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Original Article

Outbreak of Burkholderia Cepacia Infection: a Systematic Study in a Hematolo-oncology Unit of a Tertiary Care Hospital from Eastern India

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Abstract. ***Background.*** ***Burkholderia cepacia*****, an aerobic gram-negative bacillus, is a frequent colonizer of fluids used in the hospital ward. It poses little risk of infection to healthy people; however it is a known important opportunistic pathogen causing morbidity and mortality due to its intrinsic resistance to most of the antibiotics in hospitalized patients. Small hospital outbreaks are frequent.** ***B. cepacia*** **may occur as an opportunistic infection in hemato-oncology patients. Here we present an outbreak of** ***Burkholderia cepacia*** **infection in hematology ward of our institute.**

Methods. **Febrile episodes as defined by IDSA guideline, 2010 were followed, and blood for culture and sensitivity was sent in all the events. The culture was done by an automated method using Bactalert 3d Biomeriux & sensitivity pattern by Microscan Siemens method and subsequently detected by PCR based method.**

Results. **During September 2016 to February 2017 (six months), a total of 498 blood cultures were sent during febrile episodes. Out of which 60 (12%) came out to be positive for different microorganisms. Out of all positive cultures,** ***Burkholderia cepacia*** **was detected in 29 (48%) patients, which reduced drastically following the change in antibiotic administration practice. All isolates showed sensitivity to pipercillin+tazobactum, cefoperazone+sulbactum, fluoroquinolones, cotrimoxazole and carbapenems and resistance to polymyxin B and colistin. With timely intervention by appropriate intravenous antibiotics as per culture sensitivity result and change in antibiotic preparation practice, overall mortality was low 1 (4%) out of 29 culture positive episodes.**

Conclusion. **Change of antibiotic preparation practice was the key to control this outbreak, and overall mortality was low.**

Keywords: Hemato-oncology patients, Opportunistic infection, Burkholderia cepacia, Antibiotic preparation practice.

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Introduction. *Burkholderia cepacia* (*B. cepacia*) is an aerobic gram-negative bacillus which is catalase negative, non-lactose fermenting gram-negative bacterium.[1] *B. cepacia* is frequent colonizer of fluids used in the hospital ward (e.g., irrigation solutions, intravenous fluids, antiseptic

solutions).[2] *B. cepacia* poses little risk of infection to healthy people; however it is a known important opportunistic pathogen causing morbidity and mortality due to its intrinsic resistance to most of the antibiotics in hospitalized patients.[3] Small hospital outbreaks are frequent and are usually due to single contaminated source such as disinfectant, intravenous solutions, nebulizer solutions, mouthwash, and medical devices, including respiratory therapy equipment.[2,4]

Infection with *B. cepacia* is uncommon in the hematological setting. However, *B. cepacia* may occur as an opportunistic infection in hemato-oncology patients.[5] Here we present an outbreak of *B. cepacia* infection in hematology ward of our institute.

Materials and Methods. Setting and patients: Department of Haematology, Nil Ratan Sircar Medical College, and Hospital, a state-run medical college under Government of West Bengal, is a dedicated Haematology & Haemato-oncology set up where admitted patients (either neutropenic or non-neutropenic) having febrile episodes as a consequence of chemotherapy or other intensive treatments. Febrile neutropenia was defined as per Infectious Disease Society America (IDSA) 2010 guidelines.[6] Considering the immune-compromised status in these group of patients, we also routinely do perform blood culture test in any febrile episodes even in non- neutropenic state. We collaborated with the Microbiology Department of KPC Medical College and Hospital, Jadavpur within the same city of Kolkata for microbiological assessment of the specimens.

Bacterial cultures and identification. This is a retrospective analysis of total 498 febrile neutropenic episodes during the period from September 2016 to February 2017 (six months). Blood cultures were sent in all febrile episodes in Bactalert FA plus culture bottles (bioMerieux, France). A standard protocol was followed for proper storage of culture bottles as mentioned in manufacturer instructions. Blood was collected with the utmost sterility, and 10 ml blood was cultured in each BacT/ALERT bottles. Further identification of microorganisms and sensitivity pattern to antibiotics were carried out by Microscan NBC42panel (Siemens Healthcare Diagnostics, West Sacramento, CA, USA).

