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The spectrum of *Bacillus cereus* infections in patients with haematological malignancy

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Abstract:

BACKGROUND: *Bacillus cereus* is a rare but important cause of serious infections in patients with haematological malignancies. Since these infections are rapidly fatal, increased awareness, early recognition and appropriate antibiotic therapy will lead to favourable outcome.

AIM: The aim of this study is to describe the clinical spectrum and laboratory diagnosis of *B. cereus* infections in patients with haematological malignancies.

STUDY SETTING AND DESIGN: This is a retrospective observational study on the data from a tertiary care cancer hospital.

METHODOLOGY: Patients with haematological malignancy having clinical and microbiological evidence of *B. cereus* infections during 2013–2015 were included in the study. Clinical records were reviewed to assess the type of underlying haematological malignancies, the spectrum of infections caused by *B. cereus*, risk factors, antibiotic therapy and outcome. Microbiological methods used for isolation and identification of *B. cereus* as well as their antibiotic susceptibility profile were also reviewed.

RESULTS: Seven patients had *B. cereus* infection during the study period. Four patients (57.1%) had sepsis, two patients (28.6%) had skin infections with cellulitis and one patient (14.3%) had meningitis. All patients with bloodstream infections had severe neutropenia. One patient died of the infection, while others survived with appropriate antibiotic treatment.

CONCLUSION: *B. cereus*, a common agent producing acute diarrhoeal disease, can cause sepsis, invasive infections and cutaneous infections in patients with neutropenia and cancer. In patients with sepsis associated with gastrointestinal symptoms or in those with a preliminary report of Gram-positive bacilli in blood cultures, empirical antibiotic therapy should include a drug effective against *B. cereus*.

Keywords:

Bacillus cereus, infections, malignancy, neutropenia

Introduction

Bacillus cereus, a Gram-positive, aerobic, motile, spore-forming bacillus, is ubiquitous in nature. Apart from food poisoning, it causes serious and potentially fatal non-gastrointestinal tract infections. These include severe progressive pneumonia, fulminant sepsis and central nervous system infections in immunosuppressed hosts, intravenous drug abusers and neonates. Its role in nosocomial bacteraemia and wound infections after surgery is also well established, especially in patients with indwelling catheters. It can also cause cutaneous infections resembling gas gangrene after trauma.[1] Endospores of *B. cereus* are found in various environments including healthcare settings. Outbreaks have been traced to reused towels, contaminated ventilator circuits and balloons used in manual ventilation.[2-5]

Due to the intensive cytotoxic chemotherapy and other immunosuppressive treatments,

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cancer patients are prone to developing infections. Bacillus species have emerged as new Gram-positive pathogens responsible for serious infection in patients with haematological malignancies.[6] Neutropenia is extremely common in cancer patients undergoing chemotherapy. This is especially true in patients with haematological malignancies, in whom there are additional neutrophil function defects also. Bacillus species may cause serious infections, diagnostic and therapeutic dilemmas and high morbidity and mortality in this group of patients. Appropriate prophylaxis and early therapeutic intervention against possible *B. cereus* sepsis are crucially important in the treatment of haematological malignancies.[7]

The major hurdle in evaluating *B. cereus*, when isolated from a clinical specimen, is overcoming its notion as an insignificant contaminant.[1] Specific identification of these opportunistic pathogens requires additional laboratory tests, automated biochemical investigations and DNA sequencing. The isolation of Bacillus species from various clinical specimens such as blood and cerebrospinal fluid (CSF) in the division of microbiology prompted us to do a retrospective analysis of the spectrum of infections caused by these microorganisms.

Aim of the study

The aim is to describe the clinical spectrum, risk factors, therapy and outcome of *B. cereus* infections in patients with haematological malignancy who are undergoing chemotherapy and to describe the microbiological methods used for isolation and identification of *B. cereus* including antimicrobial susceptibility profile.

Methodology

Study setting and design

This retrospective observational study was conducted on the data from a tertiary care cancer hospital, in South Kerala. Patients with haematological malignancy having clinical evidence of either systemic infection or localised infection with a positive culture for *B. cereus* during 2013–2015 were included in the study. A retrospective analysis of their clinical records was carried out. The microbiological methods used for the isolation and identification of these pathogens and their antibiotic susceptibility profile were also reviewed.

Data collection

Data regarding the demographic characteristics, underlying disease, neutrophil count and other associated risk factors, clinical spectrum of these infections, antibiotic therapy and outcome were collected from medical records and assessed. Severe neutropenia was defined as absolute neutrophil count <500/cmm.

