



# Clinical and Microbiological Profile of Patients with Bloodstream Infections Caused by *Burkholderia cepacia* Complex

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## Abstract

**Introduction** *Burkholderia cepacia* complex (BCC) is an emerging pathogen causing nosocomial bloodstream infections (BSIs), and its treatment is challenging due to its multidrug resistance. In India, there is a dearth of data on BSIs caused by BCC, therefore, an updated study is required to know the clinical and microbiological profile of these patients. We aimed to study the clinical epidemiology and antibiotic susceptibility pattern of BCC isolated from blood samples in our hospital.

**Materials and Methods** This observational study was conducted from January 2019 to December 2020 at a tertiary care center in northern India. All the blood cultures were done on an automated blood culture system. All BCC isolates of BSI were identified depending on their morphological properties and biochemical reactions, and underwent the matrix-assisted laser desorption ionization time-of-flight mass spectrometry system to confirm diagnosis. Antibiotic susceptibility testing was done as per Clinical Laboratory and Standards Institute guidelines.

**Results** BCC was isolated from 30 BSI patients over a 2-year period. Sixty-six percent (20/30) of patients had cancer and a majority of them were undergoing chemotherapy. The most common predisposing factors were the use of steroids, immunosuppressive drugs, and chemotherapy (93.3%), central lines (83.3%), use of higher antibiotics (60%), and diabetes mellitus type 2 (60%). The most common species isolated were *B. cepacia* (64%) and *B. cenocepacia* (30%). Isolates showed highest sensitivity to minocycline (100%), ceftazidime (73.3%), and meropenem (70%) and the least to ticarcillin-clavulanate.

**Conclusion** BCC is an emerging pathogen causing BSIs, especially in malignancy patients. Minocycline can be a good choice for these bacteria.

## Keywords

- ▶ *Burkholderia cepacia* complex
- ▶ bloodstream infections
- ▶ antibiotic sensitivity

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## Introduction

*Burkholderia cepacia complex* (BCC) is ubiquitous in nature, present in water, soil, and plants. BCC is comprised of gram-negative non-lactose-fermenting bacteria. *Burkholderia cepacia* has emerged as an opportunistic nosocomial pathogen since the 1980s, particularly in patients with debilitating diseases. Based on molecular analysis, *Pseudomonas cepacia* has been separated from *Pseudomonas* and renamed as *B. cepaci*. BCC is a cluster of at least ten closely related genomic species, which includes *B. cepacia*, *B. multivorans*, *B. cenocepacia*, and others that can be differentiated by molecular and biochemical methods.<sup>1</sup> It can cause fatal necrotizing pneumonia and bacteremia, especially in patients with cystic fibrosis or chronic granulomatous diseases.<sup>2-4</sup> Pneumonia, meningitis, urinary tract infections, and bloodstream infections (BSIs) are caused by BCC in noncystic fibrosis patients. BCC is a new pathogen that is causing significant morbidity and mortality in hospitalized patients, owing to its high intrinsic antibiotic resistance.<sup>5</sup> It has always been a tedious task for a routine microbiological laboratory to identify the nonfermenting gram-negative bacilli (NFGNBs), and poor laboratory proficiency in the identification of BCC prevails worldwide, including our own country. For this reason, reports of disease due to this organism are rare in India.<sup>6-10</sup> Differentiation of BCC from *P. aeruginosa* is important as BCC is intrinsically resistant to aminoglycosides, first- and second-generation cephalosporins, and traditional antipseudomonal penicillins. Worldwide, among pathogenic NFGNB, BCC is the fourth most common after *P. aeruginosa*, *Acinetobacter calcoaceticus-baumannii complex*, and *Stenotrophomonas maltophilia*.<sup>5</sup> In India, there is a dearth of data on BSIs caused by BCC; therefore, in this observational study, we aimed to advance our understanding of BSIs due to BCC, by analyzing the clinical epidemiology and antibiotic susceptibility pattern of these BCC isolates in our patient population.

