Effective and Rapid Microbial Identification in Pediatric Osteoarticular Infections Using Blood Culture Bottles

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Background: The detection and identification of pathogenic microorganisms are essential for the treatment of osteoarticular infection. However, obtaining a sufficient amount of specimen from pediatric patients is often difficult. Herein, we aimed to demonstrate the effectiveness of the blood culture bottle (BCB) system in pediatric osteoarticular infections. We hypothesized that our BCB culture method is superior to the conventional swab and tissue culture methods in terms of required specimen size, incubation time, and microbial identification rate.

Methods: We analyzed the prospectively collected data of pediatric patients who underwent surgical treatment for osteoarticular infections between August 2016 and October 2019. Four needles were dipped in the infected fluid or tissue during the surgical procedure as soon as the infected area was exposed and were used to inoculate 2 aerobic pediatric BCBs and 2 anaerobic general BCBs. We also collected 2 conventional swab samples and 2 tissue samples from the identical area. The microbial identification rate and the time required for identification were compared between BCB, swab, and tissue cultures.

Results: Forty patients constituted the study group; 13 patients had osteomyelitis, 17 patients had septic arthritis, and 10 patients had both. Of these 40 patients, the microbial identification rate was higher with BCB cultures (27 [68%]) than with swab cultures (18 [45%]; $p = 0.004$) or tissue cultures (15 [38%]; $p < 0.001$). Nine samples (9 patients [23%]) were only positive in the BCB culture. Positive microbial growth was not detected with conventional culture methods when microorganisms did not grow on the BCB culture. Compared with swab culture (4.3 ± 1.1 days; $p < 0.001$) or tissue culture (4.4 ± 1.1 days; $p < 0.001$), the BCB culture reduced the time required for microbial identification (3.5 ± 0.9 days).

Conclusions: In pediatric osteoarticular infections, the BCB culture system improved the microbial identification rate, reduced the time to identification, and permitted a smaller-volume specimen, compared with traditional culture systems.

Level of Evidence: Diagnostic Level II. See Instructions for Authors for a complete description of levels of evidence.

Osteoarticular infections may cause various complications, including osteonecrosis, pathologic fracture, and early-onset osteoarthritis[1,2]. Furthermore, in growing children, osteoarticular infections may be complicated with physeal growth disturbances[3,4]. Sequelae resulting from these infections are particularly unfortunate for pediatric patients as they will likely sustain a lifelong disability. The annual estimated number of pediatric osteoarticular infection cases increased from 11,527 in 1997 to 14,786 in 2012 in the United States, which resulted in higher treatment costs[5]. The accurate identification of the pathogenic microorganism ensures prudent and targeted antimicrobial therapy, improves patient outcome, and reduces the cost of treatment[6].

The pathogenic microorganism of osteoarticular infections has been identified by the microbial culture of infected fluid or tissue using conventional agar and broth media[7,8]. However, conventional media have a limited success rate (41% to 63%) in children[9,10]. Although current molecular biological techniques such as multiplex polymerase chain reaction (PCR) or matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry may assist in detecting microorganisms[11,12], they are not widely available. The blood

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culture bottle (BCB) method is a low-cost, easy-access culture system that has proven to be effective for rapid microbial identification in periprosthetic joint infections[13-18]. However, unlike in adult patients, the amount of synovial fluid in young children is often too little to aspirate and inoculate in the BCB. In some patients with osteomyelitis, the purulent fluid is too thick and viscous to be aspirated with syringes. In addition, an insufficient amount of specimen hinders the differentiation of contaminants from true pathogens, as multiple samples cannot be acquired[19].

In August 2016, we designed a prospective study to identify an effective culture method in pediatric osteoarticular infections, in which the amount of specimen is limited. We used commercially available aerobic pediatric BCBs: pediatric BCBs differ from adult BCBs in terms of formulation and broth-to-blood volume ratio to enhance microorganism growth in a small amount of blood[20].

The purpose of this study was to demonstrate the effectiveness of the BCB culture system in identifying the causative agents of pediatric osteoarticular infections. We hypothesized that our BCB culture method is superior to the conventional swab and tissue culture methods, in terms of required specimen size, incubation time, and microbial identification rate.

