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Detection of *Burkholderia cepacia* Complex in Patients with Cystic Fibrosis

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ABSTRACT

Background: Damaging of the lung function in patients with cystic fibrosis is the most frequent cause of death in these patients. Recently, *Burkholderia cepacia* has been emerged as an important opportunistic pathogen in these patients; because of its increased isolation from patients with cystic fibrosis since late 1970s, the capacity for spread of infection among the cystic fibrosis patient community, its role in damaging lung functions, and its innate multiantibiotic resistance. These different aspects make isolation of *Burkholderia cepacia* an important task in cystic fibrosis health care settings.

Materials and Methods: We examined the capacity of *Burkholderia cepacia* selective agar (BCSA) as a medium for primary isolation of *Burkholderia cepacia* samples. Biochemical tests were used to confirm the identification.

Results: *Burkholderia cepacia* strains were isolated from 6 out of 53 respiratory samples as confirmed with biochemical tests.

Conclusion: Results of the present study suggest that BCSA can be used as a selective medium with high specificity for primary isolation and identification of *Burkholderia cepacia* complex bacteria. (*Tanaffos* 2004; 3(9): 47-52)

Key words: *Burkholderia cepacia* complex, Selective medium, Cystic fibrosis.

INTRODUCTION

Chronic infection of the lower respiratory tract is the leading cause of the morbidity and mortality in patients with cystic fibrosis (CF). Mutations altering functions of the cystic fibrosis transmembrane conductance regulator (CFTR) impair chloride transport and lead to a milieu favoring colonization by bacteria, particularly *Pseudomonas aeruginosa* and *Staphylococcus aureus*. More recently, another

microbe, *Burkholderia cepacia* has been recognized as a frequent colonizer in patients with cystic fibrosis (1-4).

In 1992, the species *Pseudomonas cepacia* was reclassified as *Burkholderia cepacia* and it was assigned as the type species for new genus *Burkholderia*. In 1997, isolates classified as *B.cepacia* were reexamined by polyphasic taxonomic approaches. Bacteria which phenotypically identified as *B.cepacia* were found to consist of at least five genetically distinct species or genomovar (5, 6).

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Strains of genomovar II have been proposed as the new species *B.multivorans*, and strains of genomovar V were found to be members of the species *B.vietnamiensis*. Strains within genomovar IV renamed as *B.stabilis*. Recently, the name *B.cenocepacia* has been proposed for the genomovar III (7).

The concern regarding the emergence of this opportunistic pathogen among CF patients is due to its increased isolation since the late 1970s (5, 8), the capacity for spread of infection among the CF patient community (6, 9, 10), its role in damaging lung functions (11), and its innate multi-antibiotic resistance (12). Thus, it is important to employ reliable diagnostic techniques for this organism.

In 1997, Henry et al. described a new selective medium for the conventional isolation of *Burkholderia cepacia* with superior characteristics over several other selective media available for this organism (13). In this study we used *Burkholderia cepacia* selective agar (BCSA) for selective isolation of *Burkholderia cepacia* complex in patients with cystic fibrosis.

MATERIALS AND METHODS

Patients

Patients attending three university hospitals of Mofid's Children Hospital, Children Medical Center and Children Infection Unit of Imam Khomeini Hospital in Tehran, Iran were included. A total of fifty-three children aged from five months to 13 years old, with the mean age of 47 months were studied. The specimens were split between throat swabs and sputum. Throat swabs (n=42) were processed by placing them in 2 ml of Phosphate Buffer Saline (PBS) and vortexing thoroughly. Fresh sputum specimens (volume range: 1-3 ml, n=11) were collected from young patients.

