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Research Article

Joint Infection Syndromic Testing: An Advanced Tool for Better Diagnostic Stewardship in Joint Infections and for enhanced Antibiotic Stewardship

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Abstract

Bone and joint infections encompass various human diseases such as septic arthritis, prosthetic joint infections, osteomyelitis, spinal infections, diabetic foot osteomyelitis. These conditions pose significant threats to health, contributing to morbidity and mortality. Diagnosis relies on clinical manifestations and identifying the causative microorganism. This study assesses the effectiveness of the BIOFIRE JI panel in detecting causative organisms, along with the automated culture method. The BIOFIRE JI panel demonstrated better pathogen identification, including the detection of resistance genes, compared to the automated culture. Early identification and concomitant detection of resistance genes marks the potential of BIOFIRE JI panel to enhance antibiotic stewardship and improve patient treatment outcomes.

Keywords:

Joint infection, synovial fluid, diagnostics, multiplex PCR, Film Array, species identification, resistance, antibiotic stewardship, syndromic panel PCR

Abbreviations

JI – joint infection

NJI- Native Joint Infection

PJI- Prosthetic Joint Infection

Introduction

Bone and joint infections are a group of human diseases that include septic arthritis, prosthetic joint infections, osteomyelitis, spinal infections, and diabetic foot osteomyelitis; resulting in morbidity and mortality and constitute a true musculoskeletal emergency when left unattended. The common presentation of joint infection

is acute monoarthritis. Identification of organisms in the synovial fluid is the standard diagnosis where the synovial fluid aspiration has been performed prior to antibiotics regimen(1). Recently, an increase in the emergence of implant-associated osteoarticular infections has been prevalent. The use of prostheses has improved the life quality of the patients, allowing to regain mobility and avoiding immobilization. This approach has complications and results in infections requiring further surgeries and prolonged antibiotic treatments(2). The increasing number of multidrug-resistant organisms further complicates antibiotic selection, so the proper etiological diagnosis of the disease is highly significant(3).

The causative organisms differ significantly between native joint infection (NJI) and prosthetic joint infection (PJI). Highly virulent monomicrobial including *Staphylococcus aureus*, *Streptococci spp.*, and gram-negative rods are usually associated with NJI (4). PJI is caused by a wide variety of pathogens and even includes low-virulence organisms such as coagulase-negative *Staphylococci*, *Cutibacterium spp*., *Viridans streptococci*, and *Enterococcus spp*(2).

The diagnosis of joint infection is based on clinical findings, laboratory tests from peripheral blood and synovial fluid, microbiological data, histological evaluation of periosthetic tissue, intraoperative inspection, and radiography (2). The 2-fold approach focuses on confirming the joint infection along with the identification of causative microorganism is beneficial. Importantly, antimicrobial susceptibility is intimately associated with the cases(2). The sensitivity of conventional gram stain method is 20–30% in septic arthritis and is even lower in PJI demanding effective strategies (4,5). The growth of microorganisms in the synovial fluid and intraoperative tissue sample culture has been influenced by multiple factors such as previous antibiotic treatments, fastidious pathogens, and the low quality of samples. This prevents an etiological identification and lead to empirical treatment. Additionally, multidrug resistance (MDR) challenges to choose a suitable antibiotic (6). The ongoing limitations in the existing methods demand a culture-independent method for pathogen detection. Molecular methods have shown great potential for improving sensitivity and reducing

the time spent on microbiological identification. 16S/18S ribosomal RNA, commercial multiplex panels, and syndromic PCR panels are being employed to address low sensitivity, though their significance in management is undetermined. The joint infection (JI) panel is a fully automated syndromic multiplex PCR panel that identifies 31 causative pathogens and 8 clinically relevant genetic resistance markers suggesting their immense translational potential. In this retrospective study, the efficiency of multiplex panel has been compared with the culture-based standards of care at a tertiary care hospital in Kerala, India.

Methods

Clinical Sample collection

59 synovial fluid samples were collected at a tertiary care hospital in Kerala, India, from patients who had clinical suspicion of native septic arthritis, or PJI. All the samples were collected either by arthrocentesis or intraoperatively under sterile conditions. Routine microbiology analysis and identification were performed using automated culture methods. Requirement of ethical approval was waived as this is a retrospective study.

