# Prolonged bacteraemia caused by VIM-1 metallo-β-lactamase-producing *Proteus mirabilis*: first report from Italy

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## Abstract

Persistent bacteraemia arising from a case of post-operative mediastinitis as a result of a *Proteus mirabilis* isolate, possessing two class 1 integrons carrying $bla_{VIM-1}$ and *aad*A2 gene cassettes located on chromosomal and plasmidic DNA, respectively, is reported. Despite the *in vitro* susceptibility to carbapenems, meropenem therapy failed, whereas the patient responded to treatment with cefepime plus amikacin. To our knowledge, this is the first report of metallo-β-lactamase production in a clinical isolate of *P. mirabilis* in Italy.

**Keywords:** carbapenem resistance, metallo-β-lactamase, proteus mirabilis, VIM-1

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Metallo-β-lactamase (MBL)-producing strains of *Proteus mirabilis* have been rarely reported in the English literature [1–3]. We describe a case of prolonged bacteraemia due to a VIM-1 producing *P. mirabilis*, that did not respond to meropenem therapy. To our knowledge, this is the first case of severe infection due to a VIM-1-producing *P. mirabilis* isolate in Italy.

A diabetic 71-year-old man underwent open heart surgery with successful replacement of the ascending aorta for acute dissection. After 10 days in an intensive care unit, the patient presented with fever and purulent discharge from the sternotomy scar. A computed tomography scan of the thorax showed multiple rim-enhancing fluid collections in the sternal region that were consistent with mediastinitis. Culture of wound swabs yielded a strain of *P. mirabilis* susceptible to carbapenems, piperacillin/tazobactam, levofloxacin, cefepime and amikacin. The semiquantitative central venous catheter tip culture and tracheal aspirate culture were negative. Urinalysis did not reveal leukocyturia, and urine cultures were negative. The patient underwent surgical debridement and was given meropenem 1 g thrice daily. However, despite the treatment, the patient developed severe sepsis. Three sets of blood cultures yielded *P. mirabilis*. The dosage of meropenem was increased to 2 g thrice daily. Surprisingly, the patient remained febrile and two blood cultures, obtained the next day, remained positive for the same *P. mirabilis*. The occurrence of bacteraemia prompted the search for endocarditis or abscesses. Neither transoesophageal echocardiography, nor total body computed tomography scanning revealed a possible cause for the therapeutic failure. Therapy was then shifted to cepefime (2 g every 8 h) plus amikacin (1 g every 24 h). The patient responded to an 8-week course of cefepime plus amikacin therapy (the latter drug was discontinued after 4 weeks). No relapse was observed after a 3-month follow-up period.

The *P. mirabilis* clinical isolate obtained from the patient was identified using the automatic system Vitek 2 (bioMérieux, Inc., Hazelwood, MO, USA), and was confirmed with API 20E (bioMérieux SA, Marcy-l'Etoile, France). The preliminary antibiotic resistance profile determined by Vitek 2 was confirmed by a broth microdilution method, using Mueller–Hinton broth, according to guidelines of the CLSI [4]. The EDTA-imipenem double-disc synergy test was performed as a screening procedure for MBL production [5]. Genomic and plasmid DNA were extracted from *P. mirabilis* using Wizard DNA extraction and purification kits (Promega, Milan, Italy). DNA probes, specific for $bla_{TEM-1}$, $bla_{SHV-1}$, $bla_{CTX-M-1}$, $bla_{PER-1}$, $bla_{VIM-1}$ and $bla_{IMP-1}$, were labelled with $^{32}P$ by a random priming technique and were used for Southern blot experiments with plasmidic and genomic DNA transferred onto nylon filters. Screening for resistance determinants ($bla_{VIM}$-type and $bla_{TEM}$-type genes) was performed by PCR analysis using the primers VIM-F and VIM-R [6] and TEM/forward (5′-ATGAGTATTCAACATTTCCG-3′) and TEM/reverse (5′-TTACCAATGCTTAATCAGTGAG-3′).

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Detection and mapping of a class 1 integron were carried out using specific primers for the conserved segments 5′-CS (5′-GGCATCCAAGCAGCAAG-3′) and 3′-CS (5′-CTCTC AAGATTTTAATGCGGATG-3′), and then amplifying the variable region containing the resistance gene cassettes. *Int*I1, *qac*ED1 and *sul*1 were amplified with specific primers [5,7]. The DNA fragments obtained by subsequent PCRs were sequenced on both strands with a ABI-Prism 310 (Applied Biosystem, Monza, Italy) automatic sequencer. Sequence analysis was performed on three independent amplification products.

The isolate was susceptible to ceftriaxone (MIC <0.06 mg/L), ceftazidime (MIC 2 mg/L), cefepime (MIC 0.125 mg/L), amoxicillin (MIC 1 mg/L), piperacillin/tazobactam (MIC 0.25 mg/L), levofloxacin (MIC 0.12 mg/L) imipenem (MIC 1 mg/L), meropenem (MIC 0.125 mg/L), ertapenem (MIC < 0.125 mg/L), tigecycline (MIC 2 mg/L). The isolate was not an ESBL producer, but showed a positive EDTA-imipenem double-disc synergy test. Southern blot analysis showed the presence of $bla_{VIM}$-type and $bla_{TEM}$-type in the *P. mirabilis* clinical isolate. The presence of class 1 integrons was detected by PCR with primers specific for 5′- and 3′-conserved segments, both in genomic and plasmidic DNA. An amplification product of 1060 bp was obtained from genomic DNA and direct sequencing revealed one gene cassette carrying a $bla_{VIM-1}$ determinant. In the latter amplicon, a gene cassette carrying the *aad*A2 gene (aminoglycoside adenylyltransferase) was detected. Both integrons were of class 1 because the integrase gene *intI1* had its own promoter region and *attI1* recombination site; the 3′-CS region presented *qacE*$\Delta$*1* and *sulI1* genes that confer resistance to quaternary ammonium compounds and sulphonamide. A large plasmid (size > 100 kb) was detected in the isolate by southern blotting and produced a positive signal after hybrydization with $bla_{TEM-1}$ probe (data not shown). The amplicon obtained by PCR was sequenced and appeared to correspond to TEM-1 $\beta$-lactamase.

