

Identification and molecular epidemiology of nosocomial outbreaks due to *Burkholderia cepacia* in cystic fibrosis patients of Masih Daneshvary Hospital, Iran

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Key words

Burkholderia cepacia complex, Diagnosis, PFGE

Summary

Introduction. *B. cepacia* complex have emerged as an important opportunistic pathogen in hospitalized and immunocompromised patients. Small hospital outbreaks are frequent and are usually due to a single contaminated environmental source. In this study we were going to investigate the role of *B.cepacia* complex in those patients suspected to involve with cystic fibrosis and evaluate responsible types in Masih Daneshvary Hospital.

Methods. One hundred specimens were collected from all admitted patients who were suspected to cystic fibrosis to Masih Daneshvary hospital during one year April 2011 till end of March 2012. All were culture and identified standard procedure. All samples were checked by API system (API20NE) and by specific PCR method for genus *Burkholderia* and *Bcc* as well. Identified

strains were finally tested by PFGE system to identifying specific involving pulse-types.

Results. Isolation and identification methods revealed 5 specimens were *B.cepacia*. The frequency of the cystic fibrosis detected at this study was lower than other similar study previously reported. All these isolates showed similar pattern by PFGE standard protocol that may have spread from a single source and could not be attributed to cross infections from patient to patients.

Discussion. Application of PFGE and identification of pulse-type is a potential tool to enhance the investigation of apparent nosocomial outbreaks of *B.cepacia*. However it needs to be adjusted with environmental findings. Implementation of educational programs and adherence to infection control policies are obviously the main element for complete elimination of an outbreak.

Introduction

The *Burkholderia cepacia* complex (BCC) is a group of strictly aerobic, gram negative BCC. also known as ‘‘*B. cepacia* like bacteria’’. It currently divided into 17 species that mainly affect people with underlying disease such as cystic fibrosis or granulomatous disease, and show antimicrobial resistant to many of antibiotics [1-3]. The term ‘‘complex’’ emerged in the literature at the beginning of the genomic era associated to taxonomy and grouping organisms that belong to different species but exhibited similar patterns according to their morphological, physiological and/or other phenotypic features. DNA–DNA hybridization with 70% and high identity on 16S rRNA gene sequences [4].

Presently, the BCC includes 17 species: *B. ambifaria*, *B. anthina*, *B. arboris*, *B. cepacia*, *B. cenocepacia*, *B. contaminans*, *B. diffusa*, *B. dolosa*, *B. lata*, *B. latens*, *B. metallica*, *B. multivorans*, *B. pyrrocinia*, *B. seminalis*, *B. stabilis*, *B. ubonensis*, *B. vietnamiensis*. Among these organisms some authors have detected *B. cepacia* more frequently than the rest especially in non-CF patients [5].

Bcc is among the pathogens most frequently reported outbreaks and has been described in several healthcare centers around the world [6-13]. These organisms have also emerged as an important cause of morbidity and mortality in hospitalized patients because of high rate of antibiotic resistance [14]. Therefore, fast detection and elimination of the source of such these outbreaks is the great importance in guiding infection control measures and preventing additional cause of infections especially at Cystic Fibrosis centers.

The aim of this study was first isolation and identification of *Bcc* and then to perform molecular typing by pulsed-field gel electrophoresis (PFGE) in order to evaluate the epidemiology of this pathogen in CF patients at Masih Daneshvary Hospital.

Methods

Sampling: One hundred specimens were collected from all admitted patients who were suspected to cystic fibrosis to Masih Daneshvary hospital during one year April

2011 till end of March 2012. Isolation and Identification: Bacterial identification was performed after culturing in BHI broth. One loop broth was then transferred to blood agar after overnight incubation. Isolates were identified using standard identification tests and with AP-I20NE system as well [15]. After species identification, isolates were stored at -20 °C as suspensions in 10% skim milk solution containing 10% glycerol.

PCR Methods: Microbial suspension was prepared for each isolated specimen and extracted using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The purity of all the extracted specimens was determined by measuring the optical density at the wavelengths of 260 and 280 nm. Primer pair sequences were ordered based on the Lynch's report (et al. 2008) [16]. He reported two sets of primer pairs: one detecting Burkholderia species and the second specific for the BCC (Tab. I).

