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Occurrence of OXA-107 and IS*Aba*1 in Carbapenem-Resistant Isolates of *Acinetobacter baumannii* from Croatia[∇]

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Carbapenem-resistant isolates of *Acinetobacter baumannii* from intensive care units at Split University Hospital, Split, Croatia, were studied. Most (100 of 106) had IS*Aba*1 inserted upstream of a *bla*_{OXA-107} gene, encoding an unusual OXA-51-type oxacillinase. Pulsed-field gel electrophoresis revealed that the isolates formed three clusters belonging to the sequence group 2 (European clone 1) lineage.

An increasing trend toward multidrug resistance, including carbapenem resistance, in *Acinetobacter baumannii* has been observed in the past two decades. Carbapenem resistance mechanisms in *A. baumannii* include the production of metallo- β -lactamases (MBLs) and carbapenem-hydrolyzing oxacillinases, 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 *A. baumannii* infections.

Since 2002, increasing numbers of carbapenem-resistant isolates of *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 *A. baumannii* from Croatia. The present study investigated the epidemiology and carbapenem resistance mechanisms of multidrug-resistant *A. baumannii* isolates endemic in this regional teaching hospital.

Between 2002 and 2007, 106 nonrepetitive isolates of *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'Étoile, France) were used to presumptively identify the

isolates as members of the *Acinetobacter calcoaceticus*-*A. baumannii* complex. Isolates were confirmed to be *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 *bla*_{OXA23}-like, *bla*_{OXA-24}-like, *bla*_{OXA-51}-like, and *bla*_{OXA-58}-like genes (10) and IS*Aba*1 (9) were used in PCR mixtures (50- μ l final volumes) containing 25 μ l of PCR master mix (Roche Diagnostics, Burgess Hill, United Kingdom), 20 μ l of ultrapure water, 1 μ l of each primer (50 μ M), and 2 μ 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*Aba*1 upstream of *bla*_{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). *A. baumannii* sequence groups were determined as described by Turton et al. (8). Pulsed-field gel electrophoresis was performed following macrorestriction with ApaI (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 μ g/ml, and the meropenem MICs were 8 to 128 μ g/ml. Cumulative percentages of *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

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TABLE 1. MICs for 106 *A. baumannii* isolates from Split University Hospital

Antibiotic	MIC ($\mu\text{g/ml}$)		
	Range	50%	90%
Imipenem	2–32	4	8
Meropenem	8–128	8	32
Ceftazidime	128–>128	128	>128
Ceftriaxone	64–>128	128	>128
Cefepime	32–>128	64	128
Ciprofloxacin	4–64	16	32
Amikacin	32–>128	64	128
Gentamicin	8–>128	128	>128
Piperacillin-tazobactam	64–>128	128	>128
Ampicillin-sulbactam	1–4	1	2
Colistin	0.19–0.50	0.19	0.38

PCR revealed that each isolate possessed a *bla*_{OXA-51}-like gene. No other class D oxacillinase genes were detected by PCR. All isolates with high-level carbapenem resistance (those for which the imipenem or meropenem MIC was ≥ 8 $\mu\text{g/ml}$ [94% of isolates]) had *ISAbal* inserted upstream of the *bla*_{OXA-51}-like gene. Pulsed-field gel electrophoresis genotyping revealed that the 106 isolates formed three related clusters (with >85% similarity) belonging to the sequence group 2 (European clone 1) lineage (i.e., that including the European clone 1 reference strain RUH 2037). Sequencing of the *bla*_{OXA-51}-like genes (both strands) from six representative isolates for which meropenem MICs were elevated revealed the presence of the unusual OXA-107 enzyme.

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 *bla*_{OXA-107} as the sole detectable carbapenemase gene, imipenem and/or meropenem resistance was associated with *ISAbal* in 94% of cases, supporting the suggestion of Turton et al. (9) that the presence of *ISAbal* upstream of a *bla*_{OXA-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 remaining therapeutic options for the patients involved. The fact that clonally related isolates were obtained over a number

TABLE 2. Cumulative percentages of 106 *A. baumannii* isolates from Split University Hospital that were inhibited by imipenem and meropenem

Carbapenem	% Inhibited at antibiotic concn ($\mu\text{g/ml}$) of:								
	≤ 0.5	1	2	4	8	16	32	64	128
Imipenem			31	71	85	93	100		
Meropenem					64	80	87	98	100

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 *bla*_{OXA-51}-like gene sequence determined in this study has been deposited in GenBank under accession number EF650033.

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