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Original Article

Identification of clinical isolates of *Acinetobacter baumannii* from Iran and study of their heterogeneity

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Abstract

Background: *Acinetobacter baumannii* has become one of the most serious causative agents of nosocomial infections due to its significant ability to survive on hospital surfaces. It is mainly an emerging opportunistic pathogen infecting patients in intensive care units. This study was aimed to identify the clinical isolates of *A. baumannii* and to investigate their heterogeneity using polymerase chain reaction (PCR)-based typing methods.
Methods: A total of 197 nonduplicate isolates recovered from a wide range of clinical samples were subjected to conventional cultural and biochemical tests. For those isolates that were preliminary identified as *A. baumannii, rpoB*-based PCR with subsequent restriction fragment length polymorphism (RFLP) using two restriction enzymes (*Tag*I and *Hae*III) was performed to investigate the genetic diversity of the strains and their presumptive relationships with different clinical presentation of the disease caused by this pathogen.
Results: In total, 50 isolates (25.4%) were identified as *A. baumannii* using conventional phenotypic methods with subsequent confirmation by *rpoB* sequencing. RFLP analysis demonstrated five different restriction enzyme patterns, designated as A−E clusters. Most *A. baumannii* isolates were categorized under Cluster A (32%). We found no significant relationship between clinical presentation and the clustering of the isolates.
Conclusion: This study showed that the *rpoB* region possesses high discriminatory power to identify the isolates to the species level. This marker showed high interspecies variability that might be useful for strain typing. The results also suggest the possibility of the existence of a predominant clone of *A. baumannii* among infected patients in Iran.

Keywords: Acinetobacter baumannii; heterogeneity; nosocomial infections; *rpoB*

1. Introduction

Although acinetobacters are strictly aerobic Gram-negative coccobacilli that are widely distributed in soil and water, they are also commonly found in the hospital environment. Over the past 20 years, *Acinetobacter* species have emerged as opportunistic pathogens that are associated with severe hospital-acquired infections.[1,2] In particular, among the various species of this genus, *Acinetobacter baumannii* is responsible for a significant proportion of nosocomial infections.[3] The management of infections caused by *Acinetobacter baumannii* is greatly hindered by its intrinsic and acquired resistance to a wide variety of antimicrobial agents. In addition to this, the number of multidrug-resistant strains has increased during the past two decades.[4] Therefore, *A.*

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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baumannii has emerged as one of the most troublesome pathogens for health-care institutions globally. To control the spread of *A. baumannii* in the hospital setting, it is necessary to identify potential reservoirs of the organism and the modes of transmission. In addition, to distinguish *A. baumannii* strains involved in the outbreaks from epidemiologically unrelated strains, a comparison of isolates at the subspecies level is required by application of molecular typing methods.[2,5]

There are several typing methods for *Acinetobacter* spp., including ribotyping, polymerase chain reaction (PCR) hybridization with species-specific probes, pulse field gel electrophoresis, and random amplified polymorphic DNA typing.[6,7] The main disadvantage of these typing methods is low reproducibility, especially in terms of global molecular epidemiology. Using sequence-based typing such as single or multilocus or whole-genome sequencing provides more reliable data to compare strain information from a local point of view or globally. However, high cost is the main disadvantage of sequence-based typing in developing countries.[6,8] From this point of view, definitive identification of *A. baumannii* strains and investigation of their heterogeneity will be of value for both clinical studies and molecular epidemiology purposes. In this study, *rpoB*-based PCR sequencing was evaluated for definitive identification of *A. baumannii* strains and investigation of their heterogeneity. The presumptive relationship between genotypes and clinical presentation of the patients was also investigated.

2. Methods

2.1. Bacterial strains and phenotypic tests

A total of 197 nonduplicate isolates from a wide range of clinical samples from laboratories of the university teaching hospitals in Ahvaz and Tehran, Iran, were collected from November 2011 to January 2013. The preliminary proposal of the work was reviewed and approved by the Institutional Review and Ethics Board of the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. We also received necessary permission for sample collection and starting the work. The sources of clinical samples and patients' medical histories are summarized in Table 1. The isolates were all those identified as *Acinetobacter* spp. based on the results of preliminary conventional phenotypic tests including growth on MacConkey agar, sugar fermentation, motility, catalase and oxidase tests, and other standard recommended tests.[9,10] For definitive identification of these isolates to the species level, molecular methods were used in the next step.

2.2. Molecular methods

The isolates were identified to the species level using species-specific *rpoB* gene-based PCR as previously described.[11] In brief, a 350-bp fragment of the *rpoB* gene was amplified from each isolate using two primers of 696F (5′-TAY CGY AAA GAY TTG AAA GAA G-3′) and 1093R (5′-CMA CAC CYT TGT TMC CRT CA-3′). To investigate the heterogeneity of *A. baumannii* isolates, restriction fragment length polymorphism (RFLP) was performed as described by other investigators, using *Tag*I and *Hae*III restriction endonucleases.[12]

2.3. Data analysis of rpoB gene sequences

The obtained sequences of the *rpoB* region of each strain were aligned with the published *rpoB* region sequences of *A. baumannii* strains retrieved from GenBank database using the JPhydit software package according to the primary structure.[13] Comparative analyses of the *rpoB* region were performed with the distance matrix, maximum parsimony, and maximum likelihood methods as implemented in the Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (MEGA4) program.[14] Tree topologies were tested by bootstrap analysis on 1000 replicates.

3. Results

A total of 50 isolates (25.4%) were identified as *A. baumannii* using conventional phenotypic methods with subsequent confirmation by *rpoB* sequencing. These 50 isolates were recovered from 20 male adults (40%), 14 female adults (28%), and 16 infants (32%). RFLP analysis demonstrated five different restriction enzyme patterns, designated as A–E clusters (designated as “isolate sequence type or seqtype”). These clusters had 98.2–100% similarity with the *A. baumannii* type/reference strain in GenBank (CIP70.34). A majority of *A. baumannii* isolates were categorized as Cluster A (32%; Table 2). Twenty-eight isolates (56%) were recovered from patients with pulmonary disease (Table 1). About half of the isolates that originated from pulmonary diseases were classified under Clusters A and B (53.57%), and all the isolates from meningitis cases, though the number was low, were classified under Clusters C and D. However, significant association between *A. baumannii* seqtype and age and sex of patients, pulmonary disease, and other clinical presentations was not seen.

A dendrogram based on maximum parsimony analysis reflecting the *rpoB* sequence-based clustering of all test strains of *A. baumannii* is shown in Fig. 1. Within the consensus tree, five clusters with distinct branches among the *A. baumannii* reference strains could be defined. The branches were supported with the highest bootstrap value (100%).

4. Discussion

In recent times, *A. baumannii* has emerged as a main opportunistic pathogen, and there is a high incidence of morbidity and mortality related to *A. baumannii* infection among immunocompromised hospitalized patients.[15] This organism is known for its involvement in hospital outbreaks and has sometimes caused interinstitutional spread.[5] Several studies have demonstrated the usefulness of *rpoB* gene sequencing for the identification and taxonomic classification of various bacterial species.[16,17] This study showed that the