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Original article

EXTENDED CULTIVATION TIME BEYOND 7 DAYS ENHANCES PROPIONIBACTERIUM ACNES ISOLATION IN SUSPECTED BONE AND JOINT INFECTIONS: A RETROSPECTIVE STUDY.

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ABSTRACT

Background

Diagnosing bone and joint infections caused by *Propionibacterium acnes* is challenging due to the lengthy cultivation period, which can extend to 14 days. This study explored the potential of a 7-day cultivation period for precise diagnosis while maintaining sensitivity.

Methods

A one-year retrospective analysis included individuals with at least one positive *Propionibacterium acnes* sample. Patients were categorized as "infection" or "no infection" based on predefined criteria. The study assessed clinical and microbiological data, including time to positive results using different cultivation techniques.

Results

Among 70 confirmed *P. acnes* cases, the median time to positivity was 6 days, compared to 9 days in 47 contaminant cases. Tissue samples from 15 infected cases (21.4%) remained positive after day 7. Beyond day 10, blind thioglycolate broth subcultures detected infection in 6 patients (8.6%). Thioglycolate broth showed the highest sensitivity at 66.3%, while anaerobic agar plates had a notable positive predictive value of 96.5%. *P. acnes* growth occurred promptly upon transfer to the microbiological laboratory.

Conclusion

Reducing the cultivation period to 7 days may increase false-negative results by 21.4%. To achieve precise identification of *P. acnes*, it is recommended to implement a 10-day biopsy specimen culturing method for bone and joint infections, including a blind subculture.

Recommendations

Based on this study, it is advisable to conduct biopsy specimen culturing for 10 days, including a blind subculture after day 7, to accurately diagnose *Propionibacterium acnes*-related bone and joint infections. This approach enhances sensitivity and reduces the risk of false negatives. Further research and validation of this cultivation protocol may improve clinical diagnosis accuracy.

Keywords: Propionibacterium acnes, *Bone and joint infections, Cultivation*
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INTRODUCTION

Propionibacterium acnes, a Gram-positive rod often found on human skin, is linked primarily to acne vulgaris due to its association with sebaceous glands in the shoulder and axilla regions [1]. However, it can lead to osteomyelitis, septic arthritis, and implant-related infections. *P. acnes* has been identified as a significant factor in shoulder infections, especially in periprosthetic joint infections (PJI), and various biofilm-related infections in cardiovascular implants, spinal osteomyelitis, and endophthalmitis, as documented in the literature [2, 3].

Diagnosing bone and joint infections caused by *P. acnes* is challenging because patients often present only with pain [4]. The use of short incubation periods in diagnostic laboratories has led to underdiagnosis. Recent research suggests extending the incubation period to a maximum of 14 days, leading to an increase in the identification of *P. acne* infections [5, 6]. However, due to financial implications, a study proposed a 7-day incubation period for orthopedic implant-related infections, with a success rate of 96.6% in identifying infections, though only one case was attributed to *P. acnes* [7].

This study focuses on a substantial cohort of patients with *P. acnes*-positive samples and compares the time required for identifying the bacterium in bone and tissue biopsy

specimens, as well as sonicated implants. The study also explores transportation durations, media types, and handling protocols for the specimens. It aims to determine whether a reduced 7-day cultivation period is diagnostically effective for identifying *P. acnes*-associated bone and joint infections without compromising sensitivity, addressing the inquiry regarding optimal cultivation duration**.**

METHODS

Study design

The investigation was carried out at Anugrah Narayan Magadh Medical College, Gaya. The establishment in question is a specialized orthopedic facility, equipped with a total of 110 beds, that places its primary emphasis on the diagnosis, treatment, and management of bone and joint infections. The medical facility conducts approximately 4,000 surgical procedures on an annual basis, encompassing 325 hip arthroplasties, 185 knee arthroplasties, and 142 shoulder arthroplasties specifically in the year 2022.

Participants

This retrospective, monocentric investigation examined a cohort of individuals who underwent orthopedic surgical interventions about osseous or articular structures within the timeframe spanning one year, from February 2022 to February 2023.

Data Collection

Clinical and demographic data about the patients' medical history were obtained from the clinical record of the orthopedic department and the Infectious Diseases Consultation service. The study cohort was stratified into two distinct categories: the "infection" group, comprising individuals who exhibited a minimum of two positive samples indicative of infection, and the "no infection" group, consisting of individuals who presented with only one positive sample, suggestive of potential contamination. The analysis was limited to cases that met the criterion of having three or more samples. The examination of infection was established through the application of microbiological criteria, with concurrent evaluation of clinical presentations.

