Use of Blood Culture Bottles to Incubate Irrigation Water from the Surgical Site May Improve Detection of Causative Organisms in Pyogenic Vertebral Osteomyelitis

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Research Article

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Abstract

Background Identification of pathogenic microorganisms are essential for pyogenic vertebral osteomyelitis (PVO). This study aimed to demonstrate the effectiveness of the blood culture bottle (BCB) system in identifying PVO causative organisms.

Materials & Methods We analyzed retrospectively collected data from patients who underwent full-endoscopic spine surgery for PVO between January 2016 and March 2019. Irrigation water generated in the surgical field was incubated in the BCB system, and compared with blood culture before surgery and tissue culture taken by the conventional method. The microbial identification rate and the time from sample collection to microbial identification of the BCB system were compared with conventional blood culture and tissue cultures using the Wilcoxon signed-rank test.

Results We included 17 patients (12 men, five women; mean age, 72.8 ± 11.9 years). Bacteria were cultured from 3 (17.6%), 13 (76.5%), and 12 (70.6%) patients by blood culture, tissue culture, and the BCB system, respectively. Tissue culture and the BCB system had significantly higher detection rates than blood culture (P = .002, P = .003), and there was no significant difference between tissue culture and BCB system (P = .655). In 15 cases (88.2%), the causative organism was identified by at least one method. In two cases, the causative organism (Escherichia coli) was only identified by the BCB system. The BCB system required amount of time for microbial identification (3.9 ± 3.0 days), compared with the time required for blood culture (5.0 ± 1.4 days, P = .180) and tissue culture (11.9 ± 15.1 days, P = .012).

Conclusions Results suggest the possibility of improving the detection rate and time to detection of causative organisms by the BCB system as an adjunct to the conventional method.

background

In pyogenic vertebral osteomyelitis (PVO), accurate identification of the pathogenic microorganism ensures prudent and targeted antimicrobial therapy and improves patient outcomes. Accurate identification also reduces relapse, the emergence of resistant microorganisms, adverse events, and the cost of treatment [1]. An accurate microbiological diagnosis is necessary to establish an effective antibiotic treatment. However, this has long been a clinical challenge. The risk of treatment failure and fear of resistant bacteria lead physicians to overuse broad-spectrum antibiotics, possibly leading to the selection and multiplication of drug-resistant microorganisms.

Conventional methods for identifying PVO-associated microorganisms include collecting blood culture before antimicrobial administration and culture of tissue in medium. According to past reports, the positive rate of blood culture is 40–80% [2, 3], and that of tissue culture is 47–70% [3–5], neither of which is a satisfactory detection rate.

In adults, when PVO is suspected, an open surgical biopsy is considered the gold standard for tissue sampling [6]. However, the infection area or abscess occurs only at the subchondral bone of the vertebral
bodies or in the intervertebral discs. Because open surgical biopsies are invasive, costly, and carry the risks of general anesthesia and major surgery, percutaneous computed tomography (CT)-guided biopsy or full-endoscopic spine surgery (FESS) and drainage is often attempted [7, 8]. However, these methods have a lower detection rate than open biopsy [8]. It is often challenging to collect appropriate specimens because the tissue collected is often necrotic, or specimens cannot be collected from relevant sites.

The method of culturing non-blood specimens in blood culture bottle (BCB) has been helpful in other fields, including articular fluid [9, 10], pleural fluid [11], ascites [12], vitreous in endophthalmitis [13], and bronchoalveolar lavage fluid in pneumonia [14]. According to the Proceedings of the Second International Consensus Meeting on Musculoskeletal Infection 2018 [15], specimens collected from patients with periprosthetic joint infection should be cultured in BCB if possible. It is also recognized that specimens should be cultured in BCB to increase the diagnosis rate.