To our knowledge, this is the first report showing a VIM-type MBL in a *P. mirabilis* clinical isolate from Italy, and the first case to be detected in Europe outside of Greece [1–3]. It is of interest that the reported strain possessed two class 1 integrons (on chromosomal and plasmidic DNA) identical to those recently described in a carbapenem-resistant *Enterobacter cloacae* strain isolated from a patient who died from pneumonia [6], suggesting the spread of these elements by gene cassette insertion into different bacterial species.

As already reported by Vourli *et al.* [3], the carbapenem MICs were below the current resistant breakpoints. However, it was surprising to observe that our strain appeared to be highly susceptible to several different $\beta$-lactams, and the single criterion to suggest a search for MBL production in this isolate was the prolonged therapeutic failure with meropenem. These findings suggest a significant variability in VIM enzyme expression levels, and a poor correlation with respect to *in vitro* susceptibility. A similar observation was recently reported in VIM 1-positive *Klebsiella pneumoniae* strains, which exhibited differences in imipenem-hydrolysing activities that were not correlated with the respective carbapenem MICs [8].

The acquisition of MBL determinants in *P. mirabilis* may be of significant concern for physicians because this organism is usually resistant to colistin [9] and is poorly susceptible to tigecycline [10], which represents an important option for treating infections caused by multi-drug-resistant Gram-negative bacilli.

This report demonstrates the dissemination of the $bla_{VIM-1}$ gene among different species of Enterobacteria. There is an urgent need for accurate routine screening methods for MBL-producing isolates, in order to identify them promptly.

## Transparency Declaration

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# Detection and characterization of *Enterobacteriaceae* producing metallo-β-lactamases in a tertiary-care hospital in Spain

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## Abstract

Carbapenem-resistant or intermediate (MIC ≥1 mg/L) clinical isolates (*n* = 12) of three species of *Enterobacteriaceae* (*Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Escherichia coli*) were characterized. The isolates harboured integrons containing the VIM-1 metallo-β-lactamase gene together with other resistance gene cassettes. In particular, the CTX-M-2 gene was detected in four of the *K. pneumoniae* isolates. The patient population was mostly paediatric and characterized by severe underlying illnesses that involved long-term hospitalization, major surgery and/or immunosuppressive and broad-spectrum antibiotic therapy.

**Keywords:** Carbapenem resistance, *Enterobacteriaceae*, *Escherichia coli*, integron, *Klebsiella pneumoniae*, metallo-β-lactamase, VIM-1

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Resistance to carbapenems may result from the presence of metallo-β-lactamases (MBLs). These are zinc-dependent β-lactam-hydrolysing enzymes with broad substrate specificity that are not inhibited by clavulanic acid, sulbactam or tazobactam. MBL genes can be chromosome- or plasmid-borne, and are often located in integrons [1]. They are usually found in nonfermenting Gram-negative bacteria and are not frequent among the *Enterobacteriaceae*. However, several recent studies have described both nonclinical [2] as well as clinical isolates of MBL-producing enterobacteria [2–6]. These included sporadic isolates, as well as isolates from clonal epidemics and multiclonal endemic conditions [6].

Carbapenem MICs are typically low and variable for MBL-producing enterobacteria. This, together with the presence of other β-lactamases, complicates their phenotypic detection in routine tests. However, they can be readily detected with specific phenotypic and molecular screening assays [7,8].

Between December 2005 and February 2008, several carbapenem-resistant or intermediate enterobacterial isolates were obtained from 12 hospitalized patients at Hospital Universitario La Paz, Madrid. Identification and susceptibility testing were conducted using the Wider (Francisco Soria Melguizo SA, Madrid, Spain.) and Vitek 2 (bioMérieux, Barcelona, Spain.) automated systems. The isolates belonged to three species (*Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Escherichia coli*). Single isolates from eight patients, and multiple isolates from the other four patients, were obtained. However, only one isolate per patient was analysed in detail. One of the patients was an adult, hospitalized in the haematology ward, whereas the others were children or infants from the paediatric hospital. Patients were from different wards and the isolates were well separated in time. Most patients had severe illnesses involving major surgery and long-term hospitalization (Table 1). Some of them had repeated infections with different microorganisms, and all had been receiving broad-spectrum antibiotic treatments. In all cases, the infections were successfully treated, although some patients had repeated septic episodes, which had to be treated with a combination of antibiotics that included ciprofloxacin. Clonal relatedness between *K. pneumoniae* isolates was analysed using enterobacterial repetitive intergenic consensus-PCR fingerprinting with the primer ERIC2 [9] and randomly amplified polymorphic DNA with the primers OPI12 (5′-AGAGGGCACA-3′) and OPA18 (5′-AGGTGAC CGT-3′) (Operon Technologies, Inc., Alameda, CA, USA). Clonality was defined using the criteria of Tenover *et al.* [10]. The results obtained with these three primers were consistent and indicated the presence of a major clone, which included isolates 2, 3, 4, 8, 9, 10 and 11 (Tables 1

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