Microbiological methods

Two blood samples each were collected under sterile precautions from every patient with suspected bloodstream infection and inoculated into automated blood culture bottles (BacT/Alert fastidious antimicrobial neutralisation [FAN] aerobic bottles – bioMerieux) for performing blood culture. One central line sample and one peripheral vein sample were collected from those patients with indwelling central line catheters and two peripheral samples in case of patients without central line. CSF from patients with meningitis was also inoculated into BacT/ALERT FAN aerobic bottles and was placed in the BacT/ALERT 3D blood culture system. They were incubated at 37°C and continuously monitored for microbial growth. The bottle inoculated with CSF flagged positive on the first day after eight hours and the blood culture bottles flagged positive after 12 h. Gram stain performed on the bottles revealed the presence of large Gram-positive bacilli arranged singly and in short chains. Subculturing was done to blood agar, which grew large grey opaque, dry, spreading, beta-haemolytic colonies on aerobic incubation [Figure 1] and was catalase positive. Gram staining showed large, Gram-positive spore-bearing bacilli arranged singly, pairs and chains. Spore staining done from the culture showed the presence of oval subterminal spores. Bacillus species was isolated from both blood culture samples of the patients with bloodstream infections.

CSF sample received for culture was turbid, and Gram stain showed numerous pus cells and large Gram-positive bacilli singly and in chains both intracellularly and extracellularly suggestive of its pathogenic role. The direct inoculation of CSF onto blood agar and chocolate agar yielded no growth.

Pus swabs were collected from the skin lesions in patients with cellulitis and Bacillus species were isolated from the samples. Significance of these isolates was confirmed by repeated isolation of the organism from culture samples.

The organisms gave a positive gelatin hydrolysis test and showed lecithinase positivity and mannitol non-fermentation in mannitol-egg yolk-polymyxin agar [Figure 2]. Based on the morphology and biochemical reactions, the organisms were presumptively identified as *Bacillus cereus*. The isolates obtained from bloodstream infections were confirmed using matrix-assisted laser desorption/ionisation-time of flight at Microbiological Laboratory, Coimbatore. Susceptibility of *B. cereus* isolates against antibiotics such as Penicillin, Cefazolin, Vancomycin, Linezolid, Ciprofloxacin, Levofloxacin, Gentamicin, Clindamycin, Imipenem

and Meropenem was tested using the disc diffusion assay on Mueller-Hinton Agar plates (HiMedia), and the diameter of the inhibition zone was determined according to the Clinical and Laboratory Standards Institute guidelines (2013 and 2014) for Staphylococcus species.

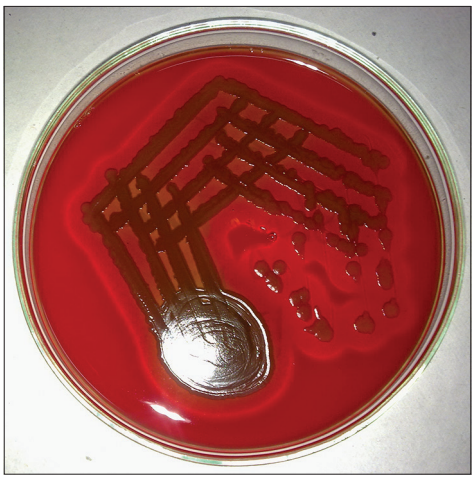

Figure 1: Blood agar plate showing large, grey, opaque, dry, spreading and beta-haemolytic colonies of *Bacillus cereus*

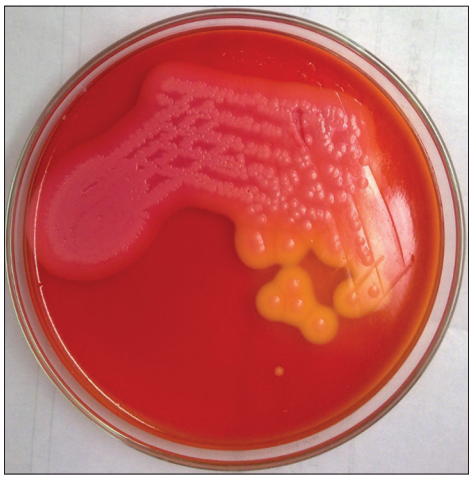

Figure 2: Mannitol-egg yolk-polymyxin agar medium showing large pink colonies of *Bacillus cereus* with precipitate