Materials and Methods

Patients

This study was approved by our institutional review board. We prospectively collected the list of patients who underwent a surgical procedure at a single tertiary-care pediatric center due to a high suspicion of musculoskeletal infection from August 1, 2016, to October 31, 2019. The inclusion criteria for this study were the diagnosis of septic arthritis and/or osteomyelitis of the extremities and patient age of <18 years at the time of the operation. We excluded patients with autoimmune diseases such as juvenile idiopathic arthritis or Behcet disease-associated arthritis, mycobacterial infections including tuberculosis and Bacille Calmette-Guérin (BCG) osteomyelitis, and chronic granulomatous disease. A pediatric infectious disease specialist (E.H.C.) reassessed the medical records of the patients with an uncertain diagnosis. Based on these criteria, 40 patients constituted the study group (Fig. 1). In 3 patients who had simultaneous infections at multiple sites, data on the site that was most severely infected were used.

There were 25 male patients and 15 female patients (Table I). The mean age (and standard deviation) was 7.2 ± 5.3 years (range, 0.1 to 17 years) at the time of the operation. All 40 patients underwent magnetic resonance imaging (MRI) prior to the operation to evaluate the possibility of causes other than infection, coexisting contiguous osteomyelitis, or septic arthritis, and the size and location of the abscess. Twenty patients (50%) had been administered intravenous or oral antibiotics within the 3 weeks prior to visiting our institution. For the 20 remaining patients (50%), we started administering intravenous cefazolin immediately after obtaining culture specimens during the surgical procedure. Infections subsided rapidly in every patient after the surgical procedure, without recurrence.

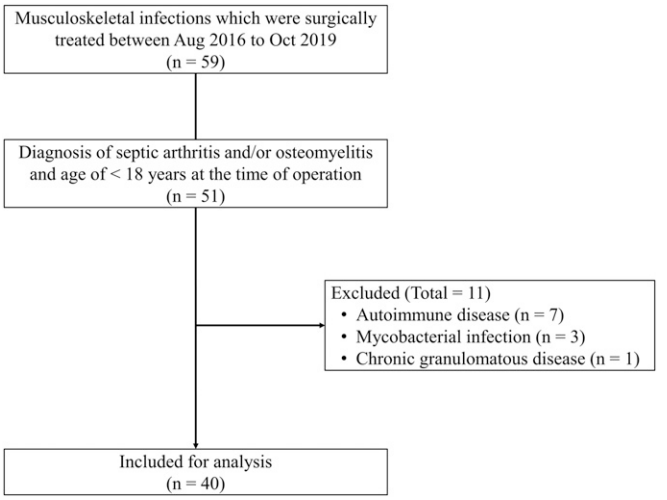

Fig. 1

Flowchart of the study population.

Sample Collection

Our procedure for microbial culture was as follows (Fig. 2). The surgical site skin was prepared with a Betadine scrub solution (7.5% povidone-iodine), followed by a solution of 0.5% chlorhexidine gluconate in 70% isopropyl alcohol and, finally, by a 10% povidone-iodine solution. The specimen was obtained immediately after the infected area was exposed: after arthrotomy in septic arthritis or after cortical fenestration in osteomyelitis with an abscess. For 10 patients with both septic arthritis and contiguous osteomyelitis, the specimen was obtained from the site where a larger amount of specimen was available (synovial fluid in 6 patients and abscess of the bone in 4 patients). First, a 15 to 30-mm length of each of four 21-gauge needles was merely dipped in the infected fluid without aspiration. After removing the plastic flip-cap from the BCBs (2 aerobic BACTEC Peds Plus/F culture vials and 2 anaerobic BACTEC Lytic/10 Anaerobic/F culture vials; Becton Dickinson), the rubber stopper on each bottle was wiped with 80% isopropyl alcohol[19]. Each needle was used to prick the rubber stopper on each bottle up to a length of about 20 to 30 mm without delay. The BCBs were manually inverted twice gently with the needle still penetrating the rubber stopper to allow contact between the needle and the broth. Then 2 swab cultures were obtained by passing a sterile swab over the infected tissue, bone, or fluid and then immediately placing the swab in the transport medium (Transystem 134C; COPAN Diagnostics). Solid samples with visual signs of inflammation, granulation, necrosis, or purulence were obtained and were placed in 2 sterile containers without medium. Eventually, 2 sets of BCBs, 2 swab samples, and 2 tissue samples were transported to the microbiology department[13]. Throughout the procedures, special care was taken to avoid contamination.

