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, gentamicin, and ciprofloxacin. 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).

TABLE 1. Epidemiological data and antimicrobial resistance profile

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Age (yr)</th>
<th>sex</th>
<th>Admission date (mo/day/yr)</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/yr)</th>
<th>Antibiotic susceptibility interpretation</th>
<th>Therapeutic treatment</th>
<th>Outcome</th>
</tr>
</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, IPM</td>
<td>12/14/04</td>
<td>20 (S) 4 (S) 18 (S) 4 (S)</td>
<td>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)</td>
<td>CST</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60/male</td>
<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)</td>
<td>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, COL</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CRO, CIP, TZP</td>
<td>4/7/05</td>
<td>21 (S) 2 (S) 21 (S) 4 (S)</td>
<td>IPM, SXT</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>56/male</td>
<td>2/28/05</td>
<td>DM, CRF</td>
<td>GW</td>
<td>SSTI, OS</td>
<td>BO</td>
<td>Unknown</td>
<td>5/16/05</td>
<td>21 (S) 2 (S) 17 (S) 4 (S)</td>
<td>Unknown</td>
<td>Favorable</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>31/male</td>
<td>5/11/05</td>
<td>—</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)</td>
<td>IPM, CST</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>86/male</td>
<td>5/10/05</td>
<td>DM</td>
<td>ICU</td>
<td>Cai</td>
<td>CAT</td>
<td>CRO, CAZ, AMK</td>
<td>5/26/05</td>
<td>21 (S) 2 (S) 17 (S) 4 (S)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>22/male</td>
<td>5/28/05</td>
<td>SK</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CRO</td>
<td>6/3/05</td>
<td>21 (S) 2 (S) 21 (S) 4 (S)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<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>SAM, CIP</td>
<td>7/9/05</td>
<td>18 (S) 2 (S) 19 (S) 4 (S)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>70/male</td>
<td>7/05/05</td>
<td>—</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CRO, CLI</td>
<td>7/9/05</td>
<td>21 (S) 2 (S) 20 (S) 4 (S)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>67/male</td>
<td>7/08/05</td>
<td>AH</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>CIP, AMK</td>
<td>7/15/05</td>
<td>21 (S) 2 (S) 19 (S) 4 (S)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>79/male</td>
<td>7/15/05</td>
<td>DV, HF</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>SAM, CIP</td>
<td>7/23/05</td>
<td>18 (S) 4 (S) 17 (S) 8 (I)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>27/female</td>
<td>8/14/05</td>
<td>—</td>
<td>ICU</td>
<td>VAP</td>
<td>BAL</td>
<td>TZP, VAN, CAZ, AMK</td>
<td>12/10/05</td>
<td>6 (R) 32 (R) 6 (R) 128 (R)</td>
<td>TZP</td>
<td>Favorable</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>31/male</td>
<td>12/5/05</td>
<td>—</td>
<td>GWS</td>
<td>SSTI</td>
<td>ST</td>
<td>IPM, VAN</td>
<td>12/11/05</td>
<td>32 (R) 6 (R) 128 (R)</td>
<td>IPM, VAN, CST</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>33/male</td>
<td>12/19/05</td>
<td>—</td>
<td>GW</td>
<td>SSTI</td>
<td>ST</td>
<td>CFZ, GEN, ERY</td>
<td>12/21/05</td>
<td>7 (R) 32 (R) 6 (R) 128 (R)</td>
<td>IPM</td>
<td>Died</td>
<td></td>
</tr>
<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, VAN</td>
<td>12/30/05</td>
<td>6 (R) 32 (R) 6 (R) 128 (R)</td>
<td>IPM</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>SSTI</td>
<td>ST</td>
<td>CFZ, GEN</td>
<td>1/2/06</td>
<td>20 (S) 2 (S) 20 (S) 2 (S)</td>
<td>IPM, CST</td>
<td>Favorable</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>56/male</td>
<td>12/9/05</td>
<td>VL</td>
<td>ICU</td>
<td>PSP</td>
<td>AF</td>
<td>IPM, VAN, SXT, RIF</td>
<td>1/6/06</td>
<td>21 (S) 2 (S) 21 (S) 2 (S)</td>
<td>IPM, VAN</td>
<td>Favorable</td>
<td></td>
</tr>
</tbody>
</table>

—, no underlying conditions; AH, arterial hypertension; SK, smoking; AMI, acute myocardial infarction; SCZ, schizophrenia; COL, colostomy; DM, diabetes mellitus; CRF, chronic renal failure; COPD, chronic obstructive pulmonary disease; DV, diverticulosis; HF, heart failure; VL, vesicular linitis.

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, colostomy; DM, diabetes mellitus; CRF, chronic renal failure; COPD, chronic obstructive pulmonary disease; DV, diverticulosis; HF, heart failure; VL, vesicular linitis.

c 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 “total” β-lactamase activity (using cephalothin [CEF] as a reporter), and carbapenemase activity (using imipenem as a reporter). EA for cephalothin (EA_{CEF}) (U/mg protein) for IPM-resistant isolates and IPM-susceptible isolates were (1.7 ± 0.3) × 10^{-7} and (2.2 ± 0.7) × 10^{-7}, respectively. EA for IPM (EA_{IPM}) (U/mg protein) were (2.7 ± 0.6) × 10^{-6} and (4.2 ± 2.0) × 10^{-7} for resistant and susceptible isolates, respectively. Hydrolysis of imipenem relative to cephalothin (AEIPM/AECCEF) was 1.5 versus 1.8 in the resistant and susceptible groups, respectively, 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 membrane protein (OMP) profiles (assumed as OprD) (1, 2, 7).

In vitro analysis for the presence of a high-level resistance subpopulation in two carbapenem-resistant (IMP-13 producing) 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 overnight 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 concentration.

Mortality among patients with IMP-13-producing (IPM-susceptible) _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, indicating the discordance between susceptibility and the presence 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 microorganisms 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 modify current CLSI breakpoints (3).

This work was partially supported by grants from UBACyT and ANPCyT to G.G. and from Fundación Roemmers to M.R. G.G. and M.R. are members of Carrera de Investigación Científica (CONICET). A.C. and G.S. were recipients of doctoral fellowships from ANPCyT.

We acknowledge Graciela Ferraro and Virginia Martino (University of Buenos Aires) for facilitating spectrophotometer use and Adriana Rosato (Department of Internal Medicine, Medical College of Virginia, Campus of Virginia Commonwealth University, Richmond) for facilitating the use of spectrophotometer. Finally, we acknowledge Nancy Hanson and Daniel Wolter (Creighton University, Omaha, NE) for kind and helpful suggestions.

REFERENCES


Gisela Santella
Arabela Cuirolo
Cátedra de Microbiología
Facultad de Farmacia y Bioquímica
Universidad de Buenos Aires
Junín 956 CP 1113
Buenos Aires, Argentina

Marisa Almazara
Susana Palombarani
Gabriela Sly
Laboratorio de Bacteriología
Hospital Interzonal General de Agudos Eva Perón
San Martín, Provincia de Buenos Aires, Argentina

Marcela Radice
Gabriel Gutfkind*
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: gutfkind@fyb.uba.ar

*Published ahead of print on 4 January 2010.