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Diagnosis of periprosthetic joint bacterial infections by culture of sonication fluid from infected implants

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Abstract

Purpose: This study compared the results of a culture method using sonication with those yielded by the conventional culture method, for patients with infected total knee arthroplasty (TKA). We also evaluated the usefulness of sonication for the identification of pathogens in infected TKA cases. **Methods:** Isolates were cultured from 13 implants that had been removed from 13 patients with infected TKA. Preoperative culture was performed on aspirated joint fluid, and during the operation, infected tissue was collected for culture. The removed prosthetic implants were cultured before and after sonication. Next, we identified the cultured bacteria using API biochemical kits and 16 S rRNA sequencing. **Results:** The cultures from preoperative joint fluid and intraoperative tissue were positive in 9 of 13 cases (69.2%). For the removed implants, 10 cases were positive before sonication. After sonication, 12 cases (92.3%) had positive cultures. The pathogen most commonly isolated from the cultures was *Staphylococcus aureus*. **Conclusions:** This study found that a culturing workflow incorporating sonication diagnosed pathogens in patients with infected TKA with higher sensitivity than did the conventional culturing method.

Keywords

arthroplasty, culture, infection, sonication

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Introduction

The incidence of total knee arthroplasty (TKA) has increased, as has the failure of this operation. Postoperative infection is one of the most common complications and accounts for 38% of reported complications.[1] The incidence of infection was reported to be 1–2% in primary TKA and 4–8% in revised TKA.[2,3] Postoperative infection occurred with a combination of three factors comprising patient health status, microorganism, and environment. Most patients who received TKA were elderly.

In the case of chronic infection after TKA, a two-stage revision arthroplasty has been recommended as a treatment.[4–7] In the first stage of revision, the femoral component removed from the patient is reused after autoclaving and applying an antibiotic-impregnated cement

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spacer. Proper antimicrobial treatment must be followed until the second stage of revision is performed. Identification of the pathogens is important for proper use of antibiotics, and most surgeons culture isolates from the surrounding tissues of infected implants to identify pathogens. However, *Staphylococcus aureus*, one of the most common pathogens causing infection, can form biofilms by attaching to implant surfaces, and biofilm-forming *S. aureus* may not be identified by traditional microbiological techniques.8 Several studies have shown that culturing after sonication of infected implants can make the identification of biofilm-forming bacteria more sensitive and accurate.

In this study, implants removed from infected patients after TKA surgery were cultured before and after sonication, and the results were compared with those obtained using the conventional culture method. The usefulness of sonication for the identification of pathogens was also evaluated.

Materials and methods

Patients

This study included 13 patients (3 men and 10 women) with prosthetic joint infections or suspicious illness with fever in the knee joint. The patients visited two university hospitals in Daegu city, from August 2015 to November 2016. Each patient received a two-stage revision arthroplasty. The patient age was 67.2 $\pm$ 7.6 years. The study protocol was reviewed and approved by the ethics committee of the institutional review board of our institution (No. 2016-10-025-001).

Preoperative and intraoperative culture

The preoperative culture was performed on aspirate of joint fluids. The intraoperative culture was performed on tissue obtained during the implant removal operation. During the operation, infected soft tissues, necrotized bone, and synovial membrane were collected for culturing by debridement after arthrotomy. We reviewed the medical records of preoperative and intraoperative histological results.

Prosthetics collection

A total of 13 implants were removed from 13 patients who underwent implant removal surgery (first stage of a two-stage revision arthroplasty). To confirm the presence of bacteria, the removed prosthesis was aseptically transferred to the Microbiology Department of Kyungpook National University School of Medicine during surgery. Clinical records, radiographs, histopathological outcomes, underlying disease of the patient, and pre- and postsurgical microbiologic results were evaluated. All 13 patients were treated with antimicrobial drugs (cephalosporin, ampicillin, or vancomycin) for 2–20 days preceding surgery.

Surgical technique

Surgical procedures were performed by two orthopedic surgeons at two institutions. In the first stage of revision arthroplasty, the necrotized bone, soft tissue, and synovium presumed to be infectious were all removed, and sterilized femoral implant was inserted with antibiotic-mixed cement, until the second stage of revision for the articulating spacer. Cement (40 g) mixed with vancomycin (4 g) and first-generation cephalosporin (4 g) was attached to the prosthetic joints in the first-stage revision arthroplasty. After the infection treatment, the second-stage revision arthroplasty was conducted. After debridement, with removal of the temporary articulating spacer, the revision arthroplasty, using a cruciate ligament-substituting knee prosthesis, or a constrained type knee prosthesis, was performed.

