Bacillus spp. among hospitalized patients with haematological malignancies: clinical features, epidemics and outcomes


Division of Haematology, Department of Internal Medicine, Uludag University School of Medicine, Uludag University Hospital, Bursa, Turkey
Department of Microbiology and Infectious Diseases, Uludag University School of Medicine, Uludag University Hospital, Bursa, Turkey
Department of Chest and Tuberculosis, Uludag University School of Medicine, Uludag University Hospital, Bursa, Turkey

Received 16 January 2006; accepted 17 May 2006

KEYWORDS
Bacillus spp.; Infections; Acute leukaemia; Lymphoma; Febrile neutropenia; Antibiotics; Cancer

Summary Between April 2000 and May 2005, 350 bacteraemic episodes occurred among patients treated in our haematology unit. Two hundred and twenty-eight of these episodes were caused by Gram-positive pathogens, most commonly coagulase-negative staphylococci and Staphylococcus aureus. One hundred and twenty-two episodes were due to Gram-negative pathogens, with a predominance of Escherichia coli, Acinetobacter baumannii and Pseudomonas aeruginosa. Bacillus bacteraemias constituted 12 of these episodes occurring in 12 patients, and accounted for 3.4% of all bacteraemic episodes. Of the 12 strains evaluated, seven were Bacillus licheniformis, three were Bacillus cereus and two were Bacillus pumilus. Seven episodes presented with bloodstream infection, three with pneumonia, one with severe abdominal pain and deterioration of liver function, and one with a catheter-related bloodstream infection. B. licheniformis was isolated from five patients who had been hospitalized at the same time. This outbreak was related to non-sterile cotton wool used during skin disinfection. B. cereus and B. licheniformis isolates were susceptible to cefepime, carbapenems, aminoglycosides and vancomycin, but B. pumilus isolates were resistant to all antibiotics except for quinolones and vancomycin. Two deaths were observed. In conclusion, Bacillus spp. may cause serious…

* Corresponding author. Address: Division of Haematology, Department of Internal Medicine, Uludag University School of Medicine, Uludag University Hospital, 16059, Bursa, Turkey. Tel.: +90 224 4428400; fax: +90 224 4428060/74.
E-mail address: ridvanali@uludag.edu.tr

0195-6701/5 - see front matter © 2006 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.jhin.2006.05.014
infections, diagnostic and therapeutic dilemmas, and high morbidity and mortality in patients with haematological malignancies. Both *B. cereus* and *B. licheniformis* may be among the ‘new’ Gram-positive pathogens to cause serious infection in patients with neutropenia.

© 2006 The Hospital Infection Society. Published by Elsevier Ltd. All rights reserved.

**Introduction**

Over the past three decades, there has been a shift in the microbiology of infections in febrile neutropenic patients from predominance of Gram-negative organisms to Gram-positive organisms.1–5 *Bacillus cereus* has emerged as one of the ‘new’ Gram-positive pathogens to cause serious infection in patients with neutropenia.6 Members of the genus *Bacillus* are aerobic or facultative anaerobic Gram-positive or Gram-variable, spore-forming rods that are ubiquitous in the environment; they may be part of the normal flora, particularly in patients hospitalized for prolonged periods.6,7 As *Bacillus* ssp. are common laboratory contaminants and have been associated with pseudo-epidemics in clinics, the initial report of a blood culture growing a *Bacillus* ssp. may create a diagnostic and therapeutic dilemma in patients with neutropenia.8 The present study describes the clinical features, epidemics and outcomes of *Bacillus* ssp. infections among patients hospitalized with haematological malignancies.

**Patients and methods**

**Demographics**

Infectious disease data from patients (fever, clinical condition, administered antibiotics, blood and other site cultures) were recorded every day. Empirical broad-spectrum antibiotics were administered to all patients who developed fever, according to guidelines for the use of antimicrobial agents in neutropenic patients with cancer.9,10 Antibiotics were administered after clinical examination and two or more separate sets of blood cultures and cultures from appropriate sites of suspected infection were collected. Chest x-rays, and other radiological and diagnostic procedures, were performed as clinically indicated. None of the patients received prophylactic antibiotic administration or selective gastrointestinal decontamination. For Bacillus bacteremia, a combination of vancomycin, imipenem and amikacin was administered initially. If the *Bacillus* ssp. were found to be sensitive to this combination, treatment was continued.

