Journal of Microbiology, Immunology and Infection (2013) 46, 195—201

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Suitable restriction enzyme for standardization of pulsed-field gel electrophoresis protocol and interlaboratory comparison of *Acinetobacter baumannii*

Kai-Ming Chang [a,b], Wei-Chang Huang [a,c], Chien-Shun Chiou [d], Gwan-Han Shen [c,e], Chen-Cheng Huang [f], Fu-Shyan Wen [a,*]

[a] *Department of Life Science, National Chung-Hsing University, Taichung, Taiwan, ROC*
[b] *Di Yi Hospital, Taichung, Taiwan, ROC*
[c] *Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, ROC*
[d] *Central Region Laboratory, Center of Research and Diagnostics, Centers for Disease Control, Taichung, Taiwan, ROC*
[e] *Institute of Respiratory Therapy, China Medical University, Taichung, Taiwan, ROC*
[f] *Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, Taichung Hospital, Executive Yuan Department of Health, Taiwan, ROC*

Received 14 March 2012; received in revised form 13 April 2012; accepted 24 May 2012

KEYWORDS
Acinetobacter baumannii;
AscI;
AsiSI;
Pulsed-field gel electrophoresis;
Restriction enzyme

Background/purpose: Interlaboratory comparison of pulsed-field gel electrophoresis (PFGE) patterns is difficult. A key reference of standardized PFGE protocol for *Acinetobacter baumannii* may address this issue. This study aimed to determine restriction enzymes with rare cutting sites on *A baumannii* genomes and evaluate their cost-effectiveness, discriminatory power, and interlaboratory consistence of band assignments.
Methods: There were 42 *A baumannii* isolates collected, including nine from three hospital outbreaks and 33 sporadic isolates. The numbers of cutting sites for the restriction enzymes were explored using the "Restriction Digest and PFGE" program. The cost-effectiveness for PFGE analysis was evaluated for the tested restriction enzymes, while its discriminatory ability was expressed through a discriminatory index and 95% confidence interval. The interlaboratory consistence of band assignments was evaluated for the 42 *A baumannii* isolates.
Results: ApaI was the most cost-effective restriction enzyme for a PFGE protocol for *A baumannii*. Both AscI and AsiSI were reasonable in terms of costs. ApaI, AscI, and AsiSI exhibited

* Corresponding author. Department of Life Sciences, National Chung-Hsing University, 250 Kuo Kuang Road, Taichung 402, Taiwan, ROC.
E-mail address: opencm@gmail.com (F.-S. Wen).

http://dx.doi.org/10.1016/j.jmii.2012.05.011

similar discriminatory indices. ApaI generated more than 40 fragments that were close and not easy to resolve, resulting in less consistence of band assignments. AscI and AsiSI generated 10—20 fragments that were clearly resolved, resulting in higher consistence of band assignments. AscI exhibited a close discriminatory power to that of AsiSI and at half of the cost of AsiSI for PFGE analysis.

Conclusion: We recommend AscI as the primary enzyme and AsiSI as the secondary enzyme for standardizing the PFGE protocol and interlaboratory comparisons of *A baumannii*.

Introduction

Acinetobacter baumannii is one of the most common pathogens of nosocomial infection in recent decades. Increasing trends in multiple drug resistance and its rapid spread in hospitals have caused a significant impact on the treatment of *A baumannii* nosocomial infections.[1] Transmission of multiple drug resistant *A baumannii* strains within and between hospitals has recently become a heavy burden to those in charge of critical care and infection control.[2]

Molecular typing is an important tool in the epidemiologic investigation of *A baumannii* infections. Several molecular typing methods, including ribotyping, randomly amplified polymorphic DNA (RAPD) analysis, amplified fragment length polymorphism (AFLP), and pulsed-field gel electrophoresis (PFGE) analysis, have been developed for subtyping bacterial isolates.[3—5] Among these, PFGE has been proven to be a powerful subtyping tool for discriminating bacterial isolates for epidemiologic investigations.

To ensure that PFGE patterns generated from different laboratories are comparable, a standardized PFGE protocol must be cost-effective, with convenient numbers of fragments, high discriminatory power and clear resolving bands in the electrophoresis gel. However, a standardized PFGE protocol for typing *A baumannii* has been rarely discussed before 2005, when Seifert and colleagues[6] proposed the standardized PFGE protocol with ApaI as the restriction enzyme.

