Joint Diseases and Related Surgery

Jt Dis Relat Surg
2024;35(2):299-304

ORIGINAL ARTICLE

Use of thioglycolate broth as a pre-analytic transport medium in the diagnosis of prosthetic joint infection

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ABSTRACT

Objectives: This study aimed to investigate whether adding tissue samples directly into thioglycolate (TG) broth yielded a greater number of anaerobic organisms than freshly sampled tissue in suspected hip and knee prosthetic joint infections (PJIs).

Patients and methods: Between January 2017 and December 2020, a total of 90 patients (46 males, 44 females; median age: 71.7 years; range, 50.8 and 87.8 years) who underwent revision hip or knee arthroplasty were included. Intraoperative samples were taken, with five placed in TG broth and five in standard containers (PC) with subsequent aerobic and anaerobic culturing conducted. Demographic and baseline data of the patients were recorded. The primary outcome was positive bacterial growth from a PJI specimen inoculated directly into TG broth at the time of collection or standard PJI specimen processing. Secondary outcomes investigated were the presence of *Cutibacterium acnes (C. acnes)* and the curative success of revision procedure.

Results: A total of 900 samples (450 PC and 450 TG) were taken from 90 revision arthroplasty patients (47 knees and 43 hips). There was no statistically significant difference in the number of positive bacterial growth samples between TG broth and standard processing (p=0.742). This was consistent with subgroup analysis analyzing *C. acnes* (p=0.666).

Conclusion: In hip and knee arthroplasty, there is no benefit in substituting or adding TG broth as a culture medium to better identify both general bacterial species and *C. acnes* infections specifically. However, the use of TG may be useful in confirming a true positive result for infection.

Keywords: *Cutibacterium acnes*, hip arthroplasty, knee arthroplasty, Prosthetic joint infection, thioglycolate.

Prosthetic joint infections (PJIs) occur in up to 1% of hip and 2% of knee arthroplasties.[1] With rise in total hip and knee arthroplasties, the number of PJIs is likely to increase.[2] Unfortunately, it still remains one of the most devastating and difficult-to-treat modes of failure after hip and knee arthroplasties. Treatment often requires revision surgery and appropriate antibiotic selection, and duration of treatment is highly dependent on the bacteria cultured.[3]

In addition to more virulent bacteria, the presence of low-grade infections can also be problematic. These low-grade infections may be a cause of aseptic loosening. We have seen evidence of this in shoulder replacements with *Cutibacterium acnes* (*C. acnes*, previously known as *Propionibacterium acnes*) frequently encountered. The *C. acnes* is a common commensal bacterium of the skin, particularly found in areas rich in sebaceous glands.[4,5] For identification of this bacterium from PJI specimens, it is recommended that culture incubation of both aerobic and anaerobic medium be extended to at least 13 days.[6,7] The use of thioglycolate (TG) broth in the identification of these low virulent infections has been used in bone and joint specimens from total

Received: August 05, 2023
Accepted: October 30, 2023
Published online: March 21, 2024

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Doi: 10.52312/jdrs.2024.1364

Citation: Lim C, Damasena I, Mclellan D, Kuster M. Use of thioglycolate broth as a pre-analytic transport medium in the diagnosis of prosthetic joint infection. Jt Dis Relat Surg 2024;35(2):299-304. doi: 10.52312/jdrs.2024.1364.

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shoulder replacements since 2013 with a potential higher yield than conventional anaerobic culture media.[8]

Previous studies have examined different culture mediums in PJI including blood culture bottles, blood aerobic and anaerobic agar, and enrichment media.[9-12] To the best of our knowledge, the benefit of direct inoculation of hip and knee PJI specimens into TG broth at the time of specimen collection has not been previously evaluated. In the present study, we aimed to investigate whether adding tissue samples directly into TG broth, thereby removing exposure to air, yielded a greater number of anaerobic organisms than freshly sampled tissue. We hypothesized that there would be no significant difference between the use of TG broth and standard culturing techniques in the detection of PJIs.

