Comparative Study of Two Molecular Methods and Culture for the Detection of Microbial Agents in Patients with Osteomyelitis and Septic Arthritis

Abstract

Introduction: Infection is a major orthopedic concern, particularly following knee replacement. Postoperative infection poses significant risks, and in trauma patients with open wounds, it can lead to severe complications. Managing these infections often requires prolonged antibiotic therapy, extended hospitalization, and, in many cases, revision surgery for joint replacement or repeated debridement in osteomyelitis—resulting in serious patient morbidity. Given the high rate of self-administered antibiotics and negative culture tests in joint infections, this study compares molecular (PCR) and culture methods for detecting microbial agents in osteomyelitis and septic arthritis.

Materials & Methods: In this cross-sectional study, 100 samples from patients with joint infections and osteomyelitis at a Hospital were analyzed using microbial culture and PCR. Demographic data (age, gender), comorbidities, and clinical signs (fever, chills, swelling, discharge, pain, limited mobility) were extracted from medical records. Data were analyzed using SPSS22.

Results & Discussion: Among 100 samples, 80 tested positive via PCR, while 55 were culture-positive. Conversely, 20% were PCR-negative and 45% culture-negative. A statistically significant difference existed between the two methods in detection rates ($P < 0.04$).

The patient factors such as old age, previous medical conditions, high BMI, osteopenia, and intraoperative factors, including failure to correct lumbar lordosis, sagittal balance, and correction of angles in primary surgery, showed increased risk for revision spine surgery.

Conclusion: PCR is faster and more accurate than culture for diagnosing osteomyelitis and septic arthritis, enabling earlier treatment intervention.

Keywords: Polymerase chain reaction, Cell culture, Osteomyelitis, Infectious arthritis.

Accepted: 36 days before printing

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Introduction

Osteomyelitis is a bone infection caused by pyogenic bacteria and mycobacteria. This infection is most commonly seen in children and usually occurs through hematogenous spread. In adults, osteomyelitis is often subacute or chronic, and typically arises secondary to an adjacent infectious focus, or as a result of direct inoculation following surgery or trauma[1–4]. More than 95% of acute hematogenous osteomyelitis cases are monomicrobial, with *Staphylococcus aureus* isolated in approximately 50% of cases. In contrast, chronic osteomyelitis is often polymicrobial, involving not only *S. aureus* but also gram-negative and anaerobic bacteria[5].

Clinical signs and symptoms of chronic osteomyelitis include purulent discharge through sinus tracts over the affected bone, pain, tenderness, local inflammatory changes at the lesion site, and low-grade fever in some patients. The disease is characterized by a prolonged clinical course, extended periods of latency, frequent relapses, and serious complications such as deformities, restricted mobility, and neurological deficits. These features make treatment extremely challenging and impose a significant economic burden[6,7].

Septic arthritis may result from the direct invasion of the joint space by various microorganisms, most often bacteria; however, viruses, mycobacteria, and fungi can also play a role.

Reactive arthritis, by contrast, is a sterile inflammatory process that can follow an extra-articular infection. Pathogenic bacteria are particularly important in septic arthritis due to their destructive nature, and failure to promptly recognize and treat the infection can lead to fatal outcomes[8–10].

Approximately 20,000 cases of septic arthritis occur annually in the United States (about 7.8 cases per 100,000 population per year), with similar incidence rates reported in Europe[4,5]. The incidence of arthritis caused by disseminated gonococcal infection is around 2.8 cases per 100,000 population per year.

Diagnostic methods for osteomyelitis and septic arthritis include joint fluid culture and PCR. Normal synovial fluid is clear, colorless, and, when expelled from a syringe, forms a thread-like structure reflecting normal viscosity. In contrast, infected synovial fluid is typically yellow-green due to elevated nucleated cell counts, with a marked predominance of polymorphonuclear leukocytes. Synovial fluid evaluation (including leukocyte count, Gram stain appearance, and microscopic examination) is considered the most valuable approach in assessing a potentially infected joint. Smear and repeat culture should also be performed depending on the differential diagnoses. Alterations in glucose and protein levels of synovial fluid are nonspecific and generally not recommended for routine evaluation[10–12].

Infection represents one of the most serious complications, requiring prolonged antibiotic therapy, extended hospitalization, and, in many cases, repeat surgical procedures such as prosthesis revision in joint replacements or repeated debridement in osteomyelitis. These interventions themselves can result in severe harm and disability. Moreover, since patients often use antibiotics indiscriminately, the rate of negative culture results in joint infections is high. Therefore, we decided to conduct a study evaluating a new molecular diagnostic method for microbial detection in these patients and compare it with conventional culture.

Materials & Methods

This cross-sectional analytical study was conducted on hospitalized patients at Baqiyatallah Hospital who had been diagnosed with joint and bone infections. The sample size was determined as 100 patients, based on the sample size calculation formula. Sampling was performed using a convenience method. After selecting the sample size and identifying eligible cases, 100 hospitalized patients diagnosed with joint infection and osteomyelitis, for whom both microbial culture and PCR had been performed, were included in the analysis.

