

## Original Article



# Biochemical Characterization and Antimicrobial Susceptibility Pattern of the Clinical Isolates of *Burkholderia cepacia* complex from Northern Part of Bangladesh

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### Abstract

**Background:** *Burkholderia cepacia* complex (Bcc) bacteria are endowed with an extraordinary metabolic diversity and emerged in the 1980s as life-threatening and difficult-to-treat pathogens among the patients suffering from cystic fibrosis (CF). Recently, these bacteria became recognized as a threat to hospitalized patients suffering from other diseases, in particular oncological patients. **Objectives:** The objectives of the present study were to isolate, speciate, reactions to different substrates and to find out the antibiogram of Bcc from different clinical samples. **Materials and Methods:** These Gram negative organisms were analyzed by BD Phoenix M50 Automated Microbiology System for carbohydrates, amino acids and proteins and other substrates to observe the reactions. Antibiogram was also observed. **Results:** Reactions to different substrates showed similarities with other studies with some variations. Antimicrobial susceptibility pattern of the Bcc isolates was found almost similar in comparison to other studies with some differences. **Conclusion:** Bcc demands an early detection and diagnosis as the incidences of infection by these organisms are increasing day by day. Therefore, this automated system can be used as a tool to facilitate early identification and antimicrobial susceptibility pattern of the Bcc bacteria in routine microbiology laboratories.

**Key words:** *Burkholderia cepacia* complex, Biochemical Character, Antimicrobials.

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## Introduction

*Burkholderia cepacia* (formerly *Pseudomonas cepacia*) was once thought to be a single bacterial species but has expanded to the *Burkholderia cepacia* complex, comprising 24 closely related opportunistic pathogenic species.<sup>1</sup> They consist of gram-negative non-lactose-fermenting bacteria that are ubiquitous in water, soil and plants. Organisms from this group, particularly *Burkholderia multivorans* and *Burkholderia cenocepacia* are important opportunistic pathogens in patients with CF and other chronic granulomatous diseases.<sup>2</sup> They are intrinsically resistant to multiple classes of antibiotics, including aminoglycosides and polymyxins. They can harbour  $\beta$ -lactamase genes, such as the gene coding for PenA, an inhibitor-resistant class A carbapenemase that structurally resembles *Klebsiella pneumoniae* carbapenemase.<sup>3,4</sup> In non-CF patients, Bcc is known to cause pneumonia, meningitis, urinary tract infections and bloodstream infections (BSIs). Information about clinical characteristics and outcome of patients with Bcc BSI is mostly derived

from small cohorts in the context of outbreaks. These indicate that patients with Bcc BSI have serious underlying diseases, receive intensive care and have undergone invasive procedures.<sup>5,6</sup> They are capable of colonizing fluids in the hospital such as irrigation solutions or intravenous fluids and serve as potential source of nosocomial infections.<sup>7-9</sup> *Burkholderia cepacia* infection is much less common, but it is notorious for being associated with cross infection and the possibility of rapid deterioration, known as the cepacia syndrome.<sup>10-12</sup> *Burkholderia* species are being recognized with increasing frequency as nosocomial pathogens. Due to their wide distribution in the natural environment, nutritional adaptability and ability to form biofilms, outbreaks of infection in hospitals are frequent. However, the source of infection is seldom identified. Several recent reports suggested a variety of infection vehicles, including ultrasound gel, nebulized medications, nasal spray, hospital water and lipid emulsion.<sup>13</sup> More than 50% strains studied in this observation were from hospital acquired infections.

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Fig: 01

## Materials and Methods

This cross-sectional study was carried out in the Laboratory Services Department (Microbiology) of Khwaja Yunus Ali Medical College Hospital, Sirajganj, Bangladesh over a period of 1 year January 2021 to December 2021. A total 14 Bcc strains were isolated from blood samples and bronchoalveolar lavage during this time period. Samples were inoculated on MacConkey agar and 5% sheep blood agar. Gram negative organisms were selected for the BD Phoenix™ M50 Automated Microbiology System. In total 45 substrates including carbohydrates, amino acids, proteins and its derivatives were used for the observation of reactions to them for identification of organisms. The same equipment was used to determine the antimicrobial susceptibility test (AST). All the procedures were performed according to the manufacturer's instruction. A sealed and self-inoculating molded polystyrene tray with 136 micro-wells containing dried reagents, serves as the BD Phoenix disposable device. The combination panel includes identification (ID) side with dried substrates for bacterial identification and an AST side with varying concentrations of antimicrobial agents, growth and fluorescent controls at appropriate well locations. The BD Phoenix system utilizes an optimized colorimetric redox indicator for AST and various colorimetric and fluorometric indicators for ID.

