Epidemic Diffusion of OXA-23-Producing Acinetobacter baumannii Isolates in Italy: Results of the First Cross-Sectional Countrywide Survey

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Carbapenem-resistant Acinetobacter baumannii (CRAb) is emerging worldwide as a public health problem in various settings. The aim of this study was to investigate the prevalence of CRAb isolates in Italy and to characterize their resistance mechanisms and genetic relatedness. A countrywide cross-sectional survey was carried out at 25 centers in mid-2011. CRAb isolates were reported from all participating centers, with overall proportions of 45.7% and 22.2% among consecutive nonreplicate clinical isolates of A. baumannii from inpatients (n = 508) and outpatients (n = 63), respectively. Most of them were resistant to multiple antibiotics, whereas all remained susceptible to colistin, with MICs values of ≤0.5 mg/liter. The genes coding for carbapenemase production were identified by PCR and sequencing. OXA-23 enzymes (found in all centers) were by far the most common carbapenemases (81.7%), followed by OXA-58 oxacillinases (4.5%), which were found in 7 of the 25 centers. In 6 cases, CRAb isolates carried both blaOXA-23-like and blaOXA-58-like genes. A repetitive extragenic palindromic (REP)-PCR technique, multiplex PCRs for group identification, and multilocus sequence typing (MLST) were used to determine the genetic relationships among representative isolates (n = 55). Two different clonal lineages were identified, including a dominant clone of sequence type 2 (ST2) related to the international clone II (sequence group 1 [SG1], SG4, and SG5) and a clone of ST78 (SG6) previously described in Italy. Overall, our results demonstrate that OXA-23 enzymes have become the most prevalent carbapenemases and are now endemic in Italy. In addition, molecular typing profiles showed the presence of international and national clonal lineages in Italy.

Acinetobacter baumannii has emerged in recent years as a leading cause of nosocomial infections, especially in intensive care units (ICUs), becoming a public health problem of major concern in several countries (1). Of note, cases of community-acquired infections are also increasingly reported (2). The ability of the organism to survive in the environment during prolonged periods of time, combined with its innate resistance to desiccation and disinfectants, make A. baumannii difficult to eradicate in the clinical setting (3).

The extensive use of antimicrobial chemotherapy, particularly carbapenems, has contributed to the emergence of carbapenem-resistant A. baumannii (CRAb), which usually exhibits a multidrug-resistant (MDR) phenotype (4). For critically ill patients with MDR infections, therapeutic options are limited, and colistin remains the last resource for treatment (5). Carbapenem resistance is mostly associated with the production of carbapenem-hydrolyzing class D β-lactamases, including the acquired OXA-23, OXA-24, OXA-58, OXA-143, OXA-235, and the intrinsic OXA-51 enzyme (6, 7). The significant contribution of OXA-type carbapenemases in A. baumannii has been emphasized, particularly when blaOXA genes are associated with ISAbα sequences, which provide strong promoters for their expression (3). Comparative typing of the outbreak strains of A. baumannii from geographically scattered European hospitals has demonstrated the occurrence of three successful clones originally named European clones I to III, which were renamed as international clones (ICs) I to III, with worldwide distribution. In addition to these major clones, a wide geographic distribution of some other clones has been reported (1). Multilocus sequence typing (MLST) analysis conducted on 496 A. baumannii strains isolated from around the world identified 17 clones, considering both clonal complexes (CCs) and sequence types (STs), distributed also in European countries, with six clones apparently restricted to Europe (8).

In Italy, hospital outbreaks caused by CRAb isolates have been repeatedly reported during the last years. CRAb isolates were found to be mostly related to the production of OXA-58 carbapenemases and belong to IC-II, while strains genetically related to IC-I and IC-III were found to be less common (9–12). More recently, CRAb isolates from Italy were characterized by different STs (e.g., ST1, ST2, ST4, ST20, ST78, ST95, ST109, ST196, and ST197) and sequence groups (SGs) (including SG1, SG2, SG5, and SG6), thus highlighting the presence of international and national clonal lineages in Italy (13–15). A high proportion of carbapenem-resistant Acinetobacter species among the bloodstream iso-
lates in Italy was recently reported by the EARS-Net surveillance system, which has been monitoring Acinetobacter resistance in Europe since 2012 (16).

In this work, we report the results of the first countrywide cross-sectional survey, promoted by the Italian Society of Clinical Microbiologist (AMCLI) and carried out in mid-2011, to investigate the prevalence of CRAB isolates in Italy and to characterize the most common resistance mechanisms. The genetic relatedness of CRAB isolates was also studied in order to trace the epidemiologic evolution of these strains.

