

# VIM-1 Metallo- $\beta$ -lactamase in *Acinetobacter baumannii*

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In 2004 and 2005, 5 metallo- $\beta$ -lactamase (MBL)-positive *Acinetobacter baumannii* isolates were found in 2 Greek hospitals. Isolates were unrelated and carried *bla*<sub>VIM-1</sub> in a class 1 integron; *bla*<sub>OXA-51</sub>- and *bla*<sub>OXA-58-like</sub> carbapenemase genes were also detected. VIM-1 MBL in *Acinetobacter* spp. causes concern, given the increasing resistance of this species.

In the last few years, resistance to antibacterial drugs has been increasing in *Acinetobacter* spp., which will likely become a substantial treatment challenge in the future (1). Carbapenems have potent activity against *Acinetobacter* spp. and are usually the drugs of choice against multidrug-resistant *Acinetobacter baumannii* isolates. *Acinetobacter* spp. may develop resistance to carbapenems through various mechanisms, including class B and D carbapenemase production, decreased permeability, altered penicillin-binding proteins, and rarely, overexpression of efflux pumps (2,3).

In Europe, carbapenem resistance in *A. baumannii* has been sporadically attributed to the production of IMP-type metallo- $\beta$ -lactamases (MBLs) and OXA-type carbapenemases (4). VIM-2-producing *Acinetobacter* spp. have been isolated in the Far East (4,5) and on 1 occasion in Germany (6). In this study, we report the appearance of the VIM-1 MBL determinant among *A. baumannii* in Greece.

## The Study

We included in the study *A. baumannii* clinical isolates from tertiary care hospitals in 2 different Greek regions (Hippokration University Hospital, Thessaloniki, and University Hospital of Larissa, Thessalia) that were positive by the imipenem-EDTA double-disk synergy test (DDST) from March 2004 to March 2005. Bacteria were provisionally identified to the genus level by the Vitek 2 automated system (bioMérieux, Marcy l'Étoile, France)

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and the ATB 32GN system (bioMérieux). Antimicrobial drug susceptibility testing of the DDST-positive isolates for  $\beta$ -lactams (aztreonam, ceftazidime, cefepime, imipenem, meropenem, and piperacillin),  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations (ampicillin/sulbactam, piperacillin/tazobactam), aminoglycosides (amikacin, gentamicin, netilmicin, and tobramycin), fluoroquinolones (ciprofloxacin and ofloxacin), and colistin was performed by Etest and Etest MBL (AB Biodisk, Solna, Sweden). The Clinical and Laboratory Standards Institute (CLSI) interpretative criteria were used (7), and *Pseudomonas aeruginosa* ATCC 27853 was used as control.

Polymerase chain reaction (PCR) testing of the synergy-positive isolates for carbapenemase genes was done by using consensus primers for *bla*<sub>IMP</sub> (8), *bla*<sub>VIM</sub> (9), *bla*<sub>SPM</sub> (10), *bla*<sub>OXA-23-like</sub> (11), *bla*<sub>OXA-24-like</sub> (11), *bla*<sub>OXA-58-like</sub> (11), and *bla*<sub>OXA-51-like</sub> (12). Pulsed-field gel electrophoresis (PFGE) of *Apa*I-digested genomic DNA was performed in the *bla*<sub>VIM-1</sub>-positive isolates, and the banding patterns were compared by using criteria proposed by Tenover et al. (13). The potential for conjugational transfer of imipenem resistance was examined in filter matings by using *Escherichia coli* 20R764 (*lac*<sup>+</sup> *rif*<sup>r</sup>) as the recipient. Donor and recipient were mixed in a 1:5 ratio, and transconjugants were selected on MacConkey agar plates containing 100  $\mu$ g/mL rifampicin and imipenem at concentrations of 0.5 to 2  $\mu$ g/mL or 2  $\mu$ g/mL ceftazidime.

Five *A. baumannii* clinical isolates that were MBL producers on the basis of DDST were detected among collections of isolates from patients hospitalized during the study period. Two of the isolates were recovered from blood cultures, 1 from bronchial secretions, 1 from a urine specimen, and 1 from cerebrospinal fluid; 2 isolates that had reduced susceptibility to carbapenems were not positive by the Etest MBL (Table). Imipenem MICs ranged from 4 to >32  $\mu$ g/mL, while meropenem MICs were 2–32  $\mu$ g/mL. *P. aeruginosa* ATCC 27853 was consistently characterized as having imipenem MIC of 2  $\mu$ g/mL and meropenem MIC of 0.5  $\mu$ g/mL. The 5 *A. baumannii* isolates were multidrug resistant; they showed resistance to all other antimicrobial drugs tested, with the exception of colistin.

