# Late Prosthetic Joint Infection and Bacteremia by *Bacillus cereus* Confirmed by 16S rRNA Sequencing and Hip Joint Tissue Pathology

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Bacillus cereus is a widespread organism in nature and a member of the *B. cereus* group of catalase-positive, aerobic, spore-forming, Gram-positive bacilli. *B. cereus* found in blood is often dismissed as a contaminant in the absence of repeated isolation from multiple cultures. Soft tissue and bone infection due to *B. cereus* have been associated with trauma, intravenous drug use, and an immunocompromised

state. We report a very late prosthetic joint infection of the hip joint and consequent bacteremia caused by *B. cereus*, which occurred 13 years after total hip replacement surgery in the absence of recent trauma or intervention. (Ann Clin Microbiol 2016;19:54-57)

**Key Words:** Arthritis, Bacillus cereus, Bacteremia, Prosthesis, 16S rRNA

### INTRODUCTION

Bacillus cereus is a widespread organism in nature and one of the *B. cereus* group, which is catalase-positive, aerobic, spore-forming gram-positive bacilli [1]. *B. cereus* rarely causes severe infections such as bacteremia or endocarditis and most of them are central line-related infections [2]. *B. cereus* from blood has been easily dismissed as a contaminant without repeated blood isolation from multiple cultures. Soft tissue and bone infection due to *B. cereus* has been associated with trauma, intravenous drug use, and immune compromised states [3]. We report very late prosthetic joint infection at hip joint and consequent bacteremia by *B. cereus*, which occurred in the 13 years after post total hip replacement operation without recent history of trauma or intervention. This is very rare case of late prosthetic joint infection by *B. cereus*, necessitating caution to discriminate true infection from contamination of *B. cereus*.

### CASE REPORT

We report very late prosthetic joint infection at hip joint and consequent bacteremia by *B. cereus*, which occurred in the 13 years after post-total hip replacement. A 61-year-old female with underlying diabetes mellitus and heart failure was admitted to the orthopedic ward for evaluation of left hip pain with an impression of prosthetic joint infection. This patient had undergone total left hip replacement due to pyogenic sequelae of hip 13 years earlier. Laboratory tests showed neutrophilia (WBC 13.40×10<sup>9</sup>/L, neutrophil 77.5%), increased erythrocyte sedimentation rate (40 mm/hr) and increased C-reactive protein (19.76 mg/dL).

On the second day of her admission, she developed fever with temperature to 38.4°C and gram-positive bacilli were isolated from one aerobic bottle in total six blood bottles (three aerobic and three anaerobic), which identified as *Bacillus* species by MicroScan Pos Combo Panel Type 39 (Siemens, Munchen, Germany). Antimicrobial susceptibility cannot be

Received 3 May, 2016, Revised 27 May, 2016, Accepted 9 June, 2016

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done due to lack of standardization. WBC or organisms was not shown in Gram stain of synovial fluid but Gram positive bacilli was cultured in synovial fluid and identified as Bacillus species. The 1522-bp portion of the 16S rRNA gene was amplified from plate-grown bacteria by using forward (AGAGTTTGATCCT GGCTCAG) and reverse (AAGGAGGTGATCCAGCCGCA) primers. The sequence was compared with National Center for Biotechnology Information GenBank entries by using the BLAST algorithm. Definite prosthetic joint infection was confirmed by B. cereus isolations from two more separate synovial fluid obtained by arthrocentesis. On the 5th day of her admis-

Fig. 1. Polymorphonuclear leukocytes and nuclear debris with fibrinous exudate (Hematoxylin-esoin stain, ×400).

sion, removal of implant with synovectomy was done and severe tissue adhesion and abscess discharge were noted at operation site. Polymorphonuclear leukocytes infiltration (Fig. 1) and clustered gram-positive bacilli in fibrinous exudate (Fig. 2) were seen in Gram stain of hip joint tissue section. Closed fractures of other parts of femur were removed by arthrotomy. Intravenous cefazolin and amikacin were given empirically during the first five days and it changed to vancomycin after B. cereus isolations. The patient showed good clinical response in eight days after definite therapy (vancomycin).

