Molecular characterization of Extensively Drug-Resistant *Acinetobacter baumannii*: first report of a new sequence type in Italy

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Running title: A new sequence type in Italy of XDR *A. baumannnii*

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ABSTRACT

The progressive increase in antibiotic resistance has made *Acinetobacter baumannii* one of the most alarming pathogen in hospital settings. The present study was aimed at investigating antimicrobial resistance patterns and clonal relatedness among *A. baumannii* isolates collected from intensive care unit (ICU) patients in a three-year period. From a collection of 103 *A. baumannii* isolates, 31 were arbitrarily selected and investigated. All isolates were resistant to antibiotics belonging to four or more categories of antimicrobial agents, carbapenems included. Genes coding for major carbapenem-hydrolyzing class D β-lactamases (CHDLs) were searched by PCR. $bla_{\text{OXA-23-like}}$ genes were the most common genes for carbepenemases found (58.1%), followed by $bla_{\text{OXA-58-like}}$ genes (3.2%). All isolates were positive for the intrinsic $bla_{\text{OXA-51-like}}$ genes, whereas no $bla_{\text{OXA-24-like}}$ genes could be detected in any isolate. Multilocus sequence typing (MLST) analysis identified 20 isolates related to sequence type (ST) 2, 9 to ST78, and 2 to ST632. While ST2 and ST78 are known endemic strains in Italy, ST632 had never been found in Italy so far. Pulsed field gel electrophoresis (PFGE) grouped *A. baumanni* isolates into three pulsotypes (A, B, and C), and the latter into further 5 C subtypes, revealing a highly relatedness among the majority of clinical isolates.

Overall, our data show the perpetration of both ST2 and ST78 strains circulation in the ICU, and the introduction of the new ST632; moreover, CHDLs encoded by $bla_{\text{OXA-23-like}}$ over $bla_{\text{OXA-51-like}}$ were found to be the main mechanism of resistance to carbapenems.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative bacterium that has emerged in recent decades as an important opportunistic pathogen worldwide (Peleg et al., 2008; Antunes et al., 2014). This bacterium, along with its close relatives with clinical importance, is not ubiquitous in nature but resides mainly in hospitals and communities (Peleg et al., 2008; Antunes et al., 2014). This ecological niche favors outbreaks of *A. baumannii* infections, especially problematic in intensive care units (ICUs). *A. baumannii* causes a diverse range of infections including pneumonia, predominantly ventilator-associated, bloodstream infections, urinary tract infections, burn and surgical-site infections, and secondary meningitis (Doyle *et al*., 2011; Antunes et al., 2014). Infections occur mainly in patients suffering from an underlying disease, immune soppression or who have undergone major surgical procedures, and rates increase significantly with longer hospitalization (Peleg et al., 2008; Antunes et al., 2014). Currently, there are eight International clones (IC) of *A. baumannii* (Karah et al., 2012), including the three predominant IC-1, 2 and 3, responsible for the vast majority of hospital outbreaks worldwide (Diancourt et al., 2010; Zarrilli et al., 2013). The predominance of certain *A. baumannii* ICs has been related to the acquisition of resistance to a wide spectrum of antibiotics that has progressively increased in recent decades (Diancourt et al., 2010). As outlined by Magiorakos et al., *A. baumannii* multi-drug-resistant (MDR) isolates were defined as non-susceptible to at least 1 agent in three or more antimicrobial categories, extensively-drug-resistant (XDR) as non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and pan-drug-resistant (PDR) as non-susceptible to all agents in all antimicrobial categories (Magiorakos et al., 2012). Since the 2007, there was a significant increase in resistance to carbapenems, which were once considered to be the mainstay against MDR *A. baumannii* infections (Kempf and Rolain, 2012). In *A. baumannii* clinical isolates, the most prevalent mechanism for carbapenem resistance is based on the overexpression of carbapenem-hydrolyzing class