Improved methodology is essential to capture the causative organisms in a case of PVO with an infected abscess in the subchondral bone or intervertebral disc with no apparent surrounding abscess on magnetic resonance imaging (MRI). We have been trying to improve the detection rate of the causative organism by using the BCB system, in which irrigation water generated during FESS and drainage is cultured from patients who are not eligible for open surgery. Herein, we aimed to demonstrate the effectiveness and usefulness in identifying the causative agents of culturing irrigation water from surgical sites using BCB.

**Materials & Methods**

This was a retrospective, single-center, case-control study. All patients permitted our institutional review board and obtained informed consent.

We analyzed the collected data of patients with PVO who underwent FESS between January 2016 and March 2021. The patients were diagnosed with PVO or had inflammation on MRI and elevated systemic inflammatory markers, strongly supporting PVO diagnosis. Open surgery was not performed due to the local abscess in the subchondral bone of the vertebral bodies or the intervertebral discs, and so the patients underwent FESS and drainage. We collected the irrigation water, which was incubated in BCB (Aerobic BACTEC Peds Plus/F culture vials and anaerobic BACTEC Lytic/10 Anaerobic/F culture vials; Becton Dickinson Sparks, Maryland, USA) system. We also collected conventional tissue culture from the disc or cartilage endplate. Two sets of blood cultures were taken before surgery.

Further, we included patients that had been treated with intravenous or oral antibiotics before visiting our institution. We excluded patients with neurological deterioration or the presence of an epidural abscess. Based on these criteria, 17 patients were included in the study. Patient demographics included age, sex, the period from consultation to operation, intervertebral level of lesions, the use of antibiotics within three weeks before their operation, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum white blood cells (WBCs), length of hospital stay, causative organism, sensitivity, the time required for microbial identification, and complications.
Surgical Technique

FESS and drainage were performed under local or general anesthesia. FESS is the least invasive disc surgery procedure [16]. We collected the irrigation water generated from the surgical field, and samples were injected into both an aerobic and an anaerobic BCB. Immediately, samples were sent to the microbiology laboratory, where the BCB were loaded into the BACTEC instrument in the appropriate, computer-assigned position. Tissue samples with visual signs of inflammation, granulation, necrosis, or purulence were placed in sterile containers without the medium. Depending on the type of organism, it was necessary to outsource the tissue to an external organization, which sent the results to our hospital.

Statistical Analysis

All descriptive statistics were calculated as the mean and standard deviation. The microbial identification rate and the time from sample collection to microbial identification of the BCB system were compared with conventional blood culture and tissue cultures using the Wilcoxon signed-rank test. Significance was set at $P<.05$. Statistical analysis was performed using SPSS ver.27 (Stata Corp, USA).

Results

There were 12 men and five women patients. The mean age was 72.8 ± 11.9 years (range, 47 to 87 years) at the time of the operation. The period from consultation to the procedure was 3.5 ± 6.3 days. The intervertebral levels of the lesions were T12/L1, 1 lesion; L1/2, 3 lesions; L2/3, 3 lesions; L3/4, 5 lesions; L4/5, 4 lesions; and L5/S1, 2 lesions, including duplicate patients. Four patients (23.5%) had been given intravenous or oral antibiotics within three weeks of visiting our institution. The mean CRP at the first visit was 7.3 ± 7.5 mg/dL, the 1-hour ESR was 73.0 ± 30.3 mm / h, and the WBCs count was 86.2 ± 37.5 × 10^3 / µL. The mean length of hospital stay was 65.8 ± 21.5 days (Table 1).

