Changing carbapenemase gene pattern in an epidemic multidrug-resistant Acinetobacter baumannii lineage causing multiple outbreaks in central Italy

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Objectives: Infections caused by multidrug-resistant (MDR) Acinetobacter baumannii are a challenging problem worldwide. Here, the molecular epidemiology and the genetic basis of antibiotic resistance in 111 MDR A. baumannii strains isolated from June 2005 to March 2009 from infected patients in 10 intensive care units (ICUs) in central Italy were investigated.

Methods: Epidemiological typing was performed by random amplification of polymorphic DNA, PCR-based sequence grouping and macrorestriction analysis. MICs of antibiotics were determined by the broth microdilution method. Genes for OXA carbapenemases, metallo-ß-lactamases and the CarO porin were searched for by PCR.

Results: Molecular genotyping identified one predominant A. baumannii lineage, related to the international clonal lineage II, accounting for 95.6% of isolates. Isolates referable to this lineage were recovered from all ICUs surveyed and were resistant to nearly all classes of antimicrobials, with the exception of tigecycline and colistin. A high percentage (60.5%) of A. baumannii isolates showed elevated resistance to imipenem (MICs ≥ 128 mg/L), concomitant with resistance to meropenem. Carbapenem resistance was associated with the presence of either blaOXA-58-like (22.8%) or blaOXA-23-like (71.1%) carbapenemase genes. Molecular typing showed that the epidemic lineage encoding OXA-23 emerged in 2007 and displaced a genetically related clone encoding OXA-58 that had been responsible for previous ICU outbreaks in the same region.

Conclusions: Emergence of the OXA-23 epidemic lineage could result from selective advantage conferred by the blaOXA-23-like determinant, which provides increased resistance to carbapenems.

Keywords: genotyping, intensive care units, OXA-23

Introduction

Acinetobacter baumannii has emerged as an important nosocomial pathogen worldwide, especially in intensive care units (ICUs). Resistance to environmental stresses, persistence in the hospital setting and a propensity to develop stable antibiotic resistance make A. baumannii a common yet difficult-to-treat pathogen. Carbapenemase have been widely used to treat serious infections associated with multidrug-resistant (MDR) A. baumannii, but a trend of increasing resistance to these drugs has been observed worldwide in the past decade. Several mechanisms are responsible for ß-lactam resistance in A. baumannii, including: (i) the production of ß-lactamases; (ii) alterations of porin-like proteins, resulting in decreased permeability to antibiotics; and (iii) the activity of efflux pumps, decreasing the intracellular concentration of antibiotics. Of the above mechanisms, carbapenem-hydrolysing ß-lactamases (carbapenemases) belonging to the molecular class D OXA enzymes have emerged globally as the main mechanism responsible for carbapenem resistance in A. baumannii. Four groups of acquired class D ß-lactamases have been described in A. baumannii, encoded by blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-58-like genes, respectively. The blaOXA-51-like genes are chromosomally located in all A. baumannii strains. The blaOXA-58-like genes are found mostly on plasmids, whereas the blaOXA-51-like genes can be either chromosomally or plasmid located. Carbapenemase gene dosage can further contribute to determining the extent of carbapenem resistance in A. baumannii.
Previous studies have reported the frequent isolation of OXA-58-producing A. baumannii in Italy, suggesting that bla\textsubscript{OXA-58}-carrying A. baumannii strains have become endemic.\textsuperscript{8,10–12} Conversely, reports concerning the bla\textsubscript{OXA-24}-like gene are limited to the description of two sporadic isolates recovered in 2000 and 2004 from two different Italian regions.\textsuperscript{9}

In a previous survey we provided evidence of the emergence in 2004–05 of the bla\textsubscript{OXA-58} gene in clinical A. baumannii isolates genetically related to the international lineage II and responsible for ICU outbreaks in the main hospitals of the Rome urban area.\textsuperscript{12} Later, the isolation of A. baumannii producing the OXA-23 carbapenemase from patients hospitalized in Rome\textsuperscript{13} and Genoa\textsuperscript{14} was reported.

The present study analysed the epidemiological evolution and changes in antibiotic susceptibility patterns for a large collection of A. baumannii isolates recovered during 2005–09 from 10 main ICUs in central Italy. Aims of the investigation were: (i) to evaluate the genetic relationships among the isolates and to strains representative of major international clonal lineages; (ii) to trace the evolution of antibiotic resistance; and (iii) to search for changes in OXA carbapenemase gene carriage among isolates.

