Metallo-beta-lactamases and resistance to carbapenems

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First isolated of Vim-1 in Enterobacteriaceae in Spain

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Objective: During the year 2003 we have investigated the carbapenem resistance in enterobacteriaceae from fecal carriers and other clinical samples.
Methods: All stool specimens submitted for bacterial culture were inoculated onto MacConkey agar supplemented with 2 mg/L of imipenem (IMAC). These IMAC plates were incubated overnight. The enterobacteriaceae strains suspected to be carrier of MBLs were identified by API 20E system (bioMérieux, Marcy l'Etoile, France). In vitro susceptibility testing were determined by the standard disk diffusion method and it was confirmed by Etest. To detect MBLs production a synergy test using disks or an Etest strip containing imipenem plus EDTA were employed. The detection of the gene blaVIM-1 and class 1 integrons was performed by PCR amplification. Nucleotide sequences of the PCR products were determined with a Beckmann 8000 sequencer. Conjugation and transformation experiments were conducted by the filter mating method and following Bio-Rad recommendations (Bio-Rad, Laboratories) respectively.
Results: Of 1043 stools studied and 4345 susceptibility tests determined in clinical samples during the year 2003 we obtained two strains, *Klebsiella penumoniae* from stool and *Escherichia coli* from urine sample, suspected to be carrier of MBLs. These strains were obtained from two patients. The in vitro susceptibility testing showed that both strains were resistant to all b-lactamics but were sensitive to aztreonam. Although imipenem was sensitive its diameter was low (22 mm). MIC to imipenem was 4–6 mg/L and 0.75 mg/L for *K. pneumoniae* and *E. coli* respectively. The synergy test using disks showed a positive result. The PCR amplification of blaVIM-1 was positive and direct sequencing of this amplification product showed 100% homology for the gen blaVIM-1 in both strains. PCR of class 1 integron yielded a 4 kb amplification product from *K. pneumoniae* and *E. coli*. The conjugal transfer of the resistance was managed with *K. pneumoniae* and *E. coli*, but in *E. coli* a previous transformation was needed.
Conclusion: The prevalence of carbapenemases in enterobacteriaceae strains is low. To our knowledge, these isolates of *K. pneumoniae* and *E. coli* are the first enterobacteriaceae species producing VIM-1 in Spain. The MBLs production should be studied when the strain showed a profile of resistance to cefotaxime, ceftazidime and cefepime but sensitive to aztreonam and imipenem.

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Metallo-beta-lactamase in clinical *Pseudomonas aeruginosa* isolate in a Portuguese hospital and identification of a new VIM-2 like enzyme

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Objectives: Metallo-beta-lactamases are responsible for carbapenems-resistant *Pseudomonas aeruginosa* . The spread of genes enconding for these enzymes among gram-negative bacteria has been described in different countries as well as in different species and is a threat to public health.The aim of this work was to identify metallo-beta-lactamases in *Pseudomonas aeruginosa* clinical isolates.
Methods: A total of 27 clinical isolates of *Pseudomonas aeruginosa* collected from February to October 2004 at an hospital in north of Portugal were found to be resistant to Imipenem. Antibiotic susceptibilities were determined by the agar disk diffusion according to the protocol recommended by NCCLS. Detection of metallo-beta-lactamases was performed by using the Imipenem-EDTA disk method and confirmed by the MBL E-test. The isolates were subjected to PCR assays with specific primers for blaIMP and blaVIM and sequence analysis were made to identify the metallo-beta-lactamase. Epidemiological typing was performed by M13 fingerprinting.
Results: Antibiotic susceptibility tests revealed high percentage of resistance to most antibiotics tested: Imipenem (96.3%), Meropenem (84%), Ciprofloxacin (74.1%), Piperacillin-tazobactam (34.6%), Ceftazidime (34.6%), Gentamicin (25.9%), Amikacin (14.8%) and Tobramicin (7.7%). In the Imipenem-EDTA test only one strain (Ps10VR) demonstrated a growth-inhibitory zone with 16 mm of diameter, suggesting production of metallo-beta-lactamases, while no remarkable distinct change was noticed in the others isolates. The MBL E-test was realized for this strain and Imipenem MIC has decreased from 16 to <1 mg/l.In PCR experiments using specific primers for blaIMP the results were negative among all strains, while in those using specific primers for blaVIM one strain generated a positive result, the same that was positive to Imipenem-EDTA test and MBL E-test (Ps10VR). The sequence analysis revealed that this strain was carrying a variant blaVIM-2 that encodes a VIM-2 like enzyme. The novel gene differs from blaVIM-2 by replacement of a G with a A at nucleotide 443 of structural gene, which results in Ser-to- Asp change at amino acid positions 136.M13 fingerprinting revealed that six clinical isolates were closely or possibly genetically related and that one VIM-2 like producing *Ps.aeruginosa* Ps10VR isolate was genetically indistinguishable.
Conclusion: The results showed a new VIM-2 like enzyme in *Pseudomonas aeruginosa*.