Selective medium

The following ingredients were combined to make BCSA (per liter of distilled water): 5.0gr of sodium chloride, 10.0gr of trypticase peptone, 10.0gr sucrose, 10.0gr lactose, 1.5gr yeast extract, 10.0ml of 0.8% phenol red, 10ml 0.02% crystal violet, and 14.0gr agar. The pH of the medium adjusted to 7.0. After autoclaving for 20 minutes at 50 lb/in², 10mg gentamicin, 600,000 unit polymixin B and 2.5mg vancomycin were added, and medium was poured approximately 22ml per plate. *B.cepacia* complex organisms in patient samples were isolated by culturing at 37°C for 5 days. Cultures were examined for growth, and specimens were categorized as to whether there was growth on the medium. The patient samples were also cultured on nutrient agar supplemented with 5-10% sheep blood agar as non-selective medium.

Phenotypic identification of *B.cepacia*

The following tests were performed for all isolated strains. Briefly, purity, morphology, and hemolysis were observed and oxidase activity (Padtan teb disks) was tested. A heavy emulsion in saline was made, and a drop was placed in Hugh and Leifson oxidation-fermentation sugar (14). Bacteria were incubated in the following sugars for up to 7 days at 35°C glucose, maltose, xylose, and sucrose. A modified lysine and ornithine decarboxylase medium (containing 5g/l glucose, 5g/l KH₂PO₄, 5g/l lysine monochloride and 0.1g/l bromocresolpurple; pH= 4.6± 0.2) and negative controls were also heavily inoculated and incubated at 35°C for two days. Organisms were also tested for the presence of betagalactosidase(O-nitrophenol-β-galactopyranoside ONPG). In addition, growth on trypticase soy agar at 35° and 42°C was observed for appearance and pigmentation.

RESULTS

Growth of *Burkholderia* was detected on BCSA medium in 8 of 53 (15.1%) specimens examined.

Among these, six were recovered from throat swabs and two from sputum specimens. *Burkholderia* species colonies were visible on almost all BCSA plates after 48 hours. *B.cepacia* complex initially appeared as pinpoint colonies which finally changed to small, gray, and sometimes dry or wrinkled colonies. There was not any delay in positive oxidase reaction for isolates finally identified as *B.cepacia*. Other isolated potential pathogens, on non-selective media during this study are listed in table 1.

Table 1. Frequency of potential pathogens in the study population

Bacteria	Number	Percent
<i>Pseudomonas aeruginosa</i>	12	24.5
<i>Staphylococcus aureus</i>	8	15.0
<i>Klebsiella</i> spp.	8	15.0
<i>Citrobacter</i> spp.	2	5.66
<i>Aeromonas</i> spp.	2	5.66
<i>Agrobacterium</i> spp.	1	1.80

Among isolates grown on BCSA medium, six isolates were identified as *Burkholderia cepacia* with biochemical tests, and two were identified as *Burkholderia gladioli*. Detailed phenotypic and biochemical reactions from isolates of each genomovar and species are shown in table 2.

Of six isolated *B.cepacia* strains, three belong to genomovar III and two belong to genomovar I. For one isolate, biochemical tests could not distinguish its exact taxonomic position in *Burkholderia cepacia* complex species due to its phenotypic overlap. Although, polymerase chain reaction (PCR) with universal primers confirmed that it was a member of the *Burkholderia cepacia* complex species, we could not find out its exact species level. Positive beta hemolysis on sheep blood agar (SBA) medium is suggestive of its relatedness to genomovar VII (*B.ambifaria*).

Table 2. Biochemical tests for BCSA positive strains

Sample Test	NCF31	NCF22	NCF4	IDS21	IDS33	CMC29	CMC23	CMC17	CMC11	CMC8	CMC5
Oxidation											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Loctose	-	+	-	-	-	-	-	+	+	-	+
Sucrose	+	-	-	-	-	-	+	-	+	-	+
Xylose	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	-	+	-	-	-	-	+	-	+	+	+
Growth at 42°C	-	+	-	-	-	-	+	-	+	+	+
Oxidase	-	+	-	+	-	-	+	+	+	+	+
Gelatin hydrolysis	-	+	-	-	-	+	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-	-	-	-	-	-	-
Growth on MC agar	+	+	+	w	+	+	+	+	+	+	w
Beta hemolysis	-	-	-	-	-	-	-	-	+	-	-
DNase test	-	-	-	-	-	-	-	-	-	-	-
Pigmentation	-	-	-	-	-	-	-	-	-	-	-