Microbiological culture of prosthetic implants before and after sonication

The removed prosthetic implant was aseptically transferred into a Stomacher bag (20 $\times$ 30 cm, BNF Korea) and then transported to a microbiology laboratory. Four hundred milliliters of sterile saline was added to the Stomacher bag with the prosthetic knee in a laminar airflow biosafety cabinet; the Stomacher was then vortexed for 30 s. Sixty milliliters of fluid was taken from the Stomacher bag and centrifuged at 6 k r/min for 5 min. A 1 mL aliquot of supernatant was collected, and the remainder was decanted. Next, the pellet in the centrifuge tube was resuspended by vortexing. Each 100 μL was spread onto four different agar plates; sheep blood agar plate (BAP), chocolate agar plate (CAP), MacConkey agar plate (Mac), and CDC blood agar plate (CDC). The Stomacher bag with the remaining 340 mL of saline was subjected to sonication (Branson 3510, Branson Ultrasonic Co., Danbury, CT, USA) for 5 min (40 kHz, 185 W) and vortexed for 30 s. Sixty milliliters from the sonication fluid was centrifuged, resuspended, and inoculated onto four different media as described above. Sheep BAPs, CAPs, and Macs were incubated aerobically at 37°C for 3 days, and CDC was incubated anaerobically at 37°C for 7 days. Identifications of bacterial colonies grown on the agar plates were performed using API biochemical kits (bioMérieux, France). Some bacterial colonies with ambiguous results from the API tests were subjected to 16 S rRNA sequencing, for more definitive identification. Analysis by 16 S rRNA sequencing was performed by colony polymerase chain reaction amplification and Sanger sequencing with the universal bacterial primers 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492 R (5′-GGT TAC CTT GTT ACG ACT T-3′) (Solgent Co., Ltd, South Korea).[9]

Results

Clinical characteristics of 13 patients

Five of 13 patients had hypertension. Of the five hypertensive patients, one had diabetes and one had gout. One had rheumatoid arthritis and one had a history of angina pectoris and thyroidectomy. One patient had a history of femur fracture operation. One had chronic renal failure and was positive for hepatitis C virus. Four of 13 patients had no underlying diseases. To diagnose infection after TKA, the physical examination checked for swelling, redness, and heat. We also determined whether the patients presented with clinical symptoms such as pain and tenderness. Laboratory tests included white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). In addition, radiography and joint fluid cell counts were considered. They were diagnosed with a late-onset chronic infection that occurred 4 weeks after the first TKA surgery.

Pre- and intraoperative microbiological tests

Of the 13 preoperative cultures of joint fluid, 9 cases (69.2%) were positive for *Staphylococcus* species (5), *Pseudomonas* species (1), *Streptococcus mitis* (1), *Streptococcus agalactiae* (1), *Enterococcus cloacae* (1), and fungus (1). Of the 10 intraoperative tissue cultures, 2 cases were positive and 8 cases exhibited no growth (Table 1). Three patients were not subjected to intraoperative tissue cultures (Not Determined).

Microbiological culture of prosthetic implants before and after sonication

Table 1 shows the clinical characteristics and microbiological culture results of 13 patients who received the first stage of revision arthroplasty. As shown in Table 1, 10 of 13 cases were culture-positive both before and after sonication. Bacteria isolated from these patients included *S. aureus*, *Pseudomonas* species, *S. agalactiae*, and *Pasteurella pneumotropica*. The isolated bacteria before and after sonication were the same in all 10 cases. Two cases were culture-positive only after sonication; bacteria isolated from these two cases were *Staphylococcus hominis* and *S. aureus*. In total, 12 of 13 cases (92.3%) were culture-positive after sonication. Regardless of sonication, one case exhibited no growth under both aerobic and anaerobic conditions.

Discussion

In this study, post-sonication cultures exhibited higher sensitivity for diagnosis of pathogens than the conventional culture method, in patients with infected TKA. In two cases, differences in cultured pathogens between the preoperative joint fluid or intraoperative tissue and the removed implant were interpreted as infections by multiple pathogens or contamination of the tissue sample. We could not entirely exclude the possibility of both infections by multiple pathogens and contamination in this study.

An infected TKA can be diagnosed by laboratory tests including CRP, WBC count, proportion of neutrophils in the joint fluid, characteristics of synovial fluid, pathologic findings for periprosthetic tissue, and microbiologic findings.[10] However, one method alone is not sufficiently diagnostic of infected TKA; it is necessary to use laboratory tests in combination with clinical symptoms. Several authors reported that diagnosis based on histopathologic findings is more sensitive than diagnosis based on microbiological findings or laboratory findings.[11,12] However, in situations where histopathological diagnosis is impossible, microbiological diagnosis is able to identify pathogens, as is necessary for appropriate antibiotic treatment.

To increase the sensitivity of microbial identification for infected implants, we applied sonication to the conventional culture method. Nine of the 13 implants studied here were culture-positive by the conventional method using preoperative joint fluid or intraoperative tissue culture, but 12 of the 13 implants were culture-positive by the sonication method. Three cases with negative results by conventional joint fluid or tissue culturing were positive after sonication.

Some studies reported that the results of the sonication method could be affected by sonication time and temperature. Monsen et al.[13] performed sonication at 22°C for 7 min (40 kHz, 350 W). In this study, sonication was performed at room temperature for 5 min (40 kHz, 185 W).