Microbial Culture

The BCBs were incubated at 35°C for up to 5 days using BACTEC FX instruments (Becton Dickinson) according to the manufacturer's instructions. Gram stain and subculture were

TABLE I Demographic and Preoperative Biochemical Characteristics of Study Subjects (N = 40)

Characteristic	Value
Age at operation* *(yr)*	7.2 ± 5.3 (0.1 to 17)
Male sex†	25 (63%)
Symptom onset to operation* *(days)*	19 ± 23 (1 to 110)
Diagnosis†	
Septic arthritis	17 (43%)
Septic arthritis with contiguous osteomyelitis	10 (25%)
Acute hematogenous osteomyelitis	7 (18%)
Subacute osteomyelitis	4 (10%)
Chronic osteomyelitis	2 (5%)
Site involved† ‡	
Joint	
Sacroiliac	1 (3%)
Hip	9 (23%)
Knee	7 (18%)
Ankle	6 (15%)
Glenohumeral	3 (8%)
Elbow	1 (3%)
Bone	
Ilium	1 (3%)
Femur	4 (10%)
Tibia	4 (10%)
Fibula	4 (10%)
Tarsal	2 (5%)
Metatarsal	1 (3%)
Humerus	5 (13%)
Radius	1 (3%)
Metacarpal	1 (3%)
Antibiotics within 3 weeks prior to operation†	20 (50%)
Body temperature* *(°C)*	37.5 ± 1.1 (36.1 to 40.1)
Laboratory parameters*	
C-reactive protein *(mg/dL)*	5.3 ± 5.2 (0.01 to 21.9)
Erythrocyte sedimentation rate *(mm/hr)*	43 ± 35 (2 to 120)
Serum WBC count *($\times 10^3/\mu L$)*	11.1 ± 5.4 (3.1 to 26.4)
Segmented neutrophils in serum *(%)*	54 ± 16 (7 to 79)
Synovial fluid WBC count§ *($\times 10^3/\mu L$)*	96 ± 80 (27 to 295)
Segmented neutrophils in synovial fluid§ *(%)*	89 ± 8 (65 to 97)
Length of hospital stay* *(days)*	22 ± 8 (7 to 45)

*The values are given as the mean and the standard deviation, with the range in parentheses. †The values are given as the number of patients, with the percentage in parentheses. ‡Data with regard to the most severely infected site were used in 3 patients who had simultaneous infections at multiple sites. §These data were obtained from 12 patients with septic arthritis.

performed using a blood agar plate (BAP) and MacConkey agar for the BCBs that showed positivity[21]. Swab specimens were inoculated on BAP and MacConkey agar, which were incubated at 35°C with 5% CO_2 for up to 2 days[22]. The tissue specimens were mixed with sterile distilled water and were homogenized with a BagMixer 100 MiniMix VP (Interscience)[22] and then were inoculated on 4 types of solid media (BAP, MacConkey agar, Brucella agar, phenylethyl alcohol agar) and 1 liquid medium (brain heart infusion broth). Subsequently, the media were incubated aerobically at 35°C for 2 days and anaerobically at 35°C for 3 days. The isolated colonies on the plates were identified using a MicroScan WalkAway (Beckman Coulter), VITEK 2 (bioMérieux), and MALDI Biotyper (Bruker Daltonik).

We defined contaminants as normal skin flora, including coagulase-negative staphylococci, micrococci, diphtheroids, or gram-negative bacilli, that grew only on 1 of 6 samples: 2 sets of BCBs, 2 swab samples, and 2 tissue samples[19,23]. Growth of the potential contaminants in ≥2 of 6 samples in immunocompromised patients or in patients with implants was considered a clinically relevant infection.

Statistical Analysis

All descriptive statistics were calculated as the mean and the standard deviation. The microbial identification rate of the BCB culture was compared with those of conventional swab and tissue cultures using the McNemar test. The time from operation to microbial identification in BCB culture was compared with those of conventional swab and tissue cultures using the Wilcoxon signed-rank test. Significance was set at $p < 0.05$. Statistical analysis was performed using Stata 15.1 (StataCorp).

Results

Table II enumerates microorganisms identified using each culture method. None of the patients had a polymicrobial infection. For cases in which *Staphylococcus hominis, Micrococcus luteus,* or methicillin-resistant *Staphylococcus epidermidis* was isolated in only 1 of 6 samples, the isolated microorganism was considered a contaminant. *Staphylococcus capitis* was isolated in a patient with an implant (intramedullary rod in the tibia) and methicillin-susceptible *S. epidermidis* was isolated in an immunocompromised patient. These cases were considered as a clinically relevant infection as the microorganisms were isolated in 2 of the 2 sets of BCBs[17,24,25]. Considering that Peptoniphilus is an obligatory anaerobe and not normal skin flora, it was considered a true pathogen even though it was isolated in only 1 of the 2 BCB sets[26,27].