Categories of Infections

The infections were classified into four distinct categories: (i) joint arthroplasty-associated infections, commonly referred to as PJI, (ii) infections related to orthopedic implants, known as implant-associated infections, (iii) septic arthritis, frequently observed in cases involving arthroscopy, and (iv) osteomyelitis. Implants were categorized into two distinct groups: small implants, which encompassed those equipped with screws or anchors, and large implants, which encompassed those equipped with plates or intramedullary nails. The microbiological diagnostics encompassed the examination of tissue or bone biopsy specimens, as well as the analysis of sonication fluid derived from extracted implants. Exclusion criteria were applied to synovial fluid samples.

Inclusion

Inclusion criteria encompassed individuals who exhibited a minimum of one affirmative specimen for *P. acnes* within the duration of their hospitalization, in direct correlation to the identical site of infection.

Exclusions

Samples that did not meet the required cultivation time were excluded from the analysis. Additionally, samples obtained from patients who had received antibiotic treatment within 24 hours before sample collection were also excluded. Exclusion criteria encompassed patients presenting with polymicrobial infections.

Ethical considerations

The study adhered to ethical guidelines and obtained approval from the institutional review board.

Microbiological Processing

The tissue, implant, and bone samples were transferred to the microbiology laboratory from the operating room for further analysis and examination. Pre-diagnostic cultures were conducted on the aforementioned samples. The diagnostic cultures involved the utilization of glass beads to vortex tissue samples, followed by the incubation of said samples on agar plates under both aerobic and anaerobic conditions. The samples were additionally enriched in aerobic thioglycolate broth. The bone samples were exclusively inoculated in aerobic thioglycolate broth. A diverse array of agar plates and media were employed to cultivate microorganisms.

In the case of explanted implants, sonication was employed as a method of extraction, followed by subsequent inoculation of the resultant fluid onto culture media. Blood culture flasks were employed for both aerobic and anaerobic cultivation purposes. The growth threshold for positivity was established as greater than or equal to 50 colony-forming units per milliliter of bacteria on agar plates.

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Microorganism Identification

Microbial identification was performed utilizing established methodologies, encompassing matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) as of 2012. Before that, the identification of *P. acnes* was based on conventional reactions.

Microscopy

A Gram stain was routinely performed on tissue and sonication fluid samples on the same day.

Time to Positivity

Time to positivity was operationally defined as the duration at which the growth of *P. acnes* became discernible on agar plates, the appearance of turbidity in the thioglycolate broth, or the manifestation of a positive signal for *P. acnes* in a blood culture bottle, subsequently confirmed on agar plates.

Statistical analysis

The diagnostic set of each patient encompassed the documentation of culture details, culture method, and Gram staining. The duration until the detection of positivity was assessed for each positive sample in both the infection and no-infection cohorts. The present study involved a comprehensive statistical analysis that aimed to assess various parameters related to culture methodologies in a medical context. These parameters included the time required for sensitivity, positivity, specificity, and predictive values. The culture methodologies under investigation encompassed direct aerobic, direct anaerobic, and enrichment techniques. The analysis was conducted across a diverse range of sample types.

RESULT

Clinical Data

In the present investigation, a total of 60 instances characterized by *P. acnes* infections were identified. Among the 370 samples analyzed, 260 of them exhibited positive results, accounting for a prevalence rate of 68.1%. However, it is important to note that out of the total 210 samples analyzed, 46 cases did not fulfill the established criteria for a confirmed infection. Among these cases, only one sample tested positive, accounting for a prevalence rate of 20.9%. The shoulder was identified as the most frequently sampled site, with a total of 70 cases, followed by the hip with 25 cases. Within the cohort of patients with infection, a total of 34 cases (48%) were diagnosed with periprosthetic joint infection (PJI). Among these cases, 11 (13.3%) were identified as implant-associated infections involving large implants, while 16 (23.3%) were associated with small implants. A total of five cases were diagnosed with septic arthritis, while three cases presented with osteomyelitis. Among the cohort devoid of infection, a total of 21 instances were observed wherein pain was attributed to mechanical causes. Additionally, 8 cases were identified as the aseptic loosening of an implant, while 15 cases were ascribed to alternative etiologies, which were conclusively diagnosed during revision surgery.