## Results

The organisms showed smooth, pinkish colonies on MacConkey agar and creamy, smooth colonies on blood agar. Gram staining from the pure culture showed typical Gram negative bacilli appearance. At the same time all the isolates showed a positive reaction for oxidase.

**Figure 1.** Colony morphologies on A) Blood agar and B) MacConkey agar media Reaction to Carbohydrate

A total 23 carbohydrate substrates were used for the identification of the isolates. Of them 17 substrates showed a homogeneous reaction by the organisms. Six other substrates showed slight variation by the organisms. The reactions are shown in Table 1.

**Table I.** Reaction to Carbohydrate of Bcc isolates

Carbohydrates	Reaction	
	Positive	Negative
D-mannitol	04	10
Adonitol	03	11
Glycine -AMC	02	12
4MU -N-Acetyl -BD - Glucosaminide	11	03
Bis (PNP) Phosphate	00	14
PNP -BD -Glucoside	02	12
Beta -allose	00	14
N-Acetyl -Galactosamine	00	14
N-Acetyl -Glucosamine	00	14
Sorbitol	00	14
Sucrose	00	14
Galacturonic acid	00	14
Maltulose	00	14
L-Rhamnose	00	14
Beta -Gentiobiose	00	14
Dextrose	02	12
D-Galactose	00	14
D-Fructose	00	14
D-Gluconic acid	00	14
D-Melibiose	00	14
L-Arabinose	00	14
Methyl -B-Glucoside	00	14
Esculin	00	14

All clinical isolates produced negative reactions to the following carbohydrates eg, sucrose, sorbitol, maltulose, rhamnose, galactose, fructose, gluconic acid, arabinose and esculin. About 10% of the organisms showed a positive reaction to the following carbohydrates namely dextrose, glycine and glucosides.

## Reaction to proteins, amino acids and metabolites

A total 12 proteins and amino acids substrates were used for the identification of the isolates. Of them 3 substrates showed a homogenous reaction by the organisms. Majority of the strains had a variety of reactions against the substrates. Their reactions are shown in Table II.

Regarding reactions to protein and its metabolites ornithine and urea showed a negative reaction by these isolates. Majority of the isolates were able to ferment lysine, alanine, glutamic acid and proline.

**Table II.** Reaction to Proteins and Amino acids of Bcc isolates

Proteins and Amino acids	Reaction	
	Positive	Negative
L-Arginine -AMC	02	12
Arginine -Arginine -AMC	01	13
Lysine -Alanine -AMC	12	02
L-Tryptophan -AMC	01	13
L-Phenylalanine -AMC	02	12
L-Glutamic acid -AMC	12	02
L-Proline -AMC	11	03
L-Leucine -AMC	05	09
L-Proline -NA	06	08
Ornithine	00	14
Urea	00	14
Glutaryl-Glycine -	00	14
Arginine -AMC		

## Reaction to miscellaneous substrates

**Citrate:** About 28.6% Bcc strains were able to utilize citrate as their utilization of energy.

**Malonate:** It is an enzyme inhibitor and inhibits utilization of succinic acid by bacteria, shutting down the Krebs and glyoxylic cycles. 16.6% isolates utilized malonate as a carbon source.

**Acetate:** It is used to test an organism's ability to utilize acetate. Only 7.14% organisms were able to utilize the acetate for their source of nitrogen.

**Alpha-keto glutaric acid:** It was the main source of energy in Krebs cycle in 35.71% strains.

**Tiglic acid:** None of the strains were able to utilize tiglic acid as a component of metabolite.

**Gamma-L-Glutamyl-NA:** A total 75% of the strains could utilize it. Reaction of Bcc to miscellaneous substrates are shown in Table III.

**Table III:** Reaction to miscellaneous substrates of Bcc isolates

Other tests	Reaction	
	Positive	Negative
Citrate	04	10
Malonate	02	12
Acetate	01	13
Alpha -ketoglutaric acid	05	09
Tiglic acid	00	14
Gamma -L-Glutamyl -NA	06	08

## Antimicrobial Susceptibility Test

In BD Phoenix M50 system AST was based on minimal inhibitory concentration (MIC) of selected 14 different antimicrobial agents. An oxidation-reduction indicator used to signify microbial metabolism in the BD Phoenix panels. The indicator changes from blue to pink as initial reduction occurs. Further reduction causes the indicator to change from pink to colorless. These microbial metabolisms inhibition indicate the susceptibility to respective antimicrobial agents.

