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.

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, α-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 ophthalmitis (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
We isolated two species of the genus *Bacillus* associated with oesophageal perforation. 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.

<table>
<thead>
<tr>
<th>Antimicrobial(s)</th>
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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, meticillin 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 (Amere 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 bacteremia. 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 bacteraemia 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 bacteraemic infections. Also, knowledge of the antimicrobial susceptibility of these bacteria is of value for the selection of appropriate therapy.

**References**