D β-lactamases (CHDLs) (Nordmann and Poirel. 2002). Main CHDLs divide into four families, the OXA-23, -24, -51, and -58, on the basis of their homology on amino acidic sequences (Poirel and Nordmann, 2006; Evans and Amyes, 2014). The gene encoding the *bla*$_{\text{OXA-51}}$-like β-lactamase is residentially located within the chromosome of all the *A. baumannii* isolates studied to date, with 95 variants (Evans and Amyes, 2014). On the contrary, the most widespread acquired carbapenemase gene is the *bla*$_{OXA\text{-}23}$ gene (and *bla*$_{\text{OXA-23}}$-like genes), being found in *A. baumannii* isolates from around the world including Italy (Evans and Amyes, 2014; Principe et al., 2014). Originally identified in Spain, the acquired *bla*$_{\text{OXA-24}}$ gene and derivatives were found in *A. baumannii* isolates scattered in different geographical areas around the world (Evans and Amyes, 2014). The acquired *bla*$_{\text{OXA-58}}$-like genes have been identified worldwide in *A. baumannii* isolates, and was associated with a hospital outbreak in Rome, Italy in 2005 (Bertini et al., 2006; Giordano et al., 2007; Evans and Amyes, 2014). Due to the increasing number of infections involving MDR, XDR and PDR *A. baumannii* clinical isolates, treatment of these infections has become a real public health issue worldwide (Peleg et al., 2008; Diancourt et al., 2010). At a national and international level, it is very important to understand the spread and clonality of circulating *A. baumannii* strains, while at a single hospital level it is fundamental to survey the effectiveness of infection preventive measures. Most common genotyping approaches for studying the epidemiology of *A. baumannii* isolates are pulse field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Therefore, the aim of this study was to determine the genetic relatedness of clinical *A. baumannii* isolates collected over a three-year period (2010-2012) from an ICU of the Policlinico Umberto I in Rome, Italy. The susceptibility to antimicrobials and the prevalence of *bla*$_{\text{OXA}}$-type carbapenemase genes among *A. baumannii* isolates were also analyzed.

MATERIALS AND METHODS

Bacterial strains

A total of 103 clinical isolates of *A. baumannii* were collected from ICU patients of the University Hospital Policlinico Umberto I from January 2010 to December 2012. The isolates were all recovered from respiratory specimens. Strains were isolated by standard methods and identified to the species level with Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and the matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry (MS) (BrukerDaltonik). From all clinical strains collected, thirty-one non-repetitive isolates were arbitrarily selected for this study. Among these isolates, 21 were from male patients and 11 from female, who were between the ages of 38 and 71 years old. The reference strain *A. baumannii* ATCC17978, a kind gift from P. Visca (University of Roma Tre, Rome, Italy), was used as control. Ethical approval was not required for the study because it was done as part of surveillance and management of health-care-associated infection.

Antibiotic susceptibility testing

The antibiotic susceptibility profiling of all *A. baumannii* isolates was performed with the Vitek 2 system and the results were interpreted using clinical breakpoints according to European Committee on Antimicrobial Susceptibility Testing (EUCAST, Version 6.0). The antibiotics tested were as reported in Table 1. The tigecyclin susceptibility was confirmed with the Etest method (AB Biodisk, Solna, Sweden). Since EUCAST still considers there is insufficient evidence to establish tigecycline breakpoints for *Acinetobacter* spp., we considered isolates with a MIC $\leq$ 2 μg/ml as susceptible, MIC 4 μg/ml as intermediate, and MIC $\geq$ 8 μg/ml as resistant.

Detection of oxacillinases (OXA)-encoding genes

105 Four families of OXA-type carbapenemases, *bla* OXA-23-like, *bla* OXA-24-like, *bla* OXA 51-like and
106 *bla* OXA-58-like, were investigated by multiplex PCR. The sequences of the primers used are reported
107 in Table S1. PCR amplicons were visualized by agarose gel electrophoresis.

108 **Pulsed-Field Gel Electrophoresis**

109 Pulsed-Field Gel Electrophoresis (PFGE) analysis was performed on *Apa*I-digested genomic DNA
110 from each *A. baumannii* clinical isolate, as previously described (Conte et al., 2014). Genomic DNA
111 was prepared in 1.5% agarose (LM-MP agarose, Roche, Italy) plugs, and DNA restriction was carried
112 out with 30 U/plug of *Apa*I for 4 hrs at 25°C. PFGE was performed in a CHEF DRII system (Bio-Rad
113 Laboratories, Richmond, CA, USA) over 20 hrs at 14°C with 5 to 30 sec of linear ramping at 200 V.
114 Lambda concatemers (New England Biolabs, Ipswich, MA) were used as size standard. Gels were
115 stained with ethidium bromide, digitalized using the Kodak Digital Science system (Kodak, Milan,
116 Italy), and analyzed with TotalLab TL120 Trace version 2006 (Nonlinear Dynamics) with a position
117 tolerance set at 1.5%. The Dice coefficient of similarity was calculated, and the unweighted pair group
118 method with arithmetic averages (UPGMA) was used for cluster analysis XLstat 7.5 (Addinsoft, USA).
119 Clones with a coefficient of similarity between 80-95% were classified in sub-clones, while those
120 >95% were considered to be identical clones.