Environmental sampling and culture. As part of the outbreak investigation, the following items were cultured: oxygen masks, stock materials of fluids for intravenous (IV) administration, venous catheters, intravenous sets, antiseptic solutions, a swab from hospital floor. These environmental samples were primarily cultured in 10 mL Brain Heart Infusion broth (HIMEDIA LAB, INDIA) and *B. cepacia* Agar Base and *B. cepacia* Selective Supplement. CODE: SR0189 and CODE: CM0995; manufactured by Oxoid Scientific (Thermo), UK.

B. cepacia complex (Bcc) species identification and assessment of clonal relatedness: We used conventional polymerase chain reaction (PCR) method to identify the organism; the details of primers used are given **table 1**, where we followed the PCR method for Burkholderia as per Lynch report.[7] He reported two sets of primer pairs detecting Burkholderia species and the second one specific for Bcc. 20 µL mixture was prepared using 200 µM of dNTP, 1.5 Mm $MgCl_2$, 50 pmol of each primer, 1.25 units of Taq polymerase and 1X PCR buffer. The PCR was performed on a gradient PCR (Verity, Applied Biosystems) by the following programme: 96°C for 4 minutes, followed by 35 cycles at 96°C, 59°C and 72°C each for 1 minute and lastly 59°C for 2 minutes.

Genotyping. DNA polymorphisms of all isolates were evaluated by PFGE (pulsed-field gel electrophoresis) with specimen I, 20 DNA patterns were compared by standard DNA marker interpreted by visual inspection. PCR products were separated on 0.8% (wt/vol) agarose gels in

Table 1. Details of primers used for identification of *Burkholderia Cepacia* complex (Bcc) species identification and assessment of clonal relatedness as per Lynch protocol.[7]

PRIMER	SEQUENCE	SPECIES	PRODUCT SIZE
Burkholderia cepacia complex (forward primer)	ATGACCAATCCGACCGATCTCAA	All	424 bp
Burkholderia cepacia complex (Reverse primer)	TCAGTGCTTGCGITNIGGGCAGTT	All	424 bp
Burkholderia cepacia complex Group K (forward primer)	GGCNGAAGACGTCTACCGG	All	117 bp
Burkholderia cepacia complex Group K (reverse primer)	TCGAAGTTGCTGCGCGAC	All	117 bp

Figure 1A

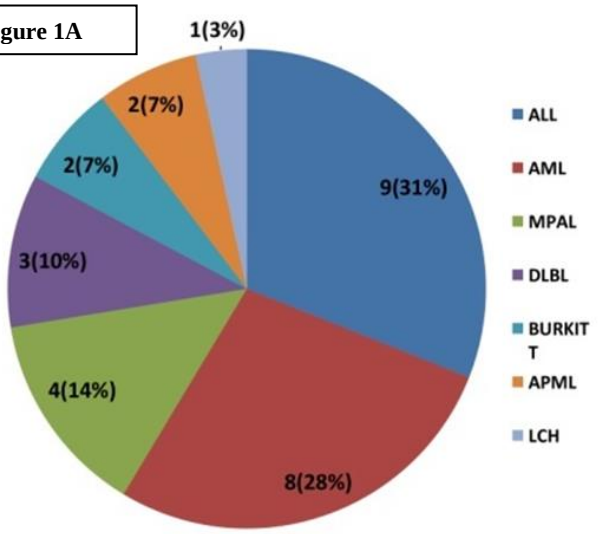
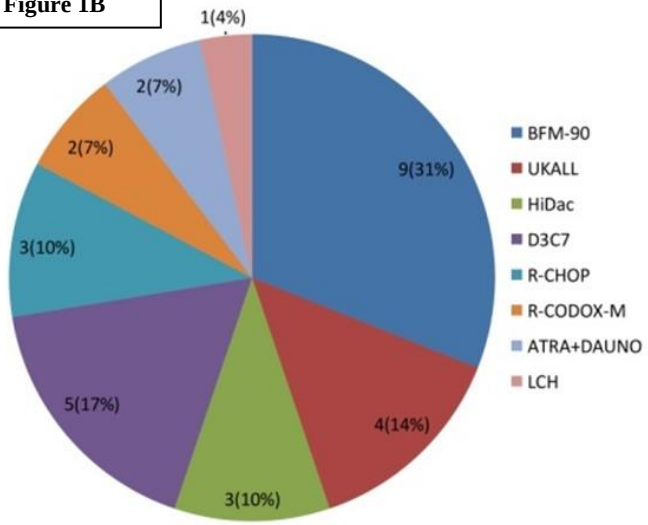