Materials and methods

Bacterial isolates

As part of the surveillance activity of the Gruppo Romano Acinetobacter baumannii (GRAB),\textsuperscript{12} 117 Acinetobacter isolates provisionally identified as A. baumannii were collected between 15 June 2005 and 24 March 2009 from 10 ICUs in central Italy and submitted to the coordinating laboratory at the National Institute for Infectious Diseases ‘Lazzaro Spallanzani’ (Rome) for further characterization (Table S1, available as Supplementary data at JAC Online). The geographical locations of the 10 hospitals from Central Italy (designated C, D, H, I, L, M, N, O, P and Q) are provided in Figure S1 (available as Supplementary data at JAC Online). Isolates were obtained either from epidemic clusters or sporadic cases. In total, 111 non-repetitive Acinetobacter isolates (i.e. one isolate per patient) were collected, all of which were from inpatients with clinically and laboratory-confirmed Acinetobacter infection. Six additional isolates were recovered from medical devices or the ICU environment of hospital N (Table S1). Acinetobacter isolates were not selected a priori because of MDR phenotype, though all but three of them were resistant to more than two of five antimicrobial classes (antipseudomonal cephalosporins, antipseudomonal carbapenems, \(\beta\)-lactam/\(\beta\)-lactamase inhibitor combinations, antipseudomonal fluoroquinolones and aminoglycosides) and were therefore considered to be MDR.\textsuperscript{7} In addition, seven index strains of A. baumannii (33, 50, 51, 57, 80, 82 and 105) from previously described outbreaks,\textsuperscript{12} two reference strains for international clonal lineages I (RUH875) and II (RUH134)\textsuperscript{15} and the prototype sequenced strain ACICU\textsuperscript{16} were included in the study for comparison purposes (Table S2, available as Supplementary data at JAC Online).

Bacteria were routinely identified to the species level by the participating centres using the Phoenix (Becton Dickinson, Sparks, MD, USA) and Vitek 2 (bioMérieux, Marcy l’Étoile, France) commercial systems. The source of Acinetobacter isolates is provided in Table S1.

Antimicrobial susceptibility testing

For ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefepime, ceftazidime, aztreonam, meropenem, trimethoprim/sulphamethoxazole and colistin, susceptibility testing was performed using the Vitek 2 system. MICs of imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, tetracycline and tigecycline were determined by the broth microdilution method, according to the CLSI protocol.\textsuperscript{17} All antimicrobials were obtained from Sigma-Aldrich (Milan, Italy), except tigecycline (Pfizer, New York, USA), Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213 were used as internal quality controls. MICs were interpreted according to the CLSI breakpoint criteria.\textsuperscript{17} No breakpoints were available for tigecycline.

Species assignment by amplified ribosomal DNA restriction analysis (ARDRA)

ARDRA was carried out with restriction enzymes Alul, CfoI, MboI,MspI and Rsal (Roche, Monza, Italy) and ribosomal DNA restriction patterns were interpreted as described previously.\textsuperscript{18,19}

Epidemiological typing

Random amplified polymorphic DNA (RAPD) analysis was performed with the primer M13 as described previously.\textsuperscript{20} Macrolide restriction analysis (MRA) of Apal-digested genomes and PGF\textsubscript{E} was carried out as described previously,\textsuperscript{21} using a CHEF mapper (Bio-Rad, Segrate, Milan, Italy). An Apal digest of genomic DNA from A. baumannii strain ACICU was included as the standard for gel run normalization. Electropherograms were analysed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) with the Dice coefficient and the unweighted pair group method with averages (UPGMA) with a 1% tolerance limit and 1% optimization. Isolates that clustered with a similarity of >85% were considered to belong to the same RAPD or MRA type.\textsuperscript{22}

To define the sequence group (SG), two multiplex PCR designs were selected to amplify specific regions of the ompA, csuE and bla\textsubscript{OXA-51-like} genes.\textsuperscript{23} Allelic profiles were interpreted according to the CLSI protocol.\textsuperscript{17} All antimicrobials were obtained from Sigma-Aldrich (Milan, Italy), except tigecycline. Isolates that clustered with a similarity of >85% were considered to belong to the same RAPD or MRA type.\textsuperscript{22}

Molecular detection of OXA carbapenemase, metallo-\(\beta\)-lactamase and carO genes