Conventional and anti-pseudomonal antibiotics were used in the AST panel. Few conventional antibiotics i.e levofloxacin, cotrimoxazole and ciprofloxacin showed sensitivity to most strains. The susceptibility to antimicrobial agents is shown in Table IV.

**Table IV:** Antimicrobial Susceptibility pattern of Bcc isolates

Antibiotics	Specimens			
	Blood		BAL	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	0	12	0	2
Levofloxacin	10	2	2	0
Colistin	0	12	0	2
Cefoxitin	0	12	0	2
Amoxicillin	0	12	0	2
Clavulanate				
Cotrimoxazole	10	2	2	0
Ceftazidime	11	1	1	1
Piperacillin	3	9	0	2
Cefazolin	0	12	0	2
Chloramphenicol	9	3	1	1
Imipenem	12	0	2	0
Meropenem	12	0	2	0
Amikacin	8	4	1	1
Ciprofloxacin	12	0	2	0

## Discussion

The BD Phoenix™ Automated Microbiology System is used for the rapid identification and antimicrobial susceptibility testing of clinical isolates. This device provides rapid identification for most aerobic and facultative anaerobic gram-positive

bacteria as well as most aerobic and facultative anaerobic gram-negative bacteria of human origin.

Biochemical properties of microorganisms are used for a complete identification of clinical isolates. Several identification systems are reported for the use of the reagent-impregnated paper discs and micro-well methods for differentiating microorganisms. Many of the tests used in the BD Phoenix ID panels are modifications of the classical methods. Fermentation, oxidation, degradation and hydrolysis of various substrates were used for the identification of the microbes. At the same time BD Phoenix System applies chromogenic and fluorogenic substrates as well as single carbon source substrates in the identification of organism.

Our study showed a variable reaction to carbohydrates in comparison to others' findings. For example, almost all organisms in our study could not ferment arabinose and sucrose which was different from other studies.<sup>14</sup>

Eighty five percent (85%) isolates in our study showed a negative degradation of phenylalanine whereas majority of the strains in the study of Harry Yudistira et al. showed a positive degradation.<sup>15</sup> Ornithine degradation pattern in our study was similar to the findings of American Proficiency Institute – 2008 2<sup>nd</sup> Test Event.<sup>16</sup>

Majority (80%) of the isolates produced a negative reaction to citrate. On the contrary, Sagar Aryal reported positive reaction to citrate.<sup>17</sup>

Malonate, acetate, alpha-ketoglutaric acid and tiglic acid were not fermented by the majority of the isolates.

Burkholderia cepacia complex bacteria are resistant to many common antibiotics and able to acquire resistance against many more. Majority of the isolates were sensitive to ceftazidime, imipenem, meropenem and ciprofloxacin. This observation was some similarity with the study of Shalini et al.<sup>18</sup> Though the combination therapy of moxifloxacin-ceftazidime had better performance.<sup>19</sup> About 85% of the isolates were sensitive to trimethoprim-sulphamethoxazole.

Identification of the organism and selection of the right antibiotic ensure a successful treatment of infectious disease. The BD Phoenix™ Automated Microbiology System could claim a successful identification of the positive organism. Combination of meropenem and ciprofloxacin showed a great response against the isolates.<sup>19</sup> In vitro study in our lab we found sensitivity to these two antimicrobials separately. Imipenem, meropenem and ciprofloxacin combination also showed better performance against this organism. In our study the majority of the strains individually produced sensitivity to these antibiotics. The conventional antibiotics were not effective against Bcc at all. Most of the strains were sensitive to ceftazidime in vitro. Though in patients' medication ceftazidime was not included. All isolates were sensitive to imipenem and meropenem. Administration of these two drugs in vivo was able to show effective result against the infection. This observation had similarity with the study of S Bonacorsi et al.<sup>20</sup> Clinical use of

conventional antibiotics i.e., ampicillin, amoxicillin-clavulanate etc. for the treatment of B. cepacia infection is highly limited. Our results from the lab and clinical administration of ceftazidime, imipenem and meropenem could be an ideal treatment for this fastidious organism.

## Conclusion

For the early detection and diagnosis of Bcc becomes easier and accurate by BD Phoenix M50 Automated System. Antimicrobial susceptibility pattern was observed by this equipment. Administration of combined antimicrobial therapy i.e, meropenem and ciprofloxacin showed a great response against the isolates.

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