Figure 1B



Figures 1A and 1B. Figure 1A shows the distribution of cases with hematological malignancies, Figure 1B reveals an account of different chemotherapy protocols used in different hematological malignancies.

1X Tris-acetate-EDTA (pH 8.0).

For every case, the MIC50 and MIC90 values against each antibiotic were recorded meticulously as per the CLSI guidelines,[8] and the sensitivity/resistance pattern was determined.

Results. This outbreak occurred in our hematology ward from September 2016 to February, 2017. All patients included in this study were having various hematological malignancies (as shown in **figure 1A**) including acute leukemia (ALL, AML, MPAL) and high-grade lymphomas (DLBCL). As shown in **figure 1B**, the patients were on different intensive chemotherapy protocols including BFM-90, UKALL, R-CODOX-M. All patients had a central venous catheter (CVC's), the majority a peripherally introduced central catheter (PICC). The dressings at the insertion site were changed regularly by using povidone-iodine solution for antisepsis. The demographic profile of the patients during febrile episodes are shown in **table 2**. Mean age, mean total leukocyte count (TLC) and absolute neutrophil count (ANC) of the patients were 21 years (range: 6-48), 1440/µl (range: 640-5000) and 620/µl (300-2800).

Total of 498 febrile episodes occurred during this period. Blood culture was positive in 60 episodes representing only 12%. There was the growth of *B. cepacia* in 29 episodes of febrile neutropenia. So, out of 60 positive culture episodes, *B. cepacia* represented 48% of all culture-positive organisms. On further analysis, *B. cepacia* alone was the major pathogen isolated. On

Table 2. Baseline characteristics of the patients (n=29) with Burkholderia infection.

Characteristics	Mean value	Range
Median age	21 yrs	6 - 48 yrs
Hemoglobin	7.1 g/dl	5.0 - 9.0g/dl
Total leukocyte count (TLC):	1440 /µl	600 - 5000 /µl
Absolute neutrophil Count(ANC):	620/µl	300 - 2800 /µl
Platelet count:	33,730/µl	7000 -100000/µl

genotyping with the forward and reverse primer used for clonal related of *B. cepacia* complex, we first got a gene product of 424 bp size that was further characterized by using the specific primer for the species identification as mentioned in **table 1** and got a gene product of 117 bp size. And, in all cases, we could confirm the clonal relatedness to Burkholderia and the species identified as Bcc.

For every case, the MIC50 and MIC90 value against each antibiotic were noted as per the CLSI guidelines. Amongst the most resistant group of antibiotics (cephalosporins), specially ceftazidime showed MIC50 and MIC90 values of 2 and 32 µg/ml, respectively (sensitive ≤8 µg/ml and resistant ≥32 µg/ml according to CLSI guideline). Likewise, the MIC50 and MIC90 values against each antibiotic were noted meticulously.

Depending upon the sensitivity pattern of microorganisms, as an institutional policy, within one hour of onset of febrile episodes, we use to start initial antibiotic therapy with either piperacillin-tazobactam or cefoperazone-sulbactam intravenously. After the culture reports