**Microbiology**

**Blood culture**

Blood cultures were taken from central lines and peripheral veins. Blood cultures were processed in BACTEC-9240 systems (Becton Dickinson, Sparks, MD, USA) with BACTEC PLUS aerobic/F and anaerobic/F vials.

**Identification and biotyping**

Organisms were identified as *Bacillus* spp. (not *B. anthracis*) in the microbiology laboratory if they were motile, catalase and cytochrome-oxidase positive, growing in aerobic conditions at 37 °C, spore-forming, Gram-positive rods. Subsequently, the Phoenix-100 (Becton Dickinson, Sparks, MD, USA) and BBL CRYSTAL (Becton Dickinson, Sparks, MD, USA) Gram-positive identification panels were used as supplementary methods for identification to species level and biotyping of these isolates.

**Antibiotyping**

Sensitivity testing of all the *Bacillus* spp. isolates was performed using disc diffusion. The National Committee for Clinical Laboratory Standards’ interpretive standards for staphylococci were used as no interpretive standards have been established for *Bacillus* spp.11 The antibiotics tested were: amikacin, tobramycin, gentamycin, penicillin, ampicillin, ampicillin/sulbactam, aztreonam, cefepime, cefoperazone/sulbactam, cefotaxime, ceftazidime, piperacillin, piperacillin/tazobactam, imipenem, meropenem, ciprofloxacin, levofloxacin, ofloxacin, tetracycline and vancomycin.

**Definitions**

**Neutropenia**

Neutropenia was defined as a neutrophil count <0.5 x 10⁹/L or <1 x 10⁹/L with a predicted decline to 0.5 x 10⁹/L within the next two days.

**Fever**

Temperature was measured orally and fever was defined as a temperature of >38.3 °C measured once or a temperature of ≥38.0 °C measured
twice, lasting for at least 1 h or measured twice within 12 h.

**Bacteraemic episode**

Patients were considered to have bacillus bacteraemia if they met one of the following criteria: (1) more than one positive blood culture for organisms without any other infectious or non-infectious causes of fever; (2) more than one positive blood culture for organisms and sepsis without any other infectious or non-infectious causes of fever; or (3) one positive blood culture and isolation of the organism from another site without any other infectious or non-infectious causes of fever.

**Bloodstream infection**

Bloodstream infection (BSI) was defined as isolation of *Bacillus* spp. from at least one blood culture in the presence of fever (>38 °C) without any other infectious or non-infectious causes of fever.

**Definite infection**

Definite infection was defined as at least two positive blood cultures drawn within a 24-h period from separate sites positive for the same species, or one positive blood culture with a microbiologically documented source of infection.

**Catheter-related bloodstream infection**

Catheter-related bloodstream infection (CRBSI) was defined as isolation of the same pathogen from the catheter tip and blood in a febrile neutropenic patient with no apparent source of BSI except the catheter.

**Exclusion of bacteraemic episode**

Patients with one positive blood culture for an organism were excluded from the study.

**Results**

**Clinical characteristics of isolated pathogens**

In total, 350 bacteraemic episodes occurred among patients treated in the authors’ haematology unit between April 2000 and May 2005. Of these, 228 were caused by Gram-positive pathogens, most commonly coagulase-negative staphylococci and *Staphylococcus aureus*. One hundred and twenty-two episodes were due to Gram-negative pathogens, with predominance of *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Twelve episodes of bacillus bacteraemias occurred in 12 patients, accounting for approximately 3.4% of all bacteraemic episodes isolated during this period. Clinical data regarding the episodes are summarized in Table I. Eight patients had acute leukaemia (four newly diagnosed, three with refractory disease and one receiving consolidation therapy), four patients had non-Hodgkin’s lymphoma (NHL; two low grade and two aggressive at advanced stage). The median age of the patients was 35 years (range 19–71 years), and eight of the 12 patients were male. Nine patients had recently received anticancer treatment (Cases 1, 2, 3, 5, 6, 8, 9, 10 and 11). Two patients with low-grade NHL were under a ‘watch and wait’ policy and were not receiving anticancer therapy. In addition, one patient with acute leukaemia (Case 12) was not started on anticancer treatment. Nine patients were neutropenic (<500 cells/mm³) at the onset of bacteraemia and four of these cases (Cases 1, 2, 3 and 9) were severe (<100 cells/mm³). The duration of neutropenia ranged between 1 and 116 days. Nine patients had long-term intravascular catheters in place before the bacteraemia occurred, and there was no association with exit site or tunnel infection at the time of their episodes of bacillus bacteraemia.

