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

Marisa N. Almuzara, Claudia M. Barberis, Carlos H. Rodriguez, Angela M. R. Famiglietti, Maria S. Ramirez, and Carlos A. Vaya

Laboratorio de Bacteriología, Instituto de Fisiopatología y Bioquímica Clínica, Hospital de Clínicas José de San Martín, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; and Instituto de Microbiología y Parasitología Médica, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Tecnológicas (IMPaM, UBA-CONICET), Facultad de Medicina, Buenos Aires, Argentina

In the literature, only three Brevundimonas diminuta environmental isolates carrying metallo-β-lactamase genes have been described. 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 1996, and a recent prior hospitalization to treat a moderate nephritic flare in August 2011. During her hospital stay, ulcers were formed. Blood cultures were also indicated. The electromyogram of the lower limbs showed motor and sensory axonal neuropathy with in-progress denervation. The patient responded with persistence of back pain, fever, and generalized muscular weakness.

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 obtained.

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

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. 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 obtained.

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-
cases (2831–2833). The presence of blaVIM-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 (4) [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.

ACKNOWLEDGMENTS

This work was supported by grants from the Secretaría de Ciencia y Técnica de la Universidad de Buenos Aires (UBACyT) to Carlos A. Vay. M.S.R. is a member of the CONICET research career.

REFERENCES


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 blaVIM-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).

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

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>MIC (µg/ml)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≥32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin–sulbactam</td>
<td>≥32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Piperacillin–tazobactam</td>
<td>≥32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≥4</td>
<td>Resistant</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ciprofloxicin</td>
<td>≥4</td>
<td>Resistant</td>
</tr>
<tr>
<td>Colistin</td>
<td>≥16</td>
<td>Resistant</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.016</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.016</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

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 blaVIM, blaIMP, and blaSPM 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 blaVIM-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 blaVIM-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 (Marseille) 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).

Since the first description of VIM-2 in southern France (Marseille) 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).

Since the first description of VIM-2 in southern France (Marseille) 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).


