Dissemination of Acinetobacter baumannii Clones with OXA-23 Carbapenemase in Colombian Hospitals

Maria Virginia Villegas,1 Juan Nicolas Kattan,1 Adriana Correa,1 Karen Lolans,2,3 Ana Maria Guzman,1 Neil Woodford,4 David Livermore,1 John P. Quinn,2,5,5* and the Colombian Nosocomial Bacterial Resistance Study Group

International Center for Medical Research and Training (CIDEIM), Cali, Colombia;1 John Stroger Hospital, Chicago, Illinois2; Chicago Infectious Disease Research Institute, Chicago, Illinois3; Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), Health Protection Agency Centre for Infections, London, United Kingdom4; and Rush University Medical Center, Chicago, Illinois5

Received 25 January 2007/Returned for modification 7 March 2007/Accepted 23 March 2007

During 2005, 66 carbapenem-resistant isolates of Acinetobacter baumannii were collected from seven tertiary-care hospitals participating in a nationwide surveillance network in Colombia. The isolates were multidrug resistant and produced the carbapenemases OXA-23 and OXA-51. Forty-five belonged to four clones while 21 were unique pulsotypes. One clone was present in two hospitals within one city, while another had spread between two hospitals in different cities. Blood, secretions, and abdominal fluids were the most frequent sites of isolation. This is the first description of widespread dissemination of OXA-23 in South America.

Acinetobacter baumannii is an important nosocomial pathogen which appears to be increasing in frequency (8). Carbapenems have been the drugs of choice for treatment of severe Acinetobacter infections, but their efficacy is increasingly compromised by resistance (19).

According to the SENTRY reports, resistance rates for nosocomial gram-negative pathogens, including A. baumannii, are higher in Latin American countries than in the United States or Europe. The prevalence of carbapenem resistance in A. baumannii isolates across Latin America in the SENTRY database in 2001 was estimated at 25% (13, 24). During 2005, carbapenem resistance rates for A. baumannii were around 40% in 12 Colombian tertiary-care hospitals (18).

Carbapenem-hydrolyzing OXA enzymes are the most important cause of carbapenem resistance in A. baumannii worldwide (23). These began to be described over a decade ago, in 1993, with the description of ARI-1, later renamed OXA-23, in an imipenem-resistant A. baumannii strain in a patient in the Edinburgh Royal Infirmary (22). The strain was isolated in 1985, before the use of imipenem in the hospital. Imipenem resistance was subsequently demonstrated to be transferable (25). Since then, carbapenem-resistant isolates of A. baumannii carrying oxacillinases have been reported worldwide (4, 14, 29). It has been recognized that most A. baumannii strains have a chromosomal carbapenemase gene (a blaOXA-51-like gene) (10), though this is expressed at a high level only if an insertion sequence, such as ISAba1, is inserted upstream (30). In addition, a minority of A. baumannii strains have further OXA carbapenemase genes that are not part of the normal genomic repertoire of the species; these include the blaOXA-23-like gene, the blaOXA-24-like gene, and blaOXA-58. Although they are less-efficient hydrolyzers of carbapenems in vitro than are the metallo-β-lactamases (MβLs), these oxacillinases can inactivate carbapenems and their presence or activation by ISAba1 is demonstrably correlated with resistance (4, 30).

Based on the high rates of resistance to carbapenems in A. baumannii strains from 10 tertiary-care hospitals in the Colombian network, an investigation into the underlying mechanisms and strain structure was undertaken.

(This report was presented in part at the 46th Annual International Conference on Antimicrobial Agents and Chemo therapy, San Francisco, CA, 2006 [13a].)

MATERIALS AND METHODS

During 2005, the research facility Centro Internacional de Entrenamiento e Investigaciones Medicas (CIDEIM) conducted a study of nosocomial multidrug-resistant A. baumannii with the participation of the Colombian Nosocomial Bacterial Resistance Study Group, which included 10 tertiary-care institutions in six cities. Centers were selected if they provided tertiary care, had microbiologists and infectious-disease physicians on site, and agreed to participate.

Epidemiological and susceptibility data for all isolates from patients in general wards and intensive care units were sent to CIDEIM. This information was analyzed with WHONET 5.3 software (26). Initial susceptibilities were determined by the automated systems used in nine participating institutions (Microscan, Dade Behring Inc, Deerfield, IL, or Vitek, bioMérieux, Lyons, France) or, at one site, by the CLSI standard disk susceptibility method (20).

