Emergence and spread of a multidrug-resistant
Acinetobacter baumannii clone producing both the
 carbapenemase OXA-23 and the 16S rRNA
methylase ArmA

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Acinetobacter baumannii is a Gram-negative organism reported worldwide as a cause of
health-care-associated infections, particularly in intensive care units (ICUs). The aim of this
study is to describe the emergence and spread of carbapenem-resistant A. baumannii (CRAB)
isolates in hospitalized patients. From March to November 2009, multidrug-resistant CRAB
isolates were obtained from 21 patients hospitalized in different wards (mostly ICUs).
Antimicrobial susceptibility was determined by using the Etest method. Carbapenem and
aminoglycoside resistance determinants were studied by PCR and sequencing. Genetic
relatedness was investigated by pulsed-field gel electrophoresis and multiplex PCR
identification of sequence groups. Clinical records of patients were examined retrospectively.
CRAB isolates were consistently resistant to multiple drugs including fluoroquinolones and
aminoglycosides, whereas they retained a susceptibility to colistin. Molecular analysis revealed
that 19 of the 21 CRAB isolates belonged to a single clone producing both the carbapenemase
OXA-23 and the 16S rRNA methylase ArmA. Based on clinical data, the patients included in
the study were classified as infected (n=13) or colonized (n=8). Colistin alone or in
combination with ampicillin–sulbactam was administered to 11 of the 13 infected patients. A
complete or partial response was obtained in eight cases, whereas a failure to respond was
observed in one patient and a relapse was observed in two patients. An A. baumannii clone
producing both OXA-23 and ArmA has been identified as an emerging and rapidly spreading
pathogen. To our knowledge, this is the first report of the ArmA enzyme in A. baumannii in Italy
and the first report of hospital dissemination of A. baumannii carrying both blaOXA-23 and
armA genes.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative organism reported worldwide as a cause of health-care-associated infections, particularly in intensive care units (ICUs) (Towner, 2009). A. baumannii does not appear to be a typical environmental organism. Nevertheless, once endemic in a

healthcare unit, it is extremely difficult to eradicate due to its marked ability to survive for prolonged periods of time throughout the clinical environment (Peleg et al., 2008).

A. baumannii produces naturally occurring β-lactamases, including a class C cephalosporinase expressed at a basal level that does not reduce the susceptibility to extended-
spectrum cephalosporins, and class D oxacillinases (including OXA-51 and closely related variants) that have low-level carbapenemase activity (Bou & Martínez-Beltrán, 2000; Hérigitier et al., 2005).
Extensive use of antimicrobial agents in hospitals may have contributed to the emergence of *A. baumannii* isolates that are resistant to a wide range of antibiotics, including aminoglycosides and fluoroquinolones. Resistance to aminoglycosides is most commonly due to enzymatic inactivation. Recently, methylation of 16S rRNA caused by the ArmA enzyme has been reported as a novel mechanism of resistance against aminoglycosides in *A. baumannii* (Doi & Arakawa, 2007).

For a long time, imipenem and meropenem have been the drugs of choice for the treatment of infections due to multidrug-resistant (MDR) *A. baumannii*. Currently, however, their efficacy has been compromised by increased dissemination of isolates showing resistance to these antibiotics (Perez et al., 2007).

Resistance to carbapenems in *A. baumannii* may occur due to different mechanisms, including the acquisition of class D oxacillinases and, less frequently, the production of class B metallo-β-lactamas (MBL) or the loss of porins (Poirel & Nordmann, 2006). Acquired oxacillinases, such as OXA-23, OXA-24/40 and OXA-58, significantly contribute to carbapenem resistance in *A. baumannii* and are mostly associated with class 1 integrons or insertion sequences (Poirel et al., 2010). The ability of OXA-23 to hydrolyse carbapenems appears to be weak, but such hydrolysis is significant when compared to the activities of other class D β-lactamases (Poirel & Nordmann, 2006).

The OXA-23 β-lactamase was first identified in one *A. baumannii* isolate from Scotland in 1995 (Scife et al., 1995). Data from 15 countries highlight the spread of OXA-23-positive *A. baumannii* isolates throughout the world (Mugnier et al., 2010). Isolates of *A. baumannii* producing the OXA-23 enzyme have also been reported in Italy (Di Popolo et al., 2011; D’Arezzo et al., 2011).