The presence of the four groups of OXA carbapenemase genes (bla\textsubscript{OXA-23-like}, bla\textsubscript{OXA-24-like}, bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-58-like}) was detected by PCR using a multiplex assay.\textsuperscript{24,25} The occurrence of the IS\textsubscript{Sa}b\textsubscript{O}1 element upstream of the bla\textsubscript{OXA-23-like}, bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-58-like} genes was determined by PCR as described previously.\textsuperscript{26} The identity of bla\textsubscript{OXA-51-like} and bla\textsubscript{OXA-23-like} amplicons was confirmed by direct DNA sequencing. PCR detection of the metallo-\(\beta\)-lactamase (bla\textsubscript{IMP-1}, bla\textsubscript{VIM} and bla\textsubscript{SIM}) was carried out as described previously.\textsuperscript{27–29}

The carO gene was amplified using PCR Ready-to-Go Beads (GE Healthcare, Milan, Italy) with primers carO-FW (5'-TGACAACTACAGCTTACTTGC-3') and carO-RV (5'-CAACCTGGCAACCATTTGTT-3'). PCR conditions for carO were 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min, with final extension for 10 min at 72°C.

Copy number determination of the bla\textsubscript{OXA-58-like} gene

Total DNA was extracted from selected A. baumannii isolates using the Wizard Genomic DNA purification kit (Promega, Milan, Italy) according to the manufacturer’s protocol. PCR amplification of the origin of replication of plasmid pACICU1 (repA27) gene was performed as described previously.\textsuperscript{23} The bla\textsubscript{OXA-58-like} gene copy number was determined using a long-range PCR assay (Qiagen, Milan, Italy) with forward primer 5'-CGATGACATGTTCAAGGGGC-3' and reverse primer 5'-GGCGTGATAACAATTCGG-3' based on the pACICU1 plasmid sequence (accession number NC_010605.1). The PCR mixtures comprised a volume of 50 μL containing 20 ng of purified DNA, 5 μL of 10x reaction buffer, 2.5 mM MγcP, 500 μM of each dNTP, 0.4 μM of each primer and 2 U of long-range PCR enzyme. Cycling conditions for long-range PCR comprised 3 min at
93°C followed by 35 cycles of 15 s at 93°C, 30 s at 55°C and 10 min at 68°C. Based on the pACICU1 sequence, isolates with one, two or three copies of the bla\textsubscript{OXA-58}-like gene cassette gave no amplicon, a 4034 bp amplicon or a 8048 bp amplicon, respectively.

## Results

### Identification of Acinetobacter genospecies

One hundred and fourteen of the 117 submitted Acinetobacter isolates were definitively identified as *A. baumannii* by ARDRA, showing the typical profiles ‘11123’ (110 isolates; 94.0%), ‘11121’ (9 isolates; study codes 122 and 133; 1.71%), and ‘1112(1+3)’ (2 isolates; study codes 215 and 170; 1.71%) with CfoI, AluI, MboI, RsaI and MspI, respectively (Table S3, available as Supplementary data at *JAC* Online). All these isolates were also PCR-positive for a *bla\textsubscript{OXA-58}*-like gene, confirming their identity as *A. baumannii*. Three isolates, provisionally identified as *A. baumannii*, were assigned to the *Acinetobacter calcoaceticus* genospecies by ARDRA, and were PCR-negative for a *bla\textsubscript{OXA-58}*-like gene (Table S1).

### A. baumannii genotyping and correlation with international clonal lineages

Dendrograms constructed following RAPD analysis and ApaI MRA showed one major cluster including 109 *A. baumannii* isolates (95.6%), defined by a similarity threshold > 94% and > 88% by RAPD analysis and MRA, respectively (Figure 1). This dominant lineage was genetically related to the *bla\textsubscript{OXA-58}*-positive clone previously isolated from ICU outbreaks in the Rome urban area (33, 80 and ACICU in Table S2)\textsuperscript{12} and to the international clonal lineage II (RUH134). Five isolates showed variant profiles (study codes 122, 133, 138, 170 and 215) and were unrelated to any international clonal lineages (Figure 1).

