Appropriate Restriction Enzymes for Pulsed-Field Gel Electrophoresis Analysis of Acinetobacter baumannii

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Background: Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory subtyping method for bacterial species. To increase discriminatory index of this method, six restriction enzymes with ApaI, SmaI, NotI, SgrA I, and AscI were evaluated. Thirty-three A. baumannii isolates were obtained at Buddhist Dalin Tzu Chi General Hospital from January to October 2006 and their identities confirmed using the VITEK® 2 system. The results of PFGE analysis showed that NotI, SfiI, SgrA I, and AscI exhibited high discriminatory index (D ≥ 0.99). The AscI generated the optimal visualization of genomic fragments and PFGE analysis with AscI also exhibited the one of highest discriminatory index. The discriminatory index of PFGE analysis with ApaI and SmaI were similar. The results reveal that the discriminatory index of AscI is superior to ApaI and SmaI. Although all enzymes produced satisfactory results, we also suggest AscI may be used in a PFGE protocol for distinguishing A. baumannii isolates.

Key words: Acinetobacter baumannii, pulsed-field gel electrophoresis, discriminatory index, restriction enzyme

Introduction

A variety of techniques are used for epidemiological typing of A. baumannii, including biotyping, antibiograms, serotyping, phage typing, bacteriocin typing, protein profiles, multilocus enzyme electrophoretic typing, plasmid profiles, and analysis by PFGE, ribotyping, and PCR-based methods. However, PFGE seems to provide highly discriminatory results and extremely useful epidemiological information [1]. To determine which restriction enzymes are most efficient, a number of factors including typability, reproducibility, and discrimination should be assessed. Typability and reproducibility are relatively easy to quantify and are often expressed as simple percentages. Thus, the typability of a method is the percentage of distinct bacterial strains which can be assigned a typing marker, and the reproducibility is the percentage of strains that give the same result on repeated testing [2].

Analysis by pulsed-field gel electrophoresis of restriction fragment length polymorphisms generated from intact chromosomal DNA has been used to compare fingerprints obtained from Acinetobacter strains following restriction with ApaI [3, 4, 5], SmaI [6], ApaI and SmaI [7], SfiI and SmaI [8] and NotI [9]. These studies have indicated considerable DNA polymorphism in the clinically important genomic species.

To improve the epidemiological survey and management of A. baumannii infection, it is important to distinguish various A. baumannii strains. Pulsed-field gel