In the 40 patients, the microbial identification rate of the BCB culture method (27 [68%]) was higher than that of the swab culture method (18 [45%]; $p = 0.004$) and the tissue culture method (15 [38%]; $p < 0.001$). In 9 patients (23%), the pathogen could be identified only with the BCB culture method, whereas the results of the other 2 culture methods were negative. None of the patients showed positive microbial growth with the swab or tissue culture method when the BCB culture results were negative. In a subgroup of 15 patients confirmed to harbor methicillin-susceptible *Staphylococcus aureus*

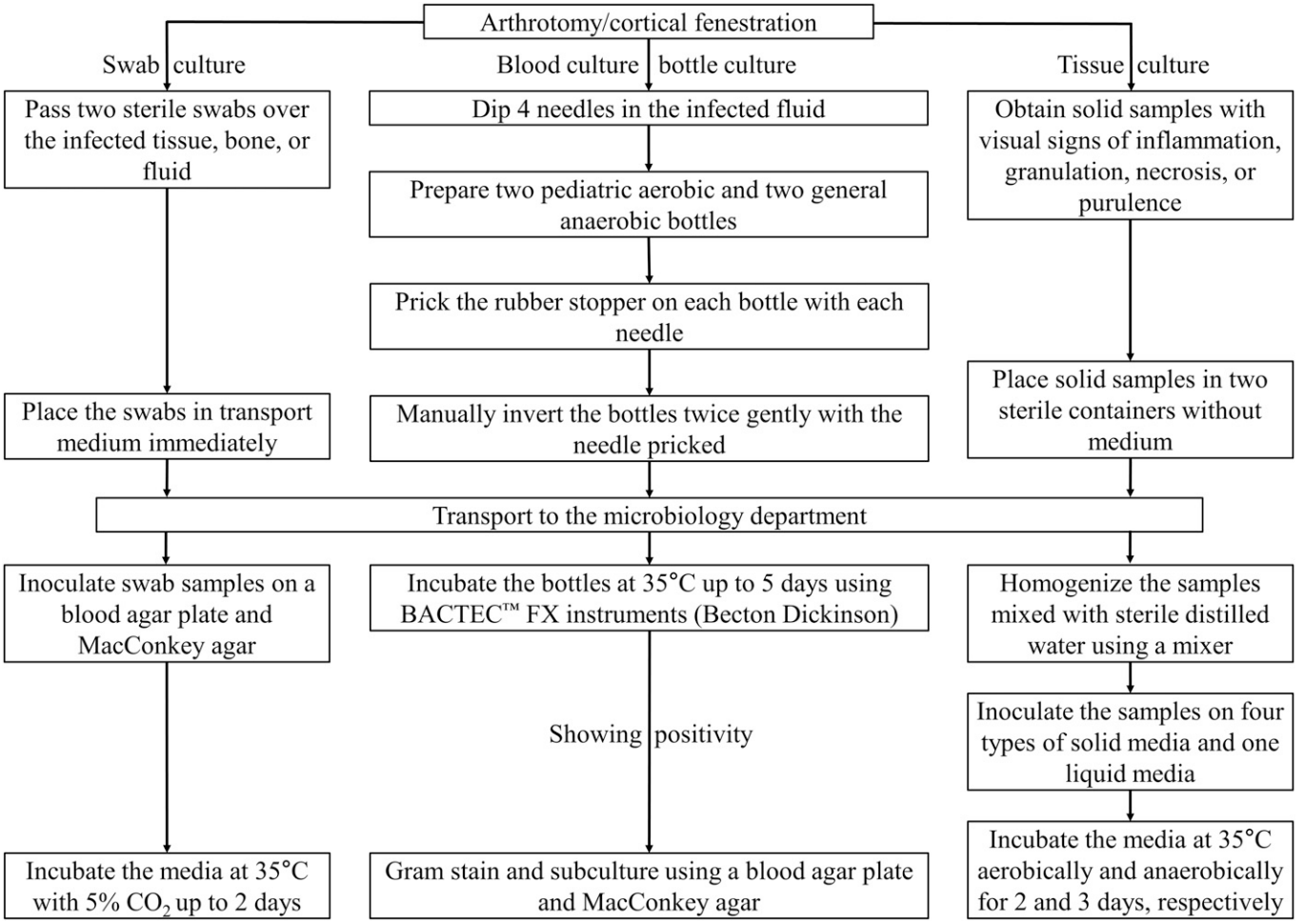

Fig. 2

Flowchart of the sample collection and microbial culture.