PATIENTS AND METHODS

This single-center, prospective, observational, non-controlled cohort study was conducted at Hollywood Private Hospital, Department of Orthopaedics between January 2017 and December 2020. Twelve fellowship trained Australian orthopedic surgeons conducted all procedures.

All consecutive patients undergoing a revision hip or knee arthroplasty during the study period were considered for recruitment in this study. Inclusion criteria were as follows: patients who had a knee or hip arthroplasty *in-situ*, those undergoing revision arthroplasty, and having samples taken at the time of revision. Exclusion criteria were as follows: an inappropriate number of samples taken, using an inappropriate sampling technique, and patients who did not undergo revision arthroplasty of a hip or knee arthroplasty. Finally, a total of 90 patients (46 males, 44 females; median age: 71.7 years; range, 50.8 and 87.8 years) who met the inclusion criteria were recruited.

Specimen collection and culturing protocol

Each case was provided with a tissue specimen sample pack to allow for a standardized collection process. This included five TG broth specimen containers (labelled TG 1-5) and five sterilized transport containers (labelled PC 1-5). Emulsification beads were already added to the PC containers. Each specimen that was taken was divided into two subsamples and one subsample placed in each of the different types of containers. This was repeated until five specimens were collected. Optimum subsample size was considered 2×2 mm and each blade was only used for the collection of that sample.

Specimen containers were transported from the operating theatre to the receiving laboratory on the same day as collection. At the time of arrival laboratory, emulsification beads were added to the 5 TG containers and 2 mL of saline was added to the 5 PC containers. Then, vortex of the containers was performed for ~30 sec to ensure adequate emulsification of tissue and allow inoculation of the following culture media - Horse Blood Agar (HBA), MacConkey (MAC), Chocolate Agar (CHOC), Colistin Nalidixic Acid Agar (CAN), and Sabouraud + CG(SAB) agar. The media were cultured under aerobic (HBA, MAC, CHOC, CAN, SAB) and anaerobic (HBA) conditions at 35ºC. For the aerobic incubation of HBA and CNA, media atmospheric conditions included 5% carbon dioxide. Aerobic cultures were read daily for five days and anaerobic media on Days 2, 5, and 14. One milliliter of emulsified fluid from each container was also inoculated into a pediatric blood culture bottle (BacT/ALERT® iPF bottle, bioMerieux) and monitored continuously for 14 days (BacT/ALERT®3D Microbial Detection System, bioMerieux). If bacterial growth was detected subculture to solid media was performed for aerobic and anaerobic incubation. Any microbial isolate detected on culture media was identified to species level utilizing standard laboratory techniques.

Outcomes

Baseline demographics, data on the presence of a sinus, aspirate results, imaging (radiographs, computed tomography, and bone scans), blood results (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]), as well as culture results using the guidelines from Parvizi et al.[13] were collected. The primary outcome was positive bacterial growth from a PJI specimen inoculated directly into TG broth at the time of collection or standard PJI specimen processing. Comparison was, then, made between the two approaches. Secondary outcomes included the number of patients that produced positive samples and the breakdown of TG versus standard processing of samples. Further analysis investigated the reason for revision surgery and an outcome of that surgery was conducted via a questionnaire to the treating surgeon.

Statistical analysis

A priori power analysis and sample size calculation were performed. Accordingly, a minimum of 500 samples (250 for the TG and standard protocols each) were required to reach statistical significance in identifying an infection

between the two cohorts with a minimum of 10% difference.

Statistical analysis was performed using the IBM SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in median (min-max) or number and frequency, where applicable. the chi-square test was performed to compare populations and subgroups. A *p* value of <0.05 was considered statistically significant.

RESULTS

A total of 90 patients who were operated by 12 surgeons were included in this study. Baseline demographic data of the general cohort and those with positive samples are shown in Table I. The most common reason for revision was infection (62.5%), followed by osteolysis (12%), loosening (9.4%), and instability (9.4%).