The aim of this study was to describe the emergence and spread of MDR *A. baumannii* isolates and analyse the molecular mechanism(s) at the root of carbapenem and aminoglycoside resistance.

**METHODS**

**Bacterial isolates.** A total of 21 carbapenem (imipenem and/or meropenem)-resistant *A. baumannii* isolates were obtained between March and November 2009 from patients hospitalized in different wards of the San Gerardo Hospital, Monza, Italy, and the Bassini Hospital, Cinisello Balsamo, Italy. Bacterial identification was routinely performed at the Microbiology Laboratory of the San Gerardo Hospital using the Vitek2 Compact Automated System (bioMérieux). Identification at the species level was then confirmed by amplification of the *bla*<sub>OXA-23</sub>-like gene and PCR amplification and sequence analysis of the 16S–23S rRNA intergenic spacer region (Chang et al., 2005).

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility testing was performed using Etest strips (bioMérieux). MICs of the following antimicrobials were determined: ampicillin–sulbactam, piperacillin–tazobactam, aztreonam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, tigecycline and colistin. When available, results were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2011). Carbapenem-resistant strains were further investigated by molecular methods to detect the presence of carbapenem and aminoglycoside resistance determinants.

**Characterization of β-lactamases.** Isoelectric focusing (IEF) analysis of crude cell extracts and detection of their activity by a substrate overlaying procedure (using 0.5 μg ml<sup>−1</sup> imipenem) were performed as described previously (Pagani et al., 2002). Known producers of β-lactamases TEM-1, TEM-2, TEM-7, TEM-8, TEM-9, TEM-12, SHV-1, SHV-2 and SHV-5 were used as controls.

Detection of naturally occurring *bla*<sub>OXA-23</sub>-like alleles was carried out by PCR as previously described (Heritier et al., 2005). Strains were screened for genes encoding acquired carbapenemases, including *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24/40</sub>-like and *bla*<sub>OXA-58</sub>-like genes, using multiplex PCR as described previously (Woodford et al., 2006). The presence of *bla*<sub>OXA-23</sub>-related sequences was determined by PCR using the primers 5′-GATGTGTCATAGAATCCGTG-3′ and 5′-TCACACAGAATACAGACGCTG-3′ (Afzal-Shah et al., 2001). The amplification conditions included an initial step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 25 s, 52 °C for 40 s, and 72 °C for 50 s, and a final elongation step at 72 °C for 6 min. PCR amplicons of *bla*<sub>OXA-23</sub>-like genes were purified using Quantum Prep PCR Kleen Spin Columns (Bio-Rad) and subjected to direct sequencing. PCR products were sequenced on both strands with an Applied Biosystems sequencer. The nucleotide sequences were analysed using the BLAST program. The presence of the insertion element ISAba1 was investigated using PCR (Turton et al., 2006). Detection of class 1 and class 2 integrons by integrase PCR was performed using the method of Koelman et al. (2001).

**Detection of the armA 16S rRNA methylase gene.** The presence of the *armA* methylase gene was investigated by PCR using specific primers as described previously (Doi & Arakawa, 2007). Thermal cycling conditions were modified as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of at 96 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, with a final extension step at 72 °C for 7 min.

**Southern blot analysis and location of *bla*<sub>OXA-23</sub> and armA genes.** Chromosomal or plasmid locations of *bla*<sub>OXA-23</sub> and *armA* genes were assessed by hybridization of I-Ceu-digested genomic DNA with *bla*<sub>OXA-23</sub>, *armA* and 16S rDNA probes followed by electrophoresis (Gene Path program 50–700 kb) (Liu et al., 1993). DNA was transferred from an agarose gel onto Hybond-N + membranes by capillary transfer and hybridized with 32P CTP-labelled probes overnight at 65 °C. The membranes were developed according to the manufacturer’s instructions (GE Healthcare).

**Pulsed-field gel electrophoresis and multiplex PCR identification of sequence groups.** Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA were analysed by means of the Gene Path procedure (Bio-Rad). Genomic DNA was digested with Apal and restricted fragments were separated on a 1.0% agarose gel using a CHEF-DR II apparatus (Bio-Rad) in 0.5 × TBE buffer. Bacteriophage λ concatamers were used as DNA size markers. The banding patterns were analysed with Fingerprinting II version 3.0 software (Bio-Rad) using the unweighted pair-group method with arithmetic mean (UPGMA). Following a standard PFGE typing protocol for *A. baumannii*, isolates with less than six fragment differences and ≥75% similarity were considered as belonging to the same PFGE clone (Seifert et al., 2005; Towner et al., 2008). A multiplex PCR targeting ompA, csuE and *bla*<sub>OXA-51</sub>-like genes was performed in order to discriminate between different sequence groups (Turton et al., 2007). *A. baumannii* RUH875 and RUH1134 were used as reference.
strains, representative of the International clonal lineages I and II, respectively.

