Occurrence of OXA-107 and IS\textsubscript{Aba1} in Carbapenem-Resistant Isolates of \textit{Acinetobacter baumannii} from Croatia\textsuperscript{v}

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Abstract

An increasing trend toward multidrug resistance, including carbapenem resistance, in \textit{Acinetobacter baumannii} has been observed in the past two decades. Carbapenem resistance mechanisms in \textit{A. baumannii} include the production of metallo-\beta-lactamases (MBLs) and carbapenem-hydrolyzing oxacillinas, efflux mechanisms, and the loss of outer membrane proteins, often in combination (6). These resistance mechanisms pose a serious therapeutic threat, since carbapenems are frequently used to treat otherwise resistant \textit{A. baumannii} infections.

Since 2002, increasing numbers of carbapenem-resistant isolates of \textit{A. baumannii} have been recovered at Split University Hospital in Split, Croatia. Meropenem is prescribed at this hospital more frequently than imipenem, while ertapenem has only recently been licensed in Croatia. There are few published data concerning carbapenem resistance in multidrug-resistant isolates of \textit{A. baumannii} from Croatia. The present study investigated the epidemiology and carbapenem resistance mechanisms of multidrug-resistant \textit{A. baumannii} isolates endemic in this regional teaching hospital.

Between 2002 and 2007, 106 nonrepetitive isolates of \textit{A. baumannii} with an unusual resistance profile were isolated consecutively from two adult surgical intensive care units (ICUs), a pediatric ICU, a neurosurgery ICU, and a general surgery ICU at different locations in Split University Hospital (a 1,651-bed university teaching hospital with facilities at three sites). The hospital serves a pediatric and adult population of ca.500,000 and is also a referral hospital for much of southern Croatia (with a total population of ca. 1 million inhabitants). Isolates were initially recovered on blood agar plates from routine blood cultures, urine samples, wound exudates, catheter tip specimens, and bronchial secretions. Conventional biochemical tests and the API 20NE system (bioMérieux, Marcy-l’Etoile, France) were used to presumptively identify the isolates as members of the \textit{Acinetobacter calcoaceticus\textendash}\textit{A. baumannii} complex. Isolates were confirmed to be \textit{A. baumannii} by the identification of an OXA-51-type enzyme (10) (see below) and, for selected isolates, by tRNA spacer fingerprinting (2).

Routine susceptibility testing used a disk diffusion method, while MICs were determined by broth microdilution with Mueller-Hinton broth in 96-well microtiter plates (1). Resistance to imipenem and/or meropenem was confirmed using Etests (AB Biodisk, Solna, Sweden). The isolates were also tested for possible MBL production by using MBL Etests. Multidrug resistance was defined as resistance to three or more antimicrobial classes.

Crude bacterial DNA templates from bacterial cells were prepared by boiling. Specific primers to detect the presence of \textit{bla}\textsubscript{OXA-23}-like, \textit{bla}\textsubscript{OXA-24}-like, \textit{bla}\textsubscript{OXA-51}-like, and \textit{bla}\textsubscript{OXA-58}-like genes (10) and IS\textsubscript{Aba1} (9) were used in PCR mixtures (50-\mu l final volumes) containing 25 \mu l of PCR master mix (Roche Diagnostics, Burgess Hill, United Kingdom), 20 \mu l of ultrapure water, 1 \mu l of each primer (50 \mu M), and 2 \mu l of the DNA template. Cycling conditions comprised 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with final elongation at 72°C for 10 min, except that an annealing temperature of 58°C was used to investigate the possible location of IS\textsubscript{Aba1} upstream of \textit{bla}\textsubscript{OXA-51}-like genes. Amplicons from selected isolates were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using an ABI Prism 377 genetic analyzer (Applied Biosystems, Warrington, United Kingdom). \textit{A. baumannii} sequence groups were determined as described by Turton et al. (8). Pulsed-field gel electrophoresis was performed following macrorestriction with Apal (5), with subsequent cluster analysis as described previously (5).

All 106 isolates were multidrug resistant (Table 1), but sulbactam and colistin nonsusceptibility was not detected. The imipenem MICs for the isolates were 2 to 32 \mu g/ml, and the meropenem MICs were 8 to 128 \mu g/ml. Cumulative percentages of \textit{A. baumannii} isolates inhibited by imipenem and meropenem are shown in Table 2. All 106 isolates were negative for MBL production according to the results of MBL Etests, but...
of Turton et al. (8). In the present study of isolates with 1 upstream of a Aba1 in 94% of cases, supporting the suggestion of Turton et al.

IMPLICATIONS FOR CLINICAL PRACTICE

The carbapenem-hydrolyzing class D β-lactamases of A. baumannii form four phylogenetic subgroups: OXA-23-like, OXA-24 like, OXA-51-like, and OXA-58-like enzymes (6). Since the discovery of OXA-51 in 2004, at least 39 related enzymes in this group have been described (3). The first description of carbapenem-resistant clinical isolates of A. baumannii in Croatia reported the presence of an OXA-69-like oxacillinase (4). The unusual OXA-107 enzyme was first detected in A. baumannii isolates from Poland and Slovenia (3) and is closely related to OXA-69, with an amino acid change at position 167 that replaces leucine with valine (3). OXA-107 is not distinguished from OXA-69 by the multiplex PCR method of Turton et al. (8). In the present study of isolates with blaOXA-107 as the sole detectable carbapenemase gene, imipenem and/or meropenem resistance was associated with ISAba1 in 94% of cases, supporting the suggestion of Turton et al. (9) that the presence of ISAba1 upstream of a blaOXA-51-like gene is associated with high-level meropenem resistance. Sequence group 2 (European clone 1) is one of two major lineages of multidrug-resistant A. baumannii that are widespread in Europe (7), including Bulgaria, Germany, Greece, The Netherlands, Norway, Poland, and Slovenia (7), but OXA-107 appears to be an uncommon enzyme and may represent a more recent evolutionary adaptation to antibiotic challenge with carbapenems.

To our knowledge, this is the first extensive characterization of clinical A. baumannii isolates producing a class D carbapenemase in Croatia. Colistin and sulbactam are often the only lines of years suggests that these strains persisted unnoticed in the hospital’s ICUs, which then served as reservoirs for patient colonization, followed by patient-to-patient transmission or common-source acquisition (e.g., through contaminated mechanical ventilation equipment). Enhanced infection control measures should be employed to limit the spread of A. baumannii strains within the hospital, and the consumption of meropenem should be restricted in order to reduce the selection pressure in the hospital environment. Further studies in other hospitals in Croatia are needed to evaluate possible interhospital spread of multidrug-resistant A. baumannii strains.

**Nucleotide sequence accession number.** The blaOXA-51-like gene sequence determined in this study has been deposited in GenBank under accession number EF650033.

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