Letter to the Editor

Full Resistance and Decreased Susceptibility to Carbapenems in IMP-13-Producing Pseudomonas aeruginosa Isolates from an Outbreak

Of 23 ceftazidime-resistant Pseudomonas aeruginosa isolates with simultaneous decreased susceptibility to carbapenems recovered from inpatients at Hospital Eva Peron, Provincia de Buenos Aires, Argentina, from December 2004 to December of 2005, 18 were positive by a double-disk phenotypic screening of metallo-β-lactamases (MBLs) using EDTA (1 μmol) (6). Decreased susceptibility was defined as to refer to isolates with MICs of 2 to 8 μg/ml or inhibition zones of 16 to 21 mm, categorized as susceptible or intermediate according CLSI breakpoints, but which clearly differed from fully susceptible isolates.

All of these isolates could be categorized as susceptible to piperacillin, piperacillin-tazobactam, and colistin. Thirteen of 18 isolates were susceptible to amikacin, while 5/18 were intermediate. They were all resistant to ceftazidime, cefepime, piperacillin, piperacillin-tazobactam, and colistin. Thirteen of 18 isolates. From them, 14 displayed decreased susceptibility to imipenem (IPM) and meropenem (MEM) (inhibition zone range, 16 to 21 mm), while 4 (consecutive) isolates were considered fully resistant to both (Table 1). Independently of their categorization, pulsed-field gel electrophoresis (PFGE) profiles using 20 U/plug SpeI nuclease (NEB) were identical after standard resolution (4).

These 18 isolates were positive when screened for the class 1 integrase gene and rendered a unique fragment corresponding to the variable region of class 1 integrons, in which imp-13 was the first cassette, followed downstream by an aminoglycoside-modifying enzyme-coding gene, aacA4 (AM931299), 99% identical to that already deposited for Tn5051 (5, 9).

Because a difference in final carbapenem resistance could be due to different mechanisms, including differential β-lactamase content and expression, crude extracts were examined after analytical isoelectrofocusing, detecting enzymatic activity with decreased susceptibility to amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CLI, clindamycin; CFZ, cefazolin; ERY, erythromycin; RIF, rifampin; CST, colistin.

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TABLE 1. Epidemiological data and antimicrobial resistance profile