Seventy-one A. baumannii isolates that had been reported as carbapenem-resistant based on an imipenem or meropenem MIC of ≥16 μg/ml (21) were available and sent to CIDEIM for further analysis. Seven of the 10 institutions sent isolates with this phenotype (Table 1).

Bacterial identification and susceptibility testing. Bacterial identification was confirmed by Vitek (bioMérieux, Lyons, France) with the GN1+ card, used according to the manufacturer's instructions. MICs were determined for imipenem (Merck Sharp & Dohme, Rahway, NJ) and meropenem (AstraZeneca, Alderley Park, United Kingdom) by the CLSI broth microdilution method (21).

Strain typing. Pulsed-field gel electrophoresis (PFGE) was performed on genomic DNA of all A. baumannii isolates as described previously (28). A CHEF Mapper system (Bio-Rad Laboratories, Fremont, CA) was used to electrophorese SmaI-digested DNA (Promega, Madison, WI) at a voltage of 6 V/cm at 14°C, with pulse times of 1 s and 30 s for 19 h. The results were analyzed with Diversity software (Bio-Rad), and band-based dendrograms were produced using Dice coefficients (7). Indistinguishable and closely related (85% to 99% related) pulsotypes were considered clonal, and the major clones from each hospital were then compared with other major clones from other hospitals and cities.
TABLE 1. A. baumannii isolates from 10 tertiary-care hospitals in Colombia

<table>
<thead>
<tr>
<th>City</th>
<th>Hospital</th>
<th>No. of carbapenem-resistant A. baumannii isolates sent to CIDEM</th>
<th>Clone no. (no. of isolates)</th>
<th>No. of unique pulsotypes</th>
<th>No. of isolates positive by PCR for gene:</th>
<th>Isolates found by WHONET to be carbapenem resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cali</td>
<td>A</td>
<td>31</td>
<td>1 (21)</td>
<td>10</td>
<td>31/31/29/29</td>
<td>29/78/268</td>
</tr>
<tr>
<td>Bogotá</td>
<td>B</td>
<td>12</td>
<td>2 (8)</td>
<td>4</td>
<td>12/12/29/29</td>
<td>62/43/69</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>2</td>
<td>2 (2)</td>
<td>2</td>
<td>2/2/29/29</td>
<td>33/3/9</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/0/29/29</td>
<td>11/3/27</td>
</tr>
<tr>
<td>Medellín</td>
<td>E</td>
<td>10</td>
<td>3 (10)</td>
<td>10</td>
<td>10/10/29/29</td>
<td>34/17/50</td>
</tr>
<tr>
<td>Pereira</td>
<td>G</td>
<td>7</td>
<td>1 (2); 4 (2)</td>
<td>3</td>
<td>7/7/29/29</td>
<td>29/15/52</td>
</tr>
<tr>
<td>Bucaramanga</td>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1/1/29/29</td>
<td>18/2/11</td>
</tr>
<tr>
<td>Barranquilla</td>
<td>J</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3/3/29/29</td>
<td>32/8/25</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>66</td>
<td>Four clones (45)</td>
<td>21</td>
<td>66/65/34/34</td>
<td>34 (avg) 182/542/7</td>
</tr>
</tbody>
</table>
intrinsic blaOXA-51-like carbapenemase gene of A. baumannii, a configuration previously associated with carbapenem resistance (27). Carbapenem resistance in these instances might be explained by other mechanisms, such as impermeability and/or another β-lactamase. MβLs were not present.

Genetic locations were investigated for the blaOXA-23-like genes in the representatives of the major A. baumannii clones. Total DNA was digested with the I-CeuI endonuclease and hybridized successively with 16S rRNA and OXA-23 probes. After digestion with the enzyme, the DNA was resolved into five to seven fragments, each of which hybridized with the 16S rRNA-specific probe (Fig. 1A and B), identifying them as chromosomal. The blaOXA-23-specific probe cohybridized with a single chromosomal fragment of isolates representing clones 1, 3, and 4 (Fig. 1C). No hybridizing chromosomal fragment was identified for clone 2.