121 **Multilocus sequence typing**

122 Multilocus sequence typing (MLST) was achieved following the Institut Pasteur's MLST scheme,
123 based on sequence analysis of internal portions of seven housekeeping genes (*cpn60, fusA, gltA, pyrG,*
124 *recA, rplB* and *rpoB*). MLST was performed with primers and conditions described on the Pasteur
125 website (http://www.pasteur.fr/mlst), with the exception of *rpoB* and *recA* genes, which were amplified
126 with the primer pair reported in Table S1, as previously described (El-Shazly et al., 2015; Wu et al.,
127 2015). Re-amplification and re-sequencing of *recA* was necessary to obtain unambiguous sequence

results. PCR products were directly purified from the reaction mixture with the QIAquick PCR purification kit (Qiagen, Italy), according to the manufacturer's recommendations. Purified products were sequenced by an external facility (Bio-Fab Research). Results were analyzed and compared with the *A. baumannii* database on the MLST website http://www.pasteur.fr/mlst.

RESULTS

Susceptibility of *A. baumannii* clinical isolates to antimicrobials

Between January 2010 to December 2012, 103 *A. baumannii* clinical isolates were recovered from respiratory specimens of ICU patients hospitalized in a teaching hospital. Thirty-one non-repetitive isolates were arbitrarily selected and analyzed in this study. Antimicrobial susceptibility testing revealed that all the isolates were resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, ciprofloxacin, trimethoprim/sulfamethoxazole, and, with the exception of one strain, to cefepime (Table 1). We found only 2 isolates with an intermediate resistance to imipenem and one isolate to ceftazidime (Table 1). The 74.2% (23/31) and 96.8% (30/31) of isolates were found to be resistant to aminoglycosides with 4 and 1 isolates susceptible to amikacin and gentamicin, respectively (Table 1). All but one clinical isolates of *A. baumannii* were susceptible to colistin (96.8%), whereas 61.3% of isolates (19/31) had a MIC value ≤ 2 to tigecycline (Table 1). Based on the current definitions for acquired resistance (Magiorakis et al., 2012), we classified all 31 isolates as XDR.

Detection of carbapenem-hydrolyzing class D β-lactamases (CHDLs)

A screening for major CHDLs in *A. baumannii* clinical isolates with multiplex PCR was performed. Among the 31 isolates, 100% (31/31) were positive for the presence of $bla_{\text{OXA-51-like}}$ genes, 58.1% (18/31) for $bla_{\text{OXA-23-like}}$ genes, 3.2% (1/31) for $bla_{\text{OXA-58-like}}$ genes, and no strain presented $bla_{\text{OXA-24-like}}$ genes (Figure 1 and Table S2). Hence, the observed XDR phenotypes consistently correlate with the presence of oxacillinases, mostly as OXA-51 and OXA-23 enzymes.

Molecular genotyping of *A. baumannii* clinical isolates

The clonal relatedness among the 31 *A. baumannii* clinical isolates was examined by PFGE and MLST. Figure 1 shows the PFGE profiles and the genetic similarity dendogram. PFGE analysis was able to

