KBM Journal of Biology (2010) 1: 10-17
doi: 10.5147/kbmjb.2010.0003

# Molecular Epidemiology of Nosocomial Infection: Analysis of chromosomal Restriction Fragment Patterns by Pulsed-Field Gel Electrophoresis

Afaf I Shehata[1*], Alwaleed A. Al-Aidan[2], and Buthainah Al-Shahrani[1]

[1] Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [2] Molecular Virology & Infectious Disease Laboratory, Research Centre, King Faisal Specialist Hospital and Research Centre, Saudi Arabia

Received: March 4, 2010 / Accepted: April 9, 2010

## Abstract

**Acinetobacter baumannii is a species of non-fermentative gram-negative coccobacilli commonly found in soil, water and other environmental samples. This bacterium is defined as being strict aerobes, nonmotile, catalase-positive and oxidase-negative. This organism was susceptible to most antibiotics in the 1970s. A. baumannii is an opportunistic pathogen that may be an important threat due to its increasing multidrug resistance and is involved in nosocomial infections that are often severe. The objective of this study was undertaken to elucidate the molecular epidemiology of A. baumannii using the most widely applicable DNA – based typing methods namely Pulsed-Field Gel Electrophoresis (PFGE). These strains comprised isolates from environmental samples, blood, wound, urine, cerebrospinal fluid and tracheal aspirates. PFGE analysis of 81 clinical isolates has been carried out by using CHEF–DR III systems from Bio – Rad and following the protocol of Gautom with some modifications. A 2.00% band tolerance and an optimization of 4.00% were selected for use during comparisons of generated fingerprints or pulsotypes after digestion with Apa I restriction enzyme. Similarity values have been generated using BioNumerics software, cluster analysis was performed by the unweighted pair – group method using arithmetic averages and DNA relatedness was calculated based on Dice coefficient. An interlinkage homology level of 80% between patterns was assumed as the cutoff for defining a close genetic relationship between strains and was used to define the cluster. As per the generated dendogram, isolates were categorized into 18 major groups designated as Strain I to Strain xvIII. Overall, PFGE was able to discriminate the 81 different Acinetobacter baumannii isolates with similarity levels of 63.63%.**

* Corresponding author: a.shib@hotmail.com

## Introduction

Bacteria of the genus Acinetobacter are increasingly being implicated in numerous outbreaks and have become a growing concern in hospitals, identifying A. baumannii as the most predominant species involved. Acinetobacter spp. can cause a wide range of clinical conditions, including pneumonia, septicemia, urinary tract infections, wound infections, endocarditis, and meningitis (Bergogne–Berezin and Towner, 1996; Mandell, 2000). Contaminated hospital equipment or colonized hands of hospital staff have previously been identified as reservoirs of this organism in epidemics (Aygun et al., 2002). Resistance to multiple antibiotics is a frequent finding with this organism (Van Looveren et al., 2004). Risk factors for acquisition of this organism include prolonged hospital stay, serious underlying disease, intravascular and intravesical catherization, and treatment with broad-spectrum antibiotics (Danes et al., 2002). Characteristics of Acinetobacter spp. may contribute to their epidemic behavior, such as the ability to acquire multiple antibiotic resistance and the ability to survive on inanimate and dry surfaces for prolonged periods of time (Dijkshoorn et al., 1987, 1996). However, it should be noted that Acinetobacters are ubiquitous organisms that can also be isolated readily from non clinical sources such as soil, drinking and surface waters, sewage, and a variety of different foodstuffs (Hanlon, 2005). In many cases, the true source of infection cannot be traced, because members of the genus Acinetobacter are widespread in the hospital environment and can be isolated from sinks, tap water, and dust or can be present as commensal organisms of human skin and respiratory tract (Gorbach et al., 1998). In order to understand the epidemiology of Acinetobacter spp., in hospitalized patients and in the hospital environment, it is therefore vital that the organism be identified to the genomic species level and then typed before epidemiological conclusions are drawn (Zarrilli et al., 2004) . The objective of this study was undertaken to elucidate the molecular epidemiology of A. baumannii using the most widely applicable DNA – based typing methods namely Pulsed Field

Gel Electrophoresis (PFGE). Eighty – one clinical A. baumannii isolates were included in this study collected from year 2001 to 2006. The strains were originally isolated from different clinical and environmental specimens by the Microbiology Laboratory of the King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Analysis by PFGE of restriction fragment length polymorphisms generated from intact chromosomal DNA has been used to compare fingerprints obtained from Acinetobacter strains following restriction with ApaI, SmaI, and NheI (Luey et al., 2007). These studies have indicated considerable DNA polymorphism in the clinically important genomic species 2 (A. baumannii), even within biotypes, and good correlation between strains from within defined outbreaks or multiple isolates from single patients. Equipment for PFGE is costly, while the preparation of intact chromosomal DNA and subsequent digestion and electrophoresis require several days. Nevertheless, PFGE seems to provide highly discriminatory results and extremely useful epidemiological information (Bergogne-Berezin and Towner, 1996).

