogens could emerge as a major problem in the clinical setting reinforces the need for the systematic surveillance of these resistance determinants. The early detection of MBL-carrying organisms is of paramount clinical importance, as it allows the rapid initiation of strict infection control practices, as well as the selection of appropriate antimicrobial agents.

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