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Emergence of metallo-β-lactamases in *Acinetobacter* spp clinical isolates from Argentina

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The class B metallo carbapenem-hydrolyzing ß -lactamases (MBLs) are enzymes of great concern because they possess activity against all ß -lactams, except monobactams. Up to now, several members of this family of enzymes have been described (IMP, VIM, SPM-1, GIM-1, SIM-1)[1]. Most of VIM and IMP genes usually reside within class 1 integrons as gene cassettes. Due to the ability to confer broad-spectrum ß -lactam resistance, the unavailability of clinically useful inhibitors, and their potential for rapid and generalized dissemination, infections produced by MBL-possessing bacteria are a matter of great concern worldwide. In Argentina, the undesirable emergence of MBLs was previously documented among *Pseudomonas aeruginosa*[2] .

We are presenting here the first two *Acinetobacter* isolates producing MBL in Argentina. The first isolate (M7978) was obtained in October 2006 from a bronchoalveolar aspirate of an 8-m-o girl in the Hospital de Pediatría "Prof. Dr. Juan P. Garrahan", a reference hospital in Buenos Aires. Also the strain was considered as colonizing, was further studied for epidemiological purpose. It was initially characterized as belonging to the *Acinetobacter lwoffii/junii* complex by API NE and Vitek systems (Biomérieux Argentina). Using the biochemical tests recommended by Bouvet and Grimont[3] the specie could not be identified. Partial sequencing (800 bp) analysis of the 16S rRNA gene of the isolate using the primer F8-27 5'GAGTTTGATCCTGGCTCAG3' was performed[4] .The sequence was compared with published ones in the GenBank database using the BLASTN algorithm. The nucleotide sequence of the amplified fragment identified the organisms as *Acinetobacter junii* (100% identity) (GenBank accession no. FJ384997)

By the disk diffusion method (CLSI), this isolate was susceptible to aztreonam, sulbactam-ampicillin, tazobactam-piperacillin, ciprofloxacin, gentamicin, and minocycline. It was

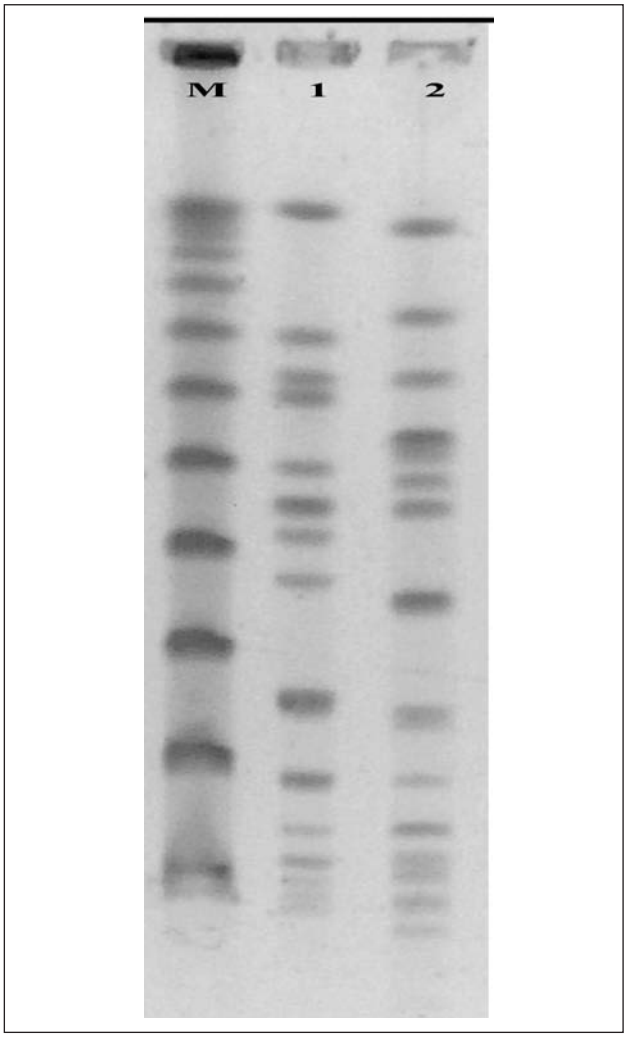

Figure 1 PFGE profiles of XbaI-digested whole-cell DNA of both *A. junii*. Lane M, XbaI-*Salmonella* Braenderup ladder; lanes 1–2, clinical isolates of IMP-1-producing *A. junii* (7978 and 9013, respectively).

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resistant to amikacin, trimethoprim-sulfamethoxazole, penicillins, cephalosporins and carbapenem. A synergy between EDTA/SMA[5] and imipenem and meropenem disks was also observed. This feature was suggestive of the presence of MBL. Phenotypic ESBL screening with ceftacidime/clavulanic and cefotaxime/clavulanic disks was also positive. Agar dilution MICs (CLSI) of meropenem and imipenem were 32 mg/L and were reduced to 8 mg/L and 4 mg/L, respectively, by adding 0.4 mM EDTA to the test (table 1).