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Age (yr)/</th>
<th>Admission date (mo/day)</th>
<th>Underlying condition</th>
<th>Ward</th>
<th>Diagnosis</th>
<th>Culture source</th>
<th>Empirical therapy</th>
<th>Date of isolation (mo/day)</th>
<th>Antibiotic susceptibility interpretation</th>
<th>Therapeutic treatment</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>65/male</td>
<td>11/26/04</td>
<td>—</td>
<td>ICU</td>
<td>ABS</td>
<td>AF</td>
<td>SAM, TZP, CIP,</td>
<td>12/14/04</td>
<td>20 (S) 4 (S) 18 (S) 4 (S) CST</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>69/female</td>
<td>12/15/04</td>
<td>AH, SK</td>
<td>ICU</td>
<td>EVS</td>
<td>BL/CAT</td>
<td>VAN, SXT, AMK</td>
<td>12/31/04</td>
<td>21 (S) 2 (S) 17 (S) 4 (S) CST</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>12/30/04</td>
<td>AMI, AH</td>
<td>ICU</td>
<td>Sepsis</td>
<td>BL</td>
<td>SAM, GEN</td>
<td>1/11/05</td>
<td>20 (S) 2 (S) 16 (S) 4 (S) CIP, SAM, GEN</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>57/male</td>
<td>3/25/05</td>
<td>SCZ, SK</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CRO, CIP, TZP, IPM, SXT</td>
<td>4/7/05</td>
<td>21 (S) 2 (S) 21 (S) 4 (S) SSTI, IPM, CST</td>
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<tr>
<td>5</td>
<td>56/female</td>
<td>2/28/05</td>
<td>DM, CRF</td>
<td>GW</td>
<td>SXT, OS</td>
<td>BO</td>
<td>Unknown</td>
<td>5/16/05</td>
<td>21 (S) 2 (S) 17 (S) 4 (S) Unknown</td>
<td>Favorable</td>
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<tr>
<td>6</td>
<td>31/male</td>
<td>5/11/05</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CAZ, VAN</td>
<td>5/15/05</td>
<td>20 (S) 2 (S) 16 (S) 2 (S) CST</td>
<td>Died</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>86/female</td>
<td>5/10/05</td>
<td>DM</td>
<td>ICU</td>
<td>CAI</td>
<td>CAT</td>
<td>CRO, CAZ, AMK, VAN</td>
<td>5/26/05</td>
<td>21 (S) 2 (S) 17 (S) 4 (S) IPM</td>
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<td></td>
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<tr>
<td>8</td>
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<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>6/3/05</td>
<td>21 (S) 2 (S) 21 (S) 4 (S) IPM</td>
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<td></td>
<td></td>
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<tr>
<td>9</td>
<td>49/male</td>
<td>5/27/05</td>
<td>COPD CRF</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>7/9/05</td>
<td>21 (S) 2 (S) 19 (S) 2 (S) IPM, CST</td>
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<td></td>
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<tr>
<td>10</td>
<td>42/male</td>
<td>7/13/05</td>
<td>DM</td>
<td>VAP</td>
<td>BAL</td>
<td>CRO, CLI</td>
<td>7/9/05</td>
<td>21 (S) 2 (S) 20 (S) 20 (S) IPM, CST</td>
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<td></td>
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<tr>
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<td>7/08/05</td>
<td>AH</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>7/15/05</td>
<td>21 (S) 2 (S) 19 (S) 4 (S) SSTI, IPM, CST</td>
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<tr>
<td>12</td>
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<td>7/15/05</td>
<td>DV, HF</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>7/23/05</td>
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<td></td>
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<td>8/14/05</td>
<td>DM</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>12/10/05</td>
<td>6 (R) 32 (R) 6 (R) 128 (R) TZP, CST</td>
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<td></td>
</tr>
<tr>
<td>14</td>
<td>31/male</td>
<td>12/5/05</td>
<td>GWS</td>
<td>SXT</td>
<td>ST</td>
<td>IPM, VAN</td>
<td>12/11/05</td>
<td>32 (R) 6 (R) 128 (R) IPM, CST, AMK</td>
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<tr>
<td>15</td>
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<td>32 (R) 6 (R) 128 (R) IPM, CST, AMK</td>
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<tr>
<td>16</td>
<td>36/female</td>
<td>12/19/05</td>
<td>DM, CRF</td>
<td>GW</td>
<td>CAI</td>
<td>BO/CAT</td>
<td>IPM, SXT</td>
<td>12/30/05</td>
<td>32 (R) 6 (R) 128 (R) IPM, CST, AMK</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>33/male</td>
<td>12/19/05</td>
<td>—</td>
<td>GWS</td>
<td>SXT</td>
<td>ST</td>
<td>CRO, CFZ, GEN</td>
<td>1/2/06</td>
<td>20 (S) 2 (S) 20 (S) 2 (S) IPM, CST</td>
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<tr>
<td>18</td>
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<td>12/9/05</td>
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<td>ICU</td>
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<td>AF</td>
<td>IPM, VAN, SXT</td>
<td>1/6/06</td>
<td>21 (S) 2 (S) 21 (S) 2 (S) IPM, CST</td>
<td>Died</td>
<td></td>
</tr>
</tbody>
</table>