No statistically significant difference was found in the number of samples collected between the cohorts with infection and those without infection. No statistically significant differences were observed in terms of gender, age, or the presence of foreign bodies when comparing the two groups. The intraoperative observations, as reported by the surgical team, did not provide adequate discriminatory value in determining the presence or absence of infection. The study observed a significantly higher prevalence of *P. acnes* isolation in the shoulder region among individuals in the infection group (n=50) compared to those in the no-infection group (n=25). In contrast, within the knee region, the prevalence of *P. acnes* isolation was observed to be higher in the group without infection (n=4) as opposed to the group with infection (n=2).

Table 1: Clinical characteristics

Parameter	Infection	No infection
Patient characteristic		
Female sex (%)	27	33
Age (yr)	57	57
Sample site (%)		
Shoulder	71.9	54.3
Hip	17.6	26.7
Spine	4.7	1.1
Knee	1.3	11.6
Other	1.3	3.3
Presence of (%)		
Any foreign body	87.6	82
Prosthesis	48	56.4
Intraoperative presentation		
Normal	10	11.8
Pus	11.9	3.3
Inflammation	57.6	54.3
Wear of the implant	1.3	1.3
Adhesion	13.3	13.9

Time to Positivity

The infection group exhibited a higher proportion of sample positivity, with 54.4% of samples testing positive after 7 days and 64.6% after 10 days. In contrast, the no-infection group had a lower proportion of positive samples, with 7.4% after 7 days and 18.1% after 10 days. This difference in proportions between the two groups was statistically significant. In the current investigation, it was noted that the group with infection demonstrated a notably reduced median duration until the initial positive sample, as compared to the group without infection. The duration required to confirm the presence of infection, as defined by the time it took to obtain a second positive sample, exhibited a notable disparity between the two cohorts. The infection group demonstrated a mean confirmation time of 6 days, whereas the no-infection group exhibited a mean confirmation time of 9 days. This discrepancy was found to be statistically significant.

Among the cohort of patients classified under the infection category, the cessation of cultures on the seventh day would have led to the omission of the infection diagnosis in 14 out of 60 cases, corresponding to a prevalence of 20.4%. On the tenth day, a previously unidentified infection caused by *Propionibacterium acnes* persisted in 5 cases, accounting for 7.6% of the total cases. However, on the eleventh day, these cultures were found to be positive, primarily due to the implementation of blind subcultures conducted after routine thioglycolate broth cultivation. Within the cohort of individuals not exhibiting any signs of infection, a majority of cases (60.7%, 28 out of 46 cases) displayed a solitary positive sample for *P. acnes*, indicating a potential contamination of the sample. Notably, these instances were predominantly observed after the seventh day.

Comparing Different Cultivation Methods

In the comparative analysis conducted on different cultivation methods in both infection and non-infection cohorts, it was noted that the implementation of thioglycolate broth for tissue biopsy specimens led to a significantly decreased median time to positivity when compared to alternative methods. Notably, no statistically significant disparities were observed among the remaining methodologies.

Sensitivity, Specificity, and Predictive Values

The utilization of thioglycolate broth as an enrichment technique for tissue and bone biopsy specimens proved to be the most efficacious in accurately discerning the presence of *P. acnes*. In tissue samples, the sensitivity of this method was determined to be 65.3%, while in bone samples it exhibited a sensitivity of 74%. The observed results demonstrated a marked improvement in comparison to the aerobic and anaerobic agar plates, exhibiting sensitivities of 4.1% and 41.1% respectively. Nevertheless, the utilization of thioglycolate broth, despite its notable sensitivity, exhibited diminished specificity in tissue (78.1%) and bone (65.7%) specimens.

The enrichment technique that demonstrated the most favorable specificity while minimizing false-positive results involved the use of aerobic agar or aerobic blood culture bottles. The study conducted a comparative analysis between tissue and sonication fluid cultures, revealing that both direct anaerobic cultivation techniques demonstrated the highest positive predictive value. Tissue cultures exhibited a positive predictive value of 95.5%, while sonication fluid cultures demonstrated a positive predictive value of 85.7%.

Gram Staining

A total of 310 samples obtained from patients diagnosed with bone and joint infections were analyzed. Among these samples, only 11 cases (4.2%) exhibited a positive outcome, indicating the presence of Gram-positive rods. There was an absence of any discernible indication of positive Gram staining in all samples collected from noninfected subjects, resulting in a positive predictive score of 100% and a negative predictive score of 36.9%.

Influence of Transportation Time

Transportation time data were obtainable for 87.9% (520 out of 590) of the samples. Among the observed cases, a noteworthy proportion of 93% of positive samples were detected within the infection group, while a slightly lower percentage of 91% was identified in the comparison group.