All of the 12 bacillus bacteraemic episodes were considered to be definite infections. All had two positive blood cultures for a *Bacillus* spp. In addition, the same pathogen was isolated from sputum in three patients and from the catheter tip in one patient. Of the 12 strains evaluated, seven were *Bacillus licheniformis*, three were *B. cereus* and two were *Bacillus pumilus*. All cases had fever at the onset of bacillus bacteraemia. One case (Case 1) also had gastrointestinal and hepatic symptoms, three cases had pneumonia (Cases 3, 6 and 12), and nine cases presented with fever only. One patient (Case 1) had severe abdominal pain, with a steady deterioration of liver function and associated jaundice [bilirubin 0.5 mg/dL on first day (0.1/0.4 direct/indirect); 40 mg/dL on second day (33/7, direct/indirect)] resulting in a fulminant course. Computed tomography of the abdominopelvic area did not demonstrate any pathology. Stool testing for *Clostridium difficile* toxin was negative. Despite the eradication of bacteraemia, she continued to deteriorate and needed mechanical ventilation. This patient died later due to ventilator-associated pneumonia. Three patients (Cases 3, 6 and 12) with pneumonia had *Bacillus* spp. isolated from sputum. Bronchoalveolar lavage was not performed in these patients. One (Case 12) developed acute respiratory distress syndrome (ARDS) within 12 h of admission and needed mechanical ventilation. In this patient, although the clearing of bacillus bacteraemia was observed on day 7, *Klebsiella pneumoniae* and *P. aeruginosa* were
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex/age</th>
<th>Underlying disease</th>
<th>Anticancer treatment</th>
<th>IVC Duration of neutropenia (days)</th>
<th>Bacillus spp.</th>
<th>Clinical assessment</th>
<th>Administered antibiotic or antifungal therapy at the time of blood culture positivity for a Bacillus sp.</th>
<th>Administered therapy for bacillus bacteraemia</th>
<th>Duration for eradication of bacillaemia (days)</th>
<th>Neutrophil count at presentation of bacillaemia/neutrophil count at the eradication of bacillaemia (cells/mm³)</th>
<th>Outcome of bacillus bacteraemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/23</td>
<td>AML — newly diagnosed</td>
<td>Remission-induction</td>
<td>Yes</td>
<td>22</td>
<td>B. cereus</td>
<td>Fever, abdominal pain, deterioration of liver function, progressive pneumonia and fatal sepsisemia</td>
<td>ABLC (empirical)</td>
<td>Vancomycin, imipenem, amikacin</td>
<td>8</td>
<td>50/6600</td>
</tr>
<tr>
<td>2</td>
<td>F/34</td>
<td>ALL — newly diagnosed</td>
<td>Remission-induction</td>
<td>Yes</td>
<td>19</td>
<td>B. cereus</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem, amikacin</td>
<td>7</td>
<td>3/303</td>
<td>Eradicated</td>
</tr>
<tr>
<td>3</td>
<td>M/71</td>
<td>AML — newly diagnosed</td>
<td>Non-intensive treatment</td>
<td>Yes</td>
<td>3</td>
<td>B. cereus</td>
<td>Fever, pneumonia*, BSI</td>
<td>Vancomycin, imipenem, amikacin</td>
<td>6</td>
<td>6/8</td>
<td>Eradicated</td>
</tr>
<tr>
<td>4</td>
<td>F/60</td>
<td>NHL — low grade</td>
<td>No</td>
<td>No</td>
<td>11</td>
<td>B. licheniformis</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem</td>
<td>6</td>
<td>3240/4610</td>
<td>Eradicated</td>
</tr>
<tr>
<td>5</td>
<td>M/48</td>
<td>AML — refractory disease</td>
<td>Salvage treatment</td>
<td>Yes</td>
<td>116</td>
<td>B. licheniformis</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem</td>
<td>5</td>
<td>395/602</td>
<td>Eradicated</td>
</tr>
<tr>
<td>6</td>
<td>M/34</td>
<td>AML — refractory disease</td>
<td>Salvage treatment</td>
<td>Yes</td>
<td>7</td>
<td>B. licheniformis</td>
<td>Fever, pneumonia*, BSI</td>
<td>Vancomycin, imipenem</td>
<td>7</td>
<td>450/300</td>
<td>Eradicated</td>
</tr>
<tr>
<td>7</td>
<td>F/35</td>
<td>NHL — low grade</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>B. licheniformis</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem</td>
<td>5</td>
<td>1300/2500</td>
<td>Eradicated</td>
</tr>
<tr>
<td>8</td>
<td>M/71</td>
<td>NHL — aggressive disease</td>
<td>Salvage treatment</td>
<td>No</td>
<td>No</td>
<td>B. licheniformis</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem, amikacin</td>
<td>5</td>
<td>168/436</td>
<td>Eradicated</td>
</tr>
<tr>
<td>9</td>
<td>M/19</td>
<td>AML — refractory disease</td>
<td>Salvage treatment</td>
<td>Yes</td>
<td>88</td>
<td>B. pumilus</td>
<td>Fever, BSI</td>
<td>Vancomycin, imipenem, ciprofloxacin</td>
<td>1</td>
<td>4/5</td>
<td>Eradicated</td>
</tr>
</tbody>
</table>
isolated from the tracheal aspirate on day 15 and the patient died due to progressive pneumonia. In eight patients (Cases 2, 4, 5, 7–11), bacteraemia presented with BSI alone. In seven of these, fever resolved after appropriate antibiotic treatment. Fever persisted in one case (Case 11), with a Hickman-catheter-related infection despite antibiotic therapy and the catheter was removed. Fever in this patient resolved after this procedure. CRBSI developed in one of 10 long-term intravascular catheters. None of the 12 episodes were polymicrobial.

