



## A Post-Operative Infection Caused by *Bacillus circulans* and *Paenibacillus macerans* in a Patient with Brain Tumour

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### Abstract

We report a case of post-operative infection due to rarely isolated bacteria - *Bacillus circulans* and *Paenibacillus macerans* - in a 49-year-old woman following resection of a tuberculoma sellae meningioma. The two bacterial pathogens were isolated from a cerebrospinal fluid specimen. These bacteria are opportunistic pathogens, which have often been ruled out as culture contaminants when isolated from clinical material.

**Key words:** neurosurgical infection, *B. circulans*, *P. macerans*

### Резюме

В статията се описва случай на пост-оперативна инфекция, причинена от рядко изолирани бактерии - *Bacillus circulans* и *Paenibacillus macerans* в 49-годишна жена след резекция на менангиома в областта на tuberculoma sellae. Двата бактериални причинители бяха изолирани от цереброспинална течност на пациента. Диференцираните бактерии са опортюнистични причинители, които често се приемат за замърсители на посевките или клиничния материал.

### Introduction

Health care-associated (or nosocomial) infections represent one of the most common complications of health care delivery. Between 5 and 10% of patients admitted to acute care hospitals acquire an infection during their hospital stay. One of the major site infections among nosocomial infections in hospitals are surgical site infection (SSI). SSI increase hospital expenses and length of stay, which cost the health care system billions of dollars annually (Logan *et al.*, 2011).

Reports of infections with non-*Bacillus cereus* group species are comparatively rare (Noskin *et al.*, 2001). A few reports of bacteremia, brain abscess, peritonitis, and meningitis due to *Bacillus* spp., mostly in immunocompromised patients, have been published in the literature (Berry *et al.*, 2002; Bert *et al.*, 1995; Galanos *et al.*, 2003; Krause *et al.*, 1999). All *Paenibacillus* species bacteria were originally classified as part of the *Bacillus* genus (Teng *et al.*, 2003). This report describes a case of SSI with two rarely isolated *Bacillus* species in a patient following cranial surgery for meningioma.

### Case Report

A 49 year-old woman underwent frontosphenotemporal craniotomy in Neurosurgical Clinic for the treatment of tuberculoma sellae meningioma. After resection of the meningioma, surgeons used duraplasty by applying fibrin glue as a dural sealant; a postoperative drainage tube was also added. Ten days after the operation patient began to experience frontal headaches, hypothalamic symptom-complex, giddiness, and became increasingly disoriented. Additionally, she had moderate paresis of the left leg, and electrolytic disorders. A cranial computerized tomography scan showed a small ischemia in the area of caudate nucleus (caput) in the right hemisphere, and data for intracranial postoperative infectious alterations in the wound. A lumbar puncture was performed, yielding xanthochromic cerebrospinal fluid (CSF), and its laboratory examination showed: increase of common proteins - 0.84 g/l; decrease of glucose (5.24 mmol/l) in comparison with its level in the serum (10,63 mmol/l). The patient's white blood cell and C-reactive protein (79.41 mg/L) levels became rap-

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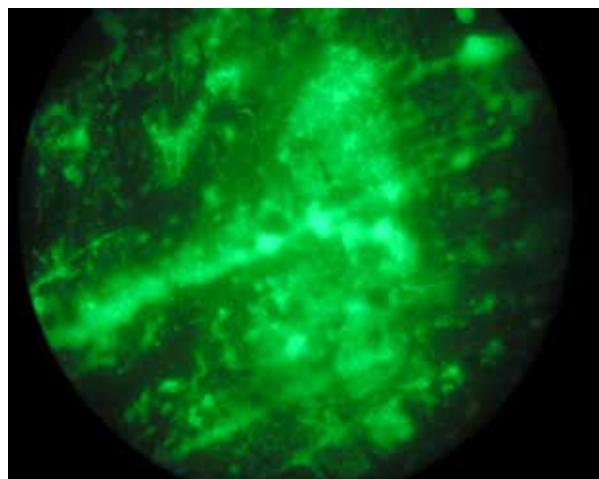
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idly elevated. A CSF sample was taken for microbiological analysis, and empirical antibiotic treatment was initiated with ceftriaxone plus amikacin. The antibiotic regimen was changed to intravenous amoxicillin-clavulanic acid five days later. The patient recovered well and was discharged four weeks after the brain tumor surgery.

The CSF specimen was centrifuged and Gram staining of its sediment was performed. Additionally, the sediment was cultured on 5% sheep blood agar, chocolate agar, and thioglycollate broth. Direct Gram staining of the CSF revealed the presence of polymorphonuclear leukocytes and round-ended Gram (+) rods. The overnight cultures yielded a growth of two types of colonies which were sporulating Gram (+) rods. They were identified as *Bacillus circulans* and *Paenibacillus macerans* via BBL Crystal™ GP identification panel (Becton Dickinson Company Ltd.) and traditional routine biochemical tests following the methods given in the Manual of Clinical Microbiology (Logan *et al.*, 2011).

Antimicrobial susceptibility was examined by broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010), using commercially prepared panel CMP2IHM Gram (+) (Sensititre, Trek Diagnostic Systems Ltd., UK). The CLSI interpretive standards, established for Staphylococci, were used since no interpretive standards have been established for *Bacillus* spp. (CLSI, 2010).

