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First Report of an Extensively Drug-Resistant VIM-2 Metallo- β -Lactamase-Producing *Brevundimonas diminuta* Clinical Isolate

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In the literature, only three *Brevundimonas diminuta* environmental isolates carrying metallo- β -lactamase genes were recently published. However, so far, no *B. diminuta* clinical isolates carrying these carbapenem resistance genes have been described. Here we report the first VIM-2 metallo- β -lactamase-producing *B. diminuta* clinical isolate obtained from an immunocompromised patient.

CASE REPORT

On 5 September 2011, a 56-year-old female patient with a history of systemic lupus erythematosus diagnosed in 1996, antiphospholipid syndrome with deep-vein thrombosis, and anticardiolipin antibodies was admitted to the Hospital de Clínicas José de San Martín with fever (38.8°C) and low back pain radiating to the left flank and pain in the left thigh trochanteric region. Also, she had difficulty walking and lower limb weakness. She presented an ulcer on her left inner thigh that involved the subcutaneous cellular tissue and with trochanteric adipose tissue commitment.

In addition, she had grade IV lupus glomerulonephritis diagnosed in 2001, for which she received 8 cycles of cyclophosphamide and 3 pulses of methylprednisolone.

She had repeated previous hospital admissions due to the presence of painful chronic ulcers in her lower limbs, which limited her mobility. Her clinical picture had been interpreted as reactivation of the underlying disease, and she was treated with corticosteroids with a favorable outcome.

She had a recent prior hospitalization to treat a moderate nephritic flare in August 2011. During her hospital stay, ulcers were treated locally with povidone-iodine (Betadine).

The laboratory findings on a peripheral venous blood sample taken on admission were as follows: white blood cell count, 12,400/mm³; hematocrit, 31%; hemoglobin count, 10.2 g/dl; platelet count, 192,000/mm³.

Because of the torpid response of the lower limb ulcers to immunosuppressive therapy (prednisone at 60 mg/day), persistent fever, and suspicion of subcutaneous cellular tissue disease, biopsy of the trochanteric adipose tissue and biopsy of the ulcer on the left inner thigh for histopathological analysis and culture were performed. Blood cultures were also indicated.

Empirical intravenous treatment with piperacillin-tazobactam at 300 mg/kg/day and vancomycin at 60 mg/kg/day was started.

The electromyogram of the lower limbs showed motor and sensory left axonal neuropathy with in-progress denervation.

The patient responded with persistence of back pain, fever, and generalized muscular weakness.

Blood cultures were negative, but in the culture of both biopsy specimens there was a pure growth of Gram-negative rods (cultures for fungi and mycobacteria were negative).

Colonies on nutrient agar were entire, convex, smooth, glisten-

ing, and opaque and presented a characteristic brown pigment on Mueller-Hinton agar after incubation for 72 h at 35°C. The organism was identified by standard biochemical tests (6) as *Brevundimonas diminuta* and by Vitek 2 System GN ID Card (bioMérieux, Marcy l'Etoile, France) as *B. diminuta*/*B. vesicularis* (with 99.9% accuracy).

PCR amplification of the 16S rRNA was performed in order to confirm the identification. A PCR product of the 16S rRNA gene, using the primers described by Weisburg et al. (15), was obtained by using the Qiagen *Taq* master mix and following the manufacturer's specifications (Qiagen). Sequencing of both DNA strands of the 1.4-kb PCR product was performed with an ABI Prism 3100 BioAnalyzer at the MacroGen, Inc., sequencing facility. The sequences were analyzed with the BLAST V2.0 software available at <http://www.ncbi.nlm.nih.gov/BLAST/>. Sequence analysis revealed 99% identity with the sequences corresponding to the 16S rRNA gene of *B. diminuta* strain ATCC 19146 (GenBank accession number EU497053.1).

The antibiotic susceptibility test was performed using the Vitek 2 System employing the AST-082 (Gram-negative susceptibility card) panel, and susceptibility to minocycline, tigecycline, and aztreonam was determined by the Etest technique (bioMérieux) on Mueller-Hinton agar in accordance with the manufacturer's specifications. The MIC results were interpreted by using CLSI categories (3).

The microorganism presented extensive drug resistance and retained susceptibility to only minocycline and tigecycline (Table 1).

