ORIGINAL ARTICLE

Trends in the molecular epidemiology of carbapenem resistant acinetobacter baumannii in a tertiary Greek hospital

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Abstract

Background: Acinetobacter baumannii is responsible for a variety of nosocomial infections, especially in intensive care unit patients. Nosocomial outbreaks due to carbapenem-resistant A. baumannii strains have been reported in many countries, including Greece. The aim of the present study was to determine the trends of molecular epidemiology of carbapenem-resistant A. baumannii isolates in a 750-bed hospital in Thessaloniki, Greece, during 2009.

Methods: The study included 39 carbapenem-resistant A. baumannii isolates collected from patients hospitalized in the General Hospital Papageorgiou during 2009. They were tested for the presence of Ambler class D carbapenemases and class 1 integrons, and they were typed by pulsed-field gel electrophoresis.

Results: The $bla_{\text{OXA-58}}$ gene was detected in all A. baumannii isolates. Among the 39 isolates, 18 were carrying a 2.2 kb integron, 18 were carrying a 2.5 kb integron, and 3 isolates had no class 1 integrons. Two different clones, each divided further into two subclones, were observed. Comparing the clones detected in 2009 with those of former years (2001-2008), a significant difference was observed: three clones have disappeared, two clones continued to circulate in the hospital, while a new subclone emerged in February 2009.

Conclusions: A change was seen in the molecular epidemiology of carbapenem-resistant A. baumannii isolates during 2009. Molecular epidemiology studies provide useful data for the distribution of resistant bacteria in order to design effective prevention and control measures. Hippokratia 2011; 15 (4): 343-345

Key words: acinetobacter baumannii, carbapenem-resistance, molecular epidemiology

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The hospital-acquired infections caused by Acinetobacter baumannii have been recently increased globally. They cause mainly respiratory infections, bacteremia, meningitis and urinary tract infections, mainly in Intensive Care Unit (ICU) patients. While carbapenems are considered the antibiotic of choice, an increase of carbapenem resistant A. baumannii has been observed during the recent years[1]. Nosocomial outbreaks due to carbapenem-resistant A. baumannii strains have been reported in Greece[2-4].

In a recent study in Papageorgiou General Hospital in Thessaloniki, Northern Greece, 164 A. baumannii isolates collected during an 8-year period (2001-2008) were tested for the presence of Ambler class A and D carbapenemases and typed by pulsed-field gel electrophoresis (PFGE); it was found that isolates were clustered into five distinct clones (A – E)[5]. Clone A isolates were carrying the $bla_{\text{OXA-58}}$ gene, as well as a 2.2 kb class 1 integron. Clone A was further subdivided into subclones A1 and A2. The subclone A1 was predominant (70.1% of the isolates) and was circulating in the hospital during most of the time of its operation (2002–2007), with a peak in 2005. In 2008 a new clone (E) had emerged.

Aim of the present study was to investigate the persistence of the known clones of carbapenem-resistant A. baumannii and the probable emergence of new ones during 2009, in order to have a follow-up in the molecular epidemiology of the microorganism in the hospital.

Material and methods

Thirty-nine non-repetitive carbapenem-resistant A. baumannii isolates collected from patients (23 male – 16 female) hospitalized in various wards of Papageorgiou General Hospital during January to December of 2009 were tested. The age of the patients ranged from 12 to 83 years (median age 62 years). Most of the isolates (26/39, 66.7%) were retrieved from patients of the ICU, while the rest were from patients in the surgery (11/39, 28.2%) and the internal medicine units (2/39, 5.1%). Isolates were obtained from intravenous catheter (n=7), blood (n=5), respiratory tract (n=4), wounds (n=4), urine (n=3), and pleural fluid (n=1), while the rest (n=15) were obtained from colonization sites (11 from throat-, 2 from skin- and 2 from rectal- swabs).