We did not detect *bla*<sub>IMP</sub>, *bla*<sub>SPM</sub>, *bla*<sub>OXA-23-like</sub>, or *bla*<sub>OXA-24-like</sub> in any of the 5 isolates, whereas *bla*<sub>VIM</sub> was detected in all of them. In 2 isolates, *bla*<sub>OXA-51</sub>- and *bla*<sub>OXA-58-like</sub> genes were also simultaneously present, while 2 more carried a *bla*<sub>OXA-51-like</sub> gene (Table). By sequencing both strands of the entire *bla*<sub>VIM</sub> amplicons (14), a *bla*<sub>VIM-1</sub> sequence identical to that available in the database was identified. Sequencing *bla*<sub>OXA-51-like</sub> amplicons identified *bla*<sub>OXA-66</sub> in all cases, while *bla*<sub>OXA-58-like</sub> alleles were classical *bla*<sub>OXA-58</sub> in both cases. In 2 isolates, 1 from each region, PCR mapping of the integron that possibly carried *bla*<sub>VIM-1</sub>, with primers 5' CS and a set of primers for genes

Table. Characteristics of *bla*<sub>VIM-1</sub>-bearing *Acinetobacter baumannii* isolates\*†

Isolate no.	Region of isolation	Material	PFGE type	Etest IMP MIC (µg/mL)	Etest MBL (IMP+EDTA)	Etest MER MIC (µg/mL)	<i>bla</i> <sub>OXA-51-like</sub> status	<i>bla</i> <sub>OXA-58</sub> status
1	Thessaloniki	Blood	Ia	4	≤1	2	+	-
2	Thessalia	Bronchial fluid	II	32	2	4	+	+
3	Thessalia	CSF	III	4	≤1	2	-	-
4	Thessalia	Blood	IV	>32	1	32	+	-
5	Thessaloniki	Urine	Ib	8	≤1	4	+	+

\*The 5 isolates exhibited resistance to all alternative antimicrobial drugs except colistin.

†PFGE, pulsed-field gel electrophoresis; IMP, imipenem; MBL, metallo-β-lactamase; MER, meropenem; CSF, cerebrospinal fluid.

*aacA*, *dhfrI*, *aadA*, *qacEΔ1*, and *sul*, showed a class 1 integron with a variable region including from 5' to 3' *bla*<sub>VIM-1</sub>, *aacA7*, *dhfrI*, and *aadA1* gene cassettes. Sequencing of the overlapping PCR amplicons showed that this class 1 integron contained the *intI1* gene with a strong P1 promoter, an inactivated (without a GGG insertion) P2 promoter, an *attI1* site, and the *bla*<sub>VIM-1</sub> gene cassette with its 59-base element identical to those reported previously in other gram-negative bacteria from Greece (15). PFGE showed that the 5 *bla*<sub>VIM-1</sub>-positive isolates did not form a genetically homogeneous group; they belonged to 4 distinct types. The 2 isolates from Thessaloniki were subtypes of the same clone (Table, Figure). In none of the 5 isolates was *bla*<sub>VIM-1</sub> transferable to the susceptible *E. coli* host after repeated conjugal experiments with imipenem or ceftazidime selection. The 5,387-bp nucleotide sequence of the integron structure reported in this study has been submitted to the EMBL/GenBank/DDBJ sequence databases and has been assigned accession no. DQ112355.

## Conclusions

This is the first report of the VIM-1 determinant in *A. baumannii* in the world. Occurrence of VIM-2 MBL among *A. baumannii* and *Acinetobacter* genomospecies 3 isolates has previously been described among clinical isolates in Korea (4,5). In Europe, IMP-type enzymes had been reported in single *A. baumannii* isolates from some European regions (4) and VIM-2 from 1 German hospital (6), while MBLs have not been detected in *A. baumannii* from the United States despite carbapenem resistance there (1). Though anticipated because of the circulation of *bla*<sub>VIM</sub> genes in several other gram-negative species in Europe and the ability of *Acinetobacter* spp. to acquire foreign DNA, this evolution is worrisome.

Retention of moderate susceptibility to carbapenems by *bla*<sub>VIM</sub>-positive *A. baumannii* isolates in our study may seem unexpected, since MBLs hydrolyze these compounds. However, MBL production in gram-negative bacteria may not substantially increase carbapenem MICs without the simultaneous operation of other mechanisms, such as impaired permeability (2,4). Furthermore, 2 of the 4 strains carrying oxacillinase genes with carbapenemase properties had imipenem MICs not higher than the CLSI

breakpoints for resistance. Recently, *bla*<sub>OXA-51-like</sub> genes have been shown to be possibly naturally occurring (12), while OXA-58 enzymes play a minor role in carbapenem resistance in *A. baumannii*, and strong promoter sequences are needed for higher levels of resistance to carbapenems (2).

