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SHORT COMMUNICATION

# Extensively drug-resistant ArmA-producing *Acinetobacter baumannii* in an Italian intensive care unit

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SUMMARY

We describe the spread of 12 carbapenem-resistant *Acinetobacter baumannii* isolates in hospitalized patients. All strains showed an extensively drug-resistant phenotype and high-level of aminoglycoside resistance, harboring the *Arm*A gene and $bla_{oxa\text{-}23}$ downstream of ISAba1 (transposon Tn2008 arrangement) where both were located on the chromosome. These strains carry a class 1 integron containing the gene cassette aacA4-catB8-aadA1. Molecular analysis revealed that all isolates belonged to the same sequence type (ST) 2 clone. The spread of ArmA-producing *A. baumannii* strains limit the treatment options showing the dramatic situation which requires novel therapies to limit high mortality rates.

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*Acinetobacter baumannii* is a non fermentative, Gram-negative, opportunistic pathogen. While it is a rare cause of infection in healthy individuals living in the community, this organism is a leading cause of nosocomial infections. The interaction of *A. baumannii* with hosts is variable, ranging from simple colonization to severe and treatment-recalcitrant infections, the latter often affecting critically ill patients admitted to the intensive care unit (ICU). The most frequent infections are pneumonia, often occurring in mechanically ventilated patients, wound infections, and catheter-related bloodstream and urinary tract infections (Lemos *et al.,* 2014; Stefani *et al*., 2008), often associated with epidemic spread. Many outbreaks have been associated with two major international clones, European clones I and II, with a wide geographical dissemination, supplemented by a remarkable ability to survive and acquire resistance against different classes of antibiotics (Karah *et al.,* 2012).

When these multidrug-resistant (MDR) strains are encountered, empirical salvage regimens may include such agents as amikacin and recently colistin (Mezzatesta *et al.,* 2014). Amikacin is an aminoglycoside that generally continues to retain good activity against *A. baumannii*. Resistance to amikacin in *A. baumanniii* is primarily mediated by structural modifications of the agent through the actions of aminoglycoside-modifying enzymes that are produced by resistant strains. In recent years, in Italy, the production of 16S rRNA methylases has been implicated in aminoglycoside resistance among gram-negative pathogens (Brigante *et al*., 2012, Milan *et al*., 2016). Notably, *A. baumannii* strains producing the ArmA 16S rRNA methylase have increasingly been reported (Doi *et al*., 2007).

The present study describes 12 carbapenem-resistant *A.baumannii* isolates from hospitalized patients of an ICU in a Sicilian hospital (Cannizzaro, Catania, Italy) during the period between October 2014 and March 2015 in which a high level of aminoglycoside resistance was detected. Eleven isolates were obtained from lower respiratory tract infections, and one from a documented bloodstream infection. Isolates were collected by standard methods, isolated in pure culture on MacConkey agar plates and identified with the API 20NE system for *A. baumannii* (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing was performed by gradient-test strips (Liofilchem, Roseto degli Abruzzi, Italy) of meropenem; imipenem; ampicillin/sulbactam; amikacin; gentamicin; ciprofloxacin; trimethoprim/sulfamethoxazole; rifampicin; colistin; and tigecycline. Susceptibility and resistance categories were assigned according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, v.6.1,2016) breakpoints.

For tigecycline (Pfizer, Rome, Italy) and colistin sulfate (Sigma Chemical Co., St. Louis, MO, USA), MICs were also determined by the standard broth microdilution method according to EUCAST Guidelines (v. 6.1, 2016); MIC breakpoint for tigecycline was by FDA (S≤2 mg/L R>8 mg/L). All isolates presented an XDR profile (Magiorakos *et al.,* 2012). Regarding tigecycline, all strains had an intermediate phenotype, only two strains were susceptible. The high level of resistance to gentamicin and amikacin (MICs between 32 and >256 mg/L), not really frequent, suggested the presence of a 16S rRNA methylase (*Table 1*), (Mezzatesta *et al*., 2013). The amplification and sequencing for

*Key words:*
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**Table 1** - *Clinical characteristics of patients and antibiotic susceptibility of ArmA-producing Acinetobacter baumannii.*