Automated Microbial Culture

Samples were inoculated into bottles in the automated microbial detection system (BACTALERT 3D, bioMerieux SA). Once the bottles flag positive, the samples from bottle were inoculated into blood agar, chocolate agar, MacConkey agar and incubated for 24 to 48 hours. The organisms were identified using automated microbial identification and susceptibility system (VITEK 2 COMPACT, bioMerieux SA). Gram staining was performed for all samples.

JI Panel Testing

In vitro diagnostic (IVD) version of the BIOFIRE JI panel was used to analyse all the 59 samples. All samples were handled in a biosafety cabinet according to the standard procedure. 200 µL of the synovial fluid was used for the analysis. The sample was added to the BIOFIRE JI PANEL sample buffer, then the same was injected

into the test pouch, and loaded into the Film Array System. Nucleic acid extraction, reverse transcription, nucleic acid amplification, and result analysis were performed automatically in 60 minutes for each sample. The presence or absence of pathogens and AMR genes were reported qualitatively.

Statistical analysis

Given the nature of the evaluation, descriptive statistics were used to compare results from BIOFIRE JI PANEL to the automated culture.

Results

Demographics

59 patient samples were analysed for the presence of causative pathogens for bone and joint infections using BIOFIRE JI PANEL. The patients ranged in age from 1 month 27 days to 86. 7 patients were aged less than 20, and 21 patients were aged 21–60. 27 patients were aged between 61 and 80 years, and 4 were aged above 80 years. 28 of the patients were females and 31 were males (**Table 1**).

Demography	**Percentage**
Age	
< 20	11.86
20-60	35.59
60-80	45.76
>80	6.77
Gender	
Males	52.54
Females	47.45

Table 1: Demographic information showing age and gender distribution of patients suspected of bone and joint infection.

22 samples were derived from patients with clinical suspicion of prosthetic joint infections and 36 from patients with clinical suspicion of native joint infections. One patient was suspected to have trochanteric bursitis (**Table 2**). From the total cohort, 30 patient samples were identified with infection.

Clinical suspicion	**Percentage**
Native joint infection	36
Prosthetic joint infection	22
Trochanteric bursitis	1

Table 2: Distribution of suspected clinical diagnosis

BIOFIRE JI PANEL results of 27 samples were compared with the results from BACT ALERT Culture. BIOFIRE JI PANEL results were obtained in 1 hour while the BACT ALERT culture took a minimum of 2 to 7 days with an average of 3.63 days.

Of the 27 samples, 7 were negative by both BIOFIRE JI PANEL and automated culture methods. The BIOFIRE JI panel showed positive pathogen identification in 17 samples, and BACT ALERT flagged positive for 13 samples.

The microorganisms identified were *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Streptococcus agalactiae, Klebsiella pneumoniae group, Streptococcus pyogenes, Proteus species, and Serratia marcescens*. 5 samples were detected with pathogens by the BIOFIRE JI PANEL that were absent in the culture. Routine culture revealed the presence of two pathogens: *Citrobacter koseri* and *Burkholderia pseudomallei* which were not present in BIOFIRE JI PANEL. A polymicrobial infection with *Enterococcus faecalis* and *Citrobacter koseri* was found in one sample. BIOFIRE JI PANEL detected resistance markers, mec A/C and MREJ (MRSA), IMP, and CTX-M in 3 different samples.

Discussion

This study analysed 59 samples using the BIOFIRE JI PANEL. The efficiency of BIOFIRE JI PANEL was compared with the BACT ALERT culture method for the identification of pathogenic microorganisms. The efficiency of diagnosis was higher in BIOFIRE JI PANEL than in the culture of synovial fluid from patients who were suspected of native or prosthetic joint infections. The number of pathogen identifications was higher, including the detection of resistance genes. These diagnoses enhance the chances for targeted antibiotic therapy in cases of septic arthritis, or an alternate diagnosis can be recommended when BIOFIRE JI PANEL is negative.