During the last few years, *A. baumannii* has been increasingly isolated from severely ill patients, and its

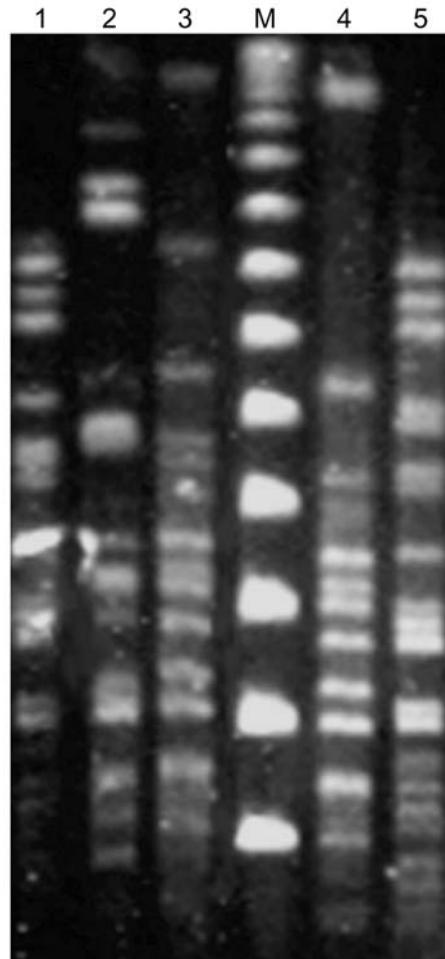


Figure. Pulsed-field gel electrophoresis of *Apal*-restricted genomic DNA of the 5 *bla*<sub>VIM-1</sub>-positive *Acinetobacter baumannii* isolates. The index numbers of the isolates are those listed in the Table. Lane M, molecular mass marker (48.5 kb).

usual cross-resistance to most available antimicrobial drugs, including carbapenems, poses substantial problems worldwide and especially in the United States (1). In New York, approximately two thirds of isolates are carbapenem resistant (3). In our region, *Acinetobacter* spp. are frequent nosocomial pathogens and are commonly multidrug resistant, which leads to the extensive use of carbapenems and, lately, polymyxins. The presence of MBLs among carbapenem-resistant *Acinetobacter* spp. from different Greek regions emphasizes the need for restricted use of carbapenems and early recognition of strains producing these enzymes. Although Etest MBL was reliable to detect VIM-2-producing *Acinetobacter* spp. in Korea (5), the assay seems unable to identify MBL-positive isolates exhibiting relatively low carbapenem MICs. Therefore, our diagnostic laboratories should screen *Acinetobacter* spp. with imipenem-EDTA DDST or alternative DDSTs, such as those using 2-mercaptopropionic acid, which appears to be more sensitive for detecting MBLs among *Acinetobacter* spp. (4). Whether carbapenems might be appropriate to treat infections with low-level carbapenem-resistant or susceptible *bla*<sub>VIM</sub>-bearing *A. baumannii* isolates has yet to be determined.

Dr Tsakris is associate professor of microbiology at the University of Athens, Greece. His research interests include the investigation of antimicrobial drug resistance mechanisms of gram-negative pathogens and the molecular epidemiology of hospital infections.

## References

1. Gaynes R, Edwards JR, National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis*. 2005;41:848–54.
2. Heritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2005;49:3198–202.
3. Quale J, Bratu S, Landman D, Heddurshetti R. Molecular epidemiology and mechanisms of carbapenem resistance in *Acinetobacter baumannii* endemic in New York City. *Clin Infect Dis*. 2003;37:214–20.
4. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo- $\beta$ -lactamases: the quiet before the storm? *Clin Microbiol Rev*. 2005;18:306–25.
5. Lee K, Lee WG, Uh U, Ha GY, Cho J, Chong Y, et al. VIM- and IMP-type metallo- $\beta$ -lactamase-producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean hospitals. *Emerg Infect Dis*. 2003;9:868–71.
6. Toleman MA, Jones RN, Walsh TR. Hospital outbreak of an imipenem-resistant VIM-2 encoding *Acinetobacter* DNA group 14TU strain in a German teaching hospital. *Clin Microbiol Infect*. 2004;10(Suppl 3):48.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI/NCCLS M100-S15. Wayne (PA): The Institute; 2005.
8. Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S, et al. PCR detection of metallo- $\beta$ -lactamase gene (*bla*IMP) in gram-negative rods resistant to broad-spectrum  $\beta$ -lactams. *J Clin Microbiol*. 1996;34:2909–13.
9. Tsakris A, Pournaras S, Woodford N, Palepou MFI, Babini GS, Douboyas J, et al. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. *J Clin Microbiol*. 2000;38:1290–2.
10. Toleman MA, Simm AM, Murphy TA, Gales AC, Biedenbach DJ, Jones RN, et al. Molecular characterization of SPM-1, a novel metallo- $\beta$ -lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. *J Antimicrob Chemother*. 2002;50:673–9.
11. Poirel L, Marqué S, Héritier C, Segonds C, Chabanon G, Nordmann P. OXA-58, a novel class D  $\beta$ -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2005;49:202–8.
12. Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2005;49:4174–9.
13. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*. 1995;33:2233–9.
14. Yan JJ, Hsueh PR, Ko WC, Luh KT, Tsai SH, Wu HM. Metallo- $\beta$ -lactamases in clinical *Pseudomonas* isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. *Antimicrob Agents Chemother*. 2001;45:2224–8.
15. Giakkoupi P, Xanthaki A, Kanelopoulou M, Vlahaki A, Miriagou V, Kontou S, et al. VIM-1 metallo- $\beta$ -lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. *J Clin Microbiol*. 2003;41:3893–6.

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