Table 2 lists microorganisms identified using blood culture method (3 [17.6%]), the tissue culture method (13 [76.5%]) and the BCB culture method (12 [70.6%]). Tissue culture and the BCB system had significantly higher detection rates than blood culture ($P=.002$, $P=.003$), and there was no significant difference between tissue culture and BCB system ($P=.655$). Both methods detected the causative organism in 15 of the 17 cases (88.2%). In the four patients who had taken antibiotics three weeks before obtaining the specimens, the microbial identification rate of the BCB system was 75% (3 patients) and tissue culture was 50% (2 patients). The detected causative organism is shown in Table 3. One of the patients had a polymicrobial infection. In two patients (11.8%), *Escherichia coli* could be identified only with the BCB system, while the results of the other two culture methods were negative. Three patients showed positive microbial growth with the tissue culture method when the blood culture and BCB system results were negative and detected *Staphylococcus aureus, Propionibacterium acnes*, and a duplicate case (*Enterococcus faecium* and *Candida albicans*). The BCB system required amount of time for microbial identification (3.9 ± 3.0 days [range, 2 to 13 days]), compared with the time required for blood
culture (5.0 ± 1.4 days [range, 3 to 6 days]; P = .180) and tissue culture (11.9 ± 15.1 days [range, 2 to 60 days]; P = .012). There were no cases in which different causative organisms were detected depending on the culture method. There was no complication due to the surgery.

**Discussion**

We demonstrated that the BCB system was superior to conventional culture methods using blood culture and tissue culture both in the microbial identification and the time required. In this study, the detection rate was as high as 88.2% by either method, even with low inflammation and local abscess.

When the patient is in poor general condition due to infection, it is essential to identify the causative organism as soon as possible. In this study, the time to identification was shorter for BCB culture than for other culture methods, which agrees with other studies [17, 18]. Early and appropriate antimicrobial therapy may contribute to improved treatment outcomes and shorter treatment periods.

If antimicrobial agents have already been administered, the detection rate of the causative organism will be lower. However, it has been reported that using BCB as a culture medium can improve organism detection in these patients. The BCB contains resins that neutralize antimicrobial agents [19], and BCB contains enriched growth media and specific media to remove antibiotics, allowing for the growth of microorganisms [20]. The dilution effect of placing an inoculum in the liquid medium in a BCB might decrease the inhibitory effects of antibiotics [9]. BCB contains lytic agents such as saponin, which may assist in releasing and recovering the microorganisms phagocytized by WBCs [21]. From the above, it is suggested that culturing infectious substances in BCB is significant for improving the detection rate of the causative organism.

In spinal infection, diagnostic culture yield is higher in open biopsies than percutaneous biopsies [22]. Open surgical biopsies are generally performed in more severe cases, requiring surgical management [1]; these cases are presumed to correlate with higher pathogen loads [22]. In our study, we excluded cases that did not result in an open biopsy, so there is a possibility that included cases contained fewer pathogens. As proof of this, *Staphylococcus aureus*, which is the most detected organism according to previous reports [3, 23], was found in only three cases in this study. Therefore, the bacteria found are different from the normal distribution of culture results in this study, as a result the identification rate of samples incubated in blood culture may tended to be lower than reported in other studies [2, 3]. Of these, the BCB system was able to identify 88.2% of causative organisms which is higher than past reports [3–5], and there were two cases in which the organism could only be detected by the BCB system. The use of BCB to incubate the irrigation water from the surgical site may improve the detection rate of causative organisms.

Although the presence of false positives cannot be accurately assessed, we believe that false positives are rare at this time because there were no cases in which the bacteria detected in conventional blood cultures and tissue culture differed from those obtained from the BCB system. To control for the possibility of the test yielding a high number of false positives due to contaminants, we verified that
organisms grown in culture were consistent with those known to cause PVO. Thus, repeat biopsies may be helpful in clinically challenging cases [24, 25].

The BCB system is a low-cost, easy-access culture system that is effective for rapid microbial identification. The advantages of this technique are that it can be used to clean and examine infected areas in cases that do not require open biopsy. It may be useful in improving detection rates based on the results of this study. This study may aid in improving the microbial diagnosis, enabling early and targeted antimicrobial therapy, and reducing complications of PVO.

This study had several limitations. First, we examined only the sensitivity of each culture method, not the specificity. This was because we only included patients highly suspected of having infections. Second, it would be better to include a description of cases in which PVO was strongly suspected. Still, we could not wholly exclude patients with non-infectious diseases from the cases. Although contamination is a problem in bacteria detection, it may be solved by increasing the number of culture bottles, as in two sets of blood cultures, etc., since more detection methods contribute to reliable identification. Third, our study included a small number of participants, which necessitates confirmation of our findings in a larger population.