## Material and Methods

### Sample collection

A total of 81 Acinetobacter isolates were investigated in this study. Bacterial strains tested were obtained from the Microbiology Laboratory of the King Faisal Specialist Hospital and Research Centre (Riyadh, Saudi Arabia), collected from year 2001 till 2006. The strains were originally isolated from different clinical and environmental specimens, e.g., blood, cerebrospinal fluid, sputum, tracheal aspirate, urine and wound. They were preserved in tryptic soy broth (TSB) supplemented with 20% (v/v) glycerol. Pure isolates were stored at -80$^0$C until used in the Research Centre, KFSH&RC.

### Confirmation of Bacterial Identification

Samples were already identified as Acinetobacter sp. in Microbiology Laboratory, Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research Centre. Frozen bacterial suspension was streaked in Tryptic Soy agar (TSA) and incubated overnight at 37$^0$C (Jawad et al., 1994 Acinetobacter baumannii grows at 440C after consecutive incubation. For Gram's staining, reagents and gram stain kit were used from BD were used (Cat. No. 8820191; Becton, Dickinson and Company, MD, USA).

### DNA Extraction

Two methods have been carried out to check which will give a better amplifiable DNA. First, using the classical Proteinase K treatment and the second method using a commercial kit, GenomicPrep Cells and Tissue DNA Isolation Kit (Catalog No. 27 – 5237 – 01; Amersham Biosciences, USA).

### DNA Extraction using Genomic Prep Cells and Tissue DNA Isolation Kit

The inoculated TSB was placed overnight at 370C shaker incubator. Centrifugation was performed for 5 minutes at 13,000 rpm at room temperature. The supernatant was decanted and the pellet was collected; and extracted using the commercially available GenomicPrep Cells and Tissue DNA Isolation Kit (Amersham Biosciences, USA; Catalog No. 27 – 5237 – 01).

For RNase treatment, 3 ul of RNase A solution (4 mg/ml) was added to the cell lysate. Mix the sample by inverting the tube 25 times and incubate at 370C for 15 – 60 minutes.

For protein precipitation, samples should be allowed to cool at room temperature. Protein precipitation solution was added to the RNase A-treated cell lysate and DNA was precipitated using 70% ethanol.

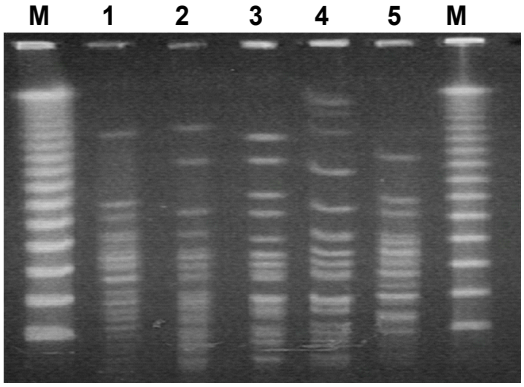


**Figure 1.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 1 to 5 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

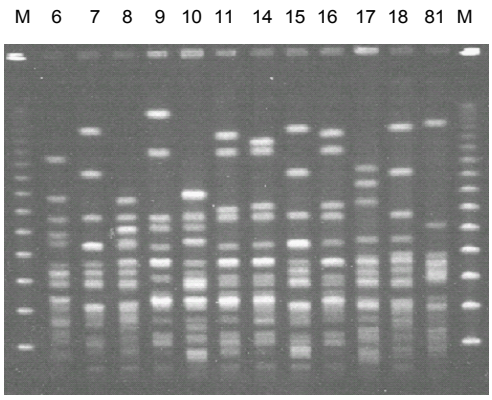


**Figure 2.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 6 to 11, 14 to 18 & 81 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

### Pulsed–Field Gel Electrophoresis (PFGE)

Bacterial extraction and purification were carried out as reported previously with some modification (Gautom, 1997). Bacterial isolates on TSA plate were incubated overnight at 37°C. A single bacterial colony is grown overnight in 3 ml tryptic soy broth at 37°C in a shaker incubator. Cells are harvested by centrifugation at 3000 ug for 10 minutes and the supernatant are then discarded. Resuspend cells in SE Buffer (25 mM EDTA [pH 8.0], 75 mM NaCl [pH 8.0]). Adjust the optical density of the cells at wavelength of 610 nm to 1.40 with SE Buffer. Mix 0.5 ml aliquot of the bacterial suspension with 0.5 ml of 2% low melting point (LMP) agarose in TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA). Pipette mixture into reusable (300 µl) large size

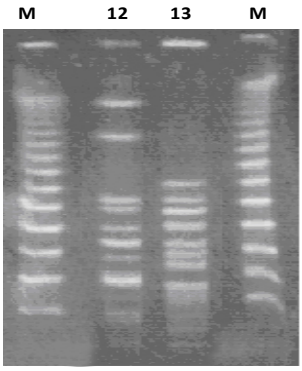


**Figure 3.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 12 & 13 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