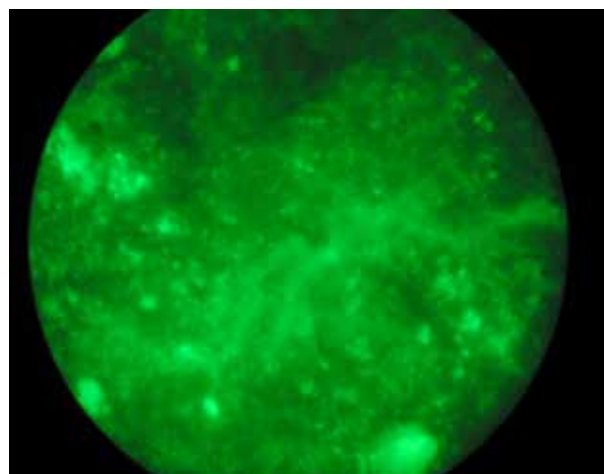
The microbiological characteristics of the two *Bacillus* spp. isolates were as follows: The colonies of *B. circulans* were spreading on blood agar, sporulating with subterminal spore, distending the cell. It grows on sheep blood agar as non-hemolytic, grey, and sticky to agar (adherent colonies). It is motile, produces catalase, and does not produce any acid from arabinose and cytochrome oxidase; BBL Crystal™ GP identification panel showed that it was 99,9% *B. circulans*. The strain was resistant to penicillin, ceftriaxone, chloramphenicol, and clindamycin, and was sensitive to amoxicillin-clavulanic acid, gentamicin, amikacin, vancomycin, levofloxacin, and meropenem. The isolate *P. macerans* was also sporulating with subterminal spore, not distending the cell. It grows as non-hemolytic, large (3-4 mm), off-white, and butyrous (in consistency) colonies; the species was motile, produces catalase and acid from arabinose and does not produce cytochrome oxidase; BBL Crystal GP panel showed that it was 99,3% *P. macerans*. This isolate was resistant to penicillin, ceftriaxone, and clinda-



**Fig. 1.** *Bacillus circulans* - an indirect fluorescent antibody test.

mycin, and was sensitive to amoxicillin-clavulanic acid, gentamicin, amikacin, vancomycin, chloramphenicol, levofloxacin, and meropenem.

Additionally, two serologic tests were performed 11 (the first serologic test) and 23 days (the second serologic test) after the onset of post-operative infection in patient, with cultures on solid media for both bacillary strains that were isolated from CSF. We performed an indirect fluorescent antibody test (FA) to measure the antibody levels of *Bacillus* spp. bacteria, as shown in Fig. 1 and Fig. 2.



**Fig. 2.** *Paenibacillus macerans* - an indirect fluorescent antibody test

Increasing antibody titers were detected: 16 from the first serologic test and 64 from the second one for *B. circulans*, and, respectively, 8 from the first test and 32 from the second for *P. macerans*, after twelve days.

Making an attempt at determining the mechanism of  $\beta$ -lactam resistance in the both *Bacillus* species, we performed  $\beta$ -lactamase testing using

two methods: a chromogenic cephalosporin test with nitrocephin paper disks (Cefinase, BBL), and Crude enzyme test with homogenates obtained by ultrasound distraction of the cells. The Crude test was performed with 5 µl of the cells and 30 µl nitrocephin solutions (500 mg/L). Beta-lactamase production has not been detected by these methods.

## Discussion

*Bacillus* spp. are aerobic Gram (+), spore-forming rods, normally found in soil, water, and decomposing organic matter (Logan *et al.*, 2011). Since *Bacillus* spp. are sometimes laboratory contaminants, isolation of the organisms from clinical specimens of sterile sites does not indicate infection unless they are detected in multiple sets (Galanos *et al.*, 2003). Our patient was considered to have a genuine, post-operative infection, despite that bacteria were isolated from CSF sample, and that we had clinical and laboratory data for central nervous system infection 10 days after operation (surgical excision of a brain tumor). These isolates were considered to be responsible for the infectious process, because direct Gram staining of the CSF demonstrated spore-forming Gram (+) bacilli, and increasing serum antibodies in double samples against both isolated bacteria were detected. The two serologic FA tests on patient for detection of antibodies can confirm the clinical diagnosis after cultures were obtained. These *Bacillus* spp. are less pyogenic microorganisms, which was the reason for late onset of this postoperative wound infection. The two bacillary strains are environmental organisms with relatively low pathogenicity, and they were probably introduced intraoperatively (the surgeons supposed it occurred by fibrin glue used for plastic surgery of dura). However, we could not establish the source of infection because we took the material for culturing of this fibrin glue from a new batch.

A few reports of infections due to *Bacillus* spp., mostly in immunocompromised individuals, have been published in the literature. *B. circulans* has been described as a cause of intravenous catheter-related bacteremia, cerebrospinal fluid shunt infections, prosthetic heart valve endocarditis, wound and joint infections (Logan *et al.*, 2011, Galanos *et al.*, 2003). *P. macerans* was due to pseudobacteremia (Noskin *et al.*, 2001, Teng *et al.*, 2003).

Most *Bacillus* spp. are sensitive to penicillin and ceftriaxone, which are a kind of empiri-

cal therapy for central nervous system infections (Weber *et al.*, 1988), but in our case *B. circulans* and *P. macerans* were resistant, and we had improvement in our patient after introduction of the intravenous antibiotics amikacin and later amoxicillin-clavulanic acid. We could not prove β-lactamase enzymes in both *B. circulans* and *P. macerans* species, but our hypotheses are that mechanisms of β-lactam resistance in *Bacillus circulans* and *Paenibacillus* are based on membrane-associated enzymes that have been rarely reported in other *Bacillus* spp. (Logan *et al.*, 2011)

Prevention of post-operative nosocomial infections is unfortunately not achieved even with the antibiotics used for prophylaxis. The most important aspect of prevention of nosocomial infections is strictly observed aseptic working.

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