## DISCUSSION

The Genus Bacillus includes B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis, B. weihenstephanensis, and B. anthracis, which are rarely associated with disease, except species of the B. cereus group. The capability for spore formation enables Bacillus spp. to exhibit incredible longevity, so Bacillus spp. can be found in virtually every type of environment [4]. B. cereus can produce  $\beta$ -lactamases and they can be resistant to penicillins and the third-generation cephalosporins, so vancomycin is generally considered the preferred antibiotic choice [5].

B. cereus is a well-known cause of food poisoning and involved in both nosocomial outbreaks and pseudoepidemics associated with disinfectants, gloves and blood culture media contaminated by B. cereus [6]. Nosocomial outbreaks due to contaminated medical equipment such as ventilators and dialysis circuits were also reported [7]. Regarding orthopedic infections,

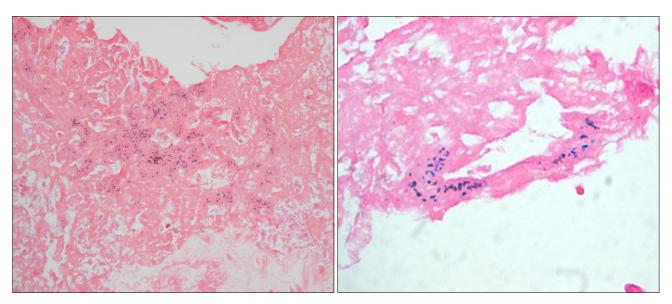


Fig. 2. Clustered Gram-positive bacilli in fibrinous exudate (Gram stain, ×400, ×1,000).

B. cereus could be suspected of causative agent when associated with trauma [8].

In this case, the isolation of B. cereus from synovial fluid was thought to be uncommon because the patient had no recent history of trauma or intervention. In this case report, the patient had hip arthroplasty thirteen years ago but majority of postop prosthetic joint infection occurs within the first 30 days of surgery (85%, median 14 days) and infections after first year were diagnosed between 2 and 10 years after the surgery [9]. One possible explanation is that the implant material had been persistently colonized with B. cereus for thirteen years via biofilm forming potential of spores and vegetative cells of Bacillus species [10]. Biofilms on surfaces of implants, which are complex communities of microorganisms embedded in an extracelluar matrix, are resistant to even adequate antibiotic therapy and result in false negative culture results [11].

Staphylococcus aureus and coagulase negative staphylococci have been known as common etiologic agents of prosthetic joint infection [12] but Bacillus spp. is not. There few reports of B. cereus infection other than food-poisoning. Akesson et al. [8] reported that B. cereus were isolated from postoperative and post-traumatic wounds in twelve patients with clinical infection signs. Dubouix et al. [13] reported forty-one B. cereus infected cases, which were suspected of terrestrial environmental contamination before admission. Gallo et al. [14] reported two culture-negative cases of orthopedic-implant-related infection that demonstration of B. cereus using a PCR-mass spectrometric technology and bacterial fluorescence in situ hybridization (FISH) of tissue. This case shows the difficulty to diagnose biofilm-associated orthopedic implant infection with conventional microbiological culture methods. The direct species-specific fluorescence in situ hybridization (FISH) can visualize B. cereus biofim in this study. In Korea there are two cases of posttraumatic B. cereus endophthalmitis [15] and one case of B. cereus pneumonia with bacteremia [3].

In conclusion, we report very late prosthetic joint infection at hip joint and consequent bacteremia by B. cereus, which occurred after 13 years post total hip replacement operation without recent history of trauma or intervention. This is rare case of very late prosthetic joint infection by B. cereus, necessitating caution to discriminate true infection from contamination of B. cereus

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## =국문초록=

# 16S rRNA 염기서열분석과 조직 병리소견으로 진단된 Bacillus cereus에 의한 인공관절 감염과 균혈증

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Bacillus cereus는 B. cereus group 중의 하나로 자연에 널리 분포하며, catalase 양성, 호기성, 아포생성, 그람양성 간균이다. 혈액에서 B. cereus가 분리되면 여러 번 시행한 배양에서 반복 분리되지 않으면 흔히 오염으로 간주되기 쉽다. B. cereus 에 의한 연조직이나 골감염은 흔히 외상이나 정맥약물 투여나 면역저하와 관련이 있다. 저자들은 고관절의 인공관절 수술 후 13년이나 지나 최근 외상력이나 중재술과 관련 없이 발생한 매우 드문 B. cereus 인공관절 감염과 패혈증을 보고 한다. [Ann Clin Microbiol 2016;19:54-57]

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