Epidemiological and clinical data. Clinical records of patients with A. baumannii infection or colonization were examined retrospectively. The following data were recorded: age, gender, admission ward, date of admission, previous hospitalization during the last year, site of infection or colonization, antimicrobial treatment before or during the infective episode, treatment outcome and clinical outcome.

Table 1. Resistance phenotype and molecular results for 21 carbapenem-resistant A. baumannii strains isolated from March to November 2009

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (mg l⁻¹)</th>
<th>PFGE clone</th>
<th>Enzyme(s) produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAM</td>
<td>TZP</td>
<td>ATM</td>
</tr>
<tr>
<td>MB01/09</td>
<td>6</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB02/09</td>
<td>24</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB03/09</td>
<td>16</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB04/09</td>
<td>16</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB05/09</td>
<td>16</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB06/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB07/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB08/09</td>
<td>32</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB09/09</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB11/09</td>
<td>16</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB12/09</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB13/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB14/09</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB15/09</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB16/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB17/09</td>
<td>12</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB18/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB19/09</td>
<td>16</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB20/09</td>
<td>16</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>MB22/09</td>
<td>12</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>MB23/09</td>
<td>24</td>
<td>&gt;256</td>
<td>48</td>
</tr>
</tbody>
</table>

Treatment outcomes were classified as follows: complete response – resolution of fever, leukocytosis and local signs and symptoms of infection; partial response – improvement of fever, leukocytosis and local signs and symptoms of infection without complete resolution; relapse – recurrence of infection with the same organism at any bodily site within 1 month of discontinuation of therapy; treatment failure – absence of resolution or worsening of signs and symptoms of infection; not assessable – due to incomplete records or death of the patient within 72 h of infection.
**Table 2. Microbiological and clinical data of patients with *A. baumannii* infection or colonization**