The choice of restriction enzymes is key to standardizing a PFGE protocol. In the past, restriction enzymes for PFGE analysis of a bacterial species were determined in a trial-and-error manner. More recently, genomic sequences of many bacterial species have been determined and are freely accessible via the Internet. Restriction enzymes with rare-cutting sites in a genome can be explored in silicon and the most cost-effective enzyme for PFGE analysis of an organism can be determined after evaluation.[7—9] Based on such concept, more suitable restriction enzymes for PFGE analysis of *Vibrio parahaemolyticus* and *Bordetella pertussis* have been proposed.[8,9]

The aim of the present study was to determine restriction enzymes with rare cutting sites on *A baumannii* genomes and evaluate their cost-effectiveness, discriminatory power, and interlaboratory consistence of band assignments for 42 clinical *A baumannii* isolates. The results could be used as key reference for standardization of a PFGE protocol for *A baumannii* and facilitate interlaboratory comparison.

Materials and methods

Setting and bacterial strains

This study was conducted at Taichung Veterans General Hospital (TCVGH), a 1500-bed tertiary care center in Taichung, central Taiwan. Forty-two clinical isolates of *A baumannii* with species-level identification by the sequence analysis of 16S-23S rRNA gene spacer regions were collected from August 2007 to February 2008, including three isolates (AB315, AB317, and AB327), two isolates (AB209 and AB338) and four isolates (AB320, AB325, AB326, and AB330) from three well-characterized hospital outbreaks (i.e., C1, C2, and C3, respectively) and 33 sporadic strains (Table 1).[10] *Salmonella enterica* subsp. *enterica* serovar *braenderup* H9812 obtained from the Central Region Laboratory, Center of Research and Diagnostics, Centers for Disease Control, Taichung, Taiwan was used as a molecular size marker.[11]

Online search of rare-cutting restriction enzymes for PFGE analysis

The number of cutting sites for restriction enzymes on the genomes of *A baumannii* strains AB0057 (GenBank accession no. CP001182.1), AB307-0294 (accession no. CP001172.1), ACICU (accession no. CP000863.1), ATCC 17978 (accession no. CP000521.1), AYE (accession no. CU459141.1), and SDF (accession no. CU468230.2) were explored using the "Restriction Digest and PFGE" program provided by the website http://insilico.ehu.es/.[12] The cost-effectiveness for PFGE analysis was evaluated for restriction enzymes with cutting sites ranging from 10 to 50 in any of the six genomes.

Preparation of chromosomal DNA

PFGE analysis of the restricted genomic DNA fragments of different *A baumannii* isolates was according to previously described methods, with certain modifications.[9,13] The bacterial isolates were grown on TSA plate (17 g pancreatic digest of casein, 3 g enzymatic digest of soybean meal, 5 g sodium chloride, 2.5 g dextrose, 15 g agar/l) at 37°C for 16—18 hours. An appropriate amount of bacterial cells were then transferred with cotton swab to test tubes (13 × 100 mm) containing cell suspension buffer (100 mM Tris-HCl, pH 8.0; 100 mM EDTA, pH 8.0). The cell concentration was adjusted to $OD_{600nm} = 0.7$.

Table 1 Characteristics of nine *Acinetobacter baumannii* isolates from three outbreaks and 33 sporadic isolates in Taichung Veterans General Hospital