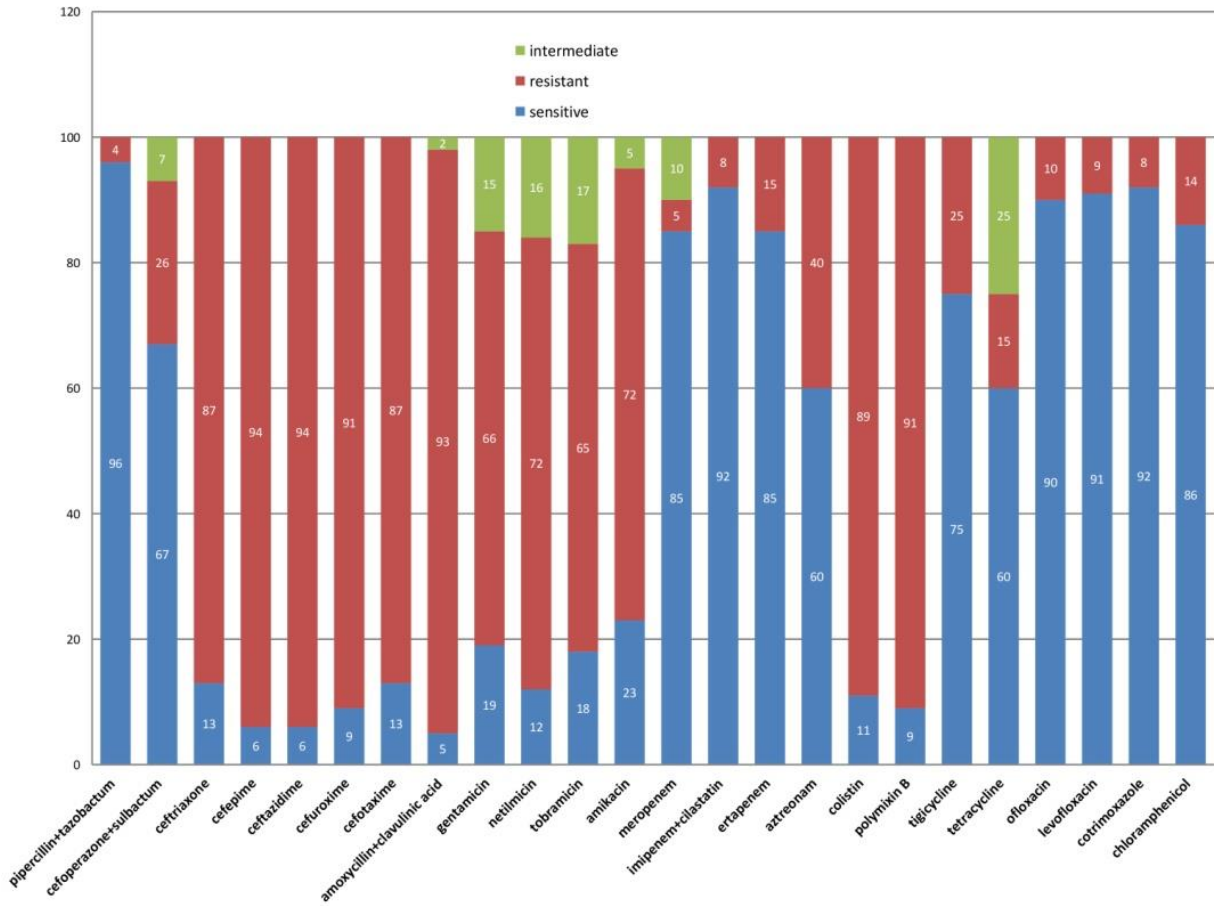


Figure 2. Sensitivity pattern of *B. cepacia* to different class of antibiotics**.**

were available, either we continued with the antibiotic started initially or changed according to the sensitivity pattern. The sensitivity pattern of *B. cepacia* to various antibiotics was analyzed in detail and analysis showed that the organism was sensitive to piperacillin-tazobactam, carbapenems, fluoroquinolones, cotrimoxazole and chloramphenicol (**Figure 2**). On further analysis of the resistance pattern of *B. cepacia*, we found that it was resistant to aminoglycosides, cephalosporins, third and fourth generation cephalosporins, colistin and polymyxin B and few had shown intermediate sensitivity pattern (**Figure 2**). For those, initially started with cefepime/amikacin, subsequently changed to the appropriate antibiotic as soon as the culture report was made available.