ICU, intensive care unit; ICU CCH, cardiosurgery intensive care unit; ICU B, intensive care unit Bassini Hospital; ICCU, intensive coronary care unit; PNEU, pneumology; GYN, gynaecology; CH, surgery; CH B, surgery Bassini Hospital; NCH, neurosurgery intensive care unit; CST, colistin; SAM, ampicillin–sulbactam; RIF, rifampicin; TGC, tigecycline; MU, million units; iv, intravenously; n, nebulized; NA, not available.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Patient</th>
<th>Admission</th>
<th>Previous hospitalization</th>
<th>Previous exposure to carbapenems and/or AG</th>
<th>Site of infection</th>
<th>Site of colonization</th>
<th>Isolation date</th>
<th>Antimicrobial therapy</th>
<th>Treatment outcome</th>
<th>Patient outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Sex</td>
<td>Ward</td>
<td>Date</td>
<td></td>
<td></td>
<td></td>
<td>Agent</td>
<td>Daily dose</td>
<td>Duration (days)</td>
</tr>
<tr>
<td>MB01/09</td>
<td>68 F</td>
<td>ICU 15/03/09</td>
<td>Yes</td>
<td>Yes (MEM)</td>
<td>Lung</td>
<td>25/03/09</td>
<td>CST</td>
<td>3 MU x 3, iv; 1 MU x 3, n</td>
<td>11</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB02/09</td>
<td>77 M</td>
<td>ICU 19/03/09</td>
<td>No</td>
<td>No</td>
<td>Blood Lung</td>
<td>31/03/09</td>
<td>SAM CST</td>
<td>3 g x 3, iv; 3 MU x 3, iv; 1 MU x 4, n</td>
<td>24</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB03/09</td>
<td>76 M</td>
<td>ICU 26/03/09</td>
<td>No</td>
<td>No</td>
<td>Blood Lung</td>
<td>02/04/09</td>
<td>SAM CST</td>
<td>3 g x 4 iv; 3 MU x 3, iv; 1 MU x 4, n</td>
<td>35</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB04/09</td>
<td>39 M</td>
<td>ICU 28/03/09</td>
<td>No</td>
<td>Yes (MEM)</td>
<td>Respiratory tract</td>
<td>06/04/09</td>
<td>SAM CST</td>
<td>3 g x 4, iv; 2 MU x 3, iv; 1 MU x 4, n</td>
<td>21</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB05/09</td>
<td>76 F</td>
<td>ICU CCH 22/03/09</td>
<td>Yes</td>
<td>No</td>
<td>Lung</td>
<td>07/04/09</td>
<td>SAM CST</td>
<td>2 g x 3, iv; 2 MU x 3, iv; 1 MU x 4, n</td>
<td>7</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB06/09</td>
<td>56 F</td>
<td>ICU 16/03/09</td>
<td>No</td>
<td>Yes (MEM)</td>
<td>Lung</td>
<td>08/04/09</td>
<td>SAM CST</td>
<td>3 g x 3, iv; 2 MU x 3, iv; 1 MU x 4, n</td>
<td>14</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB07/09</td>
<td>48 M</td>
<td>ICU 06/02/09</td>
<td>Yes</td>
<td>Yes (MEM)</td>
<td>Lung</td>
<td>08/04/09</td>
<td>SAM CST</td>
<td>3 g x 3, iv; 3 MU x 3, iv; 1 MU x 4, n</td>
<td>70</td>
<td>Relapse</td>
</tr>
<tr>
<td>MB08/09</td>
<td>58 F</td>
<td>ICU B 05/05/09</td>
<td>No</td>
<td>Yes (MEM)</td>
<td>Bladder Lung</td>
<td>23/06/09</td>
<td>SAM CST</td>
<td>3 g x 3, iv; 1 MU, iv; 1.5 MU, n</td>
<td>42</td>
<td>Discharged on day 87</td>
</tr>
<tr>
<td>MB09/09</td>
<td>78 M</td>
<td>ICU B 21/04/09</td>
<td>No</td>
<td>Yes (MEM)</td>
<td>Blood</td>
<td>29/05/09</td>
<td>None None None None None None</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>MB11/09</td>
<td>81 M</td>
<td>PNEU 25/06/09</td>
<td>Yes</td>
<td>Yes (AMK)</td>
<td>Respiratory tract</td>
<td>03/06/09</td>
<td>None None None None None None</td>
<td>NA</td>
<td>Discharged on day 25</td>
<td></td>
</tr>
</tbody>
</table>

Complete response
Death on day 23, unrelated to *A. baumannii*
Discharged on day 49
Discharged on day 20, unrelated to *A. baumannii*
Discharged on day 42
Discharged on day 46
Discharged on day 73
Discharged on day 156
Death on day 42, attributable to *A. baumannii*
<table>
<thead>
<tr>
<th>Isolate</th>
<th>Patient</th>
<th>Admission</th>
<th>Previous hospitalization</th>
<th>Previous exposure to carbapenems and/or AG</th>
<th>Site of infection</th>
<th>Site of colonization</th>
<th>Isolation date</th>
<th>Antimicrobial therapy</th>
<th>Treatment outcome</th>
<th>Patient outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB12/09</td>
<td>73 F</td>
<td>GYN 05/07/09</td>
<td>No</td>
<td>Yes (GEN)</td>
<td>Skin</td>
<td>24/07/09</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
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<tr>
<td>MB13/09</td>
<td>82 M</td>
<td>ICCU 22/06/09</td>
<td>Yes</td>
<td>Yes (MEM)</td>
<td>Bladder catheter</td>
<td>28/07/09</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MB14/09</td>
<td>55 M</td>
<td>CH 20/07/09</td>
<td>No</td>
<td>Yes (MEM)</td>
<td>Vascular catheter</td>
<td>15/08/09</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>MB15/09</td>
<td>72 M</td>
<td>CH B 20/07/09</td>
<td>No</td>
<td>Yes (GEN, MEM)</td>
<td>Lung</td>
<td>17/08/09</td>
<td>CST</td>
<td>1 MU, iv; 1.5 MU, n</td>
<td>10</td>
<td>Partial response</td>
</tr>
<tr>
<td>MB16/09</td>
<td>4 M</td>
<td>ICU NCH 30/08/09</td>
<td>Yes</td>
<td>Yes (MEM)</td>
<td>Bladder Lung</td>
<td>29/08/09</td>
<td>CST</td>
<td>2 MU × 3, iv; 1 MU × 3, n</td>
<td>7</td>
<td>Relapse</td>
</tr>
<tr>
<td>MB17/09</td>
<td>51 F</td>
<td>ICU 19/09/09</td>
<td>Yes</td>
<td>No</td>
<td>Blood</td>
<td>27/09/09</td>
<td>RIF</td>
<td>600 mg × 2, iv</td>
<td>21</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB18/09</td>
<td>72 M</td>
<td>ICU 05/09/09</td>
<td>Yes</td>
<td>Yes (MEM)</td>
<td>Lung</td>
<td>01/10/09</td>
<td>SAM</td>
<td>3 g × 3, iv</td>
<td>7</td>
<td>Treatment failure</td>
</tr>
<tr>
<td>MB19/09</td>
<td>59 M</td>
<td>ICU CCH 07/09/09</td>
<td>Yes</td>
<td>Yes (IPM, AMK)</td>
<td>Lung</td>
<td>07/10/09</td>
<td>SAM</td>
<td>3 g × 4, iv</td>
<td>19</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB20/09</td>
<td>43 M</td>
<td>ICU 12/09/09</td>
<td>No</td>
<td>No</td>
<td>Respiratory tract</td>
<td>12/10/09</td>
<td>CST</td>
<td>3 MU × 3, iv; 1 MU × 4, n</td>
<td>14</td>
<td>Complete response</td>
</tr>
<tr>
<td>MB22/09</td>
<td>38 M</td>
<td>ICU 26/09/09</td>
<td>No</td>
<td>No</td>
<td>Skin</td>
<td>14/10/09</td>
<td>SAM</td>
<td>1 g × 3, iv</td>
<td>None</td>
<td>Discharged on day 31</td>
</tr>
<tr>
<td>MB23/09</td>
<td>70 M</td>
<td>CH 18/08/09</td>
<td>Yes</td>
<td>No</td>
<td>Skin</td>
<td>26/08/09</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Discharged on day 16</td>
</tr>
</tbody>
</table>
RESULTS