Strain[a]	Nomenclature[b]	Origin	Date of Collection (mo/d/y)	Outbreak[c]
AB206	TW-ABAA16-0008	Sputum	08/13/2007	−
AB207	TW-ABAA16-0011	Sputum	08/14/2007	−
AB208	TW-ABAA16-0025	Pus (pressure sore in the sacral area)	09/05/2007	−
AB209	TW-ABAA16-0023	Sputum	09/13/2007	+(C2)
AB210	TW-ABAA16-0028	CVP tip[d]	09/16/2007	−
AB211	TW-ABAA16-0018	Sputum	09/18/2007	−
AB212	TW-ABAA16-0010	Sputum	09/21/2007	−
AB213	TW-ABAA16-0029	Sputum	09/28/2007	−
AB216	TW-ABAA16-0007	Sputum	09/29/2007	−
AB224	TW-ABAA16-0032	Sputum	10/24/2007	−
AB226	TW-ABAA16-0020	Sputum	02/18/2008	−
AB227	TW-ABAA16-0018	Blood	02/16/2008	−
AB228	TW-ABAA16-0012	Wound(sacral area)	02/22/2008	−
AB229	TW-ABAA16-0026	Sputum	02/23/2008	−
AB230	TW-ABAA16-0033	Sputum	02/23/2008	−
AB231	TW-ABAA16-0003	Sputum	02/26/2008	−
AB232	TW-ABAA16-0031	Sputum	02/29/2008	−
AB301	TW-ABAA16-0013	Sputum	08/17/2007	−
AB302	TW-ABAA16-0001	Sputum	08/20/2007	−
AB303	TW-ABAA16-0004	Sputum	08/21/2007	−
AB304	TW-ABAA16-0014	Sputum	08/22/2007	−
AB305	TW-ABAA16-0020	Urine-catheter	08/23/2007	−
AB307	TW-ABAA16-0016	Wound	08/24/2007	−
AB309	TW-ABAA16-0006	Urine-catheter	08/27/2007	−
AB310	TW-ABAA16-0009	Sputum	08/28/2007	−
AB311	TW-ABAA16-0021	Sputum	08/28/2007	−
AB313	TW-ABAA16-0015	Sputum	09/04/2007	−
AB315	TW-ABAA16-0022	Sputum	09/05/2007	+(C1)
AB317	TW-ABAA16-0022	Sputum	09/10/2007	+(C1)
AB320	TW-ABAA16-0030	Sputum	09/13/2007	+(C3)
AB323	TW-ABAA16-0027	Wound	09/17/2007	−
AB324	TW-ABAA16-0002	Sputum	09/17/2007	−
AB325	TW-ABAA16-0030	Sputum	09/18/2007	+(C3)
AB326	TW-ABAA16-0030	Sputum	09/18/2007	+(C3)
AB327	TW-ABAA16-0022	Sputum	09/19/2007	+(C1)
AB330	TW-ABAA16-0030	CVP tip[b]	09/20/2007	+(C3)
AB331	TW-ABAA16-0017	Sputum	09/21/2007	−
AB335	TW-ABAA16-0019	Sputum	09/26/2007	−
AB338	TW-ABAA16-0023	Sputum	09/23/2007	+(C2)
AB339	TW-ABAA16-0024	Sputum	09/26/2007	−
AB343	TW-ABAA16-0005	Sputum	10/21/2007	−
AB344	TW-ABAA16-0006	Sputum	02/25/2008	−

[a] Original strain designation.
[b] The nomenclature used for PFGE subtyping of *Acinetobacter baumannii* isolates is according to the standardized nomenclature for PFGE patterns in PulseNet Taiwan (http://www.pulsenetinternational.org/networks/Pages/taiwan.aspx).
[c] Outbreak-associated (+) strain (i.e., with same time-space-origin and related PFGE pattern with ApaI as the restriction enzyme) or (−) sporadic strain.
[d] CVP tip, central venous catheter tip.
PFGE = pulsed-field gel electrophoresis.

The 400 μl of cell suspension was mixed with 20 μl of proteinase K (20 mg/ml) and 400 μl of 1% molten agarose (Seakem Gold agarose; Cambrex, East Rutherford, NJ, USA), and allowed to solidify in a plug mold (H14 × W10 × D1 mm). The gel plug was then transferred to a 50 high-Clarity polypropylene conical tube containing 5 ml of cell lysis buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 1% sarcosine) and 25 μl of proteinase K (20 mg/ ml). After incubating in a water bath at 56°C and 150 rpm for 2 hours, the gel plug was washed with 15 ml of ddH_2O twice and with

15 ml of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) for four times, with each wash under the same incubating conditions for 15 minutes. The DNA-containing gel plug was stored in TE buffer at 4°C.

Restriction digestion with rare-cutting endonucleases

The restriction enzymes used were purchased from New England BioLabs, Beverly, MA, USA. The gel block was sliced into sections (W10 × H2 × D1 mm) and the DNA in each slice was digested with individual enzymes under the following conditions: ApaI at 25°C for 2 hours; AscI, AsiSI, and SgrAI at 37°C for 2 hours; and SfiI at 50°C for 2 hours.

Pulsed-field gel electrophoresis

Restricted genomic DNA fragments were separated in 1% Seakem Gold agarose gel in 0.5× TBE buffer (1 M Tris-HCl, pH 8.0; 25 mM EDTA, pH 8.0; 89 mM Boric acid) containing 200 μM thiourea. PFGE was performed with a CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA, USA), at 14°C, 200 V (6 V/cm) with the pulse time of 2.16—54.17 seconds for 19 hours. The gel was stained with EtBr (10 mg/ml) after electrophoresis and photographed using a digital camera (Kodak Electrophoresis Documentation and Analysis System 290, Kodak, Rochester, NY, USA). The band patterns were analyzed by BioNumerics (Applied Maths, Kortrijik, Belgium).

