

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

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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 *bla*IMP-1- (type carbapenemases) and *bla*VIM-2 (Verona integron-encoded metallo- β -lactamase) alleles and respectively OXA-type (oxacillinase group of β -lactamase) carbapenemases (*bla*OXA-24, *bla*OXA-51 and *bla*OXA-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 traheale (19%), sânge (3,1%), urina (3,7%), secreții de plagă (4,3%). *A. baumannii* prezintă înaltă rezistență la ciprofloxacina (93,1%), amikacina (83,8%), cefotazidima (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 *bla*IMP-1- și *bla*VIM-2, respectiv a carbapenemazelor de tip OXA (oxacilinaze) - *bla*OXA-24, *bla*OXA-51 și *bla*OXA-58. Amplificarea prin PCR a evidențiat la 60% dintre tulpini a ampliconilor

2.2kpb caracteristici pentru integronii de clasa 1. Aceste caracteristici genetice caracterizează tulpinile multirezistente de *A. baumannii* cu potențial epidemic care circulă în spital.

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 variants [8, 9]. 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- β -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_{IMP1}, bla_{VIM1}, bla_{VIM2} genes are listed in Table I.

Table I
The primers used to amplify the bla_{IMP1}, bla_{VIM1}, bla_{VIM2}

Target genes	Sequence (5' 3')	Alignment temperature t ⁰	Amplicon size
<i>bla_{IMP1}</i>	F:5' CATGGTTTGGTGGTTCTTGT3' R: 5' ATAATTGGCGGACTTTGGC3'	56 ⁰ , 30 sec	1.150kpb
<i>bla_{VIM1}</i>	F: 5' TCTACATGACCGCTCTGTC3' R: 5' TGTGCTTTGACAACGTTTCGC3'	56 ⁰ , 30 sec	0.8kpb
<i>bla_{VIM2}</i>	F: 5' ATGTTCAAACCTTTGAGTAAG3' R:5' CTAACAACGACTGAGCG3'	56 ⁰ , 30 sec	1.150kpb

The primers used to amplify the bla_{OXA23}, bla_{OXA24}, bla_{OXA51}, bla_{OXA58} genes are listed in Table II.

Table IIThe primers used to amplify the *bla*_{OXA23}, *bla*_{OXA24}, *bla*_{OXA51}, *bla*_{OXA58}

Target	Sequence (5' 3')	Amplicon size
<i>bla</i> _{OXA51}	for 5' TAATGCTTTGATCGGCCTTG3' Rev 5'TGGATTGCACTTCATCTTGG3'	353pb
<i>bla</i> _{OXA23}	for 5'GATCGGATTGGAGAACCAGA3' Rev 5' ATTTCTGACCGCATTTCCAT3'	501pb
<i>bla</i> _{OXA24}	for 5' GGTTAGTTGGCCCCCTTAAA3' Rev 5' AGTTGAGCGAAAAGGGGATT3'	246pb
<i>bla</i> _{OXA58}	for 5' AAGTATTGGGGCTTGTGCTG3' Rev 5' CCCCTCTGCGCTCTACATAC3'	599 pb

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 32mg µg/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).

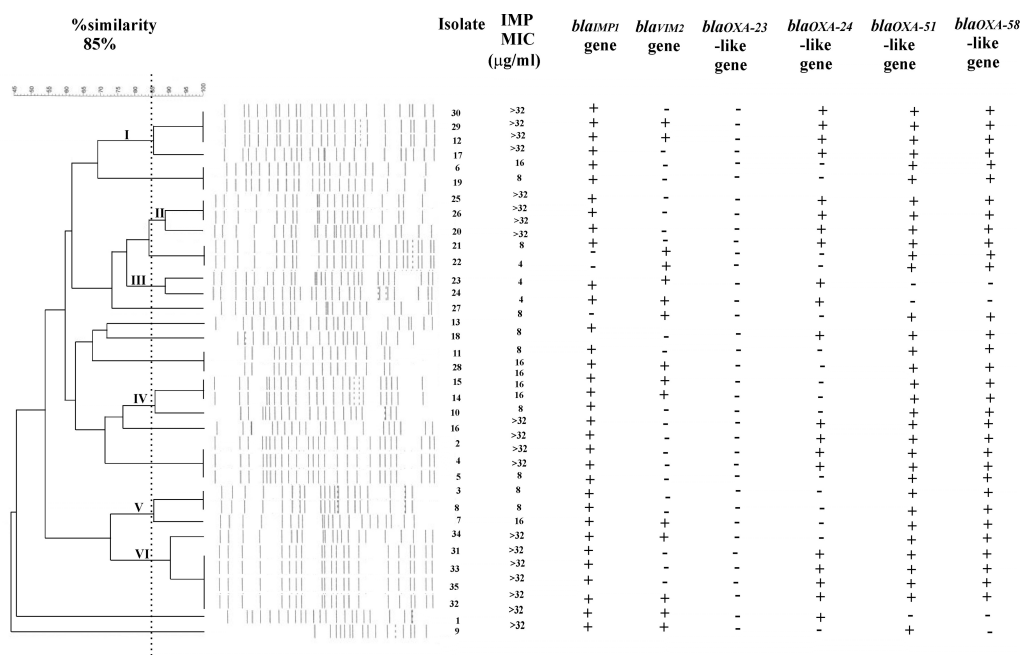


Figure 1

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 *blaIMP*-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.

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