MATERIALS AND METHODS

Study design. Twenty-five clinical microbiology laboratories from 23 Italian cities, distributed across the national territory, participated in the study (Fig. 1). Each laboratory collected consecutive nonreplicate clinical isolates of A. baumannii that exhibited MICs for imipenem and/or meropenem of $\geq$8 mg/liter from any type of clinical specimen, with the exception of surface and rectal swabs, during the period of 15 May to 30 June 2011. Both inpatients and outpatients were included in the study. Outpatients were defined as patients not hospitalized at the time of specimen collection. Isolates from patients living in nursing homes were excluded from the analysis. The collected isolates were sent to reference laboratories for confirmation of both species identification and carbapenem MICs, as previously described (23). The presence of carbapenem resistance mechanisms. For the purpose of antimicrobial susceptibility evaluation, the CRAB MICs of ampicillin-sulbactam, trimethoprim-sulfamethoxazole, ceftazidime, cefepime, imipenem, meropenem, doripenem, amikacin, gentamicin, levofloxacin, colistin, and tigecycline were determined by a reference broth microdilution method using a TREK Sensititre custom panel (TREK Diagnostic Systems, Cleveland, OH). When available, the MIC data were interpreted according to the current EUCAST breakpoints (18). In the case of cefepime, ceftazidime, and ampicillin-sulbactam, the results were evaluated according to CLSI criteria (19). Statistical differences were determined using the chi-square test, and confidence intervals (CI) were calculated by the Stata statistical software (release 13; College Station, TX, USA) as parameters.

PCR analysis for carbapenem resistance determinants. PCR assays were carried out by using specific primers to identify blaKPC, blaIMP, blaVIM, blaGIM, blaSIM, blaIMI, blaNDM, blaOXA-23-like, blaOXA-24-like, blaOXA-58-like, and blaOXA-143-like genes (20–22). The presence of ISaBa1 elements adjacent to blaOXA-58-like genes was also investigated, as previously described (23).

Molecular typing by REP-PCR, multiplex PCRs, and MLST. REP-PCR was carried out using the Acinetobacter kit of the DiversiLab microbial typing system (bioMérieux). The extraction of DNA was performed by the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA fragment separation and detection were obtained by the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and the results were analyzed and interpreted using the...
2100 expert software. Pearson’s correlation coefficient for measuring similarity and the unweighted-pair group method using average linkages (UPGMA) for clustering were used. Isolates were defined as genetically related when ≥90% similarity was found between them (24). Representatives of the major European clones I (A. baumannii strain RUH 875) and II (A. baumannii strain RUH 134) were used as controls.

Two multiplex PCRs designed to selectively amplify group 1 or group 2 alleles of the ompA, cseE, and blaOXA-51-like genes were performed. The allelic profiles were determined as described previously (25, 26). Multilocus sequence typing (MLST) of A. baumannii isolates was performed using the primers and conditions described on the Institut Pasteur website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). Sequence types (STs) were assigned using the same MLST website.

RESULTS

Proportions of CRAB isolates from inpatients and outpatients.

During the study period (15 May to 30 June 2011), a total of 571 consecutive nonreplicate clinical isolates of A. baumannii were isolated at the 25 Italian laboratories participating in the survey (inpatients, n = 508; outpatients, n = 63) (Table 1). Overall, 246 isolates (43.1%) were confirmed as CRAB (inpatients, n = 232; outpatients, n = 14), with the proportion of CRAB organisms being significantly higher among isolates from inpatients (45.7%; 95% CI, 41.4 to 50%; P = 0.38) than among those from outpatients (22.2%; 95% CI, 13.7 to 33.9%; P = 0.0014). CRAB organisms were found in all the 25 participating centers (Fig. 1), although the overall proportions ranged from 9.1 to 100%. The isolates were mostly obtained from the lower respiratory tract, followed by skin and soft tissue, urine, and blood. Of note, blood isolates from inpatients showed the highest proportion of CRAB (54%; 95% CI, 40.4 to 67%; P = 0.17). Concerning the intrahospital distribution, most isolates were obtained from ICUs (109 [47.0%]), whereas the remaining isolates were from medical (86 [37.1%]) and surgical wards (37 [15.9%]) (Table 2).

Focusing on CRAB organisms obtained from the lower respiratory tracts of ICU patients, these isolates showed the highest proportion versus other specimen types (56.0%) compared to those obtained from medical and surgical wards (34.9 and 37.8%, respectively).