Evaluation of discriminatory ability and interlaboratory consistence of band assignments

The discriminatory ability of the tested restriction enzymes to the 42 clinical *A baumannii* isolates was expressed through a discriminatory index (DI) and 95% confidence interval (CI).[14,15] The interlaboratory consistence of band assignments of the tested restriction enzymes was evaluated with 42 clinical *A baumannii* strains by three different experienced laboratories.

Results

Using the "Restriction Digest and PFGE" program, the computer searches identified nine restriction enzymes (i.e., ApaI, AscI, AsiSI, MreI, PasI, SanDI, SbfI, SfiI, and SgrAI) with 10—50 cutting sites in the six genomes. Four enzymes, MreI, PasI, SanDI and SbfI, which were either too expensive or difficult to access, were excluded for evaluation of cost-effectiveness. The minimal amount (in units) of five enzymes (i.e., ApaI, AscI, AsiSI, SfiI, and SgrAI) required for complete digestion of DNA in a plug slice was determined by testing on four *A baumannii* strains (AB209, AB210, AB211, and AB212). Plug slices from each strain were digested with 2× serial dilution amounts of each enzyme.

With ApaI as the restriction enzyme, only 2.5 U were enough for complete DNA digestion in a plug slice. The minimal amount of AscI and AsiSI were 5 U and 10 U, respectively. SfiI and SgrAI were not efficient, requiring at least 80 U for complete digestion (Fig. 1). The costs of each complete digestion of ApaI, AscI, AsiSI, SfiI, and SgrAI were $0.025, 0.488, 0.976, 1.344, and 3.904, respectively. ApaI was the most cost-effective for PFGE analysis. Both AscI and AsiSI were reasonable in terms of costs. SfiI and SgrAI were much more expensive and were excluded for evaluation of discriminatory ability and interlaboratory consistence of band assignments (Table 2).

The discriminatory ability of ApaI, AscI, and AsiSI for differentiating *A baumannii* was further evaluated with 42 clinical *A baumannii* isolates and presented by DI and 95% CI.[14,15] Two times the minimal amount for the three enzymes was used for PFGE analysis of the 42 clinical isolates. Among the three enzymes, ApaI generated the highest number of patterns for the 42 clinical isolates. However, its discriminatory power was not significantly higher than those of AscI and AsiSI, which had discriminatory levels close to ApaI (Table 2).

The PFGE with ApaI as the restriction enzyme generated more than 40 fragments, several of which were too close and difficult to resolve. By contrast, PFGE with AscI and AsiSI as the restriction enzymes generated 10—20 fragments that were not crowded and could be resolved clearly (Fig. 2).

Interlaboratory consistence of band assignments of ApaI, AscI, and AsiSI was evaluated with 42 clinical *A baumannii* isolates. With ApaI as the restriction enzyme, only eight isolates (8/42, 19.05%) revealed inter-laboratory consistence of band assignments among three different experienced laboratories. With AscI and AsiSI as the restriction enzymes, 26 isolates (26/42, 61.90%) and 22 isolates (22/42, 52.38%) revealed interlaboratory consistence of band assignments, respectively. All three strains (AB315, AB317 and AB327) from C1 outbreak and all four strains

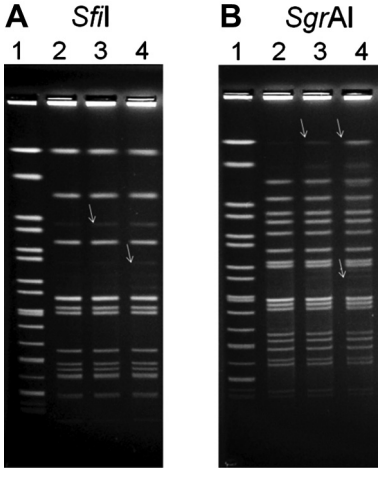

Figure 1. PFGE patterns of *Acinetobacter baumannii* generated with different restriction enzymes. (A) SfiI; (B) SgrAI, and digested with 2× serial dilution amounts of each enzyme. For each gel, Lane 1 was the size marker strain *Salmonella enterica* ser. Braenderup H9812 digested with XbaI. Lanes 2 to 4 were the strain AB212 digested with 80U, 40U, and 20U, respectively. Pulse time was set at 2.2—54.2 seconds for PFGE with SfiI and SgrAI. Arrows indicated incomplete digestions. PFGE = pulsed-field gel electrophoresis.