The difficulty of diagnosis and treatment in infected TKA is related to the acquisition of tolerance to antibiotics and the formation of biofilms.[8] Patients who start antibiotic treatment are known to be less susceptible to detection of pathogens for infected TKA. However, many patients with infected TKA have started antibiotic treatment in advance of the identification of pathogens, or surgery for the removal of the infected implant. Most patients included in this study had been treated in other clinics with empirical antibiotics before the surgery. Biofilm formation by infected pathogens has often led to difficulties in diagnosis and treatment in infected TKA. Biofilm-forming pathogens adhere firmly to implant surfaces and form mature biofilms in a few days. Because biofilms adhere strongly to implants, the traditional culture method is limited in its detection of pathogens, and antibiotic treatment often fails. The present study introduced sonication to the existing culture workflow and, in doing so, increased the sensitivity, by separating the biofilm from the implant. Tunney et al.[14,15] and Trampuz et al.[16] also reported heightened sensitivity for the sonication method and subsequent successful treatment with antibiotics. The sonication method may therefore be superior to the existing culture method for diagnosis of pathogens. Additionally, an appropriate

Table 1. Characteristics of 13 patients and microbiological culture results of the prosthetics before and after sonication.

Serial number	Sex/age	Underlying disease	Operation date (m/d/year)	Preoperative culture (date; m/d/year)	Intraoperative culture (date; m/d/year)	Before sonication	After sonication
1	F/61	Rheumatic arthritis	8/10/2015	—	—	—	—
2	F/69	Hypertension	8/12/2015	—	—	—	*Staphylococcus hominis*
3	M/72	Hypertension	8/12/2015	*Enterococcus cloacae*, *Streptococcus mitis* (7/25/2015)	—	—	*Staphylococcus aureus*
4	F/73	Angina pectoris, thyroidectomy	8/12/2015	*Staphylococcus epidermidis* (4/13/2015)	—	*Staphylococcus epidermidis*, *Staphylococcus xylosus*	*Staphylococcus epidermidis*, *Staphylococcus xylosus*
5	M/56	Lt. femur shaft old fx. C union	9/2/2015	Fungus (8/3/2015)	ND	*Pseudomonas migulae*	*Pseudomonas migulae*
6	F/75	Hypertension	9/9/2015	*Staphylococcus epidermidis* (8/24/2015)	ND	*Staphylococcus epidermidis*, *Staphylococcus xylosus*	*Staphylococcus epidermidis*, *Staphylococcus xylosus*
7	F/58	None	9/16/2015	*Streptococcus agalactiae* (9/14/2015)	—	*Streptococcus agalactiae*	*Streptococcus agalactiae*
8	M/78	Hypertension, gout	9/18/2015	*Staphylococcus aureus* (9/10/2015)	ND	*Staphylococcus aureus*	*Staphylococcus aureus*
9	F/66	None	9/23/2015	—	—	*Pasteurella pneumotropica*	*Pasteurella pneumotropica*
10	F/74	Chronic renal failure, HCV	10/19/2015	*Staphylococcus aureus* (10/15/2015)	*Staphylococcus aureus* (10/19/2015)	*Staphylococcus aureus*	*Staphylococcus aureus*
11	F/61	None	10/28/2015	*Pseudomonas* subsp. (7/11/2015)	—	*Staphylococcus cohnii* subsp. *urealyticus*	*Staphylococcus cohnii* subsp. *urealyticus*
12	F/73	None	10/30/2015	—	—	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
13	M/58	Hypertension, diabetes mellitus	11/4/2015	*Staphylococcus aureus* (11/2/2015)	*Staphylococcus aureus* (11/6/2015)	*Staphylococcus aureus*	*Staphylococcus aureus*

antibiotic therapy could be possible via the detection and identification of infection-causing pathogens.

Although we interpreted one colony grown on the plate as a positive culture, such a result may lack clinical significance. Studies have used variable criteria for positive culture results. Scorzolini et al.[17] used 5 CFU/mL in a 0.5 mL inoculation, Trampuz et al.[18] used 5 CFU/plate in a 0.5 mL inoculation, and Zalavras[19] used 20 CFU/mL. Because definite criteria for culture positivity have not been established, correlation between the criteria of culture positivity and clinical significance should be evaluated.

This study had several limitations. The number of patients was small. The design of this study was retrospective. Clinical correlation with culture results was not evaluated.

Conclusion

This study demonstrated a higher sensitivity of the sonication method for diagnosis of pathogens in patients with infected TKA than the conventional culture method. Sonication may therefore be an efficacious method for pathogen diagnosis in infected TKA.

Authors' contribution

Hee-June Kim and Shukho Kim contributed equally to this study and are co-first authors. Jungmin Kim and Hee-Soo Kyung contributed equally to this study and are co-corresponding authors.

Declaration of conflicting interests

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