Variables such as age, sex, and clinical symptoms (including fever, chills, swelling, discharge, pain, and restricted joint movement) were extracted from the patients' medical records.

The kits used in this study were as follows:

- Multiplex: Master Diagnostic kit (Spain)
- Culture: Rousha kit (Germany)
- Antibiogram: Mast kit (UK), distributed by PadTan Teb (Iran)

For laboratory procedures, 2% agarose gel was employed. To prepare the gel, an appropriate amount of agarose powder was dissolved in TBE buffer according to the size of the electrophoresis tank and gel tray. The mixture was heated using a microwave and thoroughly mixed until the agarose was completely dissolved, homogeneous, and transparent. The solution was then allowed to cool, and once the temperature reached approximately 50–60 °C, DNA Green Viewer stain—a non-carcinogenic and safe dye—was added at a ratio of 1 µL per 10 cc of gel. The gel solution was then poured into a tray with a pre-set comb, and after approximately 15 minutes, once the gel had fully solidified, the comb was removed, creating wells for sample loading.

The gel tray was then placed into the electrophoresis tank and filled with TBE buffer to a level 3–5 mm above the gel surface. Insufficient buffer volume could result in premature gel drying, while excessive volume could slow product migration and cause device overheating. In general, 6x loading dye was used, which contained bromophenol blue and xylene cyanol dyes, along with glycerol. Glycerol ensured that the samples, once mixed, became denser and settled firmly at the bottom of the wells without floating out. The dyes enabled visual tracking of nucleic acids during electrophoresis. For each sample, 1 µL was mixed with 5 µL of loading dye before being pipetted into the wells. After data collection and classification, statistical analyses were performed using SPSS software version 22. Quantitative variables were analyzed using the t-test, while qualitative variables were analyzed using the chi-square test. A p-value of <0.05 was considered statistically significant in all tests.

Results

Out of the 100 patients enrolled in this study, 46 (46%) were female and 54 (54%) were male. The age range of participants was 18 to 69 years, with a mean age of 43.41 years. Analysis of variance showed no statistically significant association between age and the occurrence of osteomyelitis or septic arthritis (P=0.65).

A total of 76 patients (76%) had a history of hospitalization, while 24 (24%) did not. Fifty-eight patients (58%) had underlying medical conditions, whereas 42 (42%) had none.

Joint involvement was observed in 57 patients, distributed as follows: 16% hip involvement, 10% knee, 7% shoulder, 8% elbow, 6% ankle, 6% combined hip and shoulder, and 4% combined hip and knee involvement.

Among patients diagnosed with osteomyelitis or septic arthritis, 54 presented with fever, 43 with chills, 65 with pain, 55 with swelling, 32 with discharge, and 32 with restricted joint movement.

Of the 100 analyzed samples from patients with osteomyelitis or septic arthritis, 80 tested positive by PCR, while 55 tested positive by microbial culture. Conversely, 20% of samples were negative by PCR, and 45% were negative by microbial culture. Statistical analysis indicated a significant difference between the two diagnostic methods in terms of positive and negative results (P≤0.04).

Specifically, the percentage of infection-positive samples was higher with PCR compared to microbial culture, and this difference was statistically significant. Similarly, the proportion of infection-negative samples was lower with PCR than with microbial culture, which was also statistically significant (Table 1).

Table 1: Determination of Positive and Negative Test Percentages Using PCR and Culture Methods

Test Method	Negative (%)	Positive (%)	P value
PCR	20	80	P<0.04
Culture	45	55	

Since all samples were obtained from patients with confirmed osteomyelitis or septic arthritis, cases that were not detected by PCR or microbial culture were considered false negatives. The incidence of false negatives was significantly lower with PCR compared to microbial culture, and this difference was statistically significant(Table 2).

Table 2: Determination of False-Negative Rates in PCR and Microbial Culture Methods

Test Method	Negative (%)	Positive (%)	P value
PCR	20	80	<0.04
Culture	45	55	–

In our study, PCR detected 80% of the samples as positive, yielding a sensitivity of 80%, compared with 55% for microbial culture. These results indicate that PCR offers superior diagnostic accuracy over culture for identifying patients with osteomyelitis and septic arthritis.

Discussion

Recent studies have reported that in many cases the infectious pathogen yields negative culture results. This means that the culture medium used cannot identify microbial agents. When unusual pathogens are suspected, the likelihood of non-detection by culture increases, which in turn raises the risk of morbidity and mortality for the patient. Furthermore, conventional culture methods require several days before results become available. Recent evidence has also shown that certain species, such as *Klebsiella*, do not grow in standard culture media, whereas PCR provides a much higher chance of detection, making it a viable alternative to culture. In addition, PCR reduces the diagnostic time to about three hours. Overall, compared to microbial culture, PCR is a more accurate method with higher sensitivity for identifying infectious agents. Thus, its application in cases such as osteomyelitis and septic arthritis can play a crucial role in timely diagnosis and the detection of causative pathogens, ultimately facilitating prompt and effective treatment. This, in turn, can lower patient mortality rates and reduce healthcare costs.