Thirteen samples were detected with causative organisms by the conventional culture, while 17 were detected by the BIOFIRE JI PANEL. Even with advanced techniques such as sonication, prolonged incubation, and the use of enriched blood culture media, the detection of pathogens by culture is time-consuming. In our reports, the culture results were obtained on day 2 to a maximum of 7 days. Meanwhile, the identification of organisms by BIOFIRE JI PANEL is rapid and takes only 60 minutes. Rapid detection of causative microbes for joint infections allows targeted antibiotic treatment. The polymicrobial nature and involvement of microbes outside the panel make BIOFIRE JI PANEL less efficient in the diagnosis of early acute PJI (6).

The sensitivity and specificity of the BIOFIRE JI PANEL were found to be reliable when compared to the culture. 16S rRNA PCR and next-generation sequencing are reported to have higher sensitivity than culture (7,8). Syndromic multiplex PCR has become a dependable diagnostic tool for the detection of infection in joint infections. Berneking et al. reported the use of a syndromic panel targeting bone and joint infection, with a sensitivity of 85% and a specificity of 89% (9). BIOFIRE JI PANEL was previously reported to have a sensitivity of 90% and a specificity of 100% (10). A negative test result in BIOFIRE JI PANEL brings down the chances of infection in patients with suspicion of native septic arthritis and prosthetic joint infection, narrowing down the empirical antibiotic treatment in less time. This recommends the selection of ideal

antibiotic to prevent antibiotic resistance and protect the infected joints from further damage (11).

Rapid and accurate diagnosis is specifically important in acute joint infections where the symptoms develop quickly. Optimal selection of antibiotic treatment has a significant impact on the outcome of the patient owing to MDR. Currently, faster diagnosis has been attained only by molecular methods, while most of these methods are commercially unavailable. Broad-range PCR methods including 16SrRNA require advanced molecular biology facilities and highly experienced personnel (12). Azad et al. compared BIOFIRE JI PANEL with targeted metagenomics sequencing where the performance of BIOFIRE JI PANEL was high, with 91% sensitivity and 100% specificity. However, the accuracy of the diagnosis was less because of the absence of microorganisms such as *S. epidermidis* and *C. acnes* in the panel, and most of the samples in this study were from chronic infections (13). The results for acute PJI and native septic arthritis were accurate, with a 95.3% agreement for the patient diagnosis (14). Common contaminants including Coagulase Negative *Staphylococci* and *C. acnes* were exempted from the panel despite their involvement in chronic implant-associated infections (15). Furthermore, clinically relevant organisms of extremely low frequency were exempted in the BIOFIRE JI PANEL. Hence, the combinatorial use of automated culture along with the BIOFIRE JI PANEL has been recommended for the detection of microorganisms involved in acute native and prosthetic joint infections.

Notably, the rapid turnaround time of BIOFIRE JI PANEL makes the availability of results in less than one hour makes it highly useful in the emergency room and intraoperatively in patients with acute joint infections. The relatively small sample size limits this study to an evaluation of sensitivity and specificity compared to the automated culture of microorganisms. This has resulted in a wide 95% confidence interval during the analysis. Even though the high cost of molecular methods has been specified as the major drawback of BIOFIRE JI PANEL, earlier diagnosis helps in the administration of timely treatment, and less time in the hospital compensates for the expense of molecular methods, including BIOFIRE JI PANEL. Even with

limitations, BIOFIRE JI PANEL, when used along with automated culture, is a rapid, convenient, and feasible method that can be an effective diagnostic tool in the detection of causative pathogens in acute native septic arthritis and prosthetic joint infections.

Conclusion

The findings indicate the high efficiency of the BIOFIRE JI PANEL in detecting pathogens associated with septic arthritis and prosthetic joint infections. Importantly, it is advisable to complement the use of BIOFIRE JI PANEL with other detection methods like automated culture, given the absence of certain causative organisms in the panel. The accelerated turnaround time and the simultaneous identification of antimicrobial resistance genes when employing BIOFIRE JI PANEL suggest potential benefits for enhanced antibiotic stewardship and improved patient treatment. However, further studies with larger sample sizes are necessary to validate the actual impact of BIOFIRE JI PANEL based diagnosis on patient management.

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