Molecular Biology Based Technique Is the Method of Choice in Identification of Burkholderia Cepacia Complex in Cystic Fibrosis Patients

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Abstract: Cystic Fibrosis (CF) is a chronic genetic disease that is inherited as an autosomal recessive disorder. Mucus glands, mainly those in the lung, are usually affected which result in the production of thick mucus in the bronchial tree. The presence of thick mucus usually lead to lung infections that caused by various types of microbial organisms leading to chronic lung disease and lung failure. The most common groups affecting lung tissues are Pseudomonas aeruginosa followed by Burkholderia cepacia complex (Bcc). Burkholderia cepacia complex (Bcc) considered as a fatal lung infections in CF patients. In this work, we used a molecular biology method which based on the recA PCR to test for its efficiency in the identification of Burkholderia cepacia compared with a biochemical method that used on the same group of patients. Primers used in this work are very specific that identify Burkholderia cepacia with no interference with other microorganisms encountered in lung CF patients. Burkholderia cepacia was identified only in two patients out of one hundred and twenty patients that were included in this study. On the other hand, in other study that performed in parallel with ours, using biochemical diagnostic methods, four patients were identified with Bcc. Our findings reflect the efficiency of molecular biology over the biochemical methods. Therefore, molecular biology techniques should be seriously considered in the identification of Burkholderia cepacia in CF patients in substitution of biochemical methods.

Key words: Cystic Fibrosis, Burkholderia cepacia, Lung

INTRODUCTION

Cystic Fibrosis (CF) is a chronic, progressive, inherited genetic disease of the Body's mucus glands that caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. CF primarily affects respiratory and digestive systems in children and young adults, sweat glands and reproductive system are also involved. In cystic fibrosis, glands that are distributed in the lung produce thick and sticky mucus which blocks air passage that usually leads to lung infection with a distinctive bacterial flora. Patients with CF usually die from respiratory failure as a result of chronic bacterial lung infections. [1,2] Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa or Burkholderia cepacia complex (Bcc), are frequently associated with increased morbidity and mortality rates in patients with CF. Over the past two decades, the incidence of B. cepacia complex infections have been increased among the CF population, this increase might partially be explained by improved diagnostic methods. [1,2,3,4] A specific panel of biochemical tests was developed that help in the identification of Bcc, these tests included oxidase, sucrose and lactose oxidation, lysine decarboxylase, and ornithine decarboxylase. [5,6] In order to maximize accuracy and identification levels in dealing with Bcc, different molecular biology methods based on epidemiological or genomovar specific aspects of Bcc were used, among these methods arbitrarily primed PCR (AP-PCR), enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), pulsed-field gel electrophoresis (PFGE) and PCR single-stranded conformational polymorphism (PCR-SSCP) were used. [7,8,9].

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Molecular identification and characterization is now widely regarded as the preferred approach. Recently, a group of novel recA gene-derived complex and genomovar-specific primer sets were developed. When used together with restriction fragment length polymorphism (RFLP) analysis of 16S rRNA, the characterization of all known genomovars found to be possible. In this work, we used a molecular biology method which based on the recA PCR to test for its efficiency in the identification of *Burkholderia cepacia* compared with a biochemical method that used on the same group of patients.

## MATERIALS AND METHODS

A hundred and twenty sputum samples were collected from CF patients from two hospitals in Irbid north of Jordan, King Abdullah Hospital, a teaching hospital affiliated with Jordan University of Science and Technology, and Princess Rahmah Teaching Hospital (PR TH), a teaching hospital affiliated with the ministry of health. These individuals were diagnosed as CF patients based on clinical symptoms and at least two sweat chloride tests. Samples were processed directly by microbiological techniques, including the growth on Muller-Hinton growth media, then were subjected to DNA extraction. DNA was extracted from bacterial cells using Wizard Genomic DNA purification Kit (Promega, Madison, WI, USA), following the manufacturer's instructions for the purification of DNA from bacterial cells. DNA was confirmed being bacterial by Polymerase Chain Reaction (PCR) testing 16S rRNA gene using universal 16S rRNA primers (UNI-1: 5'-GACTCTACGGGAGCGACGAG-3' and UNI-2: 5'-CTGATCCGATTACTAGCCATTC-3'). All bacterial DNA samples were tested for *Burkholderia cepacia* complex using PCR technique with specific primers, (BCR-1: 5'-TGACCAGCAGAGCGAACTCCGC-3' and BCR-2: 5'-CTCTTCTTCGATCGCTTC-3'), which amplified a single 1kb amplicon. PCR was performed under the following conditions. Reactions mixture: 200μM dNTP, 2.5U Taq polymerase, 5μl of genomic DNA, 150μM of each primer, 1.5mM MgCl₂ in a total volume of 50μl.