For metallo- β -lactamase (MBL) screening, a double-disk assay using EDTA-sodium mercaptoacetic acid (SMA) disks (1,900/750 μ g per disk, respectively; Britania Laboratories, Buenos Aires, Argentina) placed 15 mm (center to center) from an imipenem disk was used (5). By means of this assay, synergism between the

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TABLE 1 Antibiotic susceptibilities of the *B. diminuta* clinical isolate described in this report

Antimicrobial agent(s)	MIC ($\mu\text{g/ml}$)	Interpretation
Ampicillin	≥ 32	Resistant
Ampicillin-sulbactam	≥ 32	Resistant
Piperacillin-tazobactam	≥ 32	Resistant
Cephalothin	≥ 64	Resistant
Cefoxitin	≥ 64	Resistant
Cefotaxime	≥ 64	Resistant
Ceftazidime	≥ 64	Resistant
Cefepime	≥ 64	Resistant
Imipenem	≥ 16	Resistant
Meropenem	≥ 16	Resistant
Aztreonam	≥ 256	Resistant
Trimethoprim-sulfamethoxazole	≥ 4	Resistant
Amikacin	≥ 64	Resistant
Gentamicin	≥ 16	Resistant
Ciprofloxacin	≥ 4	Resistant
Colistin	≥ 16	Resistant
Minocycline	0.016	Susceptible
Tigecycline	≤ 0.016	Susceptible

carbapenem and EDTA-SMA disks was observed and a putative MBL present in *B. diminuta* was also indicated. Consequently, the strain was tested for MBL-encoding genes by PCR. The total DNA was isolated as described before (8) and used to carry out PCRs to test for the presence of the *bla*_{VIM}, *bla*_{IMP}, and *bla*_{SPM} genes. PCRs, sequencing, and nucleotide sequence analysis were done as described above. Sequence analysis revealed that the positive-amplification PCR product was identical to the *bla*_{VIM-2} gene. Furthermore, the *intI1* gene and the widespread aminoglycoside resistance gene *aac*(6')-Ib were found. PCR cartography and sequence analysis were done to determine the location of the *bla*_{VIM-2} gene. The results obtained showed that the gene was located in a class 1 integron followed by the *aac*(6')-Ib gene.

As a *Pseudomonas aeruginosa* isolate susceptible only to carbapenems and colistin was simultaneously recovered from the patient's urine culture, with the *B. diminuta* antibiotic sensitivity report, treatment was changed to tigecycline plus imipenem.

Histopathological examination of biopsy specimens revealed findings compatible with pyoderma gangrenosum.

The clinical picture was interpreted as *B. diminuta* superinfection secondary to her primary clinicopathological cutaneous condition.

The patient developed progressive renal function impairment (multifactorial in origin: active lupus glomerulonephritis, nephrotoxicity secondary to drugs, nephrotic syndrome) requiring dialysis and transfer to the intensive care unit. She developed hypotension, respiratory failure, and oliguria requiring the use of vasopressors (noradrenaline and dopamine) and mechanical ventilation; she went into septic shock and died a few hours later.

Since the first description of VIM-2 in southern France (Marseilles) in a *P. aeruginosa* isolate from a blood culture of a neutropenic patient in 1996 (11), this enzyme has been detected in many other species of nonfermenting Gram-negative rods, such as *Pseudomonas putida* (1, 14), *Pseudomonas fluorescens* (14), *Pseudomonas*

stutzeri (14), *Pseudomonas pseudoalcaligenes* (12), *Pseudomonas fulva* (2), *Achromobacter xylosoxidans* (14), and *Acinetobacter baumannii* (14).

So far, no *B. diminuta* clinical isolates carrying MBL genes have been described in the literature.

Scotta et al. (13) studied a total of three isolates of *B. diminuta*, among other nonfermenting Gram-negative bacilli that carry the gene encoding the VIM-13 enzyme, but these isolates were detected in the sewage water of a hospital. These were not of clinical origin. The presence of *bla*_{VIM-13} associated with a Tn1721-class 1 integron structure was detected in all *B. diminuta* isolates. This structure was located on a plasmid, suggesting that environmental bacteria represent a reservoir for the dissemination of clinically relevant MBL genes (13).

Most of the *B. diminuta* clinical isolates reported in the literature were from the blood of patients with cancer (4). These infections involved the bloodstream (one case), an intravascular catheter (four cases), the urinary tract (one case), and the pleural space (one empyema case). All organisms were resistant to multiple fluoroquinolones and cefepime but susceptible to amikacin, imipenem, and ticarcillin-clavulanate (4). *B. diminuta* was recovered from other sites, i.e., a sputum sample from a patient with cystic fibrosis (9) (who had received a course of colistin to treat *A. xylosoxidans* colonization 10 months before pneumonia onset, which could have selected the colistin-resistant *B. diminuta* isolate) and from a patient with keratitis secondary to contact lens wear (10). The location of this microorganism in soft tissue has not been reported in the literature. Additionally, this report represents the first case of a clinical isolate of this species carrying the VIM-2 enzyme.

B. diminuta is an environmental microorganism with worldwide distribution that has been isolated from water, soil, plants, and occasionally clinical specimens (6). Additionally, this species can survive in disinfectants (7).

This case could be a nosocomial soft tissue infection in an immunocompromised patient that was caused by *B. diminuta* from a contaminated disinfectant solution. Unfortunately, cultures of the solutions used for the cleansing and care of the ulcers and further epidemiologic investigation to detect the source of the organism were not performed.

We emphasize the importance of the isolation of MBL-producing, nonfermenting, Gram-negative rods whose habitat is the environment from clinical specimens.

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