

The Brazilian Journal of  
**INFECTIOUS DISEASES**

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**Letter to the Editor****Widespread dissemination of multidrug-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase and ArmA 16S ribosomal RNA methylase in a Bulgarian university hospital**

Dear Editor,

Multidrug-resistant *Acinetobacter baumannii* (MDR-Acb) has increasingly emerged as a problematic pathogen responsible for hospital-acquired infections worldwide. Carbapenems and aminoglycosides can produce a synergic effect and thus are often used together to treat MDR-Acb infections. Therefore, strains bearing resistance to these antimicrobial drugs would have a considerable clinical impact.

The aim of this study was to explore the resistance mechanisms against carbapenems and aminoglycosides and the molecular epidemiology of clinical strains of MDR-Acb isolated in the Alexandrovska University Hospital (AUH) in Sofia, over a six-year period.

A collection of 70 non-duplicate strains of MDR-Acb (resistant to representatives of at least three different classes of antimicrobial agents such as antipseudomonal penicillins, cephalosporins, carbapenems, aminoglycosides, and quinolones) was investigated. The strains were recovered between January 2005 and September 2011 from inpatients admitted to four intensive care units of the AUH. They were obtained from tracheobronchial aspirates (twenty-eight), surgical wounds (eleven), drainages (ten), blood (six), urine (six), central venous catheters (five) and cerebrospinal fluid (four). Bacterial identification was performed using BBL Enteric/Nonfermenter ID System (Becton Dickinson).

Antimicrobial susceptibility testing was performed by the Etest (LIOFILCHEM) according to the Clinical and Laboratory Standards Institute 2010 recommendations. The EUCAST-2010 MIC breakpoints for Enterobacteriaceae were applied to interpret the tigecycline MIC results. Susceptibility rates to colistin, tigecycline, trimethoprim/sulfamethoxazole, ampicillin/sulbactam, and levofloxacin were as follows: 100%, 77.1%, 64.3%, 24.3%, and 17.1%, respectively. All isolates were non-susceptible to ceftazidime and cefepime (MICs > 8 µg/mL). A total of 49 of the studied MDR-Acb isolates (70%) were carbapenem non-susceptible (MICs ranged from 8 to > 256 µg/mL).

Twenty-three strains (32.9%) isolated between 2010 and 2011 showed high-level resistance to amikacin, gentamicin, and tobramycin (MICs > 256 µg/mL).

Multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp.<sup>1</sup> detected *bla*<sub>OXA-23-like</sub> in all carbapenem non-susceptible MDR-Acb, which were confirmed by sequencing. The insertion sequence IS<sub>Aba1</sub> was positively identified by IS<sub>Aba1</sub>/OXA-23 PCR and was located upstream of *bla*<sub>OXA-23</sub> based on sequence analysis. The comparative temporal prevalence of OXA-23 producers revealed a significantly higher prevalence ( $p < 0.001$ , Student's *t*-test) among the investigated MDR-AcbB isolated during 2009-2011 than among those isolated during 2005-2008 (83.7% vs. 48.1%). None of the *A. baumannii* isolates harbored the metallo-β-lactamase genes *bla*<sub>IMP-like</sub> and *bla*<sub>VIM-like</sub>.