## Materials and Methods

### Study Design and Selection of Cases

This is an observational study conducted from January 2019 till December 2020 in microbiology laboratory of a tertiary care center of Northern India. All consecutive, nonduplicate isolates of BCC from BSIs considered clinically significant were included in the study.

### Sample Processing and Identification of Isolates

All the blood cultures were done on automated blood culture system (Becton Dickinson Diagnostics, BD Headquarters, Franklin Lakes, New Jersey, USA). Nonlactose fermenting colonies on MacConkey agar that were motile, catalase and oxidase positive, and slender gram-negative bacilli with bipolar staining were subjected to biochemical reactions like Indole production, citrate utilization, nitrate reduction, urea hydrolysis, oxidation fermentation test (Hugh and Leifson medium), decarboxylase test, and esculin hydrolysis. After confirming as BCC, tests for genomic

speciation, growth at 42°C, ONPG test, pigment production, 10% lactose utilization, were also performed. Motility is tested with hanging-drop preparation since the semisolid agar medium for detecting motility of fermentative organisms may not be suitable for this NFGNB.<sup>11</sup> Phenazine pigments (red, maroon, yellow) are produced by BCC that impart distinctive color to the colonies, which are helpful in making identification.<sup>11</sup> These isolates were confirmed by MALDI-TOF-MS using the Biotyper system according to manufacturer recommendations (VITEK MS, bioMérieux, United States).

### Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done using conventional (Kirby Bauer disc diffusion method on Müller Hinton agar) and automated method (Vitek 2)<sup>12</sup> selecting antibiotics recommended for testing against BCC by the Clinical Laboratory and Standards Institute (CLSI)<sup>13</sup>: ceftazidime, trimethoprim sulfamethoxazole, meropenem, ticarcillin-clavulanate, levofloxacin, piperacillin-tazobactam, and minocycline. Antibiotic sensitivity was interpreted as per CLSI, 2019.<sup>13</sup>

A study proforma was created, which included patient demographic data, clinical data, and predisposing factors such as (1) presence of neutropenia, (2) central lines, (3) parenteral nutrition, (4) blood products, (5) intensive care unit stay, (6) intubation/ventilation, (7) immunosuppressive drugs use, (8) chemotherapy, (9) prior use of higher antibiotics, and (10) dialysis.

### Statistical Analysis

Statistical Package of Social Sciences, version-23 (SPSS-23, IBM, Chicago, Illinois, United States) were used for descriptive statistics. Categorical data were described using numbers and percentages.

## Results

BCC was isolated from 30 BSI patients over a 2-year period. The age of the patient population ranged from 5 to 84 years, with a mean age of 38 (standard deviation: 11.5) years. The number of males and females enrolled in the study was 21 and 9, respectively, with a M:F ratio of 2.3:1. The incidence of nosocomial *B. cepacia* infections was very low in our hospital (0.32 per 1,000 admissions). The majority of patients in the study had hematological malignancy (17/30; 56%) and were mostly on chemotherapy. Other patients had coronary artery disease (5/30; 17%), solid organ malignancy (3/30; 10%), end-stage renal disease requiring dialysis (2/30; 7%), pancreatitis (2/30; 7%), and acute encephalitis (1/30; 3%).

The majority of *Burkholderia complex species* involved in clinical infections of the study were *B. cepacia* (19/30, 64%) and *B. cenocepacia* (9/30, 30%), but there was one isolate each of *B. vietnamiensis* and *B. contaminans*. The most common predisposing factors present in the study population were use of steroids, immunosuppressive drugs, and chemotherapy (93.3%) (– **Table 1**). Out of 30 patients, five patients suffered from coronavirus disease 2019 (COVID-19) pneumonia and had had bacterial coinfection with BCC. The identifications of

**Table 1** Predisposing factors present in patient population of the study

Predisposing factors	Number of patients (n, %)
Neutropenia	12 (40%)
Central lines	25 (83.3%)
Steroids/immunosuppressive drugs/chemotherapy	28 (93.3%)
Diabetes	18 (60%)
Parenteral nutrition	5 (16.7%)
Higher antibiotics used before	18 (60%)
Blood products	14 (46.7%)
Intubation/mechanical ventilation	6 (20%)
Dialysis	7 (23.3%)