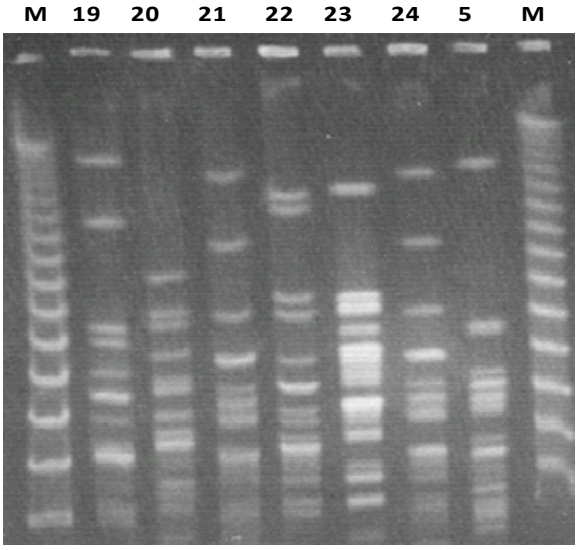


**Figure 4.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 19 to 25 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

plug mold and allow to solidify at 4°C for 30 minutes. Release the plugs into 15ml tubes containing 1 ml of Lysis Buffer (50 mM Tris – HCl [pH 8.0], 50 mM EDTA [pH 8.0], 1% sodium lauroyl sarcosine). Proteinase K (1 mg/ml) is added fresh on the day of the experiment. Incubate plugs overnight at 55°C in a waterbath. Replace the Lysis Buffer with 5 ml of sterile distilled water and incubate at room temperature for 5 minutes. Replace with 3 ml of TE [10 mM Tris – HCl [pH 8.0], 1 mM EDTA [pH 8.0]) for 5 minutes at room temperature. Final sets of four washes, 30 minutes each, are done with 3 ml of TE (10 mM Tris - HCl [pH 8.0], 1 mM EDTA [pH 8.0]) at room temperature. Place a plug slice of 3 X 5 mm wide in a 200 µl of 1X restriction buffer 4 and incubate for 30 minutes at 4°C with gentle agitation. Place the plugs in 200 µl of 1X restriction buffer 4 containing 50 Units of Apa I restriction enzyme and incubate the mixture overnight at room temperature. Wash plugs with 0.5 ml of 0.5X TBE for 37°C for 30 minutes. Insert the plugs into the wells of 1% agarose gel dissolved in 0.5X TBE. Overlay all the wells with 1% LMP agarose dissolved in 0.5X TBE and allow to solidify at 4OC for 30 minutes. 0.5X TBE running buffer was allowed to re – circulate on CHED – DR III (Bio – Rad) at least 2 hours before running the gel to maintain a temperature of 14°C. DNA restriction fragments were separated for 22 hours at 200 V, with pulse times ranging from 2.2 to 54.2 seconds at 120. Gel is then soaked in 300 ml of deionized water containing 1 µg/ml of ethidium bromide for 30 minutes, and visualized the bands using an ultraviolet illuminator.

## Results

### PFGE Analysis

PFGE analysis of 81 clinical isolates has been carried out by using CHEF – DR III systems from Bio–Rad and following the protocol of Gautom with some modifications. Figure1 to 10 show fingerprints or pulsotypes using Apa I restriction enzyme. PFGE patterns were analyzed by both computer – assisted program (BioNumerics software) and by manual or visual comparison of each banding patterns.

Figure 11 shows dendogram based on computer – assisted comparison of PFGE profiles of 81 Acinetobacter baumannii isolates using BioNumerics software. A 2.00% band tolerance and an optimization of 4.00% were selected for use during comparisons of DNA profiles. Table 2 shows generated similarity values using BioNumerics software, cluster analysis was performed by the unweighted pair – group method using arithmetic averages and DNA relatedness was calculated based on Dice coefficient. An interlinkage homology level of 80% between patterns was assumed as the cutoff for defining a close genetic relationship between strains and was used to define the cluster. As per the generated dendogram, isolates were categorized into 18 major groups designated as Strain I to Strain XVIII. Overall, PFGE was able to discriminate the 81 different Acinetobacter baumannii isolates with similarity levels of 63.63%. A total of 4 isolates were categorized into strain I: strain I-1, I-2, I-3 and I-4 represented by isolates number 10, 54, 56 and 37, respectively. Strains II-1, II-2 and II-3 were represented by isolates number 6, 8 and 3, respectively. Strain III-1, III-2 and III-3 represented by sample number 50, 53 and 27, respectively. Strain III-4 was

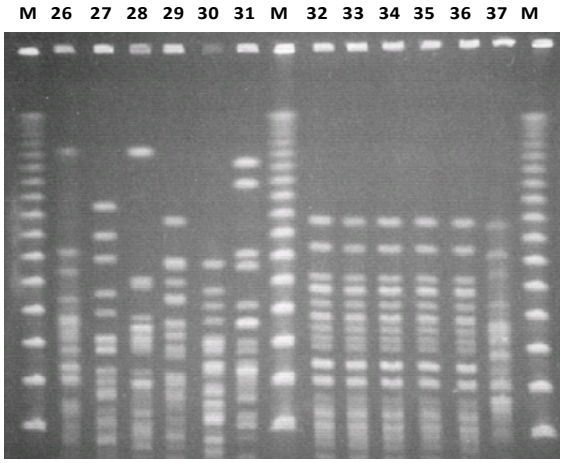


**Figure 5.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 26 to 37 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

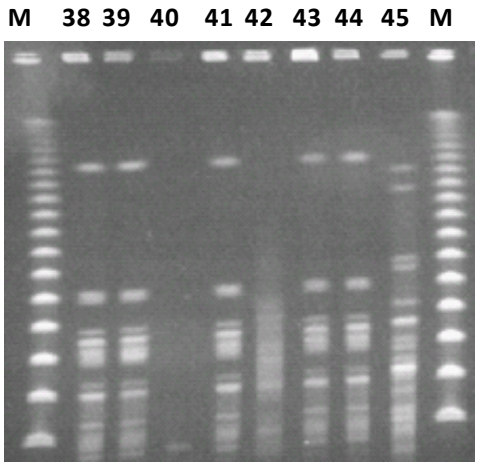


**Figure 6.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 38 to 45 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