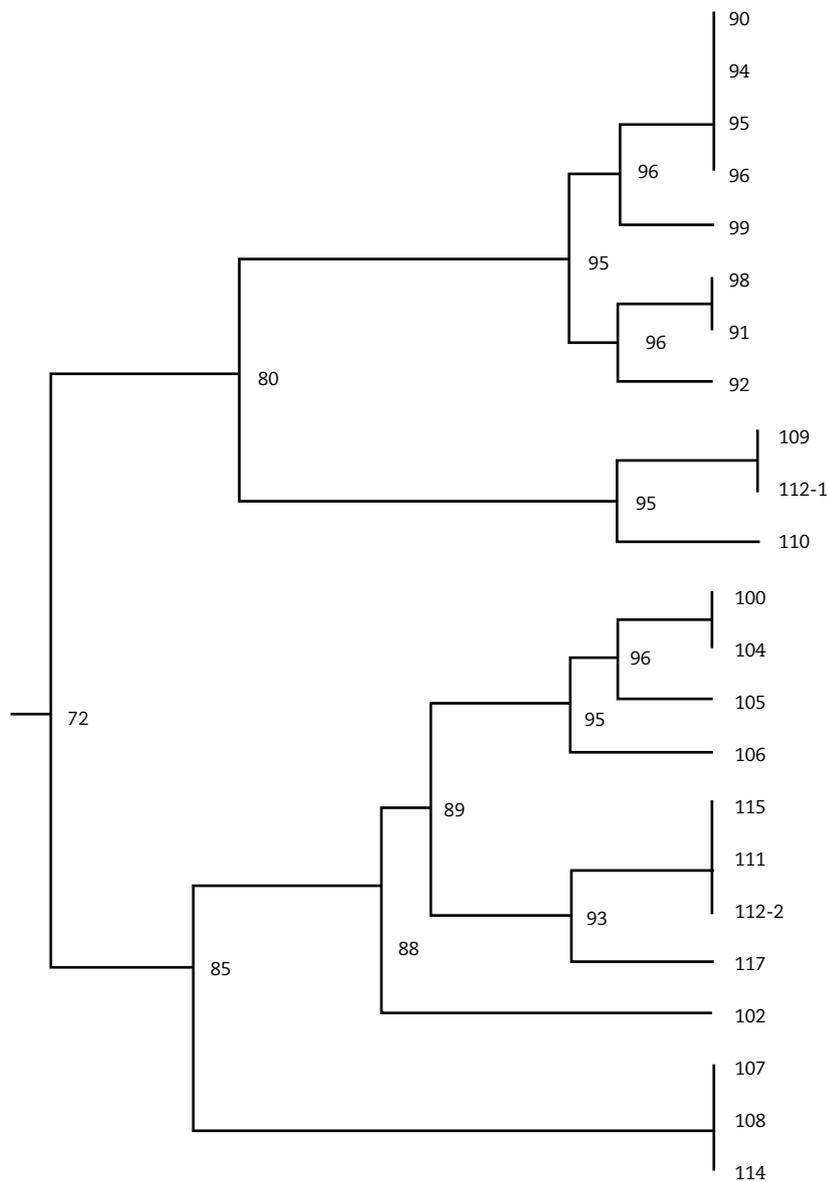
To find the molecular basis of the observed high-level resistance to aminoglycosides putative 16S rRNA methylase genes were assayed. The *armA*, *rmtA*, *rmtB*, *rmtC* and *rmtD* genes were detected by multiplex PCR using previously described primers and conditions.<sup>2</sup> All high-level resistant strains were *armA*-positive and carried the *bla*<sub>OXA-23</sub> gene. In an attempt to determine the resistance genes location, plasmid DNA was isolated using the FlexiPrep Kit (GE Healthcare) and separated by electrophoresis in 0.8% agarose gel stained with ethidium bromide. All the strains contained only a single plasmid of approximately 55 kb. As previously described, PCR assays with the plasmid DNA were performed for detection of *armA* and *bla*<sub>OXA-23</sub>.<sup>2</sup> The plasmid DNA yielded a specific 315 bp PCR product, suggesting the plasmidic location of *armA*. The negative PCR results for *bla*<sub>OXA-23</sub> indicated the probable chromosomal location of this gene.

All the OXA-23 carbapenemase and ArmA 16S rRNA methylase-producing MDR-Acb strains were analyzed by random amplified polymorphic DNA (RAPD) typing as previously described.<sup>3</sup> A high clonal relatedness (72%) was proved between the ArmA-producers ( $n = 23$ ) and indicated