A total of 900 samples were taken (450 PC and 450 TG). Breakdown of these samples is shown in Table II. There was no statistically significant difference in the testing comparing PC against TG for all samples (p=0.742) and for PC against TG for all samples that grew *C. acnes* (p=0.666). In addition, nine samples of the 470 (1.9%) taken from total knee replacements (TKRs) were positive for *C. acnes* with five (55%) in the standard protocol group and four (45%) in the TG broth group. Furthermore, 11 samples of 430 (2.6%) taken from total hip replacements (THRs) were positive for *C. acnes* with five (45%) in the standard protocol and six (55%) from the TG broth. There was no significant difference between these values.

Thirty-two of 90 patients recorded growth in samples (13 TKRs and 19 THRs). The median follow-up was 38.3 (range, 4.8 to 60.7) months. Of 32 cases, 10 (31.3%) had positive PC and TG, 10 (31.3%) had positive PC and negative TG, and 12 (37%) had negative PC and positive TG samples. There was no statistically significant difference between these values.

If a patient had a positive sample in either the TG broth or standard procedure (but not both), only one of a possible five samples was positive. In addition, all patients with positive samples in both TG broth and standard procedure had multiple positive samples in both.

A breakdown of reason for revision surgery is shown in Table III. The treatment outcomes are

TABLE I
Baseline demographic and laboratory characteristics of patients

	All				TKR				THR			
Basic demographics of surgery	n	%	Mean	Range	n	%	Mean	Range	n	%	Mean	Range
Number	90				47	52.2			43	47.8		
Mean age (year)			72.2				70.4				74.1	
Age range				50.8-88.3				59.4-81.8				50.8-88.3
Clinically infected	20	62.5			10	50			10	50		
Draining sinus	1				0				1			
Mean CRP			30.2				32.6				29.1	
Range CRP				0.7-308				1.1-165				0.7-308
Mean WBC			8.3				7.4				9	
Range WBC				3-21.5				3-10.3				3-21.5

TKR: Total knee replacement; THR: Total hip replacement; CRP: C-reactive protein; WBC: white blood cell.

TABLE II
Breakdown of all samples

	Total number		Positive all		Negative all		Positive *C. acnes*		Negative *C. acnes*	
	n	%	n	%	n	%	n	%	n	%
Total number	900	100	93	10.3	807	89.7	22	2.4	878	97.6
Standard protocol	45	50	45	48.4	405	50.3	10	45.5	440	50.2
Thioglycolate broth	450	50	48	51.6	402	49.7	12	54.2	438	49.8

TABLE III
Reasons for revisions

	All		TKR		THR	
	n	%	n	%	n	%
Infection	20	62.5	10	76.9	10	52.6
Loosening	3	9.4	1	7.7	2	10.5
Instability	3	9.4	0	0	3	15.8
Osteolysis	4	12.5	1	7.7	3	15.8
Polyethylene wear	1	3.1	0	0	1	5.3
Pain	1	3.1	1	7.7	0	0

TKR: Total knee replacement; THR: Total hip replacement.

TABLE IV
Clinical treatment outcomes

	All		TKR		THR	
	n	%	n	%	n	%
Successful revision	24	75	10	76.9	14	73.7
Lifelong Abx	2	6.3	1	7.7	1	5.3
Failed revision	1	3.1	1	7.7	0	0
Awaiting second stage	1	3.1	0	0	1	5.3
Death	1	3.1	0	0	1	5.3
Lost to follow-up	3	9.4	1	7.7	2	10.5

TKR: Total knee replacement; THR: Total hip replacement.

summarized in Table IV. One patient had recurrence of their infection after their initial procedure and underwent a two-stage revision of TKR.

DISCUSSION

The main finding of this study was that there was no statistically significant difference in the number of PJI specimens with positive bacterial growth between specimens inoculated directly in TG broth at the time of collection and standard processing. Therefore, in suspected hip and knee PJIs, there is no benefit of TG broth as a pre-analytic transport medium to better identify general bacterial species or *C. acnes* specifically. The numbers were similar and are still consistent between both total knee and total hip populations.