Antimicrobial susceptibility
A total of 21 carbapenem-resistant A. baumannii (CRAB) isolates were identified at the clinical microbiology laboratory of the San Gerardo Hospital. MIC results are summarized in Table 1. It is of note that meropenem showed consistently high MIC values (>32 mg l⁻¹), whereas imipenem MICs ranged from 6 to 32 mg l⁻¹. All isolates showed high-level resistance to piperacillin–tazobactam, last-generation cephalosporins cefotaxime, cefazidime and cefepime, and gentamicin (MIC > 256 mg l⁻¹). All but one isolate (MB16/09) were also highly resistant to ciprofloxacin and levofloxacin. Similarly, 20 out of the 21 isolates were characterized by high-level MICs of amikacin, with 19 of them showing heteroresistance. Most isolates had reduced susceptibility to ampicillin–sulbactam (MICs 12–16 mg l⁻¹), whereas MICs of tigecycline usually ranged from 2 to 4 mg l⁻¹. Colistin maintained in vitro activity in all cases (MIC ≤ 1 mg l⁻¹).

Epidemiological analysis and clinical data
Epidemiological analysis showed that the index patient with A. baumannii infection was a 68-year-old woman, admitted in March 2009 to the ICU of the San Gerardo Hospital. After 10 days of hospitalization, an MDR CRAB strain was isolated from lower respiratory tract secretions of the patient. During the next 2 weeks, several A. baumannii isolates showing the same resistance pattern were obtained from an additional five patients in the same ICU, four with infection and one with colonization. From March–April, 2009, some of these patients moved to cardiosurgical or neurosurgical ICU wards. On May 5, a patient was transferred from the neurosurgical ICU of San Gerardo Hospital to the ICU of Bassini Hospital, where a CRAB isolate (MB08/09) was recovered from the lower respiratory tract of the patient. Infection due to MDR A. baumannii occurred in two additional patients admitted to the same hospital during the following 3 months. Overall, 21 patients that were infected (n=13) or colonized (n=8) with MDR A. baumannii were identified in the two hospitals from March to November 2009 (Table 2). Previous hospitalization had occurred in 10 of the 21 cases. Fourteen patients had been previously exposed to carbapenems and/or aminoglycosides. Lower respiratory tract infections most frequently occurred in ICU patients and were associated with bacteraemia in four cases. Colistin, alone or in combination with ampicillin–sulbactam (plus rifampicin or tigecycline in one and two cases, respectively), was administered to most infected patients (n=11). The remaining two patients showing infection with strain MB09/09 did not receive antimicrobial therapy, and treatment data for MB08/09 were not available. A complete or partial response was obtained in eight patients, whereas failure of treatment or relapse was observed in one and two patients, respectively. It is of note that five out of 13 infected patients died during hospitalization. Death was attributable or related to A. baumannii infection in two cases.