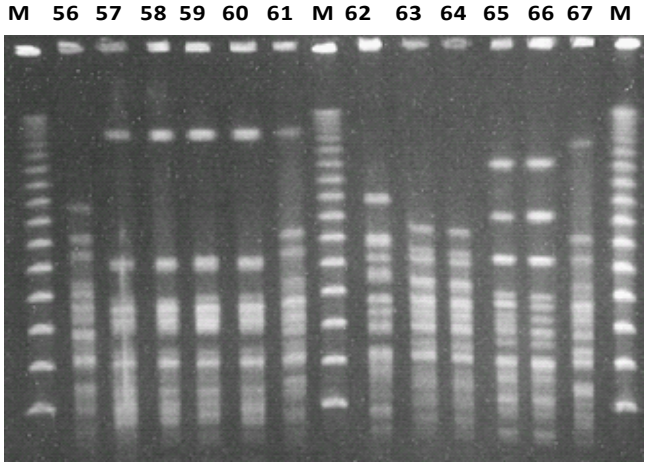


**Figure 8.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 56 to 67 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

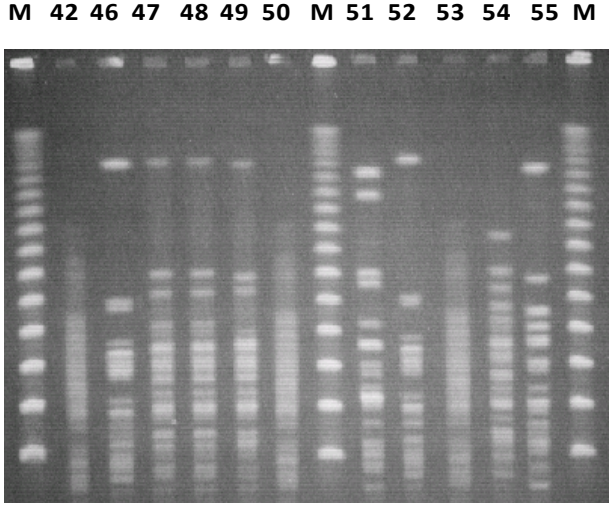


**Figure 7.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 42, 46 to 55 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

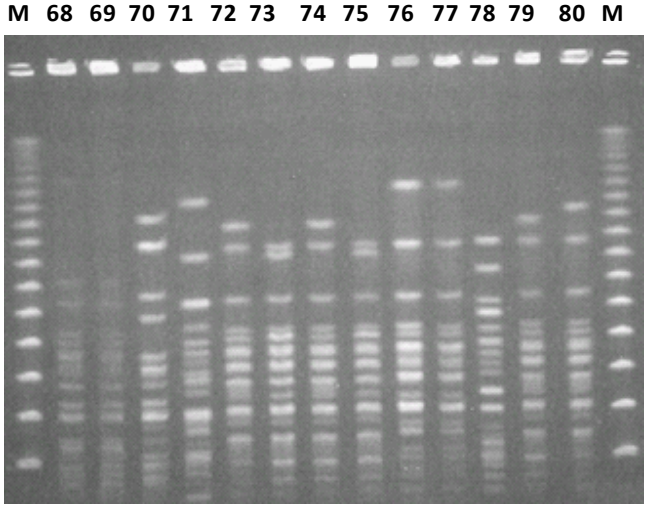


**Figure 9.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 68 to 80 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

comprised of 5 isolates (32, 33, 34, 35 & 36) that were indistinguishable from each other. Strain III-5 is represented by sample number 78. Strains IV-1, IV-2 and IV-3 were represented by sample numbers 63, 64 and 13, respectively. Strain V is represented by sample number 20. Strain VI, which is considered one of the biggest cluster in this analysis was comprised of 13 isolates (sample number 76, 77, 65, 66, 71, 73, 75, 72, 74, 79, 80, 82 and 83). Strain VI-1 is represented by sample number 76. Strain VI-2 is represented by sample number 77. Strain VI-3 is represented by samples number 65 and 66 that are indistinguishable from each other. Strain VI-4 is represented by sample number 71. Strain VI-5 is represented by samples number 73 and 75 (indistinguishable). Strain VI-6 represented by samples number 72, 74 and 79 (indistinguishable). Strain VI-7 represented by sample number 80. Strain VI-8 represented by samples number 82 and 83 (indistinguishable). Strain VII represented by sample number 5. Strain VIII-1 to VIII-3 represented by sample number 42, 68 and 69, respectively. Strain IX-1 is represented by samples number 47, 48 and 49 (indistinguishable). Strain IX-2 represented by samples number 26 and 27 (indistinguishable). Strain IX-3, IX-4 and IX-5 represented by sample number 61, 30 and 55, respectively. X-1 and X-2 represented by sample number 70 and 84, respectively. Strain XI is represented by sample number 17. XII-1 and XII-2 are represented by sample number 1 and 2, respectively. Strain XIII-1 is represented by sample number 15 and 18 (indistinguishable). XIII-2 is represented by sample number 7. Strain XIII-3 is represented by sample number 21 and 24 (indistinguishable). Strains XIII-4 and XIII-5 were represented by samples number 29 and 62, respectively. Strain XIV represented by sample number 4. Strain XV, together with Strain VI, which is also considered one of the biggest clusters in this analysis consists of 13 isolates (sample number 25, 28, 38, 39, 41, 44, 46, 52, 81, 58, 59, 60 and 57). Strains XV-1 and XV-2 were represented by samples number 25