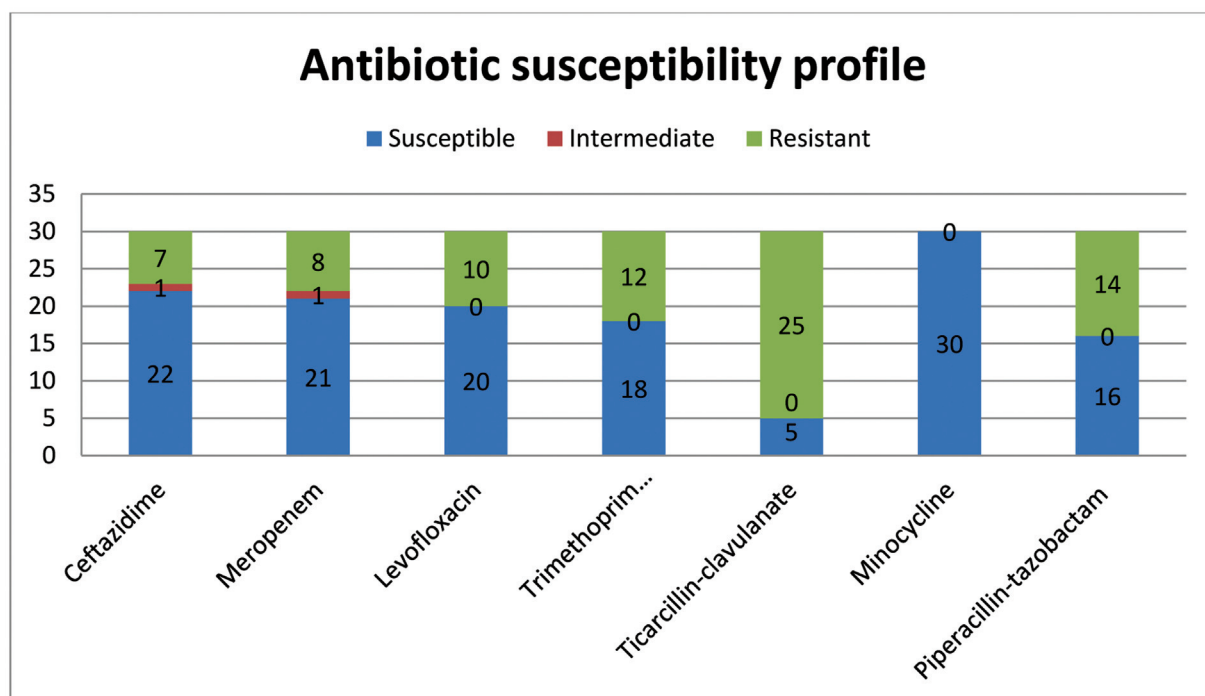
these isolates were *B. cepacia*, *B. cenocepacia*, and *B. vietnamiensis* recovered from 3, 1, and 1 patients, respectively.

All 30 isolates showed 100% sensitivity to minocycline. Ceftazidime and meropenem were found to be sensitive in 73.3% and 70%, respectively, of the isolates. However, the sensitivities of levofloxacin, trimethoprim sulfamethoxazole, and piperacillin-tazobactam were 66.7, 60, and 53.3%, respectively. Only 16.7% of isolates were sensitive to ticarcillin-clavulanate (→ Fig. 1).