(MSSA), the microbial identification rate of BCB did not differ from that of swab culture (13 [87%]; p = 0.5) or tissue culture (11 [73%]; p = 0.13). However, in the 12 patients infected with microorganisms other than MSSA, the microbial identification rate was higher with BCB culture than with swab culture (5 [42%]; p = 0.02) and tissue culture (4 [33%]; p = 0.008). In the 20 patients who had not taken antibiotics within 3 weeks prior to having specimens obtained, the microbial identification rate of BCB culture (70% [14 patients]) did not differ (p = 0.063) from that of tissue culture (45% [9 patients]). However, in the 20 patients who had taken antibiotics within 3 weeks prior to obtaining the specimens, the microbial identification rate of the BCB culture (65% [13 patients]) was higher (p = 0.016) than that of the tissue culture (30% [6 patients]). The identification rate of swab culture did not differ from that of BCB culture in the group without early antibiotics (50% [10 of 20 patients]; p = 0.125) or the early antibiotic group (40% [8 of 20 patients]; p = 0.063). Among the 22 patients whose peripheral blood culture (PBC) was obtained, only 4 patients showed positive PBC results. In the 18 patients showing negative PBC results, the microbial identification rate of the BCB culture (78% [14 patients]) was higher than those of the swab culture (39% [7 patients]; p = 0.016) and the tissue culture (39% [7 patients]; p = 0.016).

The BCB culture reduced the time required for microbial identification (3.5 ± 0.9 days [range, 3 to 7 days]), compared with the time required with swab culture (4.3 ± 1.1 days [range, 3 to 7 days]; p < 0.001) and tissue culture (4.4 ± 1.1 days [range, 3 to 7 days]; p < 0.001).

Discussion

We demonstrated that the BCB culture method was superior to conventional culture methods using swab and tissue cultures, in terms of the microbial identification rate and the time required for microbial identification. The BCB culture method was more effective than conventional methods using swab and tissue cultures even when only a small amount of specimen was available. This study may aid in improving the microbial diagnosis, enabling early and targeted antimicrobial therapy, and reducing complications of pediatric osteoarticular infections.

The improved microbial identification using BCBs in our study could be explained in light of several potential advantages of BCBs over conventional culture media. First, BCBs contain

TABLE II Microorganisms Identified Using Each Culture Method (N = 40)

Microorganisms	BCB*		Swab*		Tissue*	
	Positive in Only 1 Set†	Positive in 2 Sets‡	Positive in 1 Sample§	Positive in 2 Samples#	Positive in 1 Sample§	Positive in 2 Samples#
Gram-positive cocci						
MSSA	0	15 (38%)	4 (10%)	9 (23%)	2 (5%)	9 (23%)
Methicillin-resistant *S. aureus*	0	5 (13%)	1 (3%)	2 (5%)	0	2 (5%)
Streptococcus pyogenes (Group A streptococci)	0	2 (5%)	0	1 (3%)	0	1 (3%)
Streptococcus intermedius	0	1 (3%)	0	1 (3%)	0	1 (3%)
*S. capitis***	0	1 (3%)	0	0	0	0
Methicillin-susceptible *S. epidermidis*††	0	1 (3%)	0	0	0	0
Methicillin-resistant *S. epidermidis*	0	0	0	0	1 (3%)	0
S. hominis subsp. hominis	1 (3%)	0	0	0	0	0
Micrococcus luteus	0	0	1 (3%)	0	0	0
Peptoniphilus species	1 (3%)	0	0	0	0	0
Gram-negative bacilli						
Escherichia coli	0	1 (3%)	0	0	0	0
Total	2 (5%)	26 (65%)	6 (15%)	13 (33%)	3 (8%)	13 (33%)

*The values are given as the number of patients, with the percentage in parentheses. †The microorganism was identified in only 1 of 2 sets of BCBs. ‡The microorganism was identified in 2 sets of BCBs. §The microorganism was identified in 1 of 2 samples. #The microorganism was identified in 2 samples. **The microorganism was identified in a patient with an intramedullary rod. ††The microorganism was identified in an immunocompromised patient.

resins that neutralize antimicrobial agents[20,28]. Second, the dilution effect of placing an inoculum in the liquid medium in a BCB might decrease the inhibitory effects of antibiotics. Third, most BCBs contain lytic agents such as saponin, which may assist in releasing and recovering the microorganisms phagocytized by white blood cells (WBCs)[29]. Fourth, the use of BCBs enables surgeons to inoculate the specimen immediately into the growth media of the BCB in an operating room, whereas the specimens for tissue culture remain in a malnourished environment until they are transported to the microbiology department.