| | | | | | | MIC (mg/L) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Patient* | *Date* | *Ward* | *Specimen* | *PFGE* | *ST* | *IPM* | *MEM* | *CIP* | *CN* | *AK* | *COL* | *SXT* | *SAM* | *TGC* | *RD* | *Phenotype* |
| 1 | 15.10.14 | ICU | bronchial aspirate | A | 2 | 32 | 32 | >32 | >256 | >256 | 4 | >32 | >256 | 4 | >256 | PDR |
| 2 | 19.11.14 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | 32 | 64 | >32 | >256 | 2 | >256 | XDR |
| 3 | 20.11.14 | ICU | bronchial aspirate | A | 2 | 32 | 32 | >32 | >256 | >256 | 8 | >32 | >256 | 4 | >256 | PDR |
| 4 | 9.12.14 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 8 | >32 | >256 | 4 | >256 | PDR |
| 5 | 15.01.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 0,75 | >32 | >256 | 6 | >256 | XDR |
| 6 | 5.02.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 2 | >32 | >256 | 6 | >256 | XDR |
| 7 | 8.02.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 1 | >32 | >256 | 3 | >256 | XDR |
| 8 | 23.02.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 1,5 | >32 | >256 | 3 | >256 | XDR |
| 9 | 9.03.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 1,5 | >32 | >256 | 0,75 | >256 | XDR |
| 10 | 18.03.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 1 | >32 | >256 | 4 | >256 | XDR |
| 11 | 19.03.15 | ICU | bronchial aspirate | A | 2 | >32 | >32 | >32 | >256 | >256 | 0,75 | >32 | >256 | 4 | >256 | XDR |
| 12 | 20.03.15 | ICU | blood | A | 2 | >32 | >32 | >32 | >256 | >256 | 2 | >32 | >256 | 3 | >256 | XDR |

Legend: IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; CN, gentamycin; AK, amikacin; COL, colistin; SXT, sulfamethoxazole; SAM, ampicillin/sulbactam; TGC, tigecycline; RD, rifampicin. PDR, Pan Drug-Resistant; XDR, Extensively Drug-Resistant; ICU, Intensive Care Unit. Colistin MICs were determined using the broth microdilution method.

detection of carbapenemases (IMP, VIM, NDM and OXA) (Mezzatesta *et al.*, 2014; Gona *et al*., 2014) and aminoglycoside-modifying enzymes (ArmA, RtmA-D) were performed by PCR using previously described primers (Mezzatesta *et al*., 2013). All isolates of *A. baumannii* were positive for $bla_{\text{OXA-51}}$, the carbapenemase gene that is intrinsic to this species. The $bla_{\text{OXA-23}}$ and 16S rRNA methylases *arm*A genes were identified in all strains, whilst $bla_{\text{IMP}}$, $bla_{\text{VIM}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$ and *rtm*A-D genes were absent. Furthermore, the IS*Aba*1 sequence was always present upstream of the $bla_{\text{OXA-23}}$ gene, confirming a high carbapenemase activity.
Tn*2008* was detected in all strains; the $bla_{\text{OXA-23}}$ gene was located downstream of IS*Aba1* and upstream of the ATPase gene both in opposite orientations with respect to the transposon Tn*2008* arrangement (Lee *et al*., 2013). Detection of class 1 integrons by integrase PCR was performed using the previously described INT-5CS-f and INT-3CS-r primers (Perilli *et al*., 2008). PCR analysis of the *int*1 gene revealed class 1 integrons in all strains with the gene cassette array aacA4-catB8-aadA1 (1.6-kb), (Lin *et al*., 2010).
*A. baumannii* isolates examined for genetic relatedness with PFGE and MLST (Mezzatesta *et al.*, 2014) demonstrated the same ST2 and the same pulsotype, clone A. To characterize the localization of $bla_{\text{OXA-23}}$ and *arm*A genes we used the I-CeuI enzyme, a double-strand endonuclease encoded by a group I intron in the large subunit rRNA gene of *Chlamydomonas eugametos*. This endonuclease cuts a 26-bp sequence and specifically digests the 23S rDNA sequence in rrn operons. Thus the number of I-CeuI fragments usually represents the number of *rrn* operons (Marshall *et al*., 1992). We used the I-CeuI PFGE protocol by Lolans changing the run time to 15 h (Lolans *et al*., 2006). Our isolates showed five fragments suggesting the presence of six *rrn* operons. The hybridization signal was the same for the 16S rRNA**,** $bla_{\text{OXA-23}}$, $bla_{\text{OXA-51}}$ and *arm*A genes, suggesting that these genes are located on the chromosome in all strains.
These results demonstrate that the MDR clone ST2, previously isolated in the ICU ward of Cannizzaro hospital during 2013 (Mezzatesta *et al.*, 2014), maintained the same profile as PFGE and ST in 2014, but acquired an additional resistance determinant, the 16S rRNA methylase ArmA, encoded by the *arm*A gene, limiting the therapeutic option with aminoglycosides that are always used in combination with tigecycline or colistin. Therefore, the spread of the 16S rRNA methylase genes in *A.baumannii* should be closely monitored. Furthermore, four of our isolates were also resistant to colistin and rifampicin, exacerbating the already dramatic situation of the therapeutic options for nosocomial infections. Therefore, improved knowledge of molecular mechanisms controlling multiresistance should facilitate the development of novel therapies to combat these critical nosocomial infections causing high mortality rates.

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**Conflict of interest statement**
None of the authors have a commercial interest or other association that might pose a conflict of interest.

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