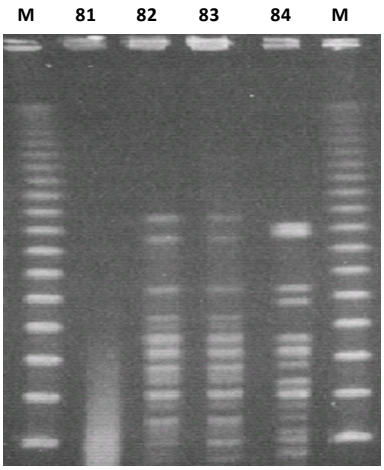


**Figure 10.** Patterns obtained by PFGE for Acinetobacter baumannii for samples 81 to 84 after running in 1% agarose gel, staining with ethidium bromide and illuminated under UV light. 50 – 1000 kb lambda ladder was used as a standard size marker.

and 28, respectively. Strain XV-3 was represented by sample numbers 38, 39, 41, 44 and 46, which are indistinguishable. Strains XV-4 and XV-5 were represented by sample number 52 and 81, respectively. XV-6 is represented by samples number 58, 59, 60 and 57 (indistinguishable). Strains XVI-1 and XVI-2 are represented by samples number 31 and 51, respectively. XVI-3 represented by samples number 11 and 14 (indistinguishable). Strains XVI-4 and XVI-5 represented by samples number 16 and 45, respectively. Strain XVII is represented by sample number 22. Strain XVIII-1 is represented by samples number 12 and 19 (indistinguishable). Strain XVIII-2 is represented by sample number 9.

For visual analysis, the Tenover classification (Tenover et al. 1995) was used to interpret the banding patterns generated. Results were summarized in table 10, which generates 25 strains. Wherein Strain A and Strain B were considered the biggest in the group comprising of 13 isolates per group. Strain A were divided into 7 (seven) subgroups, A1 to A7. Strain A1 was represented by sample numbers 38, 39, 41, 44 and 46. Strain

