PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF ANTIBIOTIC RESISTANCE PATTERNS IN ACINETOBACTER BAUMANNII STRAINS ISOLATED IN A ROMANIAN HOSPITAL

MANUELA-ANDA RADU-POPESCU1*, SILVIA DUMITRIU2, SIMONA ENACHE-SOARE3, GABRIELA BANCESCU2, AURELIAN UDRISTOIU4, MANOLE COJOCARU5, CODRUTA VAGU6

1 University of Medicine and Pharmacy “Carol Davila, Faculty of Pharmacy, Department of Microbiology, Traian Vuia 6, Sect. 2, 020956, Bucharest, Romania
2 University of Medicine and Pharmacy “Carol Davila”, Faculty of Dental Medicine, Department of Microbiology, Bucharest, Romania
3 MICROGEN- Center for Research in Microbiology, Genetics and Biotechnology, University of Bucharest, Faculty of Biology, Bucharest,
4 Targu Jiu Hospital, Targu Jiu, Romania
5 University of Medicine and Pharmacy “Titu Maiorescu”, Faculty of Medicine, Bucharest, Romania
6 “Stefan S. Nicolau” Virology Institute, Bucharest, Romania
*corresponding author: andamrp@gmail.com

Abstract
A total of 633 strains of Acinetobacter baumannii were isolated from respiratory secretions (19%), blood (3.1%), urine (3.7%) and wounds secretions (4.3%). A. baumannii exhibited high resistance rates to ciprofloxacin (93.1%), amikacin (83.8%), ceftazidime (70.4%) and 21.8% were resistant to imipenem. The genotyping PCR (polymerase chain reaction) tests performed of 35 strains confirmed the presence in these MBL-producing (metallo-β-lactamase) strains the blaIMP-1- (type carbapenemases) and blaVIM-2 (Verona integron-encoded metallo-β-lactamase) alleles and respectively OXA-type (oxacillinase group of β-lactamase) carbapenemases (blaOXA-24, blaOXA-51 and blaOXA-58). The PCR amplification has highlighted this in 60% of these strains the presence of 2.2kpb amplicons characteristic for class 1 integrons. These genetic features are accounting for the epidemic potential of multiresistant clones of A. baumannii circulating in hospitals.

Rezumat
Au fost izolate 633 tulpini de Acinetobacter baumannii din secreții tracheale (19%), sânge (3,1%), urina (3,7%), secreți de plăgă (4,3%). A. baumannii prezintă înaltă rezistență la ciprofloxacina (93,1%), amikacina (83,8%), ceftazidima (70,4%) și 21,8% rezistență la imipenem. Genotiparea prin PCR a 35 tulpini a confirmat prezența în aceste tulpini producătoare de MBL (metal β-lactamaze), a alelelor blaIMP-1- și blaVIM-2, respectiv a carbapenemelor de tip OXA (oxacillinase) - blaOXA-24, blaOXA-51 și blaOXA-58. Amplificarea prin PCR a evidențiat în 60% dintre tulpini a ampliconilor
Keywords: Acinetobacter spp., carbapenemases.

Introduction

Organisms belonging to the genus Acinetobacter are important pathogens, often causing nosocomial infections which are difficult to treat and which can be particularly severe in clinically compromised patients. There are over 20 species of Acinetobacter, though the species Acinetobacter baumannii accounts for >80% of isolates causing human diseases [1]. A. baumannii does not have fastidious growth requirements and is able to grow at various temperatures and pH conditions and develop resistance to a variety of antimicrobial agents. The risk factors for acquiring Acinetobacter infection include hospitalization, especially in intensive care units (ICUs), poor general health status, the performance of mechanical ventilation, cardiovascular or respiratory failure, previous antimicrobial therapy, and the presence of central venous or urinary catheters. In hospital settings such as ICUs, several reports have stressed the increasing frequency of the antimicrobial resistance of Acinetobacter to many drug classes, including carbapenems [2, 4]. Inherent to all A. baumannii strains are chromosomally encoded AmpC cephalosporinases [3], also known as Acinetobacter-derived cephalosporinases (ADCs) [7]. Class B carbapenemases (various IMP-type, VIM-type and SIM-1 metallo-β-lactamases) have been found in Acinetobacter spp., but worldwide most A. baumannii strains are resistant as a result of the production of OXA-type carbapenemases. Carbapenems are the standard therapy for severe Acinetobacter infections, and it is disturbing that A. baumannii isolates with resistance or reduced susceptibility are increasingly reported. Some of these, particularly those in the Far East, have IMP and VIM metallo-enzymes [10]; but more isolates, particularly those in Europe, have OXA carbapenemases [12]. These are divided into four clusters by sequence homology, comprising OXA-23, OXA-24, OXA-51, and OXA-58, respectively, and their sequence variants87]. β-Lactam resistance, including carbapenem resistance, has also been associated to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs) [5], multidrug efflux pumps [6] and alterations in the affinity or expression of penicillin-binding proteins [11, 13]. The purpose of this work was to investigate by phenotypic and genotypic methods the antibiotic resistance patterns of A. baumannii strains isolated in a Romanian hospital.
Materials and methods