The accuracy of PCR depends on several factors, including the amount of DNA present in the clinical sample and the number of live or dead bacteria contained within it. PCR inhibitors such as hemoglobin may also affect its reliability. Based on the findings of the present study, PCR was effective in the rapid diagnosis of the disease, and the occurrence of false-negative results was negligible (PCR sensitivity in this study was 80%). Reduced overall accuracy may be attributed to technical errors, poor performance of chemical reagents, insufficient DNA in the sample, chronicity of the disease, or prior antibiotic use. In such cases, PCR results may appear as false negatives. Nonetheless, PCR generally demonstrates high specificity across a variety of extrapulmonary samples, including cerebrospinal fluid, pleural fluid, and ascitic fluid. Using PCR, drug resistance can also be rapidly detected in smear-positive sputum samples or in previously cultured isolates. Moreover, molecular methods such as PCR are particularly valuable in detecting pathogens that are not culturable, thereby enabling earlier diagnosis and more timely treatment. For example, *Mycobacterium leprae*, the causative agent of leprosy, cannot be cultured, and its diagnosis relies on acid-fast staining or histopathological changes. If PCR demonstrates the presence of *M. leprae* DNA, no further testing is required, and treatment can be initiated(13). This underscores the clinical utility of molecular methods in cases where microbial culture fails to identify the infectious agent, as confirmed by the present study, which showed that molecular techniques can detect pathogens undetectable by culture or those yielding false-negative results.

Various types of PCR are also capable of identifying hepatitis B, hepatitis C, HIV, HSV, cytomegalovirus (CMV), and enteroviruses. This test is additionally used for screening purposes and for monitoring treatment response. In blood banks, PCR is routinely employed for detecting hepatitis C and HIV. At present, PCR analysis of cerebrospinal fluid is considered the gold standard for diagnosing HSV encephalitis and meningitis, with a sensitivity of 95% and specificity of 94%, and it can prevent the need for brain biopsy(14-16). Therefore, molecular methods are also highly valuable in the diagnosis of viral infections. For instance, PCR can detect CMV in plasma and cerebrospinal fluid with 95–98% sensitivity and 98–100% specificity, whereas the sensitivity of CMV culture is only about 42%. Enteroviruses, one of the most common causes of viral meningitis, can be identified in CSF by PCR within a single day, while culture requires five days.

Quantitative PCR can further be employed to monitor treatment response by measuring viral load in CMV, HIV, and HCV infections(17,18). PCR can also be used to diagnose HSV infections and determine their serotypes using saliva, serum, or CSF samples. Notably, PCR has demonstrated that HSV-2 is the principal cause of recurrent benign lymphocytic meningitis. *Varicella-zoster virus* can likewise be detected via PCR in saliva, throat swabs, tears, and skin lesions, including early non-vesicular erythematous areas. PCR has also facilitated the diagnosis of Kaposi's sarcoma, where detection of human herpesvirus type 8 in lesions is challenging by other methods, but PCR provides highly sensitive and specific confirmation.

In addition to viral pathogens, PCR is useful for detecting various parasites, including *Ascaris*, *Toxoplasma*, and *Leishmania*(19,20). Prenatal PCR testing can even identify congenital *Toxoplasma* infection(21). PCR can be performed on diverse specimen types, such as blood, amniotic fluid, and stool(22). Furthermore, Hinbaj and Schwam used PCR with primers for the *pla* gene to identify *Yersinia pestis* across subspecies from Asia, Africa, and America. Their findings confirmed the reliability of PCR for diagnostic, epidemiological, and disease-control purposes. Unlike their work, which used a uniplex PCR method, our study employed multiplex PCR, allowing simultaneous detection of multiple bacterial species, saving both time and resources(23).

In general, the findings of most studies demonstrate that molecular methods significantly improve the detection of infectious agents. The results of the present study are consistent with this evidence, showing that molecular techniques can identify more pathogens than culture alone. Therefore, incorporating molecular diagnostics can substantially aid clinicians in initiating appropriate treatment at the right time, ultimately reducing mortality rates and preventing unnecessary healthcare expenditures.

Conclusion

Based on the results of this study, the multiplex PCR method can be used for the rapid and simultaneous detection of various bacteria, particularly in patients with osteomyelitis and septic arthritis. This approach provides a reliable and accurate tool for identifying these pathogens. The sensitivity of this method is remarkably high, such that it can detect a single bacterial species among thousands of different types. It is also simple to perform, and because it allows for simultaneous detection, there is no need to conduct multiple separate PCR assays for each individual pathogen. The application of combined PCR for the rapid and concurrent detection of several infectious agents in unknown samples represents a valuable advantage of this method. Overall, the findings of this study comparing molecular and culture-based methods for diagnosing microbial pathogens in patients with osteomyelitis and septic arthritis demonstrate that molecular techniques are faster and more accurate than microbial culture. Therefore, the use of this method can play a significant role in enabling early treatment in these patients

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