156 classify the 31 isolates into three PFGE pulsotypes (PTs), named A, B, and C. The 11 isolates
157 belonging to PT A and the 9 to PT B showed identical macrorestriction patterns (Figure 1), in
158 accordance with the timeline of their isolation (Table 2). Instead, isolates belonging to PT C displayed
159 some extra bands; thus, a similarity cut-off level of 95% allowed to further distinguish 5 C subtypes,
160 referred to as C1-C5 (Figure 1).
161 MLST studies were performed using the Institut Pasteur's MLST scheme. The MLST analysis showed
162 that 64,5% (20/31) of *A. baumannii* clinical isolates were related to sequence type (ST) 2, 29.0% (9/31)
163 to ST78, and 6.5% (2/31) to ST632 (Figure 1). Hence, most of the isolates belonged to the globally-
164 distributed IC 2 (ST2) and to the IC 6 (ST78, known to be the most recurrent STs in our country
165 (Giannouli et al., 2010; Mezzatesta et al., 2012; Principe et al., 2014). Remarkably, ST632 displayed
166 differences in the *rpoB* allele and in the PFGE macrorestriction pattern in comparison to ST2 (Table S3
167 and Figure 1). To the best of our knowledge, this ST had never been found in Italy so far
168 (http://pubmlst.org/data/).
169 Interestingly, the dendrogram shows that isolates belonging to the same ST can share different PFGE
170 patterns (i.e. ST2). However, identical PFGE patterns were from corresponding STs. Clustering of *A.*
171 *baumannii* isolates achieved by PFGE was in good agreement with MLST ST assignment,
172 corroborating the discriminatory power of these techniques in epidemiological studies.
173

DISCUSSION

The prolonged survival in hospital environments and the remarkable ability to acquire resistance to multiple antibiotics makes it problematic the eradication and treatment of infections caused by MDR *A. baumannii*. In a three-year period, 103 *A. baumanni* isolates from respiratory specimens from patients hospitalized in an ICU were collected. In this paper, the antimicrobial susceptibilities and the clonal relatedness of 31 randomly selected *A. baumannii* clinical isolates were analyzed. Overall, the resistance rates were high for almost all antimicrobial agents tested (e.g. penicillins, cephalosporins, fluroquinolones, aminoglycosides and carbapenems). When the presence of four major CHDLs was investigated, we found that all isolates carried $bla_{\text{OXA-51-like}}$ genes, in agreement with the intrinsic presence of these genes in the *A. baumannii* chromosome (Evand and Amyes, 2014). Although $bla_{\text{OXA-51-like}}$ genes were reported to play a role in resistance, two isolates that carried these genes as the only carbapenemase gene presented a lower level of resistance to imipenem (MICs, 8 vs $\geq$ 16 μg/ml). Differences in MIC levels could be explained by the variability in the insertion sequences (ISs, such as IS*Aba1* and/or IS*Aba9*) upstream of the bla_{OXA} carbapenemase gene that provide the promoter for the expression of OXA enzymes (Turton et al., 2006; Figueiredo et al., 2009). Alternatively, it is possible that these two isolates possess enzyme variants with weaker activity toward imipenem. We did not detect $bla_{\text{OXA-24-like}}$ genes in any of the tested isolates. On the contrary, 18 of the 31 isolates carried $bla_{\text{OXA-23-like}}$ genes, whereas only one strain was found to be positive for to $bla_{\text{OXA-58-like}}$ genes. Interestingly, the $bla_{\text{OXA-58-like}}$-carrying strain (strain 290) was isolated in the same week with another strain (strain 288) that was found to be negative for both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$. Both isolates (strains 288 and 290) shared the same PT and ST with a strain isolated in the same ICU 30 months before (strain 939). Therefore, these observations highlight the consequence of ICU environment reservoirs (i.e. hospital size, antibiotic selection pressure, immunocompromised patients, and health care workers' behavior) for the epidemiological transmission of *A. baumannii* XDR strains. Indeed, we

198 do believe that the acquisition of two more CHDLs in epidemiologically and genetically related strains
199 (i.e. 288 and 290) substantiates the tight relationship between the persistence in hospital settings and
200 the genetic changes under antibiotic selection pressure of *A. baumannii* isolates. Accordingly, the
201 dramatic decrease in the number of $bla_{\text{OXA-58-like}}$ genes found in our isolates is in agreement with
202 previous investigations that reported an increase in $bla_{\text{OXA-23}}$ genes among *A. baumannii* isolates in
203 Italy (Minandri et al., 2012; Principe et al., 2014). Indeed, till 2008 $bla_{\text{OXA-58-like}}$ genes were the most
204 common carbapenemase genes in *A. baumannii* clinical isolates worldwide (Bertini et al., 2006;
205 Giordano et al., 2007; Minandri et al., 2012; Wu et al., 2015). Thereafter, a progressive and massive
206 transition from $bla_{\text{OXA-58-like}}$ to $bla_{\text{OXA-23-like}}$ genes was observed not only in Italian hospitals but also in
207 China (Bertini et al., 2006; Giordano et al., 2007; Principe et al., 2014; Wu et al., 2015). The higher
208 carbapenemase activity of OXA-23-like enzymes than that of OXA-58-like may provide a major
209 advantage to $bla_{\text{OXA-23-like}}$-carrying strains. Nevertheless, different therapeutic and source counteract
210 approaches at hospitals might represent the reasons for the displacement from $bla_{\text{OXA-58-like}}$ to $bla_{\text{OXA-23-like}}$ genes. Therefore, it is reasonable to believe that $bla_{\text{OXA-58-like}}$ genes will be totally replaced by
212 $bla_{\text{OXA-23-like}}$ genes in those countries in which the transition was already reported (Principe et al., 2014;
213 Wu et al., 2015).
214 Noteworthy, the most alarming finding was the one isolates was resistant to colistin, with MIC values ≥
215 16 μg/ml. Although potentially nephrotoxic and neurotoxic, colistin may be useful for treating
216 infections caused by carbapenem-resistant strains. Conversely, we found a wide spectrum of MIC
217 values for tigecycline, ranging from ≤0.5 to ≥8.0; however, EUCAST still considers there is
218 insufficient evidence to establish tigecycline breakpoints for *Acinetobacter* spp.