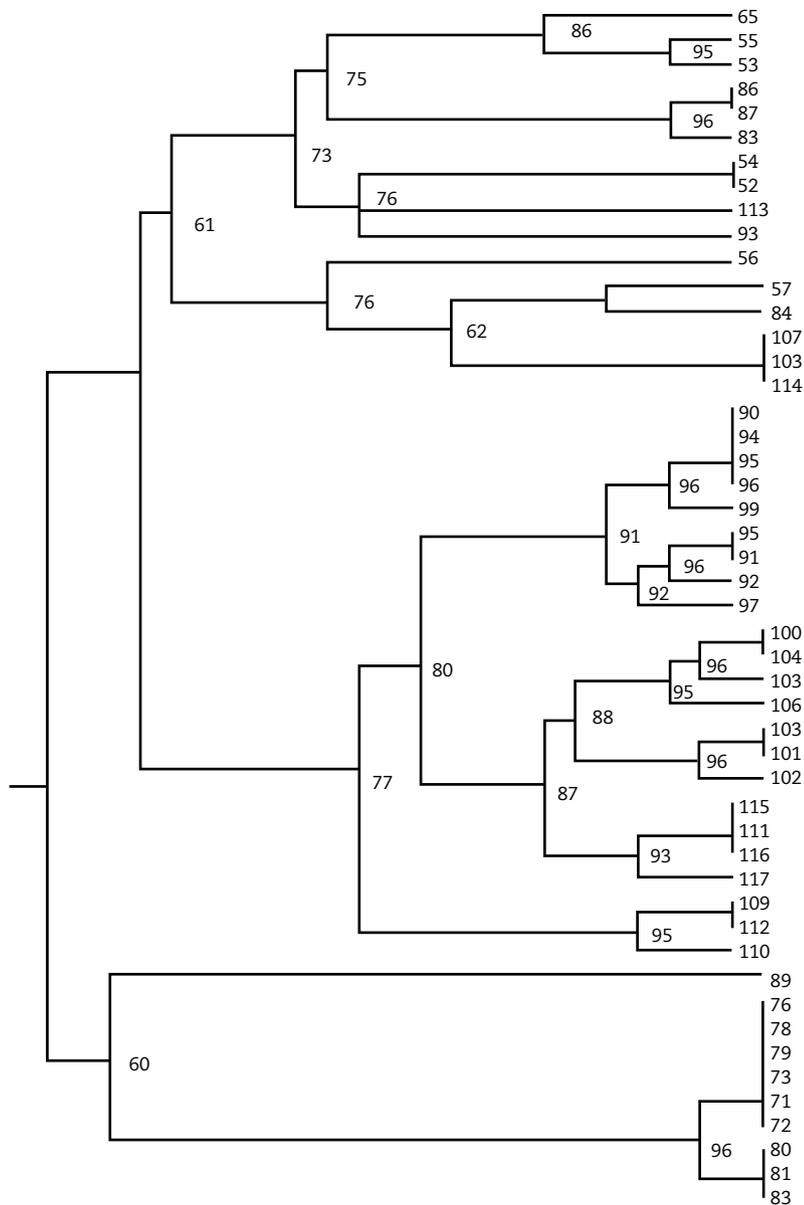
the presence of endemic clone in the AUH during 2010-2011 (Fig. 1). Three endemic clones were found among the OXA-23 producers (n = 49) isolated during 2005-2011 (Fig. 2). They were presented by 23 strains (77% clonal relatedness), 16 strains (61%), and 9 strains (96%), respectively.

To the authors' knowledge, this is the first report of co-production of *armA* and OXA-23 carbapenemase in *A. baumannii* isolates in East Europe. Recently, North

American,<sup>4</sup> Western European,<sup>5</sup> and Asian<sup>6,7</sup> studies have documented the co-existence of *bla*<sub>OXA-23</sub> and *armA*, which can pose therapeutic challenges. This study illustrates the inter-country dissemination of 16S rRNA methylase and OXA-23-producing nosocomial *A. baumannii* isolates. Moreover, the results emphasize the need to adopt surveillance programs to prevent infection by MDR-Acb in this hospital.



**Fig. 1 - Dendrogram illustrating the relationship between 23 *A. baumannii* strains, producing *armA* 16S rRNA methylase, based on analysis with the similarity coefficient (presented as percentage) of RAPD profiles generated with RAPD-4 primer (5'-AAGAGCCCGT-3').**



**Fig. 2 – Dendrogram illustrating the relationship between 49 *A. baumannii* strains, producing OXA-23 carbapenemase, based on analysis with the similarity coefficient (presented as percentage) of RAPD profiles generated with RAPD-4 primer (5'-AAGAGCCCGT-3').**

### Conflict of interest

All authors declare to have no conflict of interest.

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Tanya Strateva\*

*Department of Medical Microbiology, Medical University of Sofia, Bulgaria*

Boyka Markova, Yulia Marteva-Proevska  
*Laboratory of Clinical Microbiology, Alexandrovska University Hospital, Medical University of Sofia, Bulgaria*

Dobrinka Ivanova  
*Laboratory of Clinical Microbiology, Second Multiprofile Active Treatment Hospital, Sofia, Bulgaria*

Ivan Mitov

*Department of Medical Microbiology, Medical University of Sofia, Bulgaria*

\*Corresponding author. Department of Medical Microbiology,  
Medical University of Sofia  
2 Zdrave Street, 1431 Sofia, Bulgaria  
E-mail address: dr.strateva@abv.bg

Received 14 January 2012

Accepted 19 January 2012

1413-8670

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