Results

Patients' characteristics

Approximately, 25,000 samples from haemato-oncology patients were processed in microbiology laboratory of a tertiary care cancer hospital during the above study period. Among 10,000 culture-proven infections, significant infections due to *B. cereus* were seen in seven patients (0.07%). Age of the patients ranged between two and 15 years. Four patients (57.1%) had bloodstream infection, two patients (28.6%) had skin infections with cellulitis and one patient (14.3%) had meningitis. All four patients with bloodstream infections had severe neutropenia (absolute neutrophil count $\leq 500/mm^3$) and two of them had peripherally inserted central catheters. Three patients (42.9%) had prodromal gastrointestinal symptoms. Five patients (71.4%) had received corticosteroids before infection. No other risk factors such as indwelling urinary catheters or intrathecal chemotherapy were identified. The demographic characteristics, clinical profile, treatment and outcome of these patients are shown in Table 1.

Antimicrobial susceptibility

Antibiotic susceptibility testing showed that all isolates (100%) were resistant to Penicillin but sensitive to Vancomycin, Linezolid, Imipenem and Meropenem. Five isolates (71.4%) were sensitive to Ciprofloxacin, Levofloxacin and Gentamicin. Three isolates (42.9%) were sensitive to Clindamycin. Only one isolate (14.3%) was sensitive to Cefazolin.

Antibiotic treatment

Patient 1 was started on Ceftazidime and Amikacin after sampling blood for culture. His condition worsened rapidly and succumbed within 12 h, before blood culture reports were available. Other patients (patients 2, 3 and 4) with bloodstream infections were treated with Ciprofloxacin, Amikacin, Clindamycin or Linezolid. Patient 5 (meningitis) was treated with intravenous Vancomycin for seven days. One patient with skin and soft tissue infections was treated with intravenous

Table 1: Demographic and clinical data of patients with *Bacillus cereus* infections

Patient number	Age (years)	Sex	Underlying malignancy	Absolute neutrophil count/cmm	*Bacillus cereus* clinical syndrome	Antibiotics administered	Outcome
Patient 1	3	Male	ALL	500	Fulminant sepsis	Ceftazidime, Amikacin	Death
Patient 2	11	Female	ALL	400	Bloodstream infection	Amikacin, Ciprofloxacin	Recovery
Patient 3	3	Female	B-lymphoblastic leukaemia	300	Bloodstream infection	Clindamycin, Piperacillin/ Tazobactam and Amikacin	Recovery
Patient 4	14	Male	AML	400	Bloodstream infection	Ciprofloxacin, Linezolid	Recovery
Patient 5	15	Male	ALL	1900	Meningitis	Vancomycin	Recovery
Patient 6	4	Male	Burkitt lymphoma	800	Skin infection with cellulitis	Vancomycin	Recovery
Patient 7	2	Female	ALL	1700	Skin infection with cellulitis	Clindamycin	Recovery

ALL: Acute lymphocytic leukaemia; AML: Acute myeloid leukaemia

Vancomycin, and the other patient was treated with a seven-day course of oral Clindamycin.

Outcome

Six patients had uneventful recovery following appropriate antibiotic therapy, while patient 1 died due to fulminant sepsis before the organism was isolated.

Discussion

Aerobic Gram-positive bacilli, other than *Bacillus anthracis*, are considered to be mildly pathogenic for humans. However, *B. cereus* can produce serious and life-threatening infections in patients with haematological malignancy. When Gram-positive bacilli are found in the blood or CSF, the possibility of *B. cereus* septicaemia or meningitis has to be considered. However, there are important challenges in making the correct diagnosis. In lesional biopsy specimens and smears, the large Gram-positive rods of *B. cereus* can be mistaken for Clostridium species. This is a potentially serious error as Bacillus species are resistant to Penicillin and other beta-lactam antibiotics.[8] Although Gram stain reports are often confusing, *Bacillus cereus* can be readily differentiated from *Clostridium perfringens* by its oxygen requirement for growth and by its catalase activity. Hence, the preferred antibiotic regimen chosen for patients with Gram-positive bacilli isolated from specimen should be one of the glycopeptide antibiotics which has good activity against *B. cereus* and Clostridium species.

