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 ArmA gene and blaOXA-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 (Karab 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. baumannii 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
Aba1 sequence was always present upstream of the IS. IMP, VIM, NDM and OXA were identified in all strains, whilst IMP, VIM, OXA-23 and 16S rRNA methylases armA genes were positive for. C. Caio, G. Maugeri, T. Zingali, et al. (2014) demonstrated the same ST and the same pulsotype, clone A. To characterize the localization of blaOXA-23 and armA 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. The I-CeuI enzyme digests the 23S rDNA sequence in rrn operons. Thus the number of I-CeuI cuts a 26-bp sequence and specifically digests the 23S rDNA sequence of Chlamydomonas eugametos. The 23S rDNA sequence usually represents the number of rrn operons.

Table 1 - Clinical characteristics of patients and antibiotic susceptibility of ArmA-producing Acinetobacter baumannii.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Ward</th>
<th>Specimen</th>
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<th>ST</th>
<th>IPM</th>
<th>MEM</th>
<th>CIP</th>
<th>CN</th>
<th>AK</th>
<th>COL</th>
<th>SXT</th>
<th>SAM</th>
<th>TGC</th>
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Legend: IMP, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; CN, Gentamicin; AK, Amikacin; COL, Colistin; SXT, Amoxicillin/sulbactam; TGC, Ticarcillin; 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 blaOXA-51, the carbapenemase gene that is intrinsic to this species. The blaOXA-23 and 16S rRNA methylases armA genes were identified in all strains, whilst blaIMP, blaVIM, blaOXA-23, blaOXA-51 and rtmA-D genes were absent. Furthermore, the ISAba1 sequence was always present upstream of the blaOXA-23 gene, confirming a high carbapenemase activity.

In 2008 it was detected in all strains; the blaOXA-23 gene was located downstream of ISAba1 and upstream of the AT-Pase gene both in opposite orientations with respect to the transposon Tn2008 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 int1 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 blaOXA-23 and armA 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, blaOXA-23, blaOXA-51 and armA 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 armA 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.
References