Bacterial strains: 633 strains of *A. baumannii* were recovered from clinical infections in hospitalized patients from 2001 to 2003 in the Intensive Care Unit (ICU). All isolates were assigned to the *A. baumannii* by MicroScan Walk-Away (Dade Behring). Susceptibility testing methods: MicroScan (Dade Behring, Inc., W. Sacramento, Calif.) susceptibility tests were performed according to the manufacturers’ directions. Detection of ESBL – extended spectrum beta-lactamases was performed by double disk diffusion test, based on the synergistic antimicrobial activity between clavulanic acid/amoxicillin (AMC) and the 3rd cephalosporins (ceftazidime - CAZ and cefotaxime -CTX). Detection of metallo-ß-lactamases was performed using the imipenem/EDTA double disc synergy test according to the manufacturer’s indications [8]. In order to have a clear confirmation of the taxonomic classification of the *A.baumannii* strains, we have used molecular tools in order to evidence the genomic DNA polymorphism by in *situ* enzymatic macrorestriction and PFGE (pulse-field gel electrophoresis) analysis. Electrophoresis was performed on a 1% agarose gel (Sigma) in 0.5M Tris/borate/EDTA buffer on a CHEF DRIII PFGE system (Bio-Rad) for 18h at 14°C, with working conditions of 6 V cm⁻¹, a pulse angle of 12° and pulse times from 5 to 20s. Band profiles were interpreted by the criteria of Tenover et al. [14] with *Fingerprinting vers.II*. The primers used to amplify the *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>VM2</sub> genes are listed in Table I.

### Table I

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Sequence (5’ 3’)</th>
<th>Alignment temperature °C</th>
<th>Amplicon size</th>
</tr>
</thead>
</table>
| *bla*<sub>IMP</sub> | F:5’ CATGGTTTGGTGTTCTTGT3’  
R: 5’ ATAATTTGGGCGGACTTTTG3’ | 56°, 30 sec | 1.150kpb |
| *bla*<sub>VIM1</sub> | F: 5’ TCTACATGACCGCGTCTGTC3’  
R: 5’ TGTGCTTTGACAACGTTCGC3’ | 56°, 30 sec | 0.8kpb |
| *bla*<sub>VIM2</sub> | F: 5’ATGTCCAACCTTTTGAAGTAAG3’  
R: 5’ CTACTCAAGCAGTACTGAGCG3’ | 56°, 30 sec | 1.150kpb |

The primers used to amplify the *bla*<sub>OXA23</sub>, *bla*<sub>OXA24</sub>, *bla*<sub>OXA51</sub>, *bla*<sub>OXA58</sub> genes are listed in Table II.
Table II

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence (5’ 3’)</th>
<th>Amplicon size</th>
</tr>
</thead>
</table>
| bla\textsubscript{OXA23} | for 5’ TAATGCTTTTGATCGGCCTTG3’  
Rev 5’ TGGATTGCCACTTCACTTGG3’ | 353pb         |
| bla\textsubscript{OXA24} | for 5’ GATCGGATTGGGAGAACAGA3’  
Rev 5’ ATTTCTGACCGCATTTCCAT3’ | 501pb         |
| bla\textsubscript{OXA24} | for 5’ GGTTAGTTGGCCCCCTTAAA3’  
Rev 5’ AGTTGAGCCGAAAAAGGGGATT3’ | 246pb         |
| bla\textsubscript{OXA58} | for 5’ AAGTATTGGGGCTTGTGCTG3’  
Rev 5’ CCCCTCTGCGCTTACAC3’ | 599 pb        |

The primers used to amplify the bla\textsubscript{OXA23}, bla\textsubscript{OXA24}, bla\textsubscript{OXA51}, bla\textsubscript{OXA58} targets. 

The amplicons obtained by PCR have been analyzed by gel electrophoresis in agarose gel 0.8% (g/v) prepared in electrophoresis buffer TBE (Tris/Borate/EDTA) 1x, using the molecular weight marker of 100pb DNA (Promega, UK) containing 11 DNA fragments of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 and 1,500pb.

Results and discussion

A total of 633 A. baumannii strains were recovered from various specimens from 2001 to 2003. Specimens from which A. baumannii was isolated comprised respiratory secretions (19%), blood (3.1%), urine (3.7%) and wounds secretions (4.3%). A. baumannii exhibited high resistance rates to ciprofloxacin (93.1%), amikacin (83.8%), ceftazidime (70.4%) and 21.8% were resistant to imipenem. All resistant isolates were confirmed by the positive imipenem/EDTA double disc synergy test and by the MIC (minimum inhibitory concentration) high recorded values (4 and 32µg/mL). A number of 35 strains were selected and analyzed by molecular methods. These strains belonged to 6 pulsotypes, with a percentage of similarity ranging from 68-100% (Figure 1).
Figure 1
The representation of the similarity grouping of 35 A. baumannii and the distribution of different beta-lactamase genes among the tested strains.

The genotyping PCR tests confirmed the presence in these MBL-producing strains of the blaIMP-1 and blaVIM-2 alleles and respectively OXA-type carbapenemase (blaOXA-24, blaOXA-51 and blaOXA-58) genes. The PCR amplification has highlighted in 60% of these strains the presence of 2.2kpb amplicons characteristic for class 1 integrons.

Conclusions
This preliminary study shows the clear association with the resistance to IMP in A. baumannii strains of class 1 integrons, as well as the high prevalence and coexistence of blaIPM-1, blaVIM-2, blaOXA-24 and blaOXA-2 51 among multiple clones of clinical isolates of A. baumannii carrying carbapenemases of blaOXA-58type. These genetic features are accounting for the epidemic potential of multiresistant clones of A. baumannii circulating in Romanian hospitals.

References


Manuscript received: March 12th 2010