20 µl mixtures were prepared using 200 µM of dNTP, 1.5 mM MgCl₂, 50 pg of each primer, 2U of taq polymerase, 1x PCR buffer and different concentration of each primer. The PCR was performed on Eppendorf thermal cyclers (Mastercycler Gradient) by following program: 96°C for 4 min, then 35 cycles at 96°C, 59°C, and 72°C each for 1 min, at the end 2 min at 59°C. PCR products were analyzed on 2% agarose gel with 100 base pair ladder.

Genotyping: DNA polymorphisms of all isolates were evaluated by pulsed-field gel electrophoresis (PFGE) with SpeI (Boehringer Mannheim Biochemicals), as described elsewhere. 20 DNA banding patterns were compared by standard DNA marker interpreted by visual inspection.

PFGE was briefly performed as follows: bacterial suspension was prepared in a buffer (100 mmol l-1 Tris, 100 mmol l-1 EDTA, pH 8.0) and adjusted to absorbance values of 0.8 - 1.0 at a wavelength of 610 nm after which plugs were prepared with SeaKem Gold agarose (Lonza, Rockland, ME, USA) and proteinase K. Bac-

terial plugs were lysed (50 mmol l-1 Tris, 50 mmol l-1 EDTA, 1% sarcosine, and 0.5 mg of proteinase K, pH 8.0) and washed after which digested with forty units of Spe I restriction enzyme (Fermentase). DNA molecular weight size marker was prepared by XbaI digestion of Salmonella enterica serotype Braenderup H9812 plugs. CHEF Mapper XA System (Bio-Rad) was applied for electrophoresis.

Results

Isolation and applied identification tests revealed 5 specimens were Burkholderia cepacia. PCR results also confirmed in these specimens (Tab. II and Fig. 1). The frequency of the cystic fibrosis detected at this study was 5%. It is obviously different at various reports. Leite and coworkers studied a total of 244 CF patients from HCPA and observed a prevalence rate of BCC of 10.6%. BCC accounted for most BCC isolates in 244 examined specimens [17].

PFGE results: PFGE was performed by the standard procedures using SpeI restriction enzyme and provided one unique electrophoresis pattern (Fig. 2) meaning similar type involving in those patients suffering cystic fibrosis due to Burkholderia cepacia. Use of XbaI also revealed similar pattern among these 5 isolated organisms. The presence of uniform pattern and similar pulse-type in all these identified B. cepacia was concluded just by visual inspection using standard DNA marker since the system has been set up recently and BioNumerics had not been delivered to the molecular lab on the running date of experiment. The pattern of each sample was carefully analyzed and the experiment repeated once more, although it was necessary to interpret these results by computer software such as gel compare or BioNumerics and drawing related dendrogram.

Tab. I. Primer sequence used in this study.

Target	Primer	Sequence	Species	Product size
recA	BCC(F)	ATGACCAATCCGACCGATCTCAA	All	429(bp)
	BCC(R)	TCAGTGCTTGGCITNIGGGCAGTT		
recA	BKF(F)	GGCNGAAGACGTCTACCGG	BCC (Group K)	117(bp)
	BKF(R)	TCGAAGTTGCTGCCGAC		

Tab. II. API results for isolated specimens. All 5 isolated specimens had similar reaction to the standard strains by applied biochemical tests.

Polymixine	DNAase	Of Mannitol	Of Maltose	Of Lactose	Ornithine	Arginine	Lysine	Citrate	H ₂ S in KIA	TSI	Indole	Motility	Oxidase	Pigment	Color Gram
R	-	+	+	+	+	-	+	W-	-	ALK/ALK	-	+	+	-	Rod g- B. cepacia Standard strain (ATCC25416)
R	-	+	+	+	+	-	+	W-	-	ALK/ALK	-	+	+	-	Rod g- Isolated organisms

Fig. 1. PCR results.