Antimicrobial susceptibility of CRAB. The susceptibility results, including MIC range, MIC50, and MIC90, are summarized in Table 3. Most isolates showed combined resistance to carbapenems, fluoroquinolones, and aminoglycosides (amikacin and gentamicin), while all of them were susceptible to colistin. Of note, a small proportion of CRAB isolates showed an MIC value lower than the CLSI resistance breakpoint for ceftazidime, cefepime, and ampicillin-sulbactam (4.1, 18.3, and 19.9%, respectively). In the case of tigecycline, for which EUCAST or CLSI breakpoints are not available, the MIC50 and MIC90 values were ≤0.5 mg/liter and 1 mg/liter, respectively.

Carbapenem resistance mechanisms in A. baumannii isolates. Carbapenemase determinants were detected in the vast majority of CRAB (227/246 [92.3%]) isolates. OXA-23 enzymes (found in all centers) were by far the most common acquired carbapenemases (201/246 [81.7%]), followed by OXA-58 oxacillinases (11/246 [4.5%]), which were detected in 7 of 25 centers (Fig. 1). With respect to the different hospital areas, the proportions of OXA-23 enzymes were 73% in surgical wards, 82.6% in medicine, and 84.5% in ICUs. Overall, no significant differences were observed depending on the ward of hospitalization or specimen type (data not shown). Of note, 6 CRAB isolates carried both blaOXA-23-like and blaOXA-58-like genes. Twenty-one isolates (found in 10 centers equally distributed across the country) were positive for the presence of an ISAba1 genetic element upstream from the resident blaOXA-51-like gene. The mechanism(s) of carbapenem resistance was not identified in 19 of 246 (7.7%) isolates. The carbapenem MIC values for these isolates were as follows: imipenem MIC50 and MIC90 >16 mg/liter (MIC range, 16 to >16 mg/liter), and meropenem MIC50 and MIC90 32 and 64 mg/liter, respectively (MIC range, 16 to >64 mg/liter).

Genetic relationships among carbapenem-resistant A. baumannii isolates. Based on different antimicrobial susceptibility profiles, carbapenem resistance determinants, and geographic distribution, 47 CRAB isolates from inpatients were chosen as representatives and characterized by REP-PCR, as well as multiplex PCRs for group identification, together with 8 representative CRAB isolates obtained from outpatients. A subset of isolates was also characterized by MLST.

As shown in Fig. 2, molecular typing by REP-PCR revealed the presence of two different clonal lineages, with the dominant clone being related to IC-II. Three major subgroups, however, were distinguishable within the dominant clone when setting the cutoff value to 95% similarity. Interestingly, most isolates were grouped into a single subgroup within the dominant clone. Multiplex PCRs identified three different sequence groups (SGs), including SG1, SG4, and SG5. MLST revealed that these isolates were consistently from ST2.

Three isolates obtained from centers located in Naples, Catania, and Genoa (the Genoan isolate was from an outpatient) be-

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**TABLE 1** Numbers and proportions of carbapenem-resistant Acinetobacter baumannii isolates obtained from different clinical sources

<table>
<thead>
<tr>
<th>Source</th>
<th>No. CRAB (no. [%])</th>
<th>Source</th>
<th>No. CRAB (no. [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>50 (27 [54.0])</td>
<td>Lower respiratory tract</td>
<td>214 (105 [49.1])</td>
</tr>
<tr>
<td>Urine</td>
<td>72 (35 [48.6])</td>
<td>Skin and soft tissue</td>
<td>112 (40 [35.7])</td>
</tr>
<tr>
<td>Othera</td>
<td>60 (25 [41.7])</td>
<td>Total</td>
<td>508 (232 [45.7])</td>
</tr>
<tr>
<td></td>
<td>1 (0.0)</td>
<td></td>
<td>63 (14 [22.2])</td>
</tr>
</tbody>
</table>

**TABLE 2** Numbers and proportions of carbapenem-resistant A. baumannii isolates obtained from different wards

<table>
<thead>
<tr>
<th>Source</th>
<th>Medical ward</th>
<th>Surgical ward</th>
<th>ICU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>13 (15.1/48.2)</td>
<td>5 (13.5/18.5)</td>
<td>9 (8.2/33.3)</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td>30 (34.9/28.6)</td>
<td>14 (37.8/13.3)</td>
<td>61 (56.0/58.1)</td>
</tr>
<tr>
<td>Urine</td>
<td>20 (23.3/57.1)</td>
<td>3 (8.1/6.6)</td>
<td>12 (11.0/34.3)</td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>21 (24.4/52.5)</td>
<td>9 (24.3/22.5)</td>
<td>10 (9.2/25.0)</td>
</tr>
<tr>
<td>Otherb</td>
<td>2 (2.3/8.0)</td>
<td>6 (16.2/24.0)</td>
<td>17 (15.6/68.0)</td>
</tr>
</tbody>
</table>

**a** Other includes vascular catheters, various biological fluids (e.g., pleural, peritoneal, and cerebrospinal fluid), and the upper respiratory tract.