Plasmid DNA was extracted from the same A. baumannii isolates used in the I-CeuI experiments, and electrophoretic separation of uncut plasmid DNA detected multiple bands in each of the DNAs. Under conditions of high stringency, the OXA-23 probe hybridized with a single plasmid band in the representative of clone 2 (Fig. 2), whereas no hybridization was noted for the other clones. We conclude that blaOXA-23 was chromosomal in clones 1, 3, and 4 but plasmid mediated in clone 2.

**DISCUSSION**

During the past few years, OXA-23 enzymes have been reported in Acinetobacter strains from Brazil (6), China (GenBank accession number AY554200) (31), Ireland (3), the United Kingdom (27, 30), and Singapore (GenBank accession number AY795964). One clone with OXA-23 has spread to over 36 hospitals in southern England (5), while clones with OXA-40 (OXA-24 related) have spread widely in Spain and, more recently, in the United States (15).

We report here that OXA-23-like carbapenemases were present in A. baumannii isolates from multiple, widely separated cities in Colombia. The producers included both non-clonal and clonal isolates, with clonal spread having occurred between hospitals in the same city and between hospitals in different cities. In three of the four clones examined, blaOXA-23 was chromosomally encoded; in the fourth clone, it was plasmid encoded. Chromosomal mediation has been demonstrated or assumed for other OXA carbapenemases (4), though plasmid encoding of OXA-23 (19) and OXA-58 (1, 9) enzymes has also been reported. Chromosomal mediation of blaOXA-23 has been described previously for Proteus mirabilis (2).

Based on our data, there are two major contributors to the high prevalence of carbapenem-resistant A. baumannii isolates in Colombia. The first is the presence of OXA-23-like carbapenemases. A discrepancy in susceptibilities between imipenem and meropenem, noted for a subset of these isolates, raises the possibility of a more widespread dissemination of these OXA-23-like carbapenemases than that detected here. As clinical laboratories typically test only a single carbapenem in their automated panels, the presence of a potentially plasmid-mediated resistance mechanism in carbapenem-susceptible/intermediate isolates may go clinically unrecognized.

A second factor contributing to this high prevalence is the dissemination of resistant clones. We have previously published work on the clonal dissemination of carbapenem-resistant Pseudomonas aeruginosa in Colombian hospitals (28), where the MβL VIM-2 was detected in isolates from multiple cities. Some clones were local while others had spread between cities; in general, the prevalence of carbapenem resistance was related mostly to clonal spread. The present results illustrate similar patterns for a different species and enzyme class.

Our findings further illustrate the emerging global problems due to OXA-class carbapenemases in Acinetobacter spp. As carbapenemases have been the drugs of choice for serious Acinetobacter infection, this is a major clinical problem. Given the proclivity of Acinetobacter for nosocomial spread and contamination of the environment, enhanced infection control measures will be of major importance. This is particularly true in light of the paucity of new agents active against this pathogen.
ACKNOWLEDGMENTS

We thank the participating institutions from the Colombian Nosocomial Resistance Study Group, whose members are as follows: CIDEM, Sandra Reyes; Cali, Ernesto Martínez, Lena Barrera, Luz Marina Gallardo, Alba Lucía Bohorquez, and Nancy Villamarin; Bogotá, Carlos Alcuquiche, Aura Lucía Leal, Martha Ruiz, Mariluz Páez, Pilar Hurtado, Andrés Torres, Adela Cubides, Henry Mendoza, Alba Lucía Sanín, Nancy Botía, Claudia Rodríguez, Sandra Reina, Martha Patricia Melendez, Sonia Cuervo, Jorge Cortés, Martha Cristina Paredes, and Patricia Arroyo; Medellín, Carlos Ignacio Gómez, Jaime López, Mónica Cuartas, Ana Lucía Correa, Jorge Donado, Julián Betancourt, Juan David Villa, Ana Cristina Quiroga, Luz Teresita Correa, Eugenia Loaiza, Luz María Melguiza, Martha Valdejo, Rubén Darío Trejos, Victoria García, and Dora Rivas; Barranquilla, Ezequiel Guijarro, Rubén Darío Camargo, Adriana Marin, and Angela Mendoza; Pereira, Carmen Elisa Llano, Araceli Cano, Martha Lucía Gómez, and Liliana Villa; Bucaramanga, Claudia Bárcenas, Adriana Pinto, and Luis Ángel Villar; and Ibagüé, Claudia Chevery and Amparo Ovalle.

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REFERENCES