Identification and antibiotic susceptibility tests were

performed by the automated system WIDER (Francisco Soria Melguizo S.A.). Isolates with imipenem and meropenem MIC ≤ 4 μg/ml were considered susceptible. Isolates were screened for the intrinsic bla-$_{OXA-51}$ gene, the $bla_{OXA-58,}$ $bla_{OXA-23-like}$, $bla_{OXA-24-like}$ genes, and for the presence of the IS*Aba1* element as described in the former study[5]. In addition, the size of the integrons was determined[6]. The isolates were typed by PFGE using the *Apa*I restriction enzyme and analyzed by BioNumerics software (Applied Maths, NV). Isolates were considered to be within a clone if the similarity coefficient was ≥ 80%[7].

Results

All 39 isolates carried the bla-$_{OXA-51}$ and bla_{OXA-58} genes, while 22/39 (56.4%) harbored also the IS*Aba1* element (Table 1A). No $bla_{OXA-23-like}$, $bla_{OXA-24-like}$ genes were detected. Molecular typing results demonstrated that the 39 isolates clustered into 2 clones A (18 isolates) and E (21 isolates). Both subclones of clone A were present, A1 with 6 and A2 with 12 isolates. The presence of two distinct patterns (although will less than 20% difference) among isolates of clone E (which first appeared in the hospital in 2008), prompted to divide it into subclone E1 and E2: E1 included isolates of 2008 and 18 isolates of the present study, while 3 isolates of the present study consisted the subclone E2 (Figure 1). The first E2 isolate was recovered in February 2009. Isolates of the former described clones B, C and D, were not detected.

Isolates of subclones A1 and A2 harbored a class 1 integron of 2.2 kb, isolates of subclone E1 had a class 1 integron of 2.5 kb, while isolates of subclone E2 had no integrons. The clones and subclones per ward are seen in Table 1B.

Table 1: Characteristics (A) and distribution (B) of the *Acinetobacter baumannii* clones of the present study.

A .

Clone	*Int 1*	Size of integron (kb)	bla_{OXA-58}	IS*Aba1*
A1	pos	2.2	pos	neg
A2	pos	2.2	pos	neg
E1	pos	2.5	pos	pos
E2	neg	neg	pos	pos

B.

Clone	ICU N	Surgery N	Internal medicine N	Total N (%)
A1	4	2	0	6 (15.4)
A2	9	2	1	12 (30.8)
E1	11	7	0	18 (46.1)
E2	2	0	1	3 (7.7)
Total	26	11	2	39 (100)

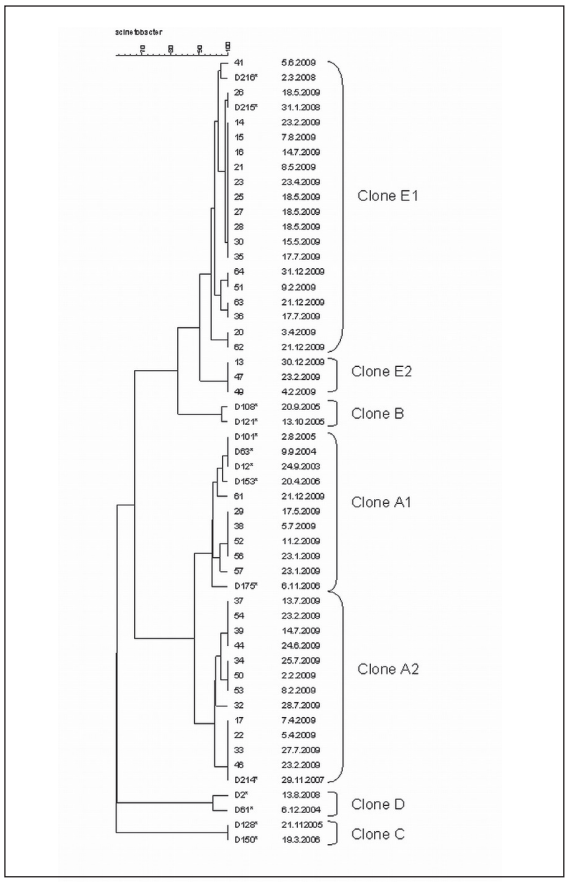

Figure 1: Pulsed-field gel electrophoresis profiles and dendrogram of *Acinetobacter baumannii* isolates of the present study. Isolates with asterisk have been recovered in the hospital during previous years. The scale indicates similarity percentage. Isolation date and site from where the samples were obtained are seen.