Table 2 Cost-effectiveness and discriminatory indices of PFGE analysis of five restriction enzymes with 10—50 recognition sites to clinical *Acinetobacter baumannii* strains

Restriction enzymes	Number of cutting sites[a]	Minimal units needed for complete digestion[b]	US$/U[c]	US$/digestion	Number of patterns[d]	DI[e]	95% CI[f]
ApaI	40—49	2.5	0.0101	0.025	35	0.991	0.977 ~ 1.005
AscI	11—14	5	0.0976	0.488	33	0.985	0.969 ~ 1.001
AsiSI	15—17	10	0.0976	0.976	32	0.986	0.970 ~ 1.002
SfiI	13—18	80	0.0168	1.344	ND	ND	ND
SgrAI	16—23	80	0.0488	3.904	ND	ND	ND

[a] Number of cutting sites present in the genomes of *Acinetobacter baumannii* strains AB0057, ABA307-0294, ACICU, ATCC 17978, AYE, and SDF.
[b] minimal amount for complete digestion determined by testing on four *Acinetobacter baumannii* strains AB209, AB210, AB211, and AB212.
[c] Cost was calculated based on the 2011 listed prices for restriction enzymes of the New England Biolabs.
[d] Obtained from PFGE analysis of 42 *Acinetobacter baumannii* isolates with the indicated restriction enzyme.
[e] DI obtained from PFGE analysis of 42 *Acinetobacter baumannii* isolates with the indicated restriction enzyme.
[f] CI obtained from PFGE analysis of 42 *Acinetobacter baumannii* isolates with the indicated restriction enzyme.
CI = confidence interval; DI = discriminatory index; PFGE = pulsed-field gel electrophoresis.

(AB320, AB325, AB326, and AB330) from C3 outbreak exhibited inter-laboratory consistence of band assignments when AscI and AsiSI were used as the restriction enzymes, but none exhibited the consistence when tested with ApaI. Both strains (AB209 and AB338) from C2 outbreak exhibited inter-laboratory consistence of band assignments when AscI was used as the restriction enzyme, but none exhibited the consistence when tested with ApaI and AsiSI (Fig. 3). To summarize, higher interlaboratory consistence of band assignments occurred when AscI and AsiSI were used as the restriction enzymes and less inter-laboratory consistence when tested with ApaI.

Discussion

In this study, ApaI is the most cost-effective restriction enzyme for a PFGE protocol for *A baumannii*, while AscI and AsiSI are also reasonable in terms of cost. These three restriction enzymes have similar discriminatory indices for differentiating *A baumannii*. AscI has a discriminatory power approximate to that of AsiSI but at half the cost of AsiSI for PFGE analysis. ApaI generates more than 40 fragments that are close and not easy to resolve, resulting in less interlaboratory consistence of band assignments. On the other hand, AscI and AsiSI each generates 10—20