In addition, the different broth formulation and broth-to-blood ratio of pediatric BCBs compared with adult BCBs may have facilitated microbial detection when the BCB was pricked with a needle harboring a weak bacterial inoculum[20]. Geller et al. also suggested that BCBs require a significantly smaller amount of fluid than conventional culture media in periprosthetic joint infections[14]. Furthermore, other studies have shown that the identification rate can be enhanced by inoculating only a small amount of synovial fluid (0.5 to 3.0 mL) in a pediatric BCB[29,30]. Our study used a much smaller amount of specimen than that used in previous reports, by pricking the BCB with a needle merely dipped in an infected fluid[29,30]. In some cases, a large amount of synovial fluid may not be ideal for microbial identification as it may also contain large amounts of antibiotics, complements, immunoglobulins, and other factors, which may be detrimental for microbial growth[31-33]. Inoculating each BCB with multiple needles might lead to more frequent microbial identification than our method of pricking each BCB with a single needle and warrants further study.

In our subgroup analyses, it remains unclear why the identification rate did not differ when the pathogens were MSSA but was higher in the BCB culture when the pathogens were non-MSSA microorganisms. Swab culture and tissue culture could also detect MSSA effectively. A previous study also showed a large disparity between BCB culture and swab culture in the detection of *S. epidermidis*, but not of MSSA, in periprosthetic joint infections[14]. This might be because MSSA can be easily obtained for culture even in poor environments, or that historically, conventional culture media were developed to offer more optimal growth conditions for MSSA, which is one of the most common human pathogens[34], than for non-MSSA microorganisms.

In the present study, the time to identification was shorter for BCB culture than for other culture methods, which concurs with the results of other studies[17,18,35,36]. This may be attributed not only to the favorable growth condition for microorganisms in BCB, but also to the automated BACTEC system that continuously monitors culture growth. The timely administration of targeted antibiotics as a result of rapid pathogen identification can optimize patient outcomes[5,6,37].

The enhanced microbial growth in BCB raises concerns as it may be accompanied by an increased rate of false-positive detection of contaminants. The most common source of

contaminants in PBC is the skin at the site where blood is obtained[19]. In our study, we obtained the specimen during a surgical procedure after preparing the skin thoroughly, reducing the possibility of contamination by normal skin flora. Some studies have shown a reduction in false-positive rates using BCB culture[14,29,30], whereas others have shown the opposite[36,38,39]. One of the strengths of our BCB culture method is that it allows multiple sets of BCB cultures and does not affect the conventional culture, as it requires a minimal amount of infected fluid. Results from multiple cultures can assist in distinguishing contaminants from true pathogens[19].

BCB culture also has certain disadvantages. First, it might not detect certain fastidious organisms such as *Haemophilus influenzae* type b (HiB) or *Neisseria gonorrhoeae* as the amount of inoculated blood is small[20,40,41]. Despite the fact that *N. gonorrhoeae* infections are rare in young children, and that HiB has become uncommon due to widespread vaccination programs[2,42,43], the additional inoculation of an appropriate supplement or blood may aid in detecting these organisms. Second, pediatric BCBs are currently available only for aerobic conditions. However, pathogens causing pediatric osteoarticular infections are mostly aerobic[2,42-44].

This study had several limitations. First, we examined only the sensitivity of each culture method, not the specificity. This was because we applied this culture protocol only to patients highly suspected of having infections, and not to others. Second, there is no gold-standard criterion for diagnosing osteoarticular infections or for distinguishing pathogens from contaminants. However, we excluded patients with non-infectious diseases from the study subjects via a meticulous review of the medical history with a pediatric infectious disease specialist. In addition, we adopted a conservative approach with regard to the sensitivity of our method, as skin flora that were identified in 1 of 6 samples were considered a false-positive. Third, as the sample size was relatively small, we could not perform various subgroup analyses.

In conclusion, the BCB culture system improved the microbial identification rate, reduced the time to identification, and permitted a smaller specimen, compared with traditional culture systems in pediatric osteoarticular infections. 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. ■

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