The results of biochemical identification also were confirmed by molecular tests based on species-specific amplification of *recA* gene. In addition, these results indicated that the results of PCR with *recA* based primers had high concordance with the results obtained by culture on BCSA medium (Data not shown).

DISCUSSION

Incomplete or incorrect identification of an organism as *B.cepacia* can lead to inappropriate segregation or cohorting of CF patients. Infection control is a significant problem of the *B.cepacia* complex due to its ability for transmission among CF patients (1, 8,15).

Identifying the cultured organisms obtained from the respiratory specimens of CF patients is not straightforward. Using commercial systems, members of the *B.cepacia* complex have been misidentified as (among others) *B.gladioli*, *Ralstonia*

pikettii, *Alcaligenes* spp., *Pseudomonas* spp., *Stenotrophomonas maltophilia*, *Flavobacterium* spp., and *Chryseobacterium* spp. Strains of these various species have likewise been misidentified as belonging to the *Burkholderia cepacia* complex (13,16,17). Techniques for identification of *B.cepacia*-like organisms must be capable of accurately identifying such a diverse variety of gram-negative non-fermenters. In addition, this method must be quick and easy to perform.

B.gladioli and *Ralstonia* spp. are organisms not belonging to *B.cepacia* complex but have the capability of growing on BCSA (5, 18). In our study, the most common isolated non-*cepacia* organism recovered from BCSA medium was *B.gladioli*. This organism can be relatively easily distinguished from *B.cepacia* through a simple oxidase test.

In contrast with *B.gladioli*, *B.cepacia* complex bacteria generally considered to be oxidase positive (19). However, this reaction is delayed and weak with some commercial oxidase systems like Pathotec cytochrome oxidase strip, and this deletion can be regarded as an indicative characteristic for identification of *B.cepacia* complex bacteria. A slow reaction occurs between 10 and 30 seconds and is usually weaker in color development than that of the *Pseudomonas aeruginosa* positive control, which provides a quick and strong reaction within 10 seconds (20).

Burkholderia cepacia complex are one of the few non-fermenters that can be positive for both lysine and sucrose utilization tests. Therefore, positive results for these tests (combined with growth on BCSA, a negative result for DNase) can be useful as a screening tool (18).

There was no *Burkholderia cepacia* growth detectable on non-selective media perhaps as a result of overgrowth of other faster growing bacteria, like *Pseudomonas* spp. or *Klebsiella* spp.

Overall, accurate cultural detection of the *B.cepacia* complex organisms is important for patients with cystic fibrosis, both in terms of their management and infection control purposes. The problems of reliable detection in CF microbiology are further exacerbated by the quality and quantity of the sputum specimens, whether primary diagnostic laboratories perform selective isolation methods for *B.cepacia*. The use of sputum specimens of compromised quality or the use of inadequate or inappropriate selective methods may eventually translate into the transmission of this organism to patients with cystic fibrosis who are not colonized with *Burkholderia cepacia* complex organisms.

We recommend that isolates identified by some primary tests as being a member of the *Burkholderia cepacia* complex be screened by the following tests: growth on the BCSA, lysine and ornithine decarboxylase, sucrose oxidation hemolysis, pigment production, and growth at 42°C. We also recommend the use of this medium routinely for isolation of this group of bacteria in CF patients.

In conclusion, our current study is in accordance with the results obtained by other groups, working with BCSA medium (13,17,18); indeed, we found this medium superior to other selective and non-selective media in terms of rapidity and inhibition of other *B.cepacia*-like organisms.

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