Multiplex PCRs for SG assignment yielded the 111 allelic profile (corresponding to SG 1) for 101 of 109 *A. baumannii* isolates (92.7%), in line with their genetic relatedness to the international clonal lineage II. The remaining eight *A. baumannii* isolates, which were genetically related to the international clonal lineage II, belonged to SG 4 (study codes 162, 163 and 164) or showed a new combination of amplicons in SG analysis (study codes 207, 217, 221, 233 and 234). Table S3 summarizes the relationship between SG, RAPD type and MRA type for each isolate.

### Antimicrobial susceptibility

The MDR phenotype was observed for 111 of 114 (97.4%) *A. baumannii* isolates. These were characterized by resistance to piperacillin (97.4%), piperacillin/tazobactam (95.6%), cefepime (97.4%), ceftazidime (97.4%), aztreonam (100%), imipenem (93.8%), meropenem (86.0%), amikacin (97.4%), gentamicin (77.2%), ciprofloxacin (97.4%), levofloxacin (97.4%) and piperacillin (97.4%).

MRA. The dendrograms were generated using BioNumerics software (Applied Maths) using UPGMA and the Dice coefficient. Broken lines denote isolates belonging to the same RAPD or MRA type.\textsuperscript{22} Study codes (see Table S1) are shown on the right. Reference strains (see Table S2) are highlighted with grey shading.

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**Figure 1.** Molecular typing of *A. baumannii* isolates. Clustering relationships inferred from RAPD analysis with the primer M13 and **M13 RAPD**. **ApaI MRA**.
trimethoprim/sulfamethoxazole (95.6%) (Figure 2a). Remarkably, a significant percentage of A. baumannii isolates was susceptible to ampicillin/sulbactam (48.3%), and all isolates were susceptible to colistin (Figure 2a). The distribution of MICs of tigecycline and imipenem for the 114 A. baumannii isolates is shown in Figure 2(b and c). MIC₅₀ values of tigecycline and imipenem were 0.5 mg/L and 128 mg/L, respectively. Of note, 60.5% of carbapenem-resistant A. baumannii isolates showed high-level resistance to imipenem with MICs ≥128 mg/L. Ninety-eight A. baumannii isolates were resistant to both imipenem and meropenem (86.0%), but nine (7.9%) were susceptible to meropenem and resistant to imipenem while seven (6.1%) were susceptible to both drugs.

**Genetic determinants for carbapenem resistance**

Carbapenem resistance was associated with the presence of two carbapenem-hydrolysing oxacillinase genes, namely bla⁰⁴⁸⁵⁻⁻⁵⁸⁻⁻ like (81 of 114 isolates; 71.1%) and bla⁰⁴⁸⁵⁻⁻⁵⁸⁻⁻ like (26 of 114 isolates; 22.8%) (Table 1). None of the isolates carried both determinants (Table S3). The ISAb₁ element was always present upstream of the bla⁰⁴⁸⁵⁻⁻ gene, but it was never detected upstream of the bla⁰⁴⁵⁻⁻ gene and bla⁰⁴⁵⁻⁻ genes. No amplifications were obtained with primers targeting the bla⁰⁴⁸⁵⁻⁻, bla⁰⁴⁵⁻⁻, bla⁰⁴⁵⁻⁻ and bla⁰⁴⁵⁻⁻ genes. The seven carbapenem-susceptible A. baumannii isolates were negative for all ß-lactamase genes, except for a bla⁰⁴⁵⁻⁻ gene (Table 1).

**Changes in carbapenemase gene carriage and influence on carbapenem resistance**

Molecular typing revealed that bla⁰⁴⁵⁻⁻- and bla⁰⁴⁵⁻⁻-positive isolates had identical or similar RAPD and MRA patterns, and all were genetically related to international clonal lineage II (Figure 1). The distribution of bla⁰⁴⁵⁻⁻- and bla⁰⁴⁵⁻⁻-positive isolates per year is shown in Figure 3(a). In April 2007...
the first A. baumannii isolate testing positive for a bla_{OXA-23}-like gene was isolated from hospital N. The OXA-23 determinant became prevalent in 2008 and replaced the OXA-58 determinant thereafter. Only two bla_{OXA-58}-like-positive isolates were recovered in 2008 and none in 2009 (Figure 3a).

The levels of imipenem and meropenem resistance associated with the two predominant carbapenemases were remarkably different. All 81 bla_{OXA-23}-like-positive isolates were resistant to both imipenem and meropenem, whereas only 17 of 26 bla_{OXA-58}-like-positive isolates were resistant to both drugs (Table 1). The presence of an OXA-23 carbapenemase determinant was associated more frequently with high-level imipenem resistance (MIC ≥ 128 mg/L for 63 of 81 isolates, 77.8%) compared with an OXA-58 determinant (MIC ≥ 128 mg/L for 6 of 26 isolates, 23.1%) (Figure 3b).