The presence of contaminants is often considered, if only a single positive sample is present. However, in the context of supportive clinical and laboratory findings, and the presence of certain pathogens, a single positive sample may also represent a true PJI. In this study, of 32 patients, 12 had only a single positive TG sample and a further 10 had only a single positive standardly processed sample. No patient had both a single positive sample present in both media. Therefore, potential contamination (with a single positive sample) only occurred in either native or TG, and never in both transport media. This supports a theory that a single positive sample across both media is more suggestive of a contaminant.

In addition, some of these single positives were *C. acnes*, which is rarely considered a contaminant. Therefore, the use of both media may play a role in aiding the sensitivity of identifying a pathogen in low virulent circumstances. Building on this concept, all patients that had positive samples in both media had multiple positive samples. Therefore, growth in both media may provide additional specificity information in both confirming the presence of true PJI and identifying the pathogen.

Since the collection of data for this study, Parvizi et al.[13] developed new criteria for the definition of a PJI. A positive PJI was defined as the patient having one major, or three minor criteria with major criteria as follows: *(i)* two positive periprosthetic

(tissue or fluid cultures with matching organisms), or *(ii)* a sinus tract communicating to the joint. Minor criteria were as follows: *(i)* elevated CRP >100 mg/L in acute PJI, or >10 mg/L in chronic PJI and ESR >30 mm/h in chronic PJI, *(ii)* increase synovial fluid white blood cell count >10,000 cells/uL or ++ or greater change on leukocyte esterase test strip of synovial fluid, *(iii)* increase in synovial fluid polymorphonuclear neutrophils (PMN) >90% in acute PJI, or >80% in chronic PJI, *(iv)* positive histological analysis of periprosthetic tissue >5 neutrophils (PMNs) per high-power field and *(v)*, a single positive periprosthetic tissue or fluid culture. Further research can focus on relevance of using the Parvizi criteria and TG broth as pre-analytic transport medium to see whether there is any clinically significant difference in the identification of PJIs.

There are some key strengths of this study. First, it was sufficiently powered to identify the primary outcome measure. Second, the prospective nature of the study with direct comparison between the two sample populations limits the number of confounders between the two culturing techniques. Third, it is the first study to investigate the use of TG broth as pre-analytic transport medium in the identification of *C. acnes* in hip and knee arthroplasty.

Nonetheless, there are some limitations to this study including missing data and lost-to-follow-up, in particularly the retrospective collection from each surgeons' records. This was insufficient or incomplete, such that we were not able to complete the Parvizi criteria for all patients as a comparison. In addition, a questionnaire to the treating surgeon may provide opportunity for embellishment of outcomes. Finally, it is difficult to draw reliable conclusions on the clinical importance of these positive samples. There is much debate over what the criteria for a positive PJI is and what constitutes a contaminant. Many consider a single positive sample as a contaminant, while others would be more suspicious of PJI with the identification of certain bacteria.

In conclusion, our study results showed no statistically significant difference between the use of TG broth as pre-analytic transport medium and standard culturing procedures in the identification of PJI for hip and knee arthroplasty. There was also no statistically significant difference between rate of identification of *C. acnes* infections. Further research may focus on investigating the use of clinical outcome measures with TG broth in the identification of PJIs.

Ethics Committee Approval: The study protocol was approved by the Hollywood Private Hospital Research Ethics Committee (date: 08.02.2016, no: HPH446). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Was responsible for data collection, statistical analysis, interpretation of the data and writing of the manuscript: C.L.; Was responsible for ethical consideration of the paper, interpretation of the data and editing of the manuscript: I.D.; Was responsible for data collection, interpretation and editing of the manuscript: D.M.; Was responsible the initial concept of the paper, interpretation of the data and editing of the manuscript: M.K.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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