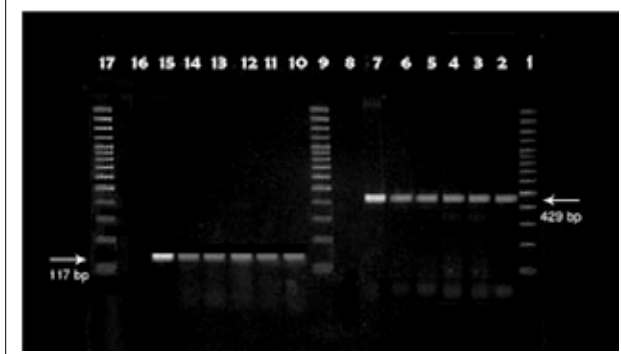
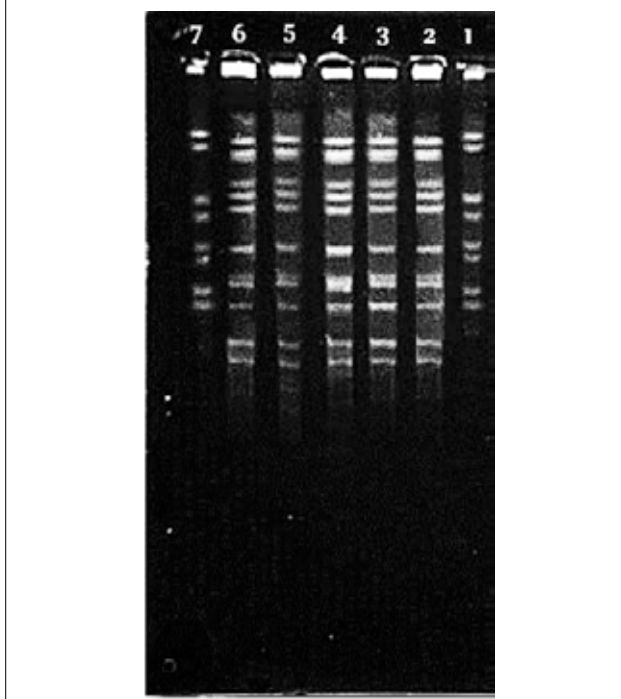


Fig. 2. PFGE results.



Discussion

In 2001, Agodi (et al. 2001) indicated a prevalence of Bcc infection of 9.5% in a study performed in four Italian CF Centers [18]. The frequency rate is obviously different in various reports. The possibility of patient-to-patient transfer of *B. cepacia* in the CF community has always been one of great concerns. Because patients colonized with Bcc may remain colonized for a long period of time. In some patients, however, Bcc was not isolated for several years and then their first strain reappeared up to 10 years later. In some of these patients, it cannot be excluded that reacquisition from the environment occurred.

PFGE has the excessively high discriminatory and reproducible for the epidemiological study of BCC comparison with other typing method [19]. Based on the

generated PFGE results by *SpeI* restriction enzyme all specimens had analogous PFGE pattern with 100% genetic similarity in this study.

It is possible that the observed infections may have spread from a single source since having identical PFGE pattern. This suggested hypothesis was supported by the isolation results because no other *Burkholderia* species was identified during the study over a period from April 2011 till end of March 2012. The unusual species distribution may be attributed to environmental sources besides to cross infections from patient to patients, although having similar pattern. Several epidemiological studies have shown most cases of BCC infection transmitted between CF patients [20]. However, occasionally detected *B. cepacia* distribution during one year of study may represent the acquisition from the environment as well as other sources cross infections from patient to patients.

It is frequently reported *B. cepacia* has high transmissibility rate and are the responsible microorganism for the mostly nosocomial infections among BCC. *B. cepacia* infection may result a significant illness, particularly in the cystic fibrosis patients, where patients are at greater risk of nosocomial infection due to their underlying illness. Therefore this finding suggests the importance of adequate patient follow-up in those hospitals that are the referral for CF centers.

Conclusion

Application of PFGE and identification of pulse-type is a potential tool to enhance the investigation of apparent nosocomial outbreaks of *B. cepacia*. However it needs to be adjusted with environmental findings. Implementation of educational programs and adherence to infection control policies are obviously the main element for complete elimination of an outbreak.

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