**b** Others include vascular catheters, various biological fluids (e.g., pleural, peritoneal, and cerebrospinal fluid), and the upper respiratory tract.
longed to a previously described national clonal lineage (27). MLST and SG analyses revealed that these isolates belong to ST78 and SG6. Notably, different carbapenem resistance determinants were detected in these isolates, including the overexpression of \( \text{bla}_{OXA-51} \) associated with the IS\( \text{Aba}1 \) genetic element (Catania and Genoa) and the simultaneous presence of \( \text{bla}_{OXA-23} \) and \( \text{bla}_{OXA-58} \) genes (Naples).

**DISCUSSION**

This survey was conducted in 25 Italian centers distributed across the country in order to take an overall picture of \( A. \text{baumannii} \) in both hospitalized and community patients. All clinical isolates identified as \( A. \text{baumannii} \) during the survey were included in the study and investigated to evaluate both epidemiologic features and antibiotic resistance phenotypes; however, for specimens from nonsterile sites, we could not distinguish between infection and colonization based on the clinical data provided by participating centers. Focusing on carbapenem-resistant CRAB isolates comprised 45.7% of all \( A. \text{baumannii} \) isolates from Italian hospitals during the survey. Similarly, a recent study (2008 to 2009) in 16 countries (14 of which are in Europe) reported an overall rate of imipenem-resistant strains of 48.9% (28). A lower rate (34%) was observed from the National Healthcare Safety Network (NHSN) surveillance system from U.S. hospitals between 2006 and 2008 (29). In another U.S. survey, however, nonsusceptibility to carbapenems increased from 22% in 2002 to 52% in 2008 (30). In 2012, data on \( A. \text{cinetobacter} \) spp. were included for the first time in the EARS-Net surveillance report (16), showing a high percentage of CRAB (83.3%; 95% CI, 78 to 88%) isolates, as well as combined resistance to multiple antibiotics (78%) for the Italian centers. That report, however, analyzed only invasive isolates recovered from hospitalized patients. In our survey, carried out during 2011 in different centers, the proportion of CRAB organisms among blood isolates was lower (54%) than that reported in the EARS-Net data. Furthermore, due to a limited number of invasive isolates, our data showed a broad confidence interval (95% CI, 40.4 to 67%) that might account for this difference. It is to be noted that colistin fully maintained its activity against CRAB isolates, and tigecycline was characterized by low MIC\(_{50}\) and MIC\(_{90}\) values (0.5 and 1 mg/liter, respectively).

From an epidemiological point of view, our data demonstrate that similarly to carbapenem-resistant Enterobacteriaceae (CRE), CRAB organisms are commonly found in Italy, with a widespread distribution across the national territory. To date, no guidelines have been issued in Italy to prevent \( A. \text{cinetobacter} \) infections, but the present findings suggest to implement them as soon as possible. Compared with the results obtained by the CRE nationwide surveillance (carried out simultaneously in the same Italian centers), CRAB organisms appear to have a hospital distribution comparable to that of carbapenem-nonsusceptible \( K. \text{pneumoniae} \) isolates (232 versus 234, respectively) (31). Notably, a high number of CRAB isolates were from the lower respiratory tracts of ICU patients, whereas blood isolates accounted for a number lower than the CREs (27 versus 40, respectively). Similarly to CREs, however, CRAB isolates were not restricted to ICU settings but affected all major hospital areas, a finding that should be carefully considered when planning infection control strategies. Overall, similar numbers of carbapenem-resistant isolates were found among outpatients (14 CRAB versus 21 CRE isolates). However, \( A. \text{baumannii} \) obtained from outpatients was mainly from skin and soft tissue specimens, but \( K. \text{pneumoniae} \) was mostly from urine specimens.

In this nationwide survey, carbapenem resistance in \( A. \text{baumannii} \) was primarily associated with the OXA-23 enzyme (found in all centers), which was the most commonly acquired carbapenemase. On the contrary, OXA-58 oxacillinases, previously reported to be prevalent in Italy (10, 13, 14), were detected in few centers. Two CRAB strains belonging to IC-II now almost exclusively carrying the \( \text{bla}_{OXA-23} \) gene carriage had been already observed among IC-II CRAB isolates responsible for ICU outbreaks in the main hospitals in central Italy, with \( A. \text{baumannii} \) strains producing OXA-23 appearing in 2007 (33).