The origin of pACICU1 replication (repAci1) was detected in all A. baumannii isolates carrying a bla_{OXA-58}- or bla_{OXA-23}-like gene, as well as in two carbapenem-susceptible isolates that tested negative for carbapenemase genes (study codes 133 and 136) (Table 1).

### Resistance to meropenem in the bla_{OXA-58}-like-positive isolates is associated with increased levels of resistance to other antibiotics

An intriguing observation was the variable level of resistance to meropenem among the 26 bla_{OXA-58}-like-positive imipenem-resistant isolates. One copy of the bla_{OXA-58}-like gene was identified in 22 of 26 isolates, irrespective of their meropenem resistance or susceptibility (Table S3). Two or three copies of the bla_{OXA-58}-like gene were detected in four isolates (study codes 110 and 174, and study codes 123 and 137, respectively).

### Table 1. Relationship between presence of bla_{OXA-23}-like or bla_{OXA-58}-like carbapenemase genes, MEM and IPM resistance, and presence of A. baumannii pACICU1 origin of replication

<table>
<thead>
<tr>
<th>carbapenemase gene</th>
<th>resistance phenotype</th>
<th>n (%)</th>
<th>MEM</th>
<th>IPM</th>
<th>Ori pACICU1</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla_{OXA-23}-like</td>
<td></td>
<td>81 (71.1)</td>
<td>81</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>bla_{OXA-58}-like</td>
<td></td>
<td>17 (14.9)</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>7 (6.1)</td>
<td>0</td>
<td>0</td>
<td>2 (1.8)</td>
</tr>
</tbody>
</table>

MEM, meropenem; IPM, imipenem; Ori pACICU1, origin of pACICU1 replication.

Isolates showing an intermediate level of susceptibility to IPM or MEM are reported as resistant.
OXA-58-like-positive isolates. Of note, the MDR lineage accounting for 95.6% of all isolates. Of note, the of carbapenem-resistant A. baumannii. According to GRAB as a consequence of the 2004–05 survey,12 the number of MDR A. baumannii strains by means of molecular epidemiology tools. Despite the urgent infection control measures recommended by GRAB as a consequence of the 2004–05 survey,12 the number of carbapenem-resistant A. baumannii isolates from 10 ICUs in central Italy continued to increase between 2005 and 2009. Epidemiological typing highlighted the emergence of an epidemic MDR lineage accounting for 95.6% of all isolates. Of note, the epidemic lineage was genetically related to the carbapenem-resistant A. baumannii strains responsible for multiple outbreaks occurring in the Rome urban area during 2004–05.12 and belonged to the A. baumannii international clonal lineage II. Accordingly, all isolates assigned to the epidemic A. baumannii lineage belonged to SG 1. Moreover, the epidemic lineage was isolated from all ICUs surveyed and was characterized by resistance to nearly all classes of antimicrobials, in addition to carbapenems. The present study revealed that carbapenem resistance was driven by the dissemination of two OXA-type carbapenemase genes, namely bla<sub>OXA-23</sub> (71.1% of isolates) and bla<sub>OXA-58</sub> (22.8% of isolates). However, by comparison with data from our 2004–05 survey,12 the prevalence of bla<sub>OXA-58</sub>-carrying isolates appeared to be drastically reduced (22.8% in 2005–09 versus 80.7% in 2004–05), while the OXA-23-like determinant become prevalent in 2008 and completely replaced the OXA-58-like determinant thereafter. The bla<sub>OXA-23</sub> carbapenemase gene has increasingly been reported worldwide.13 In several studies,14,36 the bla<sub>OXA-23</sub>-like- or bla<sub>OXA-58</sub>-like-positive A. baumannii isolates belonged to apparently unrelated MRA types and were distinct from the worldwide clonal lineages I, II and III. Only recently, the bla<sub>OXA-23</sub>-like gene has been detected among A. baumannii isolates genetically related to the international clonal lineages I or II. The present study revealed that isolates carrying bla<sub>OXA-23</sub>-like or bla<sub>OXA-58</sub>-like genes have a strict genetic correlation and both belonged to the international clonal lineage II, as did the bla<sub>OXA-58</sub>-positive clone responsible for ICU outbreaks in the Rome urban area during 2004–05.12 It can therefore be speculated that acquisition of the bla<sub>OXA-23</sub>-like determinant by the new epidemic lineage may provide A. baumannii with a selective advantage over the pre-existing bla<sub>OXA-58</sub>-positive clone by increasing resistance to both imipenem and meropenem. Given the high carbapenemase activity of OXA-23, the presence of the bla<sub>OXA-23</sub>-like gene could determine full resistance to bactericidal activity of all <i>β</i>-lactams.6,30–32 These results suggest that overexpression of efflux systems could be contributing to meropenem resistance in the bla<sub>OXA-58</sub>-like-positive isolates.