Because of the emergence of an MBL-producing *Acinetobacter* strain, a retrospective screening with EDTA/SMA discs was conducted to assess the MBL status among *Acinetobacter* isolates in our hospital, prior to WHONET-Argentina Network mandatory surveillance. Among the 186 preserved clinical strains of *Acinetobacter* spp. isolated during 2005, a second isolate (M9013) that displayed EDTA/SMA and carbapenem discs synergy was recognized. M9013 was obtained from the urinary tract of a 14-years old boy undergoing imipenem therapy and was identified as *A. lwoffii/junii* complex by both, API NE and Vitek System. Biochemical scheme[3] as well as sequence analysis of the 16S rRNA gene (840 bp) identified M9013 as *A. junii.* (100% identity) (GenBank accession no. FJ392119)

M9013 showed by disk diffusion a different resistance profile that M7978: it remained susceptible to carbapenem, aztreonam, sulbactam-ampicillin, piperacillin, tazobactam-piperacillin, cefepime, gentamicin and minocycline, and was resistant to amikacin, trimethoprim-sulfamethoxazole, ampicillin, cephalotine and ceftazidime. M9013 ESBL screening was also positive, as in M7978. MICs of carbapenem confirmed imipenem susceptibility (4.0 mg/L) but showed intermediate susceptibility to meropenem (8.0 mg/L). These MICs were reduced to 0.5 mg/L and 1 mg/L, respectively, by adding EDTA (table 1).

Crude ß-lactamase extracts of M7978 and M9013, showed significant carbapenem hydrolysis in a bioassay method, confirming the enzymatic nature of carbapenem resistance[5]. IEF showed that the clinical isolates produce two active bands at pI 6,9 and >9.0 (table 1)[6]. MBL were identified in both strains as belonging to the IMP family by PCR, using primers previously described[6] (VIM and SPM families had been rule out by the same method). Sequencing the complete gene defined a bla_{IMP-1}. A PCR assay with primers 5´-CS/3´-CS[5] resulted in a unique amplicon of 2.0 kb that carried the $bla_{IMP\ 1}$ gene and *aadA5* cassettes once sequenced. Screening by PCR for other commonly found carbapenemases in *Acinetobacter*[6], such as oxacillinase (table 1) revealed the presence of OXA-58 in both strains, but not the OXA-51 typically of *A. baumannii.* PCR screening for commonly found ESBLs genes (TEM, VEB, PER, SHV, CTX-M)[2,6] was also performed on both isolates due to the positive phenotypic ESBL screening. No amplicons were obtained. As well, neither extra IEF active bands reminding ESBLs were visualized by IEF. Thus, clavulanate (alone) MIC was performed. Susceptibility to clavulanate (MICs 2 mg/L) was observed on both isolates and could give reason for false positive phenotypic disc-based ESBL tests[7].

Table 1 **Antimicrobial susceptibly (MICs in mg/L), IEF of ß-lactamases, and PCR of antimicrobial resistance determinants in *bla*IMP producing *Acinetobacter***

	M7978	M9013
i) MICs		
Ampicillin-sulbactam	0.5	0.5
Ceftazidime	256	256
Cefepime	>256	16
Aztreonam	8	4
Imipenem	32	4
Imipenem/EDTA†	4	0.5
Meropenem	32	8
Meropenem/EDTA†	8	1
Clavulanate	2	2
ii) IEF (pI)§	6.9 + 9.0	6.9 + 9.0
iii) PCR		
bla_{IMP}	+	+
bla_{SPM}	-	-
bla_{VIM}	-	-
bla_{VEB}	-	-
bla_{TEM}	-	-
bla_{PER-2}	-	-
bla_{OXA-51}	-	-
bla_{OXA-58}	+	+

† 0.4mM final concentration

Molecular typing by pulsed-field gel electrophoresis (PFGE) (DNA digested with *Xba*I) illustrated that the bla_{IMP-1}-producing isolates belong to different profiles, ruling out nosocomial spread of *A. junii* MBL-producer in the Institution (figure 1)[8] .Several attempts to transfer the carbapenem resistant trait of both *Acinetobacter* isolates to an ampicillin susceptible *Escherichia coli* were unsuccessful.

This the first report of a MBL found in *Acinetobacter* spp. in Argentina. In addition, these are the first MBLs detected in our institution. Mean mostly of MBL plague in Argentina is due to VIM-producing strains, unexpectedly, emergence in *Acinetobacter* harbored a bla_{IMP} MBL. Additionally to MBL, a second carbapenem-hydrolyzing enzyme (OXA-58) was detected in both strains. Of concern, even in the presence of these two carbapenemases, isolate M9013 remained susceptible to carbapenem, in particular imipenem. Phenotypic test were also helpless in ascertain the indisputably ESBLs status of the isolates: *Acinetobacter* spp. could frequently present clavulante susceptibility, thus obscuring clavulanate based ESBL screening tests[7] .

Hence, the emergence of MBL-producing *Acinetobacter* in Argentina constitutes a public health concern. We would like to underline the importance of searching for the

presence of these enzymes to accurately guide and preserve the antimicrobial armamentarium.

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