Further studies on the detailed diagnostic performance of the BCB culture system and the modification of our current protocol for enhancing microbial growth are warranted.

**conclusions**

In cases of infectious spondylitis without obvious abscess, where the causative organism is difficult to identify, the possibility of improving the detection rate and reducing the time required for microbial identification of the causative organism was suggested by culturing the irrigation water from the surgical site using a BCB as an adjunct to the conventional collection of blood culture and tissue culture. This technique can be performed simultaneously with washing the infection site and should be considered for addition in the future because of its convenience and versatility.

**abbreviations**

PVO; pyogenic vertebral osteomyelitis, BCB; blood culture bottle, CT; computed tomography, FESS; full-endoscopic spine surgery, MRI; magnetic resonance imaging, CRP; c-reactive protein, ESR; erythrocyte sedimentation rate, WBCs; white blood cells

**Declarations**

**Ethics approval and consent to participate:** The study was approved by our Institutional Review Board and was conducted according to the principles of the Declaration of Helsinki. Ethical approval was obtained from the Mito Kyodo General Hospital's institutional ethics committee (application no.21-16). Informed consent was obtained from all subjects and/or their legal guardians.
Consent for publication: Informed consent was obtained from all participants in this study.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: Not applicable

Funding: Not applicable

Authors’ contribution: Shun Okuwaki wrote and prepared the manuscript, and Masaki Tatsumura participated in the study design and analysis of the data. All authors reviewed and approved the manuscript.

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Tables

Table1 Demographic data
**Characteristic** | **Value**  
--- | ---  
Age (range) years | 72.8±11.9\(\text{47-87}\)  
Men (%) | 12\(\text{70.6}\)  
Women (%) | 5 (29.4)  
Period from consultation to operation in days (range) | 3.5 ± 6.3\(\text{0-20}\)  
**Level** |  
T12/L1 | 1  
L1/2 | 3  
L2/3 | 3  
L3/4 | 5  
L4/5 | 4  
L5/S1 | 2  
Antibiotics within 3 weeks prior to operation (%) | 4\(\text{23.5}\)  
**Laboratory parameters** |  
C-reactive protein (range) mg/dL | 7.3 ± 7.5\(\text{0.08-29.1}\)  
Erythrocyte sedimentation rate (range) mm/hr | 73.0 ± 30.3\(\text{17-132}\)  
Serum white blood cell count (range) \(\text{X10}\text{3/\(\mu\)L}\) | 86.2 ± 37.5\(\text{43-147}\)  
Length of hospital stay (range) days | 65.8 ± 21.5\(\text{37-107}\)  

**Table 2** Detection rate of causative bacteria for each culture method

|          | Blood culture | Tissue culture | BCBsystem | Total  |
|----------|--------------|---------------|-----------|--------|
| Positive (%) | 3(17.6) | 13(76.5) | 12(70.6) | 15(88.2) |
| Negative (%) | 14(82.4) | 4(23.5) | 5(29.4) | 2(12.8) |

BCB; blood culture bottle

**Table 3** Detected species
| Organism                                      | Blood culture | Tissue culture | BCB system | Total |
|-----------------------------------------------|---------------|----------------|------------|-------|
| group B β-hemolytic streptococci (*Streptococcus agalactiae*) | 0             | 3              | 3          | 3     |
| *Escherichia coli*                            | 0             | 2              | 4          | 4     |
| *Staphylococcus aureus*                       | 1             | 3              | 2          | 3     |
| *Candida albicans*                            | 0             | 2              | 1          | 2     |
| *Staphylococcus schleiferi*                   | 1             | 1              | 1          | 1     |
| *Streptococcus constellatus*                  | 1             | 1              | 1          | 1     |
| *Enterococcus faecium*                        | 0             | 1              | 0          | 1     |
| *Propionibacterium acnes*                     | 0             | 1              | 0          | 1     |

BCB; blood culture bottle