One patient of acute myeloid leukemia on day10 of induction chemotherapy with *B. cepacia* infection died during the study period. This patient had also CVC induced thrombosis and possible fungal infection in lungs as suggested by ground glass pattern of infiltration in high-resolution computed tomography (HRCT). In all other 28 episodes, patients responded to appropriate antibiotics as per culture and sensitivity pattern. To identify the possible sources of infection culture samples were sent from the hospital floor, walls, and antiseptic solutions. No growth of *B. cepacia* was identified from any site.

Our tertiary care teaching hospital with hematology-oncology care facility catering to people of low socio-economic status mostly, have many resources constraints such as-provision/facility of no separate room for the patients undergoing intensive chemotherapy sharing multiple beds in a single room, common bathroom, very low nurse-patient ratio. On further departmental inquiry for the possible cause of this outbreak, it was revealed that the health care providers used to prepare the intravenous antibiotics at late night supposed to be given in the next morning and store in the refrigerator. Since Bcc is an organism with a capacity to grow in antiseptic solutions, it might have grown in prepared antibiotic solutions. So we considered these practice could have precipitated the *B. cepacia* outbreak. This practice was stopped with our intervention following which Burkholderia outbreak came down sharply from 48% to 9% in the next three months (**Figure 3**).

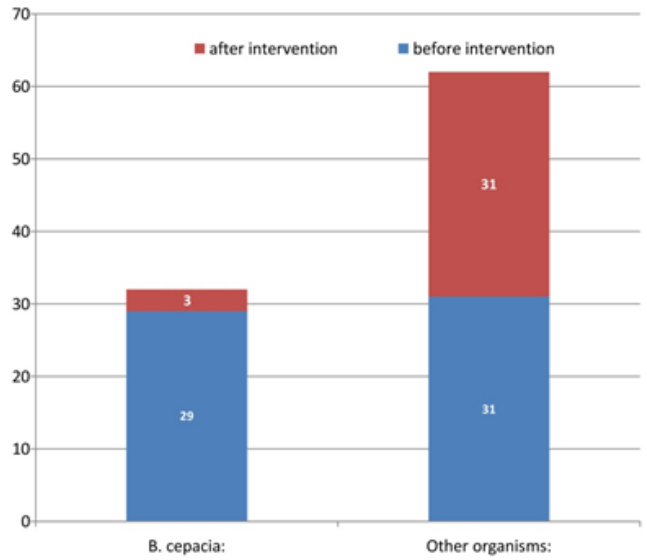


Figure 3. Change in *Burkholderia* outbreak before and after intervention (change in antibiotic preparation practice).

Discussion. In our study, we found that *B. cepacia* infection is an opportunistic gram-negative infection in patients with hematological malignancy receiving chemotherapy. Such outbreaks are not uncommon.[9] Fever with chills and rigor was the universal manifestation in all episodes as initial presentation. In a recent study in febrile neutropenia with hematological malignancy done by Mandal PK et al[10] from eastern part of India showed that, eight commonest isolates are Pseudomonas aeruginosa (14.10 %), methicillin-resistant Staphylococcus aureus (MRSA-12.82 %), Acinetobacter species (11.53 %), coagulase-negative Staphylococcus (10.25 %), Klebsiella pneumoniae (8.97 %), Escherichia coli (8.97 %), ESBL E. coli (6.41 %), methicillin-sensitive S. aureus (MSSA-6.41 %).

B. cepacia is a gram-negative organism and is a common infection in patients with cystic fibrosis[11] with lung involvement or chronic granulomatous disease, usually due to contamination of medical devices or products. Its intrinsic resistance to many disinfectants, antiseptic solutions, and antibiotics makes infection control particularly problematic.[12] Though many reports of Bcc outbreaks are there in intensive care units, hemodialysis clinic, and also oncology departments, but reports regarding hematology patients are few.[8,12,13] The very pertinent issues regarding these organisms is that the virulence of sepsis is no different from other gram-negative organism and considering the prolonged hospital stay of our patients the organism may become multi-antibiotic resistant.[14] Moreover Burkholderia outbreak can occur due to the growth of the organism in saline, antiseptic solutions as established by others.[2,4]

Another very interesting fact about *B. cepacia* infection is that cross-transmission is the very common mode of spread although all the studies are in patients with cystic fibrosis.[15] While investigating an outbreak of *B. cepacia* bacteremia in a tertiary care center by Abdelfattah R et al.[16], *B. cepacia* was isolated from the blood cultures of 14 patients resulting from contamination of the gel applied to the ultrasound probe used to guide the insertion of a central venous catheter. Gill JS et al[17] from a tertiary care hospital in India reported 17 cases of cardiac pacemaker pocket infection including multidrug-resistant *B. cepacia* (n=3) infection and concluded that every hospital should formulate their antibiotic policy based on the pattern of the hospital flora and their drug sensitivity.

