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.¹ 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.² They are intrinsically resistant to multiple classes of antibiotics, including aminoglycosides and polymyxins. They can harbour β-lactamase genes, such as the gene coding for PenA, an inhibitor-resistant class A carbapenemase that structurally resembles Klebsiella pneumoniae carbapenemase.³ 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.⁴,⁵ They are capable of colonizing fluids in the hospital such as irrigation solutions or intravenous fluids and serve as a potential source of nosocomial infections.⁶,⁷ 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.⁸,⁹ 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.¹⁰ More than 50% strains studied in this observation were from hospital acquired infections.

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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 PhoenixTM 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 1. Reaction to Carbohydrate of Bcc isolates

| Carbohydrates                | Reaction |
|------------------------------|----------|
| D-mannitol                   | Positive | Negative |
| Adonitol                     | 04       | 10       |
| 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                      | 02       | 12       |
| 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            | 00       | 14       |

Table III: Reaction to miscellaneous substrates of Bcc isolates

| Other tests                  | Reaction |
|------------------------------|----------|
| Citrate                     |          |
| Malonate                    | 02       |
| Acetate                     | 01       |
| Alpha -ketoglutaric acid    | 05       |
| Tiglic acid                 | 00       |
| Gamma -L-Glutamyl -NA       | 06       |

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         | Blood Sensitive | Blood Resistant | BAL Sensitive | BAL Resistant |
|---------------------|-----------------|-----------------|---------------|--------------|
| Ampicillin          | 0               | 12              | 0             | 2            |
| Levofoxacin         | 10              | 2               | 2             | 0            |
| Colistin            | 0               | 12              | 0             | 2            |
| Cefoxitin           | 0               | 12              | 0             | 2            |
| Amoxicillin-Clavulanate | 0       | 12              | 0             | 2            |
| 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.

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. Ornithine degradation pattern in our study was similar to the findings of American Proficiency Institute – 2008 2nd Test Event.

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

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. Though the combination therapy of moxifloxacin-ceftazidime had better performance. 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.

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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