Environmental survey and control of the epidemic

Five of the bacteraemias (Cases 4–8) occurred concomitantly and this outbreak appeared to be related to the use of non-sterile cotton wool. The authors were alerted by the microbiology laboratory about the unusual frequency of Bacillus spp. isolation in the haematology unit. Extensive environmental sampling (including patients' skin, bedding, disinfectants and the tops of the blood culture bottles) was performed in the haematology unit. The source was found to be contaminated non-sterile cotton wool, used during skin disinfection with povidone iodine for venepuncture. The hospital committee for prevention of nosocomial infection organized further cultures of cotton wool from other parts of the hospital but did not establish contamination in any other unit.

Antibiotic susceptibility

All B. cereus and B. licheniformis isolates were resistant to clindamycin, penicillin and other β-lactam agents, whilst aztreonam, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, ofloxacin, tetracycline and vancomycin remained fully active. B. pumilus isolates were resistant to all antibiotics except for ciprofloxacin, levofloxacin, ofloxacin and vancomycin. Recurrent bacteraemia did not occur in any case.

Mortality

Two deaths (Case 1 and 12) were related to bacillus bacteraemia. In these cases, bacillus bacteraemias were not attributable factors for the death, but were thought to be major factors in the course of disease. Unfortunately, in these cases, autopsy could not be performed.

Discussion

Patients with haematological malignancies develop several episodes of fever and infection during
chemotherapy-induced bone marrow suppression. Leukaemic patients, in particular, are at increased risk of bacteraemia due to severe and long-lasting neutropenia, disruption of physical defence barriers and alterations in microflora.\(^{1-4}\) *Bacillus* spp. are more usually associated with food poisoning, or they are dismissed as contaminants in clinical samples. However, serious infections may occur, including sepsis, meningitis, endocarditis, endophthalmitis, respiratory infection (pneumonia, abscess, pleuritis), surgical wound infections and severe bacteraemia in cancer patients.\(^{6,12-28}\) Although most incidents attributed to *Bacillus* spp. are associated with *B. cereus*, *B. licheniformis* and members of the subtilis group, i.e. *B. licheniformis*, *B. subtilis* and *B. pumilus*, can also cause food-borne gastroenteritis, septicaemia, peritonitis, ophthalmritis and CRBSI.\(^{29-33}\)

The objective of this study was to investigate *Bacillus* spp. infections in febrile neutropenic patients with haematological malignancies. Although most incidents attributed to *Bacillus* spp. are associated with *B. cereus*, seven of the 12 bacteraemias in this series were due to *B. licheniformis*, and two of these presented with pneumonia; one of which resulted in ARDS. In the study cases, *B. cereus* was the cause of pneumonia in one case, fever in two cases, and abdominal pain with fatal deterioration of liver function in another case (Case 1). In the latter patient, neutropenic enterocolitis findings were not demonstrated. *B. licheniformis* caused pneumonia in two cases and fever in five cases. *B. pumilus* caused fever in one case and CRBSI in another case who required catheter removal. In the study patients, CRBSI was only documented in this episode.