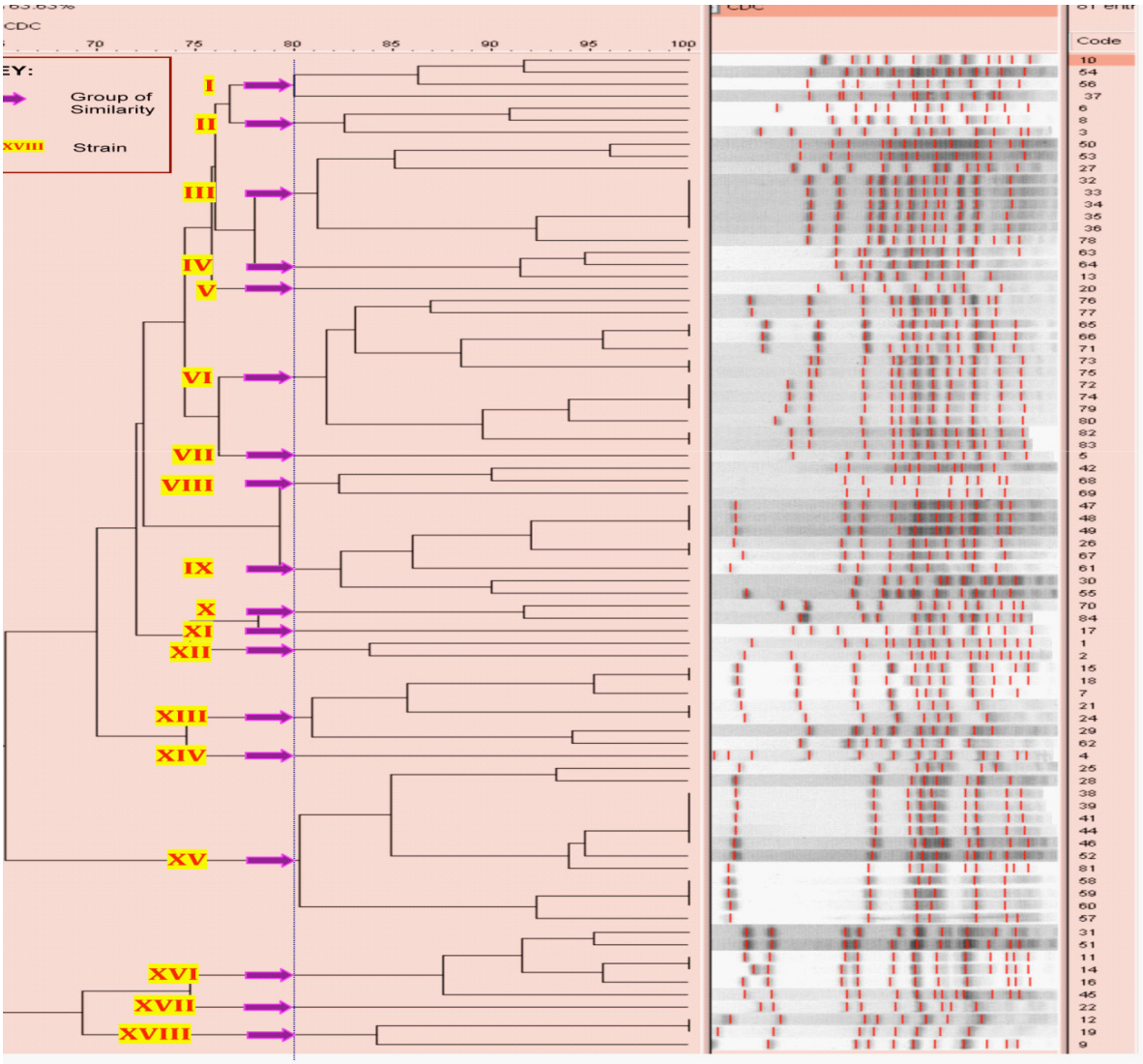


**Figure 11.** Dendogram based on pulsed – field gel electrophoresis (PFGE) of 81 samples of Acinetobacter baumannii. The Dendogram is shown on the left with the percent – homology score indicated on top (0% - 100%). The actual PFGE banding patterns are given on the immediate right of the Dendogram. Dice (Opt: 4.00%) (Tol 2.0%-2.0%).

A2 is represented by samples number 58, 59 and 60. Strains A3 to A7 were represented by samples number 52, 81, 28, 25 and 57, respectively. Strain B was classified into 8 (eight) subgroups; B1 represented by samples number 72, 74 and 79. Strain B2 represented by samples number 73 and 75. Strain B3 is represented by samples number 82 and 83. Strain B4 is represented by sample number 80. Strain B5 was represented by samples number 65 and 66. Strains B6 to B8 were represented by samples number 71, 77 and 76, respectively. Strain C was classified into 3 (three) subgroups: C1 represented by samples number 32, 33, 34, 35 and 36. Strains C2 and C3 were represented by samples number 78 and 27, respectively. Strain D was classified into 4 (four) subgroups: D1 represented by samples number 15 and 18. D2 represented by sample number 7; D3 represented by samples number 21 and 24; and D4 represented by sample number 29. Strain E were divided into 3 (three) groups: E1 represented by samples number 47, 48 and 49; E2 represented by samples number 26 and 67; and E3 represented by sample number 61. Strain F was classified into 5 (five) subgroups: F1 represented by samples number 11 and 14; F2 to F5 were represented by samples number 16, 31, 51 and 45, respectively. Strain G was classified into 2 (two) groups: G1 represented by samples number 12 and 19; G2 is represented by sample number 9. Strain H was classified into 3 (three) subgroups: H1 to H3 were represented by samples number 10, 54 and 56, respectively. Strain I was classified into subgroup I1 to I3, that are represented by samples number 6, 8 and 3, respectively. Strain J was classified into subgroups J1 to J3, which are represented by samples number 63, 64 and 13, respectively. Strain K was classified into subgroups K1 to K3, which are represented by samples number 42, 68 and 69, respectively. Strain L was classified into 2 (two) subgroups: L1 and L2, which are represented by samples number 70 and 84, respectively. The rest of the 13 samples were considered unique, and assigned to Strain M to Strain Y that are represented by samples number 1, 2, 20, 5, 17, 4, 22, 30, 50, 55, 62, 53 and 37, respectively.

Numerical Index of the Discriminatory Ability of Typing System was calculated using the Simpson's Index of diversity (Hunter and Gaston et al. 1988) and is given by the following equation:

$$D = 1 - \frac{1}{N(N-1)} \sum_{J=1}^{s} n_j (n_j - 1)$$

Where N is the total number of strains in the sample population, s is the total number of types described, and nj is the number of strains belonging to the jth type. The equation can be applied both to a direct comparison of the discriminating power of typing methods and to analysis of the discriminating power of combined typing schemes. Table 1 shows the discriminating indiex for PFGE. It can be seen that the discriminatory power of PFGE (0.914). Typability (100%) was also obtained for this methods used in this study (Table 2). This is done by dividing the samples that is typeable against the total number of samples. Table 2. Showed t Groupings of 81 samples for PFGE analysis .

**Table 1.** Discrimination indiex for typing method used in this study PFGE.

| Method | No. of types | Typability | Discrimination index |
|---|---|---|---|
| PFGE | 18 | 100% | 0.914 |