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Detection of a new VIM-type metallo-beta-lactamase (VIM-11) in a *Pseudomonas aeruginosa* clinical isolate from Italy

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Objectives: A one-year prospective study of multidrug-resistant Gram-negative microorganisms was carried out in a neonatal intensive care unit (NICU) in Palermo, Italy, within the framework of an infection control programme. All the newborns that were admitted to the NICU for at least 24 h – namely 211 cases – were enrolled in the study. All the imipenem-resistant *Pseudomonas aeruginosa* isolates from all kinds of specimens were tested for metallo-beta-lactamase (MBL) production.
Methods: Antimicrobial susceptibility testing was performed by both diffusion and microdilution and interpreted according

to the latest NCCLS documents. The presence of carbapenemase was screened by means of the MBL-etest, and further investigated by following carbapenem hydrolysis and its inhibition by EDTA. PCR and sequencing were carried out by standard procedures. An enzyme endowed with carbapenemase activity was cloned on a phagemid vector pPCR script Cam SK+ and transformed in *Escherichia coli* XL10 ultracompetent cells using the ligation kit polishing protocol.

Results and Conclusions: 54.5% (115/211) of newborns were colonised by MDR Gram-negatives. 16.1% (34/211) were colonised by an imipenem-resistant strain of *P. aeruginosa*. Twenty-one of these latter strains shared the same PFGE profile and all showed clear positivity with the MBL etest. Imipenem hydrolysis by spectrophotometric analysis of crude sonic extracts confirmed that imipenem was hydrolysed at a rate of 5×10^{-8} mol/min/mg, whereas on adding EDTA 2 mM the kinetics was 7.2×10^{-10} mol/min/mg.A polymerase chain reaction (PCR) performed with either bla(VIM) or bla(IMP) primers yielded positive results only with the bla(VIM) primers; a specific PCR yielded a product of about 800 bp that - after sequencing - was found to code for a polypeptide differing from VIM-4 in one amino acid, namely G31S. The new enzyme was named VIM-11 at the www.lahey.org site and received the accession number AY635904.

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JAP-1, a new member of subclass B3 metallo-beta-lactamase from *Bradyrhizobium japonicum*

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Objectives: The most common cause of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase. Among these, metallo-beta-lactamases (MBLs) are zinc-dependent enzymes that have been the focus of increasing attention because of their ability to hydrolyze nearly all known beta-lactams and of the lack of clinically-useful inhibitors. However, several MBLs have been reported also from bacterial species that are primarily members of the environmental microbiota (e.g. CAU-1 from *Caulobacter crescentus* and THIN-B from *Janthinobacterium lividum*). In the genome of *Bradyrhizobium japonicum* USDA 110, a nitrogen-fixing symbiont of legumes among the most important in agriculture and plant biotechnology, an open reading frame was found (ORF blr 6230) that encodes a protein sharing 28–40% sequence identity with subclass B3 MBLs. The functional properties of the enzyme, named JAP-1, were investigated.