In our survey, the coexistence of \( \text{bla}_{OXA-23} \)-like and \( \text{bla}_{OXA-58} \)-like genes was found in 6 isolates obtained from inpatients hospitalized at 5 different centers. A similar finding was previously reported (14, 34), but the potential clinical implications remain unknown, and further investigations are needed to clarify this point.

**TABLE 3 Susceptibility results obtained from carbapenem-resistant \( A. \text{baumannii} \) isolates \((n = 246)\)**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC data (mg/liter)</th>
<th>Interpretation*^a^</th>
<th>% S</th>
<th>% R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>Range 4 to &gt;16</td>
<td>MIC(_{50}) &gt;16</td>
<td>0</td>
<td>95.1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2 to &gt;64</td>
<td>MIC(_{50}) &gt;64</td>
<td>1.2</td>
<td>96.4</td>
</tr>
<tr>
<td>Doripenem</td>
<td>2 to &gt;8</td>
<td>MIC(_{50}) &gt;8</td>
<td>0</td>
<td>98.0</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤4 to &gt;16</td>
<td>MIC(_{50}) &gt;16</td>
<td>2.9</td>
<td>97.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤1 to &gt;4</td>
<td>MIC(_{50}) &gt;4</td>
<td>5.3</td>
<td>94.7</td>
</tr>
<tr>
<td>Colistin</td>
<td>≤0.5 to &gt;2</td>
<td>MIC(_{90}) ≤0.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>4 to &gt;4</td>
<td>MIC(_{90}) &gt;4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≤0.5/9.5 to &gt;4/76</td>
<td>MIC(_{90}) &gt;4/76</td>
<td>4.1</td>
<td>95.1</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>≤8/4 to &gt;32/16</td>
<td>MIC(_{90}) &gt;32/16</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4 to &gt;128</td>
<td>MIC(_{90}) 64</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>8 to &gt;32</td>
<td>MIC(_{90}) 32</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.12 to 4</td>
<td>MIC(_{90}) 0.5</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*^a^MICs interpreted according to EUCAST criteria (18). S, susceptible; R, resistant.

*^b^Values separated by slashes are the respective concentrations of drugs in the indicated combination.
CRAb isolates in most cases were found to belong to IC-II, whereas other clonal lineages were sporadically detected. This picture appears to be the most common in both the United States and Europe (8, 35). Molecular epidemiology surveys conducted in the United States on CRAB isolates obtained from 1995 to 2008 showed that IC-II isolates had spread to many centers as early as 1996 and became the dominant lineage (35, 36). Interestingly, strains assigned to IC-II are known to form biofilms and adhere to abiotic surfaces more efficiently than strains assigned to IC-I (mainly characterized by elevated resistance to desiccation). The association of biofilm formation with the ability to acquire different antimicrobial resistance determinants might have favored their spread and persistence in the hospital environment (37).

In Italy, the IC-II appears to be widely distributed since 2004, even though different clones and STs have often been described (13–15). Noteworthy, a single clone of CRAb belonging to the IC-II/ST2 lineage was most recently reported in an ICU in Palermo, Sicily (11). Our results demonstrate that this evolution occurred at a countrywide level, with the ST2 clone able to disseminate in Italian hospitals. Similarly, a high predominance of the ST2 clone was recently reported in several European countries, including Poland, Romania, Latvia, and the Czech Republic (38–41). In these cases, however, interesting differences were observed among countries with respect to the associated carbapenem resistance determinants, thus demonstrating a multidirectional evolution of the IC-II/ST2 clonal lineage. It is worth noting that most outpatients were grouped into a single subgroup within the dominant clone. This finding, together with the low rate of carbapenem resistance, seems to confirm the different origins of these isolates.

Three A. baumannii isolates belonged to the ST78 clone, previously known as the SMAL clone or the Italian clone, and are now assigned to the worldwide clone 6 (1, 42). The ST78 genotype was first reported from Naples in 2006 and then detected in several Italian hospitals, as well as in other Mediterranean countries (34). Similarly to ST2, ST78 strains have been shown to have a higher adherence to both biotic and antibiotic surfaces, with ST78 also showing an elevated resistance to desiccation (37, 42). Our survey demonstrates that ST78 strains acquired different resistance determinants, which may contribute to the persistence of this genotype in Italian hospitals.

In conclusion, our data indicate that resistance to carbapenems is more and more common among A. baumannii isolates, and they demonstrate that the OXA-23 enzyme has become the most prevalent carbapenemase, which is now endemic in Italy. This further evolution of carbapenem-resistant A. baumannii strains confirms the need to continuously monitor this worrisome bacterial pathogen.

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