Apart the carbapenem-resistance, *A. baumannii* isolates showed high rates of co-resistance to other antimicrobials tested, except colistin. Briefly, the MICs of all isolates were as following: amoxicillin/clavulanic acid >32/16 μg/ml, piperacillin/tazobactam 128/4 μg/ml, ceftazidime 8 to >32 μg/ml, amicacin >64 μg/ml, meropenem ≥ 16 μg/ml. Isolates of subclones A1, A2 and E1 were resistant to gentamicin (MIC ≥ 16 μg/ml), while isolates of subclone E2 were susceptible to gentamicin (MIC ≤ 4 μg/ml), and they did not have a class 1 integron. Isolates of E1 and E2 were susceptible also to tobramycin (MIC≤ 4 μg/ml). Concerning cefepime, isolates of subclones A1 and A2 were resistant (MIC 8 to >16 μg/ml) while isolates of subclones E1 and E2 were sensitive (MIC ≤ 8 μg/ml). The MIC of imipenem varied from ≤ 4 μg/ml to ≥ 16 μg/ml.

Most isolates were recovered from the ICU patients. The distribution of the clones per ward is seen in Table 1B. In ICU all clones were present. No difference was observed between isolates obtained from infection and colonization sites (Figure 1).

Discussion

A great difference in the epidemiology of *A. baumannii* was observed in 2009. While during 2001-2008 five distinct clones (A-E) were present, with subclone A1 being responsible for a large outbreak in 2005, isolates of 2009 belonged into two clones, A and E, with the majority of them (46.1%) belonging to subclone E1 (former clone E, which had emerged in 2008). An additional subclone (E2) emerged in the hospital in February 2009, however, causing only sporadic cases. Isolates belonging to clones B, C and D were not detected in 2009.

Three major subgroups of acquired carbapenem-hydrolyzing class D-lactamases have been identified in *A. baumannii* and are represented by the OXA-23, OXA-24/OXA-40, and OXA-58-lactamases. Currently isolates carrying genes encoding OXA-23 and OXA-24/OXA40 lactamases have never been reported in Greece, while there are many reports of isolates carrying the $bla_{\text{OXA-58}}$ gene[2]. All 39 isolates of the present study carried the $bla_{\text{OXA-58}}$ gene, together with the intristic gene bla-$_{\text{OXA-51}}$. Acquired class D β-lactamase genes are mostly associated to class 1 integron or to insertion sequences[8]. A variety in the size of class 1 integrons was seen in the present study: 2.2 kb in clone A, 2.5 kb in subclone E1, while they were not detected in isolates of subclone E2. The difference in integrons reflects the difference seen in resistance to aminoglycosides[9]. Isolates of clone E (E1 and E2) had the insertion sequence IS*Aba1*. This element has been suggested to provide the entire promoter sequences for $bla_{\text{OXA-58}}$ expression[10].

A significant reduction in the number of isolates belonging to the previous epidemic *A. baumannii* clone was observed, probably due to strict application of infection control measures. Despite these measures, carbapenem-resistant *A. baumannii* strains continued to circulate in the hospital in 2009, although not in such large scale as in previous years, especially in 2005. The present study showed that a change was seen in the genetic clones and their dissemination, probably driven through selective pressure. The molecular epidemiology provides useful data for the extent and distribution of multi-drug resistant bacteria in order to design effective prevention and control measures.

Acknowledgement

The technical support of Elpida Gavana is highly appreciated.

Disclosure Statement

The authors have no conflicts of interest to declare.

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