### Discussion

A progressive decline in susceptibility rates to <i>β</i>-lactams has been documented in A. baumannii during the last decade.13 This emphasizes the need to monitor the diffusion of MDR A. baumannii strains by means of molecular epidemiology tools. Despite the urgent infection control measures recommended by GRAB as a consequence of the 2004–05 survey,12 the number of carbapenem-resistant A. baumannii isolates from 10 ICUs in central Italy continued to increase between 2005 and 2009. Epidemiological typing highlighted the emergence of an epidemic MDR lineage accounting for 95.6% of all isolates. Of note, the emerging lineage was genetically related to the carbapenem-resistant A. baumannii strains responsible for multiple outbreaks occurring in the Rome urban area during 2004–05.12 and belonged to the A. baumannii international clonal lineage II. Accordingly, all isolates assigned to the epidemic A. baumannii lineage belonged to SG 1. Moreover, the epidemic lineage was isolated from all ICUs surveyed and was characterized by resistance to nearly all classes of antimicrobials, in addition to carbapenems.

The present study revealed that carbapenem resistance was driven by the dissemination of two OXA-type carbapenemase genes, namely bla<sub>OXA-23</sub> (71.1% of isolates) and bla<sub>OXA-58</sub> (22.8% of isolates). However, by comparison with data from our 2004–05 survey,12 the prevalence of bla<sub>OXA-58</sub>-carrying isolates appeared to be drastically reduced (22.8% in 2005–09 versus 80.7% in 2004–05), while the OXA-23-like determinant become prevalent in 2008 and completely replaced the OXA-58-like determinant thereafter. The bla<sub>OXA-23</sub> carbapenemase gene has increasingly been reported worldwide.13 In several studies,14,36 the bla<sub>OXA-23</sub>-like- or bla<sub>OXA-58</sub>-like-positive A. baumannii isolates belonged to apparently unrelated MRA types and were distinct from the worldwide clonal lineages I, II and III. Only recently, the bla<sub>OXA-23</sub>-like gene has been detected among A. baumannii isolates genetically related to the international clonal lineages I or II. The present study revealed that isolates carrying bla<sub>OXA-23</sub>-like or bla<sub>OXA-58</sub>-like genes have a strict genetic correlation and both belonged to the international clonal lineage II, as did the bla<sub>OXA-58</sub>-positive clone responsible for ICU outbreaks in the Rome urban area during 2004–05.12 It can therefore be speculated that acquisition of the bla<sub>OXA-23</sub>-like determinant by the new epidemic lineage may provide A. baumannii with a selective advantage over the pre-existing bla<sub>OXA-58</sub>-positive clone by increasing resistance to both imipenem and meropenem. Given the high carbapenemase activity of OXA-23, the presence of the bla<sub>OXA-23</sub>-like gene could determine full resistance to bactericidal activity of all <i>β</i>-lactams.6,30–32 These results suggest that overexpression of efflux systems could be contributing to meropenem resistance in the bla<sub>OXA-58</sub>-like-positive isolates.