## Discussion

The majority of data on nosocomial BCC infections in the literature are limited to epidemics,<sup>4</sup> whereas our data

show no epidemics and only sporadic cases of BCC infections. Ku et al studied BCC bacteremia and found BCC infection to be commoner in male patients (66.7%).<sup>14</sup> Similarly our study also had a male preponderance with 70% of infected patients being male. The most common species of *Burkholderia complex* isolated in the study were *B. cepacia* and *B. cenocepacia*, which is similar to other studies.<sup>15,16</sup> The most frequent risk factors in the study population were use of steroids, immunosuppressive drugs, and chemotherapy, which could be because most of the patients in the study had malignancy, which itself is an immunocompromised state. Besides, these patients received chemotherapy as well as lots of immunosuppressive drugs that cause low immunity in the patients. Hence, they were vulnerable to opportunistic infections such as *Burkholderia*, which is itself nonharmful to healthy people. Five patients suffered from COVID-19 pneumonia and had had bacterial coinfection with BCC. Long-term use of steroids also causes an increase in the rate of infections due to deranged cellular immunity, which could be cause of such infections in both cancer and COVID-19-positive patients. Other risk factors revealed in these patients were invasive procedures, mostly central line insertions like chemo ports as well as peripherally inserted central catheters. As central lines are essential in malignancy patients for long-term chemotherapy infusions, infection of central lines might happen during the insertion procedure as well as during the maintenance period. Biofilm formation in the central lines might cause bacteria to harbor and cause infections in the central lines associated with BSIs. Blood products through central lines might help in the formation of biofilms that might cause infections in central lines in these cases. Neutropenia will be caused by

**Fig. 1** Results of antimicrobial susceptibility testing of isolates of *Burkholderia cepacia* complex causing bloodstream infection.

chemotherapy due to myelosuppression during the treatment. Diabetes itself causes an infection due to the deranged blood sugar level in patients. All the above predisposing factors that have been reported in earlier studies<sup>14,17</sup> were also found to be present in our cases that might have resulted in *Burkholderia* infections.

BCC is intrinsically resistant to many antibiotics. It has been well documented that it has intrinsic resistance to aminoglycosides, first- and second-generation cephalosporins, and traditional antipseudomonal penicillins. The multidrug resistance of *B. cepacia* has been credited to an impermeable selective outer membrane, an efflux pump mechanism, and/or the production of an inducible chromosomal  $\beta$ -lactamase.<sup>18–20</sup> In the current study, antibiotic susceptibility tests revealed that minocycline was the most effective antibiotics against BCC with susceptibility percentage (100%), followed by ceftazidime (73.3%), meropenem (70%), levofloxacin (66.7%), and trimethoprim sulfamethoxazole (60%). They exhibited moderate sensitivity to piperacillin-tazobactam (53.3%), while BCC isolates showed high resistance to ticarcillin-clavulanic acid (83.3%). The study done by Kady et al reported *B. cepacia* isolates to be 100% susceptible to meropenem, ceftazidime, and piperacillin-tazobactam, followed by cefepime (87.5%), cotrimoxazole, and minocycline (50%). All strains (100%) were resistant to both ciprofloxacin and ticarcillin-clavulanate.<sup>21</sup> Omar et al in their study found that *B. cepacia* isolates were 88.5% susceptible to meropenem and 60% to ceftazidime. All are 100% resistant to both co-trimoxazole and ciprofloxacin.<sup>22</sup>

Comparing the results of this study most of the isolates were sensitive to minocycline meropenem and ceftazidime as suggested by the CLSI guidelines, with varying sensitivity to other antimicrobials tested. On the other hand, this study showed high resistance (83.3%) to ticarcillin-clavulanic acid, which was almost similar to Kady et al (100%). From various studies finally, we could conclude that there were variations in the results of drug susceptibility that may be because of various antibiotic policies followed in different hospitals. So, there is need to properly isolate and do antibiotic sensitivity testing for each strain for better patient management.

## Conclusion

As BCC is intrinsically resistant to many antibiotics, laboratory diagnosis with identification of these bacteria becomes mandatory. Moreover, due to the lack of advanced diagnostic facilities to confirm the etiological agent, they are reported as NFGNB or simply as *Pseudomonas species*. Thus, to facilitate diagnosis and appropriate antibiotic treatment, all NFGNB grown in clinical specimens should be speciated. Our study highlights BCC as an emerging pathogen causing BSIs, especially in malignancy patients. Minocycline can be a good choice for these bacteria.

### Conflict of Interest

None declared.

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