**Table 2.** Groupings of 81 samples for PFGE analysis.

| Sample No. | PFGE Profile (BioNumerics) |
|---|---|
| 1 | XII |
| 2 | XII |
| 3 | II |
| 4 | XIV |
| 5 | VII |
| 6 | II |
| 7 | XIII |
| 8 | II |
| 9 | XVIII |
| 10 | I |
| 11 | XVI |
| 12 | XVIII |
| 13 | IV |
| 14 | XVI |
| 15 | XIII |
| 16 | XVI |
| 17 | XI |
| 18 | XIII |
| 19 | XVIII |
| 20 | V |
| 21 | XIII |
| 22 | XVII |
| 24 | XIII |
| 25 | XV |
| 26 | IX |
| 27 | III |
| 28 | XV |
| 29 | XIII |
| 30 | IX |
| 31 | XVI |
| 32 | III |
| 33 | III |
| 34 | III |
| 35 | III |
| 36 | III |
| 37 | I |
| 38 | XV |
| 39 | XV |
| 41 | XV |
| 42 | VIII |
| 44 | XV |
| 45 | XVI |
| 46 | XV |
| 47 | IX |
| 48 | IX |
| 49 | IX |
| 50 | III |
| 51 | XVI |
| 52 | XV |
| 53 | III |
| 54 | I |
| 55 | IX |
| 56 | I |
| 57 | XV |
| 58 | XV |
| 59 | XV |
| 60 | XV |
| 61 | IX |
| 62 | XIII |
| 63 | IV |
| 64 | IV |
| 65 | VI |
| 66 | VI |
| 67 | IX |
| 68 | VIII |
| 69 | VIII |
| 70 | X |
| 71 | VI |
| 72 | VI |
| 73 | VI |
| 74 | VI |
| 75 | VI |
| 76 | VI |
| 77 | VI |
| 78 | III |
| 79 | VI |
| 80 | VI |
| 81 | XV |
| 82 | VI |
| 83 | VI |
| 84 | X |

## Discussion

Acinetobacter spp. has unique characteristics among nosocomial gram – negative bacteria that favor their persistence in the hospital environment. This organism spreads easily in the environment of infected or colonized patients and can persist in that environment for many days, a factor that may explain their propensity for causing extended outbreaks. However, it should be noted that acinetobacters are ubiquitous organisms that can also be isolated readily from nonclinical sources such as soil, drinking and surface water, sewage, and a variety of different foodstuffs. There appears to be a significant population differences between the genomic species found in clinical specimens and those found in other environments, and it is therefore

vital that acinetobacters be identified to the genomic species level and then typed before epidemiological conclusions can be drawn. The increased incidence of Acinetobacter baumannii infection required clarification of a possible mode of transmission. The predominant of one genotype in patient or environmental specimens seemed to suggest transmission from common sources. Bacterial typing schemes based on genotypic analysis of multiple isolates within a particular species to identify characteristics that may subdivide the strains into smaller groupings. Such analyses have several uses – to investigate outbreaks that may in turn influence or focus epidemiological investigations, to examine sequential isolates from a single patient to determine whether infection is recurring or the patient has suffered a relapse, to establish whether certain strains are associated with specific clinical syndromes, and therefore, have unusual pathogenic mechanisms, and in a wider context, to increase our understanding of the epidemiology of infection. Nevertheless, typing is most often used to differentiate dissimilar isolates rather than to confirm a relationship between two different strains. The basic premise of all typing schemes is that strains isolated from an epidemiological cluster arise from a common precursor and therefore, that these strains will share certain characteristics that can distinguish them from epidemiologically unrelated strains of the same species. At least three criteria are necessary for the evaluation of typing schemes: (1) Typability – the ability to obtain a definite result for each isolate tested, (2) reproducibility – the ability to achieve the same result whenever and wherever the same strain is tested, and (3) discrimination – the ability to distinguish between epidemiologically unrelated strains.

PFGE technique was shown to be most suitable method for differentiating strains from hospital outbreaks. Because PFGE fingerprints are highly reproducible, interpretation is fairly straightforward. However, major disadvantage of PFGE is the difficulty of comparing results obtained from different laboratories. In addition to the expense of the PFGE apparatus, the total time required to perform the test is a disadvantage of this method. Plugs containing the DNA extraction procedures take about 2 to 3 days, although more rapid methods have been developed. The electrophoresis time is also lengthy, 24 hours is a typical running time, and a fair amount of technical expertise is necessary. To prevent mechanical breakage of chromosomal DNA, all extraction steps must be carefully performed with preparations embedded in agarose.

In this study, evaluation of genomic fingerprinting methods performed by computerized comparison of digitized fingerprinting patterns (in PFGE analysis) is easier and gives an accurate analysis for large numbers of samples tested. Data analysis by computer offers the possibility of comparison of large numbers of patterns, formation of databases, and cluster analysis. Although visual analysis for PFGE is also possible, however For PFGE analysis, a guest commentary (Tenover et al., 1995) proposes a set of guidelines for interpreting DNA restriction patterns generated by PFGE. The authors are investigators from the United States who, over the last several years, have correlated epidemiologic data from dozens of outbreaks with strain typing results produced by PFGE. These guidelines are intended to be used by clinical microbiologists in hospital laboratories to examine relatively small sets of isolates (typically, < 30) related to putative outbreaks of disease.

In this study, to give an assessment in whichPFGE typing method is the most efficient several factors must be considered that includes: reproducibility, Typability and discrimination. Reproducibility is the percentage of strains that give the same result on repeated testing. Typability of a method is the percentage of distinct bacterial strains which can be assigned a positive typing marker. PFGE give 100% typability.

The discriminatory power of a typing method is its ability to distinguish between unrelated strains. Numerical Index of discriminatory ability of typing system was calculated using Simpson's index of diversity. It can be seen that the discriminatory power of PFGE (0.914) . In conclussion, our data suggest that Pulsed – Field Gel Electrophoresis (PFGE) can cluster epidemiologically unrelated strains of Acinetobacter baumannii into distinct populations; and the three factors or criteria for an effective assessment of typing method has been met. At this stage, having a properly optimized laboratory protocol for PFGE technique that can generate comparable and reproducible results is a necessary first step. In choosing a typing scheme for epidemiological studies, one should aim for as large discriminatory index as possible. The acceptable level of discrimination will depend on a number of factors, but an index of greater than 0.90 would seem to be desirable if the typing results are to be interpreted with confidence.