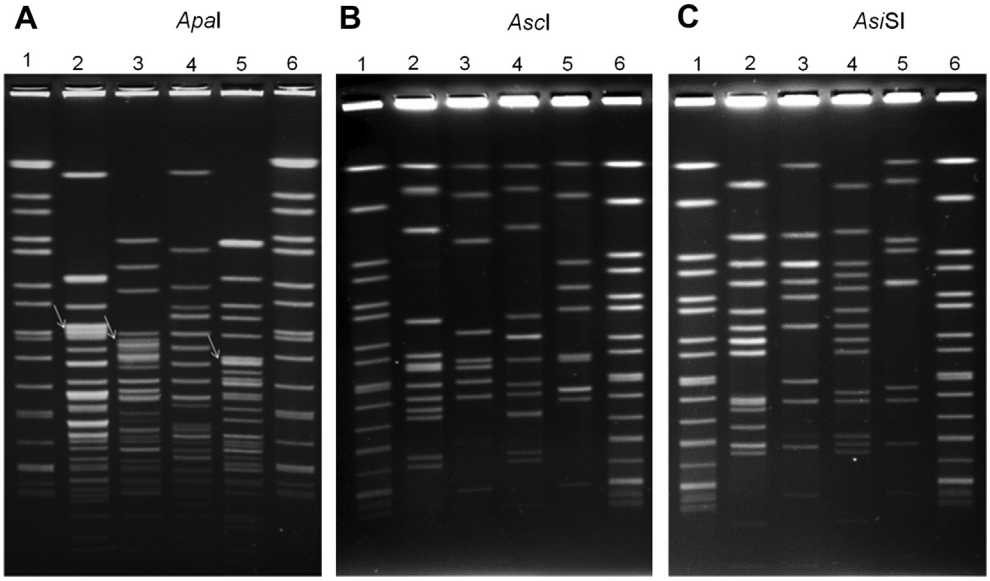

Figure 2. PFGE patterns of *Acinetobacter baumannii* generated with different restriction enzymes. (A) ApaI; (B) AscI; (C) AsiSI. For each gel, Lanes 1 and 6 were the size marker strain *Salmonella enterica* ser. Braenderup H9812 digested with XbaI. Lanes 2 to 5 were the strains AB209, AB210, AB211, and AB212, respectively. Pulse time was set at 2.2—35 seconds for PFGE with ApaI and 2.16—54.17 seconds for PFGE with AscI and AsiSI. Arrows indicated some crowded fragments with ApaI as the restriction enzyme. PFGE = pulsed-field gel electrophoresis.

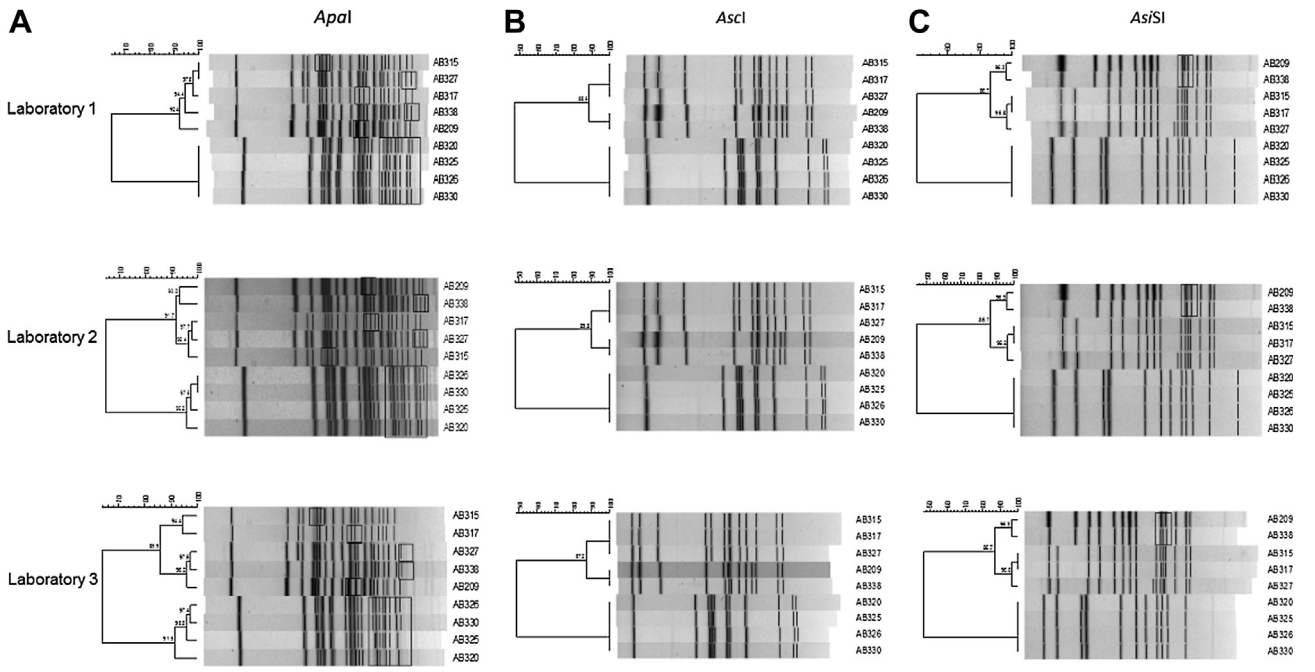

Figure 3. Interlaboratory consistency of band assignments of PFGE-generated fingerprints for nine *Acinetobacter baumannii* strains from three well-characterized hospital outbreaks with (A) *Apa*I; (B) *Asc*I; (C) *Asi*SI as the restriction enzymes by three different experienced laboratories **(1—3)**. Boxes indicated band assignments that were not consistent among laboratories. PFGE = pulsed-field gel electrophoresis.

fragments that are not close and easy to resolve, resulting in higher inter-laboratory consistence of band assignments.

Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex is needed to determine epidemiology during infectious outbreaks, because it obscures possible differences in the biology and pathology of the individual species. Several genotypic methods have been developed for species identification of *Acinetobacter*. Of those, sequence analysis of the16S-23S rRNA gene spacer region is commonly adopted, as in the present study.[10]

The cost-effectiveness and discriminatory ability are important considerations when evaluating the more suitable restriction enzymes for a standardized PFGE protocol. Wang and colleagues[9] suggested using NotI as the first restriction enzyme for standardizing the PFGE protocol of *Vibrio parahaemolyticus* because PFGE with NotI was discriminatory and cost-effective. To date, there has been no published literature evaluating the cost-effectiveness and discriminatory ability of restriction enzymes for standardizing the PFGE protocol of *A baumannii*.

In the present study, ApaI is the most cost-effective restriction enzyme for PFGE analysis ($0.03/digestion). However, AscI and AsiSI are likewise reasonable in terms of costs ($0.49 and 0.98/digestion, respectively). These three restriction enzymes have similar discriminatory indices for differentiating *A baumannii*. As a result, AscI and AsiSI were reasonable alternatives to standardize the PFGE protocol of *A baumannii* in terms of cost-effectiveness and discriminatory ability.

The Band assignment is always subjective and makes interlaboratory comparisons of PFGE patterns problematic. To resolve such problem, optimal restriction enzymes used as key references for standardization of a PFGE protocol for *A baumannii* should provide convenient numbers of fragments and should be easy to resolve clearly in an electrophoretic gel. *Apa*I is currently the most frequently used enzyme for PFGE analysis of *A baumannii*. In this study, *Apa*I generated more than 40 fragments that are so crowded, even overlapping, and not easy to resolve in an electrophoretic gel, resulting in higher percentage of subjective band assignments and less inter-laboratory consistence. Thus, ApaI is not an ideal enzyme to standardize the PFGE protocol of *A baumannii* for interlaboratory comparisons.

SmaI is also used by several researchers for PFGE analysis of *A baumannii*.[16,17] SmaI has 76—101 cutting sites in the six *A baumannii* genomes (data not shown) and is, therefore, not a good choice for PFGE analysis of this organism. By contrast, in the present study, AscI and AsiSI generated convenient numbers of fragments that are resolved clearly in an electrophoretic gel. Most DNA fragments generated by AscI and AsiSI have sizes ranging between 20.5 and 1135 kb (range of size reference markers). Besides, most of the bands can be assigned unambiguously among persons and laboratories. These characteristics result in higher inter-laboratory consistence of band assignments compared to ApaI. AscI also has a high level of discriminatory power approximate to that of AsiSI but at half the cost of AsiSI for PFGE analysis. Thus, this study suggests AscI as the primary enzyme and AsiSI as the secondary enzyme for standardization of the PFGE protocol for *A baumannii* to make interlaboratory comparison possible.

Most recently, two molecular typing methods have been proposed for the epidemiologic investigation of *A baumannii* infections: multilocus sequence typing (MLST) and

multiple locus variable-number tandem repeat analysis (MLVA).[18,19] However, each method has its own shortcomings. Although PFGE is the gold standard of molecular typing for the epidemiologic investigation of *A baumannii* infection, it is laborious, time-consuming, and problematic in terms of inter-laboratory comparisons when ApaI is used as the enzyme for restriction of chromosomal DNA.[20] MLST is relatively expensive and is more suitable for population genetic structure studies than for epidemiologic typing.[18] Although MLVA typing scheme for *A baumannii* has been proven to be fast, portable, and highly discriminatory, its data are presented by PCR amplicon sizes for VNTR loci or by repeat numbers converted from amplicon sizes.[19] The sizes of PCR amplicons determined in different laboratories using the same protocol may have significant discrepancies, making the MLVA data unreliable for comparison among laboratories. In addition, the VNTR loci can be evolved rapidly, which is advantageous for disease outbreak investigation but not for global surveillance of *A baumannii* infections.

PFGE still has its advantages as regards global surveillance and building a database for long-term monitoring of *A baumannii* transmission in hospitals and communities. A comparison of PFGE fingerprints has been proven reliable among PulseNet laboratories, which use standardized PFGE protocols for subtyping bacterial isolates for food-borne disease surveillance.[21] When using AscI as the primary enzyme and AsiSI as the secondary enzyme instead of ApaI for standardization of the PFGE protocol for *A baumannii*, interlaboratory comparisons become possible. This facilitates early identification of common source outbreaks and global surveillance of *A baumannii* infections.