Molecular detection of resistance determinants and clonal relatedness
All isolates revealed the presence of β-lactamases by exhibiting isoelectric points (pIs) > 9.0 (which is coherent with the expression of an AmpC-type cephalosporinase) as well as pIs typical of OXA-23 and OXA-51 enzymes (pI 6.7 and 7.0, respectively). Imipenemase activity was detected at pI 6.7 by the substrate overlaying procedure. These isolates were consistently positive by PCR for the presence of blaOXA-23 and blaOXA-51 genes. The blaOXA-23/44, blaOXA-58, blaIMP and blaVIM genes were not detected in any isolate. DNA sequencing confirmed the presence of the blaOXA-23 gene. ISAbal was always present upstream of the blaOXA-23 gene. Nineteen isolates, all of which were characterized by heteroresistance for amikacin when using the Etest, were positive for the 16S rRNA methylase armA gene (Table 1).

According to established criteria, macrorestriction analysis by PFGE revealed that most of carbapenem-resistant isolates (19/21) were closely related, with 16 of them showing identical patterns (clone A), and three having a difference of two bands (subtype A1). These isolates were related to the International clone II (≥ 75% similarity). The two remaining isolates, MB05/09 and MB16/09 were assigned to clone B and clone C, respectively. Notably, clone B was closely related (> 95% similarity) to the SMAL clone, recently responsible for outbreaks in acute care and long-term care facilities in northern Italy (Nucleo et al., 2009), whereas clone C was related (≥ 75% similarity) to the International clone I (data not shown).

The I-Ceul technique showed the chromosomal location of blaOXA-23 in MB12/09, MB05/09 and MB16/09 isolates (representative of A, B and C PFGE clones, respectively) as well as that of the armA gene in case of the MB12/09 isolate. As expected, multiplex PCR, performed using primers targeting ompA, csiE and blaOXA-51-like genes, assigned isolates belonging to PFGE clones A and C to the previously characterized sequence type groups 1, and 2, respectively, whereas clone B showed the trilocus sequence-based typing (3LST) group 6 pattern (data not shown), thus confirming the results obtained by the macrorestriction analysis. PCR analysis of the intI gene revealed the presence of class 1 integrons in all strains belonging to clone A.

DISCUSSION
The present study describes the emergence and spread of a clonal strain of carbapenem-resistant A. baumannii in two Italian hospitals. Genetic features and molecular epidemiology were also investigated.

Following the index case, an ICU patient admitted on March 2009, 18 additional patients were demonstrated to be
infected or colonized with the same clone over an 8 month period. Of the 18 patients, five were hospitalized in the same ICU during the following 2 weeks, thus demonstrating the ability of the strain to rapidly disseminate in a high-risk setting. During the following months, the outbreak strain was isolated from patients hospitalized in the same ICU as well as in different wards. In these cases, an epidemiological link to the outbreak source was consistently found. The outbreak strain was also detected from patients admitted to another hospital of the same geographical area in three cases, the first of them occurring in a patient coming from the neurosurgical ICU. Taken together, our data demonstrate the ability of CRAB isolates to be transmitted within a hospital setting as well as between different hospitals. It is of note that cross-contamination within the same hospital has been commonly reported, whereas only few cases of cross-contamination between different hospitals have been documented (Quale et al., 2003; Manikal et al., 2000).