- a Results for carbapenem-resistant isolates are shown in boldface. Screening for MBL was performed using disks containing 1 μmol EDTA 15 mm from disks containing imipenem, meropenem, and ceftazidime.
- b —, no underlying conditions; AH, arterial hypertension; SK, smoking; AMI, acute myocardial infarction; SCZ, schizophrenia; COL, colectomy; DM, diabetes mellitus; CRF, chronic renal failure; COPD, chronic obstructive pulmonary disease; DV, diverticulosis; HF, heart failure; VL, vesicular linitis.
- c ICU, intensive care unit; GW, general ward/surgery.
- d ABS, abdominal sepsis; EVS, endovascular sepsis; VAP, ventilator-associated pneumonia; SSTI, skin and soft tissue infection; OS, osteomyelitis; CAI, catheter-associated infection; PSIP, post-surgical peritonitis.
- e AF, abdominal fluid; BL, blood; CAT, catheter; BAL, bronchoalveolar lavage; BO, bone; ST, soft tissue.
- f SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; IPM, imipenem; VAP, vancomycin; SXT, trimethoprim-sulfamethoxazole; AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CLI, clindamycin; CFZ, cefazolin; ERY, erythromycin; RIF, rifampin; CST, colistin.
- g DD, disk diffusion; R, resistant; S, susceptibility; I, intermediate.
an iodometry-based overlay system (8); no differences could be seen between both groups of isolates, and no suggestion for any other acquired β-lactamase or overexpression of the chromosomal AmpC could be inferred. No significant difference could be found between both groups of isolates concerning spectrophotometric enzymatic activity (EA), expressed as "to-
molar AmpC could be inferred. No significant difference
subpopulation in two carbapenem-susceptible (IMP-13 pro-
was 1.5 versus 1.8 in the resistant and susceptible groups, respec-
tively, suggesting there is no difference in the level of expression
of carbapenemases between IPM-resistant and IPM-susceptible
isolates.

Resistant isolates also lacked a 46-kDa band in outer mem-
brane protein (OMP) profiles (assumed as OprD) (1, 2, 7).

In vitro analysis for the presence of a high-level resistance
subpopulation in two carbapenem-susceptible (IMP-13 pro-
ducing) isolates (isolates 3 and 7) showed that both MEM and
IPM at 16 μg/ml could select fully resistant subpopulations at frequencies ranging from 2 × 10^{-8} to 1.6 × 10^{-7} from over-
night cultures when cultured on antibiotic-containing agar
plates. Independently of the selecting antibiotic, they displayed
resistance to both carbapenems. Not a single colony could be
obtained by single-step selection from reference strains (ATCC 9027) or clinical isolates without IMP-13 at this con-
centration.

Mortality among patients with IMP-13-producing (IPM-sus-
ceptible) P. aeruginosa infections treated with IPM alone or in combination was 5/8. As this analysis is retrospective, whether subpopulations of fully resistant microorganisms that resulted in treatment failures may have also emerged in those patients (as actually occurs in vitro) cannot be ruled out.

We suggest MBL producers with MICs in the susceptible range should be clearly reported (categorized) differently, indi-
cating the discordance between susceptibility and the pres-
ence of a resistance marker, even if their resistance levels do not anticipate therapeutic failure. This categorization would help to redefine the current knowledge on the epidemiological analysis comparing the evolution of patients infected by micro-
organisms classified as susceptible to carbapenems, but which
prove to be IMP producers. Furthermore, this approach may contribute by providing data for discussing if, eventually, the presence of this associated resistance mechanism should mod-
ify current CLSI breakpoints (3).

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characterization of a novel metallo-beta-lactamase gene, blalnlmp-13, harb-
oured by a novel Tns5051-type transposon disseminating carbapenemase

Gisela Santella

Arabela Cuírolo

Cátedra de Microbiología

Facultad de Farmacia y Bioquímica

Universidad de Buenos Aires

Junín 956 CP 1113

Buenos Aires, Argentina

Marisa Almuzara

Susana Palombarani

Gabriela Sly

Laboratorio de Bacteriología

Hospital Interaltonal General de Agudos Eva Perón

San Martín, Provincia de Buenos Aires, Argentina

Marcela Radice

Gabriel Gutkind

Cátedra de Microbiología

Facultad de Farmacia y Bioquímica

Universidad de Buenos Aires

Junín 956 CP 1113

Buenos Aires, Argentina

*Phone: 5411-49648285
Fax: 5411-45083645
E-mail: gutkindg@fibyiba.ar

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