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Risk-factors for the acquisition of imipenem-resistant *Acinetobacter baumannii* in Spain: a nationwide study

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ABSTRACT

Potential risk-factors for the acquisition of imipenem-resistant *Acinetobacter baumannii* were investigated in a cohort study in 25 Spanish hospitals. The clonal relationship among isolates was determined by pulsed-field gel electrophoresis (PFGE). In total, *A. baumannii* was isolated from 203 patients, with imipenem-resistant (MIC_{90} 128 mg/L) isolates being obtained from 88 patients (43%), and imipenem-susceptible isolates from 115 patients (57%). A wide clonal distribution was observed among the imipenem-resistant isolates, but spread of the same clone among centres was not demonstrated. The results indicated that imipenem-resistant *A. baumannii* is a widely distributed nosocomial pathogen in Spain and reaches an alarming frequency in some centres. Independent risk-factors for the acquisition of imipenem-resistant *A. baumannii* were a hospital size of >500 beds (multivariate OR, 6.5; 95% CI, 1.8–23), previous antimicrobial treatment (multivariate OR, 4.3; 95% CI, 1.6–11), a urinary catheter (multivariate OR, 2.7; 95% CI, 1.1–6.7) and surgery (multivariate OR, 2; 95% CI, 1.07–3.8).

Keywords *Acinetobacter baumannii*, antibiotic resistance, imipenem, molecular epidemiology, nosocomial infection, risk-factors

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INTRODUCTION

Acinetobacter baumannii is an important nosocomial pathogen in developed countries which is difficult to both control and treat because of its prolonged environmental survival and its ability to develop resistance to multiple antimicrobial agents [1,2]. In general, imipenem is the agent most active against *A. baumannii*. In a study in 49 USA hospitals, in which 111 episodes of bacteraemia caused by *A. baumannii* were analysed, imipenem was active *in vitro* against all the isolates (MIC_{90} 1 mg/L) [3]. However, reports of imipenem-resistant *A. baumannii* (IMP-R *A. baumannii*) strains have been rising steadily during the past few years, and these isolates are often multidrug-resistant [4–8]. It is likely that multiple mechanisms account for carbapenem resistance in *A. baumannii* [9,10]. These data are disturbing because inappropriate empirical antimicrobial treatment increases mortality in patients with bacteraemia [11,12].

Previous studies of risk-factors for the acquisition of IMP-R *A. baumannii* have focused on a single city or institution [4,6,7]. These studies have suggested that previous use of third-generation cephalosporins or carbapenems, admission to a ward with a high density of patients infected with IMP-R *A. baumannii*, and a high workload all contribute to acquisition of carbapenem-resistant *A. baumannii* [4,6,7]. Recently, a case-control study suggested that the risk-factors for nosocomial IMP-R *A. baumannii* infection were

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previous stay in an intensive care unit (ICU), and previous exposure to imipenem or third-generation cephalosporins [13]. However, more data regarding the risk-factors for IMP-R *A. baumannii* colonisation/infection are needed in order to prevent infection and to optimise therapy.

The present study investigated the risk-factors for the acquisition of IMP-R *A. baumannii* as part of a nationwide study in Spain. Genotypic analysis using pulsed-field gel electrophoresis (PFGE) was used to determine whether inter-hospital spread of imipenem-resistant isolates had occurred.

METHODS

Participating hospitals

Members of the Spanish Group for Nosocomial Infection (GEIH) from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) were asked to participate in the GEIH-Ab 2000 project, which was designed to investigate the epidemiology, mechanisms of resistance and clinical implications of *A. baumannii* in Spanish hospitals. The 28 participating hospitals serve a population of 11 million (*c.* 25% of the total population of Spain). Twenty-six (92.8%) of the participants were public hospitals, 14 (50%) were university hospitals, and 19 (67.8%) had active transplant programmes. Eleven (39.3%) had >1000 beds, ten (35.7%) had 500–999 beds, and seven (25%) had <500 beds.

Study definitions

Every new case of colonisation or infection caused by *A. baumannii* during November 2000 in the participating hospitals was included. For each case, only the first isolate was included.

The breakpoint for defining imipenem-susceptible *A. baumannii* (IMP-S *A. baumannii*) was an MIC ≤ 4 mg/L, and a breakpoint of ≥8 mg/L was used for IMP-R *A. baumannii*, i.e., intermediate and resistant isolates [14,15]. *A. baumannii* was considered to be acquired nosocomially if the specimen was obtained >2 days after the admission of the patient to hospital. The clinical significance (colonisation or infection) of each *A. baumannii* isolate and the type of infection were assessed according to CDC criteria [16,17].

Risk-factor analysis

The following data were collected: age, gender, presence or absence of underlying conditions, hospital size (i.e., >500 or <500 beds), type of hospital ward (ICU, medical, surgical or paediatric), treatment with antimicrobial agents, number and classes of antimicrobial agent, intravenous, arterial or urinary catheter, nasogastric tube, parenteral nutrition, mechanical ventilation, surgical procedures, and ICU and hospital stay before infection/colonisation. Patients were followed until discharge or death, or until 30 days after the specimen had been obtained if the patient was still hospitalised.

Microbiological studies

All isolates identified presumptively as *A. baumannii* in each participating hospital were sent to a reference laboratory (Hospital Clinic, Barcelona), where identification was performed initially with the API 20 NE system (bioMérieux, Marcy l'Etoile, France) and confirmed by amplified ribosomal DNA restriction analysis [18].

PFGE was used to determine whether inter-hospital spread of imipenem-resistant isolates had occurred, following the methodology described by Gautom [19], with 20 U of *Apa*I used for restriction endonuclease digestion. DNA fragments were separated in an agarose 1% w/v gel and electrophoresed in 0.5× Tris-borate-EDTA buffer at 6 V/cm on a contour-clamped homogeneous electric field apparatus (CHEF DRIII; Bio-Rad Laboratories, Richmond, CA, USA). Pulse times were from 5 to 8 s for 20 h. The gels were stained with ethidium bromide and photographed under UV light. Isolates were assigned to clonal groups according to the criteria of Tenover *et al.* [20].

Antimicrobial susceptibility to imipenem (Merck Sharp & Dohme, Madrid, Spain) was determined by microdilution following NCCLS recommendations [15].

Statistical analysis

Data were recorded on standardised forms and entered into a database. Analyses were performed using SPSS software, v. 11.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared by Fisher's exact test. If continuous data were distributed normally, the ANOVA test was used; otherwise, non-parametric two-sample tests were used. Variables with a p value <0.1 in the univariate analysis were included in the multivariate analysis. Multivariate stepwise (forward) logistic regression analysis was performed to determine independent risk-factors for acquiring imipenem resistance. The optimal variables, on the basis of clinical significance, were included in the final model. Co-linearity was discarded. All statistical analyses were two-sided, and significance was set at $p < 0.05$.

RESULTS

Clinical and microbiological findings

During the study period, 240 isolates identified presumptively as *A. baumannii* were sent to the reference laboratory. Nineteen isolates were excluded: one was not *Acinetobacter* spp.; 15 were identified as *Acinetobacter* genospecies 3 [21]; and three were *Acinetobacter* spp. other than *A. baumannii*. Thus, in total, 25 (89.2%) of the 28 participating hospitals had 221 patients with *A. baumannii* colonisation or infection during the study period. The clinical features and epidemiology of these patients, as well as the clonal diversity, antimicrobial susceptibility and type-1 integron content of the isolates, have been described previously [22–24]. The present study of