Case Report

Combined *Bacillus licheniformis* and *Bacillus subtilis* infection in a patient with oesophageal perforation

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Species of the genus *Bacillus* are a common laboratory contaminant, therefore, isolation of these organisms from blood cultures does not always indicate infection. In fact, except for *Bacillus anthracis* and *Bacillus cereus*, most species of the genus *Bacillus* are not considered human pathogens, especially in immunocompetent individuals. Here, we report an unusual presentation of bacteraemia and mediastinitis due to co-infection with *Bacillus subtilis* and *Bacillus licheniformis*, which were identified by 16S RNA gene sequencing, in a patient with an oesophageal perforation.

**Case Report**

A 71-year-old male visited the emergency department with chest pain that was first noticed after swallowing tablets 3 h before admission. The pain progressively worsened and was associated with dyspnoea. On examination, his heart rate was 95 beats min⁻¹, respiratory rate was 30 breaths min⁻¹, blood pressure was 130/70 mmHg, body temperature was 36 °C and arterial O₂ saturation was 94 %. His medical history included a mild drinking habit and past pulmonary tuberculosis. He was also taking medicine for chronic obstructive pulmonary disease (COPD). The results of laboratory tests performed on admission were haemoglobin 14.3 g dl⁻¹, white blood cell (WBC) count 9.76 × 10⁹ cells l⁻¹ (73.7 % segmented neutrophils, 15.9 % lymphocytes, 3.1 % monocytes, 6.6 % eosinophils), C-reactive protein (CRP) <0.3 mg dl⁻¹ and creatine kinase myocardial band (CK-MB) 2.2 mmol l⁻¹. A computed tomography (CT) scan of the chest showed a pleural effusion in the left lower lobe and an oesophageal perforation was suspected. He was also taking medicine for chronic obstructive pulmonary disease (COPD). The results of laboratory tests performed on admission were haemoglobin 14.3 g dl⁻¹, white blood cell (WBC) count 9.76 × 10⁹ cells l⁻¹ (73.7 % segmented neutrophils, 15.9 % lymphocytes, 3.1 % monocytes, 6.6 % eosinophils), C-reactive protein (CRP) <0.3 mg dl⁻¹ and creatine kinase myocardial band (CK-MB) 2.2 mmol l⁻¹. A computed tomography (CT) scan of the chest showed a pleural effusion in the left lower lobe and an oesophageal perforation was suspected. We believe that the tablets were the cause of this. Sputum and blood were cultured and empirical antimicrobial therapy with ceftriaxone, clindamycin, and gentamicin was administered. The next day, the pain continued, the CRP had increased to 12.57 mg dl⁻¹ and his body temperature was 38.4 °C. A chest tube was inserted and blood-tinged fluid was drained. Over time, the CRP increased steadily to 29.53 mg dl⁻¹. Three sets of blood cultures and culture of the pleural fluid from the chest tube were performed. On day 4, the patient underwent an exploratory thoracotomy. In the mediastinum, pus and inflammatory tissue were observed and the pus was cultured; however, no oesophageal perforation was found. At the end of the operation, a chest tube was inserted and pus-containing fluid was drained. Colonies identified as members of the genus *Bacillus* were isolated from blood and pleural fluid cultured on days 1 and 2 and subcultures were performed on blood agar. Subsequently, the antimicrobial regimen was changed to moxifloxacin and clindamycin. After hospitalization, the patient had a persistent fever above 38 °C and the CRP remained high at 26.07 mg dl⁻¹.

The subcultures grew various colonies of Gram-positive bacilli and the cultures taken on day 1 grew transparent, spreading colonies (designated colony 1). All three sets of blood cultures taken on day 2 grew two kinds of colonies: transparent, spreading colonies (designated colony 2) and whitish medium-sized colonies (designated colony 3). The pleural fluid grew only a whitish medium-sized colony resembling colony 3.

Abbreviations: CRP, C-reactive protein; WBC, white blood cell.
The pus cultured on day 4 grew different mucoid and colourless colonies (designated colony 4) of Gram-positive bacilli. On day 7, because the WBC count, CRP and body temperature were elevated, a repeat blood culture was performed, which grew small, white, χ-haemolytic colonies (designated colony 5) and greyish medium-sized colonies (designated colony 6). The Gram stain revealed all the colonies to be Gram-positive bacilli.

In total, six different colonies were isolated from cultures from days 1–7. Of these, colony 3 was observed twice in diverse culture specimens. Fig. 1 shows the culture process and observed colonies. Initially, antimicrobial susceptibility testing was performed by using the disk diffusion method according to the CLSI guidelines, the results of which are shown in Table 1.

The isolated bacilli were subcultured three consecutive times. As colony 3 was observed simultaneously in both blood and pleural fluid cultures, the colony was identified further using 16S rRNA sequence analysis performed by Macrogen (Seoul, South Korea). The colony was identified as *Bacillus subtilis* (99% sequence similarity). Sequence analysis of the other five colonies identified them as *Bacillus licheniformis* (99% sequence similarity).

The patient underwent percutaneous endoscopic gastrostomy (PEG), which allowed the oesophageal perforation to be located. On day 12, repeated blood cultures grew no bacteria. On day 14, the antimicrobial regimen was changed to clindamycin and teicoplanin, after which the WBC count and CRP started to decrease gradually.