### Table 2.

Analysis of MICs (mean ± SD, median and mode values) of representative antimicrobials for 26 bla<sub>OXA-58</sub>-like-positive A. baumannii isolates showing MEM-R and MEM-S phenotypes

<table>
<thead>
<tr>
<th>MEM susceptibility phenotype (no. of isolates)</th>
<th>IPM</th>
<th>TGC</th>
<th>AMK</th>
<th>GEN&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LVX</th>
<th>CIP</th>
<th>TET</th>
<th>EtBr</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>55.1 ± 43.2</td>
<td>1.44 ± 0.72</td>
<td>90.2 ± 79.6</td>
<td>10.2 ± 8.9</td>
<td>11.5 ± 4.2</td>
<td>48.0 ± 33.9</td>
<td>24.9 ± 8.4</td>
<td>64.0 ± 0</td>
</tr>
<tr>
<td>median</td>
<td>64</td>
<td>2</td>
<td>64</td>
<td>4</td>
<td>8</td>
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<td>32</td>
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<td>32</td>
<td>4</td>
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<td>64</td>
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<td>S (9)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>27.5 ± 9.0</td>
<td>1.22 ± 0.66</td>
<td>16.2 ± 20.1</td>
<td>3.4 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.9 ± 2.7</td>
<td>15.1 ± 7.4</td>
<td>16.0 ± 0</td>
<td>39.1 ± 14.1</td>
</tr>
<tr>
<td>median</td>
<td>32</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>8</td>
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<td>16</td>
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</tbody>
</table>

| MEM, meropenem; R, resistant; S, susceptible; IPM, imipenem; TGC, tigecycline; AMK, amikacin; GEN, gentamicin; LVX, levofloxacin; CIP, ciprofloxacin; TET, tetracycline; EtBr, ethidium bromide. |
| aStudy code 138 (Table S1) showed a very high level of resistance to GEN (>512 mg/L). |
| bMIC of GEN calculated excluding the isolate with study code 138. |
| cMIC of GEN calculated including the isolate with study code 138. |
resistance to both imipenem and meropenem. Interestingly, all Acinetobacter baumannii isolates carrying the blaOXA-23-like gene possessed the pACICU1 replication origin but lacked the plasmid-borne blaOXA-58-like gene. This holds true also for two carbapenem-susceptible isolates (study codes 133 and 136), and thus precludes the establishment of any association between the presence of the repAci1 origin of replication and the presence of the carbapenemase genes.

Analysis of imipenem susceptibility during the 5 year investigation period (2005–09) demonstrated a gradual yet significant increase in resistance, coherent with the spread of isolates positive for blaOXA-23-like carbapenemase, while the pattern and extent of resistance to other drugs did not show remarkable differences. Of note, OXA-23 clonal isolates showed 100% resistance to meropenem and high imipenem MICs (≥128 mg/L). Conversely, meropenem activity was superior to that of imipenem in nearly 35% of OXA-58 clonal isolates. Since the OXA-58 enzyme hydrolyses imipenem more efficiently than meropenem, differences in enzymatic activity can partly explain the higher in vitro activity of meropenem observed in the blaOXA-58-like-positive group. However, additional mechanisms can determine the extent of carbapenem resistance in A. baumannii, including modification of the CarO porin for carbapenem influx, and plasmid and/or carbapenemase gene copy number. In the present study, resistance to meropenem was not linked to blaOXA-58-like gene copy number per plasmid or to loss of integrity of the CarO porin, at least in the blaOXA-58-like-positive isolates. In addition, broad-specificity multidrug efflux systems can mediate resistance to a wide range of antibiotics, including carbapenems. Interestingly, we observed that resistance to meropenem was associated with increased levels of resistance to several antibiotics and ethidium bromide, all known for being substrates of multidrug efflux systems. Therefore, it can be hypothesized that overexpression of efflux pumps in the blaOXA-58-like-positive isolates would confer resistance to meropenem, while concomitantly increasing the extent of resistance to imipenem and other antibiotics.

By comparison with our previous survey (2004–05), significantly decreased resistance to tigecycline was observed (MIC50 2 mg/L in 2004–05 versus 0.5 mg/L in 2005–09), concomitant with increased resistance to gentamicin (50.0% resistance in 2004–05 versus 77.2% in 2005–09). Colistin continued to exert excellent activity (100% susceptibility), whereas ampicillin/subactam remained effective for approximately half of MDR A. baumannii isolates from central Italy.

In conclusion, this study updates current knowledge on the prevalence of MDR A. baumannii in Italy and highlights the emergence and epidemic spread of a carbapenem-resistant epidemic lineage carrying the blaOXA-23-like determinant. The emerging OXA-23 lineage replaced a pre-existing OXA-58 clone, probably because of the selective advantage provided by increased resistance to carbapenems. This temporal succession of carbapenemase gene carriage appears to parallel the more recent clinical use of meropenem compared with imipenem.

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Transparency declarations
None to declare.

Supplementary data
Tables S1 to S3 and Figures S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
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