We report on the sequence type and beta-lactamase content of 174 carbapenem-resistant *Acinetobacter baumannii* isolates recovered from clinical specimens during 2010 and 2011 in a tertiary care hospital in central Greece. Carbapenem resistance was associated mainly with carriage of the *bla*_{OXA-23} gene (in 72.4% of the isolates). To our knowledge, this is the first description of *A. baumannii* strains producing OXA-23 in Greece. During 2011, in our hospital they rapidly ‘replaced’ the previously predominant OXA-58-positive *A. baumannii* strains.

Previous studies have documented the predominance of oxacillinase (OXA)-58-producers among carbapenem-resistant *Acinetobacter baumannii* in Greek hospitals [1]. Here, we report the isolation of OXA-23-producing *A. baumannii* from clinical specimens taken in 2010 and 2011 at the University Hospital of Larissa in central Greece. To the best of our knowledge, this is the first description of OXA-23-producing *A. baumannii* in this country.

We therefore in December 2011, after the annual evaluation of the infection control committee, studied retrospectively single carbapenem-resistant *A. baumannii* clinical isolates (i.e. one per patient) obtained during July to December 2010 (n=47) and those from January to December 2011 (n=127). The isolates were recovered from various clinical specimens including, in descending frequency, bronchial secretions, blood, pus and urine. When necessary, species identity was confirmed with VITEK 2 (bioMérieux, France) and/or detection by PCR of *bla*_{OXA-51}, the intrinsic carbapenemase gene of *A. baumannii* [5]. MICs of various antimicrobials, including imipenem and meropenem, were re-determined by the Etest (bioMérieux, Sweden). Resistance status was defined according to the latest European Committee on Antimicrobial Susceptibility Testing breakpoints [6].
All 174 isolates were typed by three loci-sequencing typing [5,7]; allele numbers and sequence types were assigned based on the information in the respective databases. Identification of CHDL-encoding genes and mapping of their genetic environment were performed by PCR assays and sequencing of the respective amplicons, as described elsewhere [8,9].

**Characteristics of the carbapenem-resistant *A. baumannii* population**

Of the 174 carbapenem-resistant *A. baumannii* isolates, 125 (71.8%) carried *bla*$_{OXA-23}$, 48 (27.6%) carried *bla*$_{OXA-58}$ and one (0.6%) was positive for both genes. The majority of *bla*$_{OXA-48}$ carriers (n=42) were isolated during 2010. Five of the *bla*$_{OXA-23}$ carriers were isolated in late 2010 from patients in the intensive care unit. The first two were isolated from patients in October 2010. The first patient was in their midzos and the second in their early 70s. Both patients had no previous medical history of underlying disease such as diabetes, cancer or immunosuppression before being hospitalised. These two patients had been treated for at least two weeks before the isolates were cultured and their infections (both bacteraemia) were considered as hospital acquired, as was the case for the remaining patients infected with OXA-23-producing *A. baumannii*. Medical records of the first two patients did not indicate prior hospitalisation in the past six months or any travel abroad. It is therefore possible that OXA-23-positive *A. baumannii* had already circulated in the hospital.

Environmental screening was not carried out. We cannot provide reliable hypotheses regarding potential routes of introduction of OXA-23 producers to the hospital. Among the carbapenem-resistant *A. baumannii* isolates detected in 2011, the *bla*$_{OXA-23}$ carriers predominated (120/127; 95%): 43 were isolated from patients in the intensive care unit and the rest from various medical (n=60) and surgical wards (n=17). Of the other seven isolates, recovered in a sporadic fashion throughout 2011, six were positive for *bla*$_{OXA-48}$ and one carried both *bla*$_{OXA-48}$ and *bla*$_{OXA-23}$. According to the three loci-sequencing typing, 24 *bla*$_{OXA-48}$-positive isolates belonged to sequence type (ST) 106, 23 to ST201 and one exhibited a novel allelic profile (2-1-2). ST101 was prevalent among *bla*$_{OXA-23}$ carriers (n=101), the remaining 24 isolates being classified as ST201. The isolate harbouring both carbapenemase genes belonged to ST106. The isolation frequency of the isolates is shown in the Figure.

Apart from resistance to at least one carbapenem (imipenem and/or meropenem), all 174 isolates exhibited resistance to multiple drugs including penicillin-inhibitor combinations, oxycimino-cephalosporins, aminoglycosides and fluoroquinolones. All were susceptible to colistin. The *bla*$_{OXA-23}$-positive isolates were generally inhibited by higher concentrations of meropenem as compared with *bla*$_{OXA-48}$ carriers. This is consistent with previous findings indicating that OXA-23 confers to *A. baumannii* higher levels of carbapenem resistance, as compared with OXA-58, although both enzymes exhibit relatively weak carbapenemase activities [10]. The genetic environment of *bla*$_{OXA-23}$ gene was investigated in 20 isolates representing all sequence types and beta-lactamase combinations found. In all cases, *bla*$_{OXA-23}$ occurred as part of the previously characterised Tn2006 transposon bracketed by two ISAb1 (insertion sequences) in opposite orientation [9].

**Discussion**

During 1999 to 2009, *A. baumannii* strains carrying the *bla*$_{OXA-58}$ carbapenemase gene predominated among carbapenem-resistant isolates of this species in the hospital flora in various Mediterranean countries including Italy, Greece, Lebanon and Turkey [1,11]. Since 2009, isolation of *A. baumannii* producing the OXA-23 carbapenemase has been increasingly reported in European countries [12-14]. Of note, a massive ‘replacement’ of OXA-58 *A. baumannii* by OXA-23 producers, similar to that observed here, has recently been described in Italian hospitals [15]. It was hypothesised that the higher carbapenem MICs of the *bla*$_{OXA-23}$-positive isolates may provide a selective advantage in the hospital setting. Yet, the change of the *A. baumannii* population seen in our study was too abrupt to be explained by the operation of carbapenem selective pressure alone. Whatever the reasons for the shift in

**Figure**

Isolation frequencies of carbapenem-resistant *Acinetobacter baumannii* strains, University Hospital of Larissa, Thessaly, Greece, July 2010–December 2011 (n=174)
the *A. baumannii* population, the spread of strains producing OXA-23 seems to reflect a global trend towards a predominance of *bla*OXA-23 carriers belonging to the international clonal lineages I and II [16,17]. Spread of multidrug-resistant strains producing OXA-23 may aggravate the therapeutic problems caused by this species by further limiting treatment options.

It is highly likely that similar changes have also occurred in the *A. baumannii* population in tertiary care hospitals in the major urban centres of Greece, Athens and Thessaloniki. The available resources however, have been almost exclusively allocated to detecting and containing carbapenemase-producing *Enterobacteiceae*. Yet, we believe that a low-cost study involving a limited number of sentinel hospitals would provide a comprehensive picture regarding the *A. baumannii* strains circulating in this country.

Hospital personnel have been informed about our findings and infection control measures have been reinforced.

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**References**