Previous studies have shown that some risk factors favour a fulminant course and poor outcome from *B. cereus* bacteraemia. These include leukaemia, relapsing cases or during the induction phase of chemotherapy, presence of neutropenia, receiving systemic corticosteroids or third-generation cephalosporins, and recent hospitalization.\(^{14}\) Chemotherapeutic regimens based upon cytarabine plus an anthracycline or high-dose cytarabine administration for acute myeloid leukaemia are associated with severe and long-lasting neutropenia and severe mucositis that are major risks for infections.\(^{1,4}\) Seven of the study patients (58%) had acute myeloid leukaemia with regimens based upon cytarabine plus an anthracycline or high-dose cytarabine administration (salvage treatment, late consolidation). In this series, approximately 80% of the *Bacillus* spp. infections were diagnosed in the presence of neutropenia. Although the majority of the study patients were neutropenic at the onset and at the time of bacterial eradication, the eradication of *bacillus* bacteraemia was achieved in all of them, except the one patient with CRBSI. Recurrent bacteraemia was not observed in any of the patients. These data confirm the importance of neutropenia as a risk factor for rare infections in patients with haematological malignancies. Although the number of cases in this study is limited, the authors believe that the duration of neutropenia does not influence the eradication of *bacillus* bacteraemia. In these cases, both *B. cereus* and *B. licheniformis* isolates were resistant to penicillin, clindamycin and other β-lactam agents, including third-generation cephalosporins, but were susceptible to carbapenems, quinolones, fourth-generation cephalosporins, aztreonam, aminoglycosides and vancomycin. *B. pumilus* isolates were resistant to all antibiotics except quinolones and vancomycin. In all patients, bacillus bacteraemias were eradicated after 5–11 days of therapy, with vancomycin, imipenem and ceftiofur demonstrating effective antimicrobial activity.

*Bacillus* spp. have been reported to cause nosocomial outbreaks and dissemination among hospitalized patients. In an epidemic context, *B. cereus* has been identified on many occasions and has been associated with environmental reservoirs such as contaminated air filtration systems, ventilator equipment, dressings, gloves, hands of healthy staff, intravenous catheters, alcohol-based hand-wash solutions, specimen collection tubes, blood culture media and linens.\(^{6,18,19,28,34-37}\) It has been speculated that construction work increases the amount of bacteria in the air.\(^{28,38}\) Also, a recent case-control study suggested that there was an association between the ingestion of tea and invasive *B. cereus* disease among immunocompromised patients.\(^{39}\) In the present study, when five of the cases occurred together, environmental sampling recovered similar organisms from cotton wool used in the haematology unit. The association between environmental recovery and patient illness is likely to be clinically relevant and biologically plausible; however, the authors were unable to perform a formal epidemiological study with genomic identification to demonstrate a link between these isolates. The original source of contamination could not be identified.

Mortality rates due to *Bacillus* spp. infections reported in the medical literature are extremely high, but a lower rate of nearly 5% was reported in 2001.\(^{33}\) *B. cereus* is the most frequently isolated species.\(^{13}\) Two deaths occurred in the present study. In these cases, bacillus bacteraemias were thought to be major contributing factors. While the mechanism of pathogenesis remains unclear,
it may involve toxins produced by *Bacillus* spp. 

In view of the current results and previous data regarding *Bacillus* spp. infections, the authors suggest that when immunosuppressed patients develop acute abdominal pain, jaundice and hyperbilirubinemia with rapid deterioration of hepatic function, *Bacillus* infections may present with pneumonia, and they may contribute to a fatal outcome in patients with acute leukemia and neutropenia. Therefore, if there is a preliminary report of a Gram-positive bacillus from blood culture or sputum, antibiotic regimens that cover this organism should be considered for patients with neutropenia and suggestive clinical symptoms.

In conclusion, although *Bacillus* spp. are ubiquitous in the environment, infections are uncommon in immunocompromised patients. They are capable, however, of causing serious infections, diagnostic and therapeutic dilemmas, and high morbidity and mortality in these patients. The isolation of a *Bacillus* sp. in the blood culture from a patient with haematological malignancy and fever should not be viewed routinely as a contaminant, but should be assessed as a potential pathogen, especially during myeloplasia. Finally, in addition to *B. cereus*, *B. licheniformis* may be another ‘new’ Gram-positive organism to add to the list of potential pathogens for neutropenic patients.

References