## References


Aygun G, O Demirkiran, T Utku, B Mete, S Urkmez, M Yilmaz, H Yasar, Y Dikmen, and R Ozturk (2002) Environmental Contamination During a Carbapenem-Rresistant Acinetobacter baumannii Outbreak In An Intensive Care Unit. J Hosp Infect 52: 259–62.

Bergogne-Berezin E and KJ Towner (1996) Acinetobacter Spp. As Nosocomial Pathogens: Microbiological, Clinical, and Epidemiological Features. Clinical Microbiology Reviews. 9: 148–165.

Danes C, MM Navia, J Ruiz, F Marco, A Jurado, MT Jimenez de Anta, and J Vila (2002) Distribution of B-lactamases in Acinetobacter baumannii cClinical isolates and the effect of Syn 2190 (AmpC inhibitor) on the MICs of different B-Lactam antibiotics. J Antimicrob Chemother 50: 261–264.

Denton M, NJ Todd, KG Kerr, PM Hawkey and JM Littlewood (1998) Molecular Epidemiology of Stenotrophomonas maltophilia Isolated from Clinical Specimens from Patients with Cystic Fibrosis and Associated Environmental Samples. J of Clin Microbiol 36: 1953–1958.

Dijkshoorn L, H Aucken, P Gerner-Smidt, P Janssen, ME Kaufmann, J Garaizar , J Ursing and TL Pitt (1996) Comparison Of Outbreak and Non-Outbreak Acinetobacter baumannii Strains By Genotypic and Phenotypic Methods. J Clin Microbiol 34: 1519–1525.

Dijkshoorn L, W Van Vianen, J Degener, and M Michel (1987) Typing of Acinetobacter calcoaceticus strains lisolated from hospital patients by Cell Envelope Proteins Profiles. Epidemiol. Infect 99: 659–667.

Erdenizmenli M and A Grossato (2007) Rapid Pulsed–Field Gel Electrophoresis for typing of Acinetobacter Species. Department of Histology, Microbiology and Medical Biotechnology. University of Padua, Padova, Italy.

Gautom RK (1997) Rapid Pulsed–Field Gel Electrophoresis for typing of Escherichia coli O157:H7 and other Gram Negative organisms. J Clin Microbiol 35: 2911–2980.

Gerner–Smidt P and I Tjernberg (1993) Acinetobacter in Denmark II. Molecular Sstudies of the Acinetobacter calcoaceticus - Acinetobacter baumannii Complex. Acta Pathol Microbiol Immunol Scand 101: 826–832.

Goering RV (1993) Molecular Epidemiology of Nosocomial Infection: Analysis of chromosomal restriction fragment patterns by Pulsed–Field Gel Electrophoresis. Infection Control and Hospital Epidemiology 14: 595–600.

Gorbach SL, JG Bartlett, and NR Blacklow (1998) Infect Diseases (2nd eds.). pp. 1877–1878.

Hanlon GW (2005) Under the Microscope: The Emergence of Multidrug Resistant Acinetobacter Species: A Major Concern in the Hospital Setting. Letters in Appl Microbiol 41: 375–378.

Hunter PR and MA Gaston (1988) Numerical Index of the Discriminatory Ability of Typing Systems: an Application of Simpson's Index of Diversity. J Clin Microbiol 26: 2465–2466.

Jawad A, P Hawkey, J Heritage and A Snelling (1994) Description of Leeds Acinetobacter Medium, a New Selective and Differential Medium for Isolation of Clinically Important Acinetobacter Spp., and Comparison with Herellea agar and Holton's agar. J Clin Microbiol 32: 2353–2358.

Kropec A, J Hubnerand, and F Daschner (1993) Comparison of Three Typing Methods in Hospital Outbreaks of Acinetobacter calcoaceticus Infection. J Hosp Infect 23: 133–141.

Luey CK, YW Chu, TK Cheung, CC Law, MY Chu, DT Cheung and KM Kam (2007) Rapid pulsed-field Gel Electrophoresis Protocol for Subtyping of Streptococcus Suis Serotype 2. J Microbiol Methods 68: 648–650.

Mandell GL, JE Bennett, and RD Dolin (2000) Principles and Practice of Infectious Diseases, Fifth Edition; pp. 2339.

Tenover FC, RD Arbeit, RV Goering, PA Mickelsen, BE Murray, DH Persing, and B Swaminathan (1995) Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing. J Clin Microbiol 33: 2233–2239.

Van Looveren M, H Goossens and the ARPAC Steering Group (2004) Antimicrobial Resistance of Acinetobacter spp. in Europe. Clin Microbiol Infect 10: 684–704.

Zarrilli R, M Crispino, M Bagattini, E Barretta, A Di Popolo, M Triassi and P Villari (2004) Molecular Epidemiology of Sequencial Outbreaks of Acinetobacter baumannii in an Intensive Care Unit Shows the Emergence of Carbapenem Resistance. J Clin Microbiol 42: 946–53.

KBM Journal of Biology - ISSN 1948-5883. Published By KBM Scientific Publishing, LP (www.kbm-scientific-publishing.org)