All possible sources from our ward were cultured, and no other causative source was identified and thus, strengthened our proposal of overnight refrigeration of prepared intravenous antibiotics was a possible cause for the outbreak of *B. cepacia*.

We very meticulously noted the MIC50 and MIC90 values against every antibiotic as per CLSI guidelines. Though other studies showed sensitivity to ceftazidime,[11,18] but our study showed resistance to this antibiotic including many other cephalosporins as shown in **figure 3**. As shown in the study by Vardi et al[12], the organisms had shown resistance to polymyxin B, Imipenem, and aminoglycosides; our study also showed a similar type of resistance to the antibiotics except for imipenem (including all other carbapenems) which had shown excellent sensitivity. All the patients received empirical antibiotics with Cefoperazone-Sulbactum or Piperacillin-Tazobactum and became afebrile within three days of intravenous antibiotic therapy. We didn't have to escalate to carbapenems in any of our patients. Antibiotics were continued for 10-14 days depending on the clinical scenario, and the repeat blood culture report was negative. As shown in **table 2**, the patients in the study had a mean ANC of 600/µl (range: 300-2800). Thus, many patients had no neutropenia at the onset of fever. And this signifies that patients may get infected with opportunistic organisms like *B. cepacia* due to the immune-compromised status

related to primary hematological malignancy and/or chemotherapy, even if the patient may not be in neutropenic phase.

When we analyzed our outcome regarding mortality related to this outbreak, it was not disappointing. Only one patient with *B. cepacia* sepsis suffering from acute myeloid leukemia died of sepsis on day 10 of her chemotherapy with DA 3+7 standard protocol. The death cannot surely be attributed to *B. cepacia* sepsis as she also had developed possible fungal pneumonia with multi-organ failure.

We work in a tertiary care university hospital with hematology-oncology care facility catering to people of low socio-economic status, mostly. Due to obvious reasons, there are many resource constraints such as- provision/facility of no separate room for the patients undergoing intensive chemotherapy, multiple beds in a single room with very little inter-bed distance, common toilet facility, very low nurse-patient ratio. On trying to search for the possible cause of this outbreak, it was suddenly discovered that the health care providers used to reconstitute the intravenous antibiotics and store them in the syringes late at night in anticipation of administration of the drug on the next morning. The reconstituted antibiotics used to be stored in the refrigerator. Since Bcc is an organism with a capacity to grow in antiseptic solutions, it might have grown in prepared antibiotic solutions. So we hypothesized that this practice could have precipitated the Bcc outbreak. In our urgency to save the lives of the patients, the practice was immediately discontinued, and the intervention resulted in the incidence coming down sharply from 48% to 9% in the next three months (**Figure 3**). On hindsight, we realized that in our enthusiasm to act fast, we had not sent the suspected fluid for culture and sensitivity. And, we successfully managed to control the propagation of this outbreak further by changing our antibiotic preparation practice.

Conclusions. *B. cepacia*, an opportunistic infection initially reported in patients of cystic fibrosis with lung involvement or chronic granulomatous disease, usually is due to contamination of medical devices or products. Its intrinsic resistance to many disinfectants, antiseptic solutions, and antibiotics makes infection control particularly problematic. Many reports of Bcc outbreaks are from intensive care units, hemodialysis clinic, and also oncology departments, but reports regarding hematology patients are few. Burkholderia sepsis in any clinical ward is a matter of concern, as it may be considered as a surrogate indicator of deficient barrier nursing care and breach of principles of asepsis. By continued surveillance and active supervision of this unusual outbreak could be checked and thus protected the at-risk immune-compromised patients in the hemato-oncology ward.

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