Efflux Pump AdeABC Assessment in Acinetobacter baumannii Strains Isolated in a Teaching Hospital

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Abstract

Over the past twenty years the worldwide clinically impact of Acinetobacter baumannii (A. baumannii) demonstrated its etiopathogenetic relevance. During a previous retrospective study in a teaching hospital, between January 2011 and February 2015, we observed increasingly infections caused by A. baumannii associated with antibiotic multi-resistance. Tigecycline, the first member of the glyccycline class, is an effective option for the treatment of such infections even if, due to its increased clinical use, tigecycline resistant isolates have recently emerged. In A. baumannii several mechanisms are associated with a tigecycline decrease susceptibility, among these, expression efflux pump AdeABC and the presence of insertion sequence (IS) in the adeRS operon. About that, we decided to analyze adeB and adeS genes in 24 MDR A. baumannii clinical isolates, selected on the different tigecycline phenotype. The study of adeB and adeS genes was performed by an in-house polymerase chain reaction (PCR) and by Sanger sequencing method. According to literature adeB and adeS genes were detected in all MDR A. baumannii isolates tested. Therefore our attention has focused on two resistant tigecycline clinical strains (ACI 2313 and ACI 1213), with a MIC value >8. In particular the ACI 2313 strains, showed the presence of an IS in the adeS gene. Then, adeS sequence analysis identified ISAba1 insertion. Moreover, adeB gene expression was evaluated by an in-house SYBR Green I-based real-time RT-PCR. We found an over expression of adeB gene in ACI 2313 strain, according to IS presence on adeS gene, while the lack of adeB overexpression in ACI 1213, still resistant to tigecycline, could be due to different resistance mechanisms.

Keywords: Tigecycline; A. baumannii isolates; Glycycycline; Antibiotic resistance

Introduction

Acinetobacter baumannii (A. baumannii) is an opportunistic pathogen that commonly causes nosocomial infections, as pneumonia, bloodstream and urinary tract infections, particularly in the intensive care unit [1]. Multi drug resistant (MDR) A. baumannii isolates have been reported worldwide and their increasing prevalence has led to limited therapeutic choice [2].

Tigecycline, the first member of the glycycline class of antibacterial agents, remain effective option for the treatment of these infections. However, due to its increased clinical use, tigecycline resistance is recently emerging [3].

Several studies have indicated that tigecycline resistance of A. baumannii is associated with the over expression of AdeABC efflux system [4,5]. A two component system containing adeS and AdeR, a sensor kinase and a response regulator respectively, are responsible for modulating AdeABC efflux pump [6]. Moreover, nucleotide/ amino acid variations as well as the presence of insertion sequences (IS), such as ISAbA1, in the adeRS operon have been related to the over expression of the adeABC efflux pump, decreasing A. baumannii susceptibility to tigecycline [7]. However, the exact mechanisms of resistance and the relationship between the level of expression of efflux pumps and the minimal inhibitory concentration (MIC, mg/liter) of tigecycline have not yet been clearly elucidated. Also, whether clinical isolates with resistance to tigecycline, originating from the same geographic locations, possess similar mechanisms of resistance is still unclear.

During a retrospective study in a teaching hospital, between January 2011 and February 2015, we evaluated distribution and antibiotic resistance of A. baumannii strains isolated from patients admitted to four hospital units (medical units, surgical units, cardiac intensive care unit and the intensive care unit). A. baumannii isolates were collected from several sites such as blood culture, bronchial aspirate, bronchoalveolar lavage, central venous catheter, urine, and bladder catheter tip. Data collected showed an increasingly infections caused by A. baumannii associated with antibiotic multi-resistance (unpublished data). In particular on 83 strains, isolated in the last year, the percentages of MDR and pan drug resistant (PDR) A. baumannii were 75% and 13% respectively.

Objective

Since the observed high frequency of multi drug resistant A. baumannii in our hospital, the aim of this study was to assess efflux pump AdeABC in 24 MDR A. baumannii strains, selected on the different tigecycline phenotype.

Study Design

Twenty-four clinical isolates of A. baumannii, collected at "Mater Domini" University Hospital of Catanzaro, Southern Italy, from January 2013 to February 2015, were selected according to tigecycline phenotype (0.5 ≤ MIC ≥ 28). Isolates were identified by using VITEK 2 system (bioMérieux) and by mass spectrometry MALDI-TOF MS
Acinetobacter calcoaceticus-baumannii (ACB complex) are prevalent mechanism in tigecycline resistant A. baumannii clinical isolate with overexpression of efflux pumps in resistance to clinically relevant antibiotics [11]. AdeABC efflux pump has been well characterized, it is apparently not well expressed in wild-type strains [12], and contributes significantly to acquire multidrug resistance in worldwide clinical isolates, including resistance to tigecycline increasingly reported since 2007 [13-17]. However tigecycline is one of the few remaining therapeutic options for treating infections caused by MDR A. baumannii. Previous report [4] suggested that decreased susceptibility to tigecycline in the complex Acinetobacter calcoaceticus-Acinetobacter baumannii is associated with overexpression of efflux pump AdeABC.

Recently, the overexpression of AdeABC was referred as the prevalent mechanism in tigecycline resistant A. baumannii clinical isolates, and a linear relationship was found between adeB gene expression levels and tigecycline MICs [17]. Our data showed that, even if adeB were detected in all MDR A. baumannii strains tested, differences in adeB gene expression have been found.
Indeed, in the 2 clinical isolates, sharing a MIC value >8, we determined substantial differences; in particular, in just ACI 2313 isolate, adeS showed a higher relative expression.

The AdeABC efflux pump is regulated by a two component system, AdeS sensor kinase and AdeR response regulator, encoded by the adeRS operon. It has been reported that overexpression of the AdeABC system is due to mutation in adeRS operon, included the presence of insertional sequence, such as ISAba1, one of the most frequent IS insertional sequence, such as ISAba1, one of the most frequent IS.

Conflicts of interests

The authors declare that they have no competing financial interests.

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