Amplification profile: initial denaturation at 96°C for 3 minutes followed by 35 cycles consisting of 1 min of denaturation at 96°C, 1 min of annealing at 56°C, and 1.5 min of extension at 72°C. The PCR was completed with a final extension step at 72°C for 10 min. The PCR products were electrophorased on 1.5% agarose gel containing ethidium bromide. The size of each product was determined using 1-Kb DNA ladder. DNA bands were visualized under ultra violet light. Pictures were taken with a special UV camera.

## RESULTS AND DISCUSSION

Figures: 1 and 2 demonstrate samples of our results that obtained in this work. We found that, out of one hundred and twenty patient’s samples, fifty seven were gram negative, and only two of the fifty seven belonged to the *Burkholderia cepacia* complex (Bcc).

**Discussion:** Cystic Fibrosis (CF) is a chronic genetic disease affects mostly the lung. It is the most common lethal genetic disease in white populations. The most common bacterial pathogens in young children with cystic fibrosis (CF) are *Staphylococcus aureus* and *Haemophilus influenzae* followed by *Burkholderia cepacia* complex. The precision in identification of *Burkholderia cepacia* is crucial since patients colonized with this infection undergo a special treatment in term of the kind of medicine that receive and special isolation procedures especially when we consider patient to patient spread within and outside the hospital which might play a role in increasing the prevalence of cystic fibrosis among population. A good number of patients with CF who become infected with *B. cepacia* usually experience a rapid deterioration in pulmonary function and general clinical status which might lead to death. The above stated facts emphasize the importance of rapid and accurate identification of *Burkholderia cepacia* complex (Bcc) in CF patients.

In routine clinical laboratories, the identification of putative Bcc isolates is generally performed using biochemical techniques. The accurate laboratory identification of Bcc and related bacteria from CF patient's sputum has been challenging. In many cases *B. cepacia* complex isolates were not identified and were missed by routine laboratory testing. **Burkholderia cepacia** is difficult to isolate because it usually grows slowly when compared to other organisms frequently found in sputum samples of CF patients, such as *Pseudomonas aeruginosa*. Several types of selective media have been developed for the purpose of isolation of Bcc from CF patients. These media significantly improve the ability to recover Bcc from CF respiratory secretions compared with non-selective media, however, these media may require up to 72 hours for growth to become visible. In addition, these selective media support the growth of other gram-negative non-fermenting bacilli such as *Comamonas acidovorans*, and *Stenotrophomonas maltophilia*. Thus, presumptive isolates require further characterization by a set of biochemical multi-test systems. A number of commercial kits are available for the identification of non-fermenting bacilli, these are frequently applied to non-*Pseudomonas* isolates from CF respiratory samples, but do not have equivalent accuracy for the
identification of Bcc. Because identification of Bcc by commercial kits alone is complicated, a group of experts from the international \emph{B. cepacia} working group has developed a short panel of biochemical tests that can be used as an adjunct in the identification of Bcc. This panel of biochemical tests still misidentified many isolates of Bcc group due to the complex taxonomy of Bcc and related species that colonize the CF respiratory tract, also certain species of the Bcc have phenotypes that would be considered atypical based on conventional parameters for Bcc. \cite{6,14,15,16,17} To overcome the problems mentioned by using biochemical methods, or to confirm results obtained by these methods, more accurate procedures were used which based on the identification of bacterial DNA. Many different molecular biology approaches were in use which mostly based on the identification of 16S rRNA genes of Bcc, but these methods couldn’t discriminate between different species or genomovars of this group, since the variation within the rRNA operon is obviously too small to separate all members of Bcc. Because of this limitation, these methods were less frequently used to determine the genomovar species. \cite{16,17,18,19,20,21,22} Discriminatory limitations of 16S rRNA methods lead to the use of more discriminatory methods, involved the use of recA gene methods, the recA gene polymorphisms enable both differentiation of Bcc from other closely related bacteria, and its sorting into genomovars, by the use of DNA-hybridization studies. The primers used with recA genes were very specific to Bcc and no interference with other organisms encountered in CF patients sputum were reported, also they gave a clear amplicon in PCR techniques. \cite{17,22,23,24}.

In this work we had the real opportunity to compare the use of molecular biology methods that based on the identification of \emph{Burkholderia cepacia} complex members using recA specific primers with those based on the selective media/biochemical testing methods that performed in our laboratory which conducted side by side with this work on the same group of patients. Using selective media/biochemical
based technique they could detect four cases of CF with Bcc but using recA specific primers we could clearly show that the number of patients with Bcc were only two out of one hundred and twenty patients. Thus using biochemical testing we talk about a good percentage of misdiagnosed CF *Burkholderia cepacia* carriers if compared with molecular biology methods. We hope that future studies will take this into consideration, which might lead to a more clear idea about the true prevalence of *Burkholderia cepacia* carriers in population. Since molecular biology methods could give more precise results in detecting Bcc in patients displaying intermittent colonization with *Burkholderia cepacia* especially when culturing methods give negative results molecular methods would be the method of choice especially if we use sputum samples directly from the patients.

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