219 The genetic relatedness of 31 *A. baumannii* was investigated. The PFGE was used to study the clonal
220 spread of isolates, whereas MLST discriminates the genetic relatedness between isolates. Molecular
221 typing by PFGE grouped *A. baumanni* isolates into three pulsotypes (A, B, and C), and the latter

pulsotype into further 5 C subtypes. This analysis revealed a tight relatedness among isolates, in accordance with the timeline of their isolation.

The MLST analysis (Pasteur scheme) showed that genotype ST2 was the predominant among *A. baumannii* isolates, followed by genotype ST78. ST2 corresponds to IC 2 and is characterized by a wide geographical distribution (Peleg et al., 2008; Diancourt et al., 2010; Karah et al., 2012; Mezzatesta et al., 2012; Minandri et al., 2012; Zarrilli et al., 2013; Principe et al., 2014; El-Shazly et al., 2015). Conversely, ST78 belongs to the IC 6 and is known as the 'Italian clone' since it was frequently found in Italian hospitals (Giannouli et al., 2010; Carretto et al., 2011; Zarrilli et al., 2013; Giannouli et al., 2013; Principe et al., 2014). Analysis of the temporal distribution of genotypes shows that ST2 was persistent in the ICU over the three-year period analyzed, whereas ST78 had a major prevalence in late 2010 and early 2011 (Table 2). Indeed, both STs were reported to possess common virulence-associated phenotype typical of epidemic genotypes (Giannulli et al., 2013). Remarkably, in mid-2012, we detected for the first time in Italy 2 isolates belonging to genotype ST632. Both ST632 (isolates 229 and 237) clustered within the C4 subtype, carried $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ genes, and displayed almost the same antibiotic resistance profiles as the other isolates. Six isolates belonging to ST632 were identified in Thailand in 2011, mainly from upper respiratory tract specimens (http://pubmlst.org/data/). To the best of our knowledge, no data about the virulence-related traits are available in the literature for isolates belonging to ST632, which warrant further investigation.

Overall, our data show the persistence of XDR ST2 and ST78 in an ICU of Policlinico Umberto I and the occurrence of a ST detected at a national level for the first time. Moreover, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ genes were found to be the most common types of OXA carbapenemases. Moreover, a very alarming aspect was the finding of one isolate belonging to ST78 with a reduced susceptibility to colistin, lowering significantly the therapeutic options for ICU infected patients. These findings

emphasize the need to keep on the surveillance of *A. baumannii* isolates and improve the success of the preventive measures to control the spread of infections.

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314

315 **FIGURE LEGEND**

316 **Figure 1.** Dendrogram representing PFGE profiles, MLST results, and *bla*$_{OXA}$ content of *A. baumannii*
317 clinical isolates. Left to right: isolate number, presence (+) or absence (-) of *bla*$_{OXA}$, sequence type
318 (ST), pulsotype (PT), PFGE profiles using *Apa*I as restriction enzyme, and genetic similarity
319 dendogram. The scale indicates the percent of similarity and broken lines show the 80 and 95% genetic
320 similarity cut-off, respectively.