Methods: ORF blr 6230 was amplified from *Bradyrhizobium japonicum* USDA110 by PCR using custom primers, and the amplification product was cloned into the T7-based expression vector pET-9a to yield the recombinant plasmid pET-JAP-1. *E. coli* BL21(DE3)[pET-JAP-1] strain was used for protein production. The enzyme was subjected to biochemical characterization and the kinetic properties investigated by spectrophotometry.

Results: ORF blr 6230 encodes a putative protein of 294 residues and a predicted molecular mass of 32 kDa. JAP-1 exhibits the highest sequence identity with CAU-1 and FEZ-1 (from *Legionella gormanii*) enzymes (40 and 34% respectively). *E. coli* BL21(DE3)[pET-JAP-1] produced a imipenemase activity (sp. act. 155 nmol/min.mg of protein) that was inhibited >95% after incubation in presence of 5 mM EDTA. The purified JAP-1 enzyme efficiently hydrolyzed penicillins, cephalosporins and carbapenems.

Conclusion: The MBL homologue encoded by the *B. japonicum* USDA 110 chromosome is a functional MBL of subclass B3 that exhibits a broad substrate profile. This is the first example of a MBL found in the bacterial species belonging to the Rhizobiales order.

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The diverse integron structures disseminating VIM genes in Poland

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Objectives: Recently we have characterized a novel blaVIM-4 metallo-beta-lactamase (MBL) gene cassette from clinical strains isolated from hospitalized children in Warsaw, Poland. This novel gene cassette was harboured on class 1 integron and found in 11 strains of *Pseudomonas aeruginosa* with various PFGE patterns. The aim of this study was to genetically examine other carbapenem-resistant *P. aeruginosa* and *P. putida* clinical strains for similar integrons.

Methods: Phenotypic screening involved the MBL Etest strip (AB BIODISK). Biochemical analysis used crude extrats examining imipenem (IMP) and meropenem (MEM) hydrolysis preincubated with 20 mM EDTA. PCR analysis was performed using primers specific for blaIMP and blaVIM genes and class 1 integrons. Sequencing was carried out using Perkin-Elmer Biosystems 377 DNA sequencer and analysed using DNAstar.

Results: The Etest produced for the majority of isolates a positive phenotypic test for MBL: IMP MIC > 256 mg/l; IMP/EDTA 1–8 mg/L. Isolates were resistant to all beta-lactams, and some additionally to aminoglycosides. The crude extracts of isolates showed hydrolysis of IMP and MEM, which were inhibited over 90% with EDTA. Screening for MBL using PCR analysis gave a positive result for the presence of the blaVIM gene. Two isolates of *P. putida* had an integron containing MBL gene blaVIM-4/blaVIM-4 partial repeat, then aacA4 (or aadB), OXA-2, orfD followed by qacEdelta1. *P. aeruginosa* isolate 266/03 had restriction pattern of blaVIM-4 as previously isolated in our hospital. Two *P. aeruginosa* isolates: 414/03 from our hospital and 303/03 from other hospital in Warsaw, had MBL gene blaVIM-2 as isolated previously in Warsaw. However, they had different additional genes within the class 1 integron.

Conclusions: Since 1998, *P. aeruginosa* strains with blaVIM-4 MBL genes have become endemic in children hospitalized in our institute. The source of blaVIM-4 gene cassette are probably the *P. putida* strains. Diverse integrons found in *P. putida* and *P. aeruginosa* isolates are responsible for the spread blaVIM genes in Poland.

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Diverse transposons are responsible for the dissemination of Japanese metallo-beta-lactamase alleles: Report from the SENTRY Surveillance Programme

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Objectives: MBL's were initially characterised in Japan, usually of the IMP type and found in *Pseudomonas aeruginosa* , *Acinetobacter* or *Serratia marcescens* and have been endemic in Japan for the last decade. However, little work has been done to characterize the integron structures or classify the mobile