**Discussion**

Members of the genus *Bacillus* are Gram-positive or Gram-variable, spore-forming, aerobic or facultatively anaerobic rod-shaped bacilli with rounded or squared-off ends. They are ubiquitous in the environment and are usually found in decaying organic matter, dust, soil and deep water. Some species of the genus *Bacillus* reside in the human gut and form part of the skin flora (Mandell et al., 2010). Because species of the genus *Bacillus* are common laboratory contaminants, isolation of these organisms in blood cultures does not always indicate infection. In fact, except for *Bacillus anthracis* and *Bacillus cereus*, most species are rarely considered human pathogens. However, as early as 1963, the literature has documented serious infections by these normally non-pathogenic organisms (Farrar, 1963). The reported spectrum of *Bacillus* infections includes food poisoning, wound infections, closed-space infections and severe systemic infections (Farrar, 1963).

In cases of *Bacillus* bacteraemia, the majority of patients have a haematological malignancy, such as leukaemia or lymphoma (Banerjee et al., 1988). In a report of 140 cases of *Bacillus* bacteraemia in immunocompromised patients, the most common species were *B. cereus* and *B. subtilis* (Beebe & Koneman, 1995).

Bacteraemia caused by species of the genus *Bacillus*, especially *B. licheniformis*, has been reported in several immunocompetent individuals; it has also been reported in association with a postoperative neurosurgical infection (ventriculitis) (Young et al., 1982), post-traumatic ophthalmomisis (Maucour et al., 1999) and prosthetic valve endocarditis (Santini et al., 1995). Five cases associated with indwelling central venous catheters have also been reported (Blue et al., 1995). As noted by previous reviewers, serious infections caused by non-*anthracis* species of *Bacillus* often develop post-surgery, in association with trauma or in burn cases, and predisposing conditions include alcoholism and diabetes (Farrar, 1963; Pearson, 1970). Our patient was considered to have true bacteraemia, as bacteria grew from multiple blood cultures, pleural fluid...
and pus. The chest pain was due to acute mediastinitis associated with oesophageal perforation.

We isolated two species of the genus *Bacillus*, *B. subtilis* and *B. licheniformis*. As *B. subtilis* was isolated from a blood culture on day 2 and pleural fluid on day 3, it can be considered as the culprit of this episode. On day 4, *B. licheniformis* was isolated from a pus culture in a sterile area (an operating room), which led us to also consider it as a responsible pathogen.

Similar colonies found on days 1 and 2 (colonies 1 and 2) were identified as *B. licheniformis*, although the results of the antimicrobial susceptibility test were different for these colonies. The antimicrobial susceptibilities of the *B. licheniformis* strains isolated from colonies 1, 2, 4, 5 and 6 differed. This may have resulted from subtle differences in the substrains of *B. licheniformis*, as the third repeat of the blood cultures identified different substrains of *B. licheniformis* in both culture bottles.

The *B. licheniformis* strains isolated from colonies 1, 2, 5 and 6 were the most likely pathogens causing transient bacteremia because they were isolated from sets of cultures performed separately. However, the possibility of contamination cannot be ruled out completely.

A case reported in 1999 involved bacteremia with multiple species of the genus *Bacillus* following self-inoculation with an organic drain cleaner. Since the cleaning product contained spores of *B. licheniformis*, *Bacillus pumilus* and several other species of *Bacillus*, multiple strains were isolated (Hannah & Ender, 1999). Similarly, three bacilli (*B. licheniformis*, *B. pumilus* and *Paenibacillus polymyxa*) were isolated from an 18-year-old patient with Munchausen’s syndrome who had a history of self-injecting soil (Galanos et al., 2003).

Several reports of the antimicrobial susceptibility of species of the genus *Bacillus* other than *Bacillus cereus* have been published (Weber et al., 1988; Blue et al., 1995; Coonrod et al., 1971). In 1971, detailed antimicrobial susceptibility data for a large number of species of the genus *Bacillus* were reported, showing that *Bacillus subtilis* was very susceptible to penicillin G, ampicillin, metacinil and cephalothin (Coonrod et al., 1971). According to other studies, *B. licheniformis* may or may not be susceptible to β-lactam antibiotics (Blue et al., 1995; Weber et al., 1988) but it is usually susceptible to carbapenems, glycopeptides, aminoglycosides, quinolones, chloramphenicol, peptolides and fusidic acid. *B. licheniformis* has also been found to be resistant to penicillin, fosfomycin, macrolides and nitroimidazoles (Ameur et al., 2005; Santini et al., 1995). Our patient was initially treated with β-lactam antimicrobials but the treatment was changed to the use of quinolones and then teicoplanin due to persistence of the bacteraemia. Based on an *in vitro* study (Weber et al., 1988), the drug of choice for *Bacillus* infections appears to be vancomycin.

Herein, we report an unusual presentation of bacteremia and mediastinitis due to *Bacillus subtilis* and *Bacillus licheniformis*, which were identified by 16S rRNA gene sequencing, in a patient with an oesophageal perforation. Species of the genus *Bacillus*, though usually thought to be non-pathogenic, should not be ignored as a possible human contaminant as they can cause significant bacteremic infections. Also, knowledge of the antimicrobial susceptibility of these bacteria is of value for the selection of appropriate therapy.

### References


**Table 1. Antimicrobial susceptibility of the colonies isolated**

<table>
<thead>
<tr>
<th>Antimicrobial(s)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</table>

S, Susceptible; R, resistant. All colonies were identified as *B. licheniformis*, except for colony 3, which was identified as *B. subtilis*. Although colonies 1, 2, 4, 5 and 6 were the same species, the antimicrobial susceptibility test results were different, which implies that the colonies represent different substrains.