B. cereus isolates are commonly reported as contaminants as the spores are ubiquitously present in the hospital atmosphere. Clinician's awareness about *B. cereus* as a potential pathogen in predisposed patients needs to be improved. *B. cereus* isolated from the clinical specimen from a sterile body site, especially in an immunosuppressed host, should not be disregarded as a contaminant. There are well-documented risk factors favouring a fulminant course and poor outcome of *B. cereus* bacteraemia which include an underlying diagnosis of leukaemia, the presence of neutropenia, receiving systemic corticosteroids or third-generation cephalosporins, recent hospitalisation and undergoing a recent lumbar puncture procedure with intrathecal chemotherapy. Clinicians and microbiologists should be attentive to these risk factors. Ceftazidime monotherapy, which is used frequently as empiric therapy for patients with neutropenia who have unexplained fever, is unlikely to eradicate *B. cereus* bacteraemia and its sequelae.[9] Therefore, use of alternative antibiotic regimens, such as Vancomycin along with Carbapenem in patients with associated gastrointestinal symptoms or in those with a preliminary report of Gram-positive bacilli in blood cultures, should be considered.[7]

This study also highlights the importance of inoculating normally sterile body fluids into highly enriched media which help to improve the yield of clinically significant isolates with a reduced time to detection. Routine culture of CSF from the patient with meningitis yielded no growth. Bacillus species was recovered from the automated blood culture bottle into which the CSF was inoculated. The enrichment method is a valid alternative that can be used as a routine procedure, allowing more accurate detection of etiological agents, thereby enabling more adequate and efficient treatment for the patient.[10] The use of automated blood culture systems has been shown to be superior to conventional cultures and also gave excellent results when used for the culture of other sterile body fluids. We used the fastidious antibiotic neutralisation (FAN) bottles which have demonstrated superior recovery compared to either the standard blood culture bottles or routine culture in other studies.[11]

Conclusion

B. cereus is a rare but important bacterial pathogen in neutropenic cancer patients producing cutaneous infections, sepsis and meningitis. Risk of sepsis appears to be greater in patients with severe neutropenia. Appropriate microbiological methods and early therapeutic intervention result in a good clinical outcome. Inoculating normally sterile body fluids in enrichment media such as automated blood culture bottles improves the yield of clinically significant isolates and reduces the time to detection.

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Conflicts of interest

There are no conflicts of interest.

References

1. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. Clin Microbiol Rev 2010;23:382-98.
2. Dohmae S, Okubo T, Higuchi W, Takano T, Isobe H, Baranovich T, *et al. Bacillus cereus* nosocomial infection from reused towels in Japan. J Hosp Infect 2008;69:361-7.
3. Gray J, George RH, Durbin GM, Ewer AK, Hocking MD, Morgan ME. An outbreak of *Bacillus cereus* respiratory tract infections on a neonatal unit due to contaminated ventilator circuits. J Hosp Infect 1999;41:19-22.
4. Van Der Zwet WC, Parlevliet GA, Savelkoul PH, Stoof J, Kaiser AM, Van Furth AM, *et al.* Outbreak of *Bacillus cereus* infections in a neonatal Intensive Care Unit traced to balloons used in manual ventilation. J Clin Microbiol 2000;38:4131-6.
5. Gaur AH, Shenep JL. The expanding spectrum of disease caused by *Bacillus cereus*. Pediatr Infect Dis J 2001;20:533-4.
6. Ugai T, Matsue K. Association between neutropenia and brain abscess due to *Bacillus cereus* bacteremia in patients with hematological malignancies. Leuk Lymphoma 2014;55:2947-9.
7. Inoue D, Nagai Y, Mori M, Nagano S, Takiuchi Y, Arima H,

et al. Fulminant sepsis caused by *Bacillus cereus* in patients with hematologic malignancies: Analysis of its prognosis and risk factors. Leuk Lymphoma 2010;51:860-9.

8. Khavari PA, Bolognia JL, Eisen R, Edberg SC, Grimshaw SC, Shapiro PE. Periodic acid-Schiff-positive organisms in primary cutaneous *Bacillus cereus* infection. Case report and an investigation of the periodic acid-Schiff staining properties of bacteria. Arch Dermatol 1991;127:543-6.
9. Gaur AH, Patrick CC, McCullers JA, Flynn PM, Pearson TA, Razzouk BI, *et al. Bacillus cereus* bacteremia and meningitis in immunocompromised children. Clin Infect Dis 2001;32:1456-62.
10. Daur AV, Klimak F Jr., Cogo LL, Botão GD, Monteiro CL, Dalla Costa LM. Enrichment methodology to increase the positivity of cultures from body fluids. Braz J Infect Dis 2006;10:372-3.
11. Bourbeau P, Riley J, Heiter BJ, Master R, Young C, Pierson C. Use of the BacT/Alert blood culture system for culture of sterile body fluids other than blood. J Clin Microbiol 1998;36:3273-7.