In conclusion, we recommend AscI as the primary enzyme and AsiSI as the secondary enzyme for standardization of the PFGE protocol and interlaboratory comparison for *A baumannii*.

References

1. Yum JH, Yi K, Lee H, Yong D, Lee K, Kim JM, et al. Molecular characterization of metallo-beta-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter genomospecies* 3 from Korea: identification of two new integrons carrying the bla(VIM-2) gene cassettes. *J Antimicrob Chemother* 2002;**49**: 837—40.
2. Nemec A, Krizova L, Maixnerova M, Diancourt L, van der Reijden TJ, Brisse S, et al. Emergence of carbapenem-resistance in *Acinetobacter baumannii* in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. *J Antimicrob Chemother* 2008;**62**:484—9.
3. Seifert H, Schulze A, Baginski R, Pulverer G. Comparison of four different methods for epidemiologic typing of *Acinetobacter baumannii*. *J Clin Microbiol* 1994;**32**:1816—9.
4. Gonçalves CR, Vaz TM, Araujo E, Boni R, Leite D, Irino K. Biotyping, serotyping and ribotyping as epidemiological tools in the evaluation of *Acinetobacter baumannii* dissemination in hospital units, Sorocaba, Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 2000;**42**:277—82.
5. Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, et al. Multi-center study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of Acinetobacter spp. *J Clin Microbiol* 1997;**35**:3071—7.
6. Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, et al. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005;**43**:4328—35.
7. Chiou CS, Liao TL, Wang TH, Chang HL, Liao JC, Li CC. Epidemiology and molecular characterization of *Streptococcus pyogenes* recovered from scarlet fever patients in central Taiwan from 1996 to 1999. *J Clin Microbiol* 2004;**42**:3998—4006.
8. Lee MS, Lee YS, Chiou CS. The suitable restriction enzymes for pulsed-field gel electrophoresis analysis of *Bordetella pertussis*. *Diagn Microbiol Infect Dis* 2006;**56**:217—9.
9. Wang YW, Chern LL, Cam PD, Chiou CS. Evaluation of restriction enzymes for standardizing pulsed-field gel electrophoresis protocol for rapid sub-typing of *Vibrio parahaemolyticus*. *Diagn Microbiol Infect Dis* 2008;**61**:251—5.
10. Chang HC, Wei YF, Dijkshoorn L, Vaneechoutte M, Tang CT, Chang TC. Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. *J Clin Microbiol* 2005;**43**:1632—9.
11. Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, Graves L, et al. Establishment of a universal size standard strain for use with the pulsenet standardized pulsed-field gel electrophoresis protocols: converting the National databases to the new size standard. *J Clin Microbiol* 2005;**43**: 1045—50.
12. Bikandi J, Millan RS, Rementeria A, Garaizar J. In silico analysis of complete bacterial genomes: PCR, AFLP-PCR and endonuclease restriction. *Bioinformatics* 2004;**20**:798—9.
13. Matushek M, Bonten M, Hayden M. Rapid preparation of bacterial DNA for pulsed-field gel electrophoresis. *J Clin Microbiol* 1996;**34**:2598—600.
14. Hunter PR. Reproducibility and indices of discriminatory power of microbial typing methods. *J Clin Microbiol* 1990;**28**: 1903—5.
15. Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol* 2001;**39**:4190—2.
16. Durmaz R, Otlu B, Koksal F, Hosoglu S, Ozturk R, Ersoy Y, et al. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, *Escherichia coli* and Klebsiella spp. *Jpn J Infect Dis* 2009;**62**: 372—7.
17. Gouby A, Carles-Nurit MJ, Bouziges N, Bourg G, Mesnard R, Bouvet PJ. Use of pulsed-field gel electrophoresis for investigation of hospital outbreaks of *Acinetobacter baumannii*. *J Clin Microbiol* 1992;**30**:1588—91.
18. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multi-resistant clones from an ancestral susceptible genetic pool. *PLoS ONE* 2010;**5**. e10034.
19. Pourcel C, Minandri F, Hauck Y, D'Arezzo S, Imperi F, Vergnaud G, et al. Identification of variable-number t*andem-repeat (VNTR) sequ*ences in *Acinetobacter baumannii* and inter-laboratory validation of an optimized multiple-lo*cus VNTR analysi*s typing scheme. *J Clin Microbiol* 2011;**49**: 539—48.
20. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; **21**:538—82.
21. Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, et al. Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to food-borne disease outbreaks and emerging food-borne diseases. *Food-borne Pathog Dis* 2006;**3**:36—50.