Based on the molecular analysis of CRAB isolates, it was most likely that carbapenem resistance was attributable to the presence of the \(\text{bla}_{\text{OXA-23}}\) gene, even though other resistance mechanisms were present, for example \(\text{bla}_{\text{OXA-51}}\)-like gene expression and the presence of outer-membrane proteins. Moreover, the heterogeneous carbapenem MIC values suggest that expression of OXA-23, and/or the contribution of additional resistance mechanisms, is variable in different isolates. The detection of the \(\text{bla}_{\text{OXA-23}}\) gene was partially unexpected since CRAB outbreaks described in Europe (including Italy) during recent years were mostly characterized by the production of OXA-58 carbapenemase. In a study conducted in the Mediterranean area from 1999 to 2009, the \(\text{bla}_{\text{OXA-58}}\) gene was widely prevalent in Italian strains, whereas the \(\text{bla}_{\text{OXA-23}}\) gene was found only in strains causing outbreaks in Genoa, Italy, and Kocaeli, Turkey (Di Popolo et al., 2011). Most recently, however, D’Arezzo et al., (2011) analysed genetic determinants of carbapenem resistance in \(A.\) baumannii isolates from Rome (Central Italy), demonstrating the dissemination of two OXA-type carbapenemase genes, \(\text{bla}_{\text{OXA-23}}\) (71.1% of isolates) and \(\text{bla}_{\text{OXA-58}}\) (22.8% of isolates). Compared with a previous survey conducted in the same hospital in 2004–2005, the prevalence of \(\text{bla}_{\text{OXA-58}}\)-carrying isolates appeared to be drastically reduced, with the OXA-23-like determinant becoming prevalent in 2008 and completely replacing the OXA-58-like determinant thereafter (D’Arezzo et al., 2011). Our data confirm the increasing role of OXA-23 production in determining nosocomial outbreaks of \(A.\) baumannii in Italy, and the associated resistance of CRAB isolates to aminoglycosides (including amikacin). Molecular analysis revealed the presence of the 16S rRNA methylase gene \(\text{armA}\) in 19/21 cases. It is of note that \(\text{armA}\) has been most commonly found in members of the family \(\text{Enterobacteriaceae}\), even though its presence has been documented in \(A.\) baumannii as well, mostly in East Asia (Lee et al., 2006; Yu et al., 2007). The present study describes the dissemination of CRAB isolates producing both the OXA-23 carbapenemase and ArmA in a hospital setting. To our knowledge, this is the first report of the ArmA enzyme in Italy, and the dissemination of \(\text{bla}_{\text{OXA-23}}\) and \(\text{armA}\)-carrying \(A.\) baumannii in a hospital setting.

Molecular studies based on PFGE analysis and multiplex PCR for sequence type group identification revealed that the intrahospital and interhospital spread of CRAB isolates was caused by an outbreak strain assigned to the International clone II. The two remaining study isolates (MB05/09 and MB16/09) did not belong to the clonal strain but were closely related to the SMAL clone (3LST group 6) and to the International clone 1, respectively. It is of note that the 3LST group 6 has been described recently in a tertiary care hospital in Naples, southern Italy (Giannouli et al., 2010).

In all isolates, the \(\text{bla}_{\text{OXA-23}}\) gene was located on the chromosome and was associated with an upstream insertion sequence, \(\text{ISA}_{\text{ba}}\), which is responsible for its mobilization and expression (Mugnier et al., 2009). On the contrary, the presence of class 1 integrons was demonstrated only in clonal strains, thus indicating the potential epidemic behaviour of \(A.\) baumannii strains producing both OXA-23 carbapenemase and ArmA 16S rRNA methylase.

Treatment options and outcomes are critical in cases of CRAB infection. Current recommendations indicate intravenous colistin as a first choice treatment, considering the addition of aerosolized colistin therapy in patients with tracheobronchitis or ventilator-associated pneumonia (Garnacho-Montero & Amaya-Villar, 2010). Sulbactam is also regarded as being effective against most \(A.\) baumannii strains (Peleg et al., 2008). Clinical information regarding the use of combined treatment is scarce but a synergistic activity between colistin and rifampicin has been reported (Giamarellos-Bourboulis et al., 2001). The use of tigecycline against \(A.\) baumannii is off-label and its clinical efficacy remains a matter of controversy (Neonakis et al., 2011).

In the present study, most of the infected patients received combined therapy including both colistin (intravenous and nebulized) and ampicillin–sulbactam. Overall, this approach led to a complete or partial clinical response in 8/11 cases (72.7%), limiting the spread of \(A.\) baumannii multidrug-resistant strains. A similar response (76.9%) was been reported by Bassetti et al. (2008) using colistin plus rifampicin but the superiority of combined therapy over colistin alone remains uncertain (Bassetti et al., 2008).

The present study demonstrates the ability of CRAB isolates to acquire additional resistance determinants, further narrowing the antimicrobial spectrum that can be used to combat them. In addition, the study highlights the role of patient–patient transfer in the spread of antimicrobial drug resistance, thus emphasizing the need for hospitals to isolate and screen for multidrug-resistant pathogens in patients admitted to hospitals from other institutions.

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REFERENCES