DISCUSSION

In this extensive study encompassing a cohort of 60 cases afflicted with *P. acnes* infections, the current findings reveal a median duration of 6 days (with a maximum range of 11 days) required for the confirmation of infection. The implementation of a shortened cultivation period of 7 days, as previously recommended in scientific investigations, would have failed to detect diagnoses in 21.4% of the patient population [8]. Hence, in instances characterized by a substantial probability of *P. acnes* infections, the imperative lies in the elongation of the cultivation period to ensure precise identification. This finding is consistent with a prior investigation that advocated for a standardized cultivation duration of 10 days to ensure accurate identification of *P. acnes* infections, a recommendation that is corroborated by our dataset [9]. A 10-day cultivation period with a blind subculture in thioglycolate broth is additionally recommended for cases exhibiting a robust suspicion of *P. acnes* infection. This approach is warranted as it has been observed that ceasing cultures at day 10 would result in the oversight of 7.6% of cases.

The median duration until the initial positive sample was detected was found to be 5 days, a finding that aligns with observations reported in prior investigations [10]. Nevertheless, our investigation revealed a notable acceleration in the growth rate of *P. acnes* within infected samples as opposed to contaminated samples, particularly in the presence of thioglycolate broth. The utilization of thioglycolate broth enrichment over 10 days proved to be the most efficacious technique in the identification of *P. acnes*. However, it is important to note that this method also exhibited an elevated susceptibility to bacterial contamination, as evidenced by the presence of such contamination in 61.7% of samples within the non-infected cohort after the seventh day of incubation [11]. Hence, it is imperative to establish an equilibrium between the levels of sensitivity and specificity.

The reliability of Gram staining in the exclusion of bone and joint infections was found to be limited, as all instances of positive results were observed exclusively in cases with confirmed infection. The process of sonication of implants, although recognized for its heightened sensitivity, exhibited diminished sensitivity in our study when juxtaposed with tissue cultures, potentially attributable to abbreviated cultivation durations. The superior performance of direct agar plating, with a duration of 2 to 3 days, in comparison to blood culture bottles lasting 7 days, has presented a significant challenge to the prevailing perspective in terms of sensitivity [12].

The impact of transportation duration from the operating theater to the microbiology laboratory on the time required for *P. acnes* growth to reach positivity was found to be statistically insignificant. This suggests that *P. acnes*, when existing in the form of biofilm, demonstrates resilience against alterations in its surrounding environment.

CONCLUSION

The study has determined that an extended incubation period of 10 days is imperative for the successful identification of *Propionibacterium acnes*. It is not recommended to reduce the cultivation period to 7 days in patient populations with an increased incidence of *P. acnes* infections, such as individuals diagnosed with shoulder PJI or vertebral osteomyelitis. A comprehensive analysis reveals that a notable proportion, precisely 21.4%, of the P. acnes infections would have successfully evaded detection if the cultivation period had been curtailed to a mere 7-day duration. The application of thioglycolate broth as an enrichment methodology in tissue biopsy specimens has exhibited a noteworthy level of sensitivity. The study findings demonstrated that the utilization of direct incubation on anaerobic agar plates yielded the most favorable positive predictive value. The potential influence of an extended duration of transportation on the temporal progression towards positivity of *P. acnes* growth seems to

exhibit minimal significance. This observation indicates that *P. acnes*, which resides within the biofilm of musculoskeletal infections, exhibits the ability to persist for extended periods, even in an environment that necessitates meticulous adherence to its specific needs.

Limitations of the study

One notable limitation of the study pertains to the disparate incubation durations of various culture media. This incongruity poses challenges in terms of facilitating direct comparisons. However, it is important to acknowledge that rectifying this issue is arduous given the retrospective nature of the study design.

Recommendations

Based on the findings of this study, it is recommended that for the precise diagnosis of bone and joint infections caused by Propionibacterium acnes, a cultivation period of 10 days, including a blind subculture after day 7, should be implemented when using biopsy specimen culturing methods. This approach is suggested to ensure optimal sensitivity and reduce the risk of false-negative results in the diagnosis of P. acnes infections. Further research and validation of this cultivation protocol may be beneficial for improving diagnostic accuracy in clinical practice.

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List of abbreviations

PJI: Periprosthetic Joint Infections

MALDI-TOF: Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

P. acnes: Propionibacterium acnes

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The study was not funded.

Conflict of interest

The authors report no conflicts of interest in this work.

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