321

Table 1. Susceptibility profiles of *A. baumannii* strains. Isolates were designated susceptible (S), intermediate (I), or resistant (R) according to EUCAST antibiotic breakpoint guidelines. Minimum inhibitory concentrations (MICs, μg/ml) are shown in brackets. Abbreviations: AK, Amikacin; GM, Gentamicin; A/AA, Amoxicillin/clavulanic acid; AMP, Ampicillin; P/T, Piperacillin/Tazobactam; CPE, Cefepime; CTX, Cefotaxime; CAZ, Ceftazidime; CIP, Ciprofloxacin; T/S, Trimethoprim/Sulfamethoxazole; IMP, Imipenem; CST, Colistin; TGC, Tigecycline.

Antibiotic/ Isolate	**AK**	**GM**	**A/AA**	**AMP**	**P/T**	**CPE**	**CTX**	**CAZ**	**CIP**	**T/S**	**IMP**	**CST**	**TGC**
4	R (≥64)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥32)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	I (8)	S (≤0.5)	S (1)
8	I (32)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥64)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	S (2)
9	S (8)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	S (8)	R (≥64)	I (16)	R (≥4)	R (≥320)	I (8)	S (≤0.5)	S (≤0.5)
17	I (32)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	I (16)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	R (≥16)	S (2)
36	S (16)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	I (16)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	S (2)
76	R (≥64)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥64)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	I (4)
83	R (≥64)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥64)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	I (4)
93	R (≥64)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥64)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	S (2)
136	R (≥64)	R (≥16)	R (≥32)	R (≥32)	R (≥128)	R (≥64)	R (≥64)	R (≥64)	R (≥4)	R (≥320)	R (≥16)	S (≤0.5)	S (2)
144	R	R	R	R	R	R	R	R	R	R	R	S	I

	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)
146	R	R	R	R	R	R	R	R	R	R	R	S	I
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)
147	R	R	R	R	R	R	R	R	R	R	R	S	I
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(6)
150	R	R	R	R	R	R	R	R	R	R	R	S	R
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(≥8)
155	R	R	R	R	R	R	R	R	R	R	R	S	I
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)
184	R	R	R	R	R	R	R	R	R	R	R	S	I
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)
203	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(2)
229	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(0.75)
230	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(2)
237	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
270	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(2)
288	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
290	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
326	R	R	R	R	R	R	R	R	R	R	R	S	S
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(2)
340	R	R	R	R	R	R	R	R	R	R	R	S	S

	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1.5)
351	R	R	R	R	R	R	R	R	R	R	R	S	I
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)
436	S	R	R	R	R	R	R	R	R	R	R	S	S
	(16)	(≥16)	(≥32)	(≥32)	(≥128)	(≥32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
702	I	R	R	R	R	R	R	R	R	R	R	S	I
	(32)	(≥16)	(≥32)	(≥32)	(≥128)	(≥32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(3)
880	S	R	R	R	R	R	R	R	R	R	R	S	S
	(16)	(≥16)	(≥32)	(≥32)	(≥128)	(≥32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
884	R	R	R	R	R	R	R	R	R	R	R	S	R
	(≥64)	(≥16)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(≥8)
899	S	R	R	R	R	R	R	R	R	R	R	S	S
	(16)	(≥16)	(≥32)	(≥32)	(≥128)	(32)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(1)
939	S	S	R	R	R	R	R	R	R	R	R	S	I
	(≤2)	(4)	(≥32)	(≥32)	(≥128)	(≥64)	(≥64)	(≥64)	(≥4)	(≥320)	(≥16)	(≤0.5)	(4)

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Table 2. Timeline of *A. baumannii* strain isolation. Strain number, sequence type (ST), and bla_{OXA} gene content. $bla_{OXA\text{-}51\text{-}like}$ genes found in all isolates are not reported.

Year	**2010**	**2011**	**2012**
January	939-ST2	9-ST78	184-ST2-23
		17-ST78	
February			203-ST2-23
March			
April	436-ST78		229-ST632-23
	702-ST78		230-ST2-23
			237-ST632-23
May	880-ST78		270-ST2-23
June			288-ST2
			290-ST2-23-58
July	884-ST2-23	76-ST2-23	
		83-ST2-23	
August		93-ST2-23	326-ST2-23
September			340-ST2-23
October		136-ST2-23	351-ST2-23
		144-ST2	
November	4-ST78	146-ST2-23	
		147-ST2-23	
		150-ST2-23	
		155-ST2-23	
December	8-ST78		
	36-ST78		
	899-ST78		