

# Identification of cultivable and non-cultivable organisms causing intraarticular and bone infections using molecular diagnostic techniques

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**Abstract**

*Introduction: Osteomyelitis is pathologically defined as inflammation of bone and bone marrow due to infection with a microbial pathogen. Septic arthritis is pyogenic infection of the joint either due to direct extension from local tissue infection or more commonly as a result of bacteremia. Microbiological diagnosis in these cases can be made by culture on enriched media and PCR. As PCR detects bacterial DNA, it does not rely on the presence of viable bacteria in the sample for identification. The rapidity of PCR along with its higher sensitivity and specificity enables faster and more accurate diagnosis and treatment*

*Objectives: To identify the cultivable pathogens by aerobic culture and to detect cultivable and non-cultivable organisms causing intraarticular and bone infections using broad range PCR*

*Material & Methods: Synovial fluid and bone tissue biopsy were processed, Gram stain was performed. Culture of synovial fluid was done using BacTec system and samples were subcultured on chocolate agar, blood agar. The growth was identified by standard tests and antibiotic susceptibility pattern was assessed by modified Kirby-Bauer method and Vitek 2 compact system. All samples were stored at -20°C for PCR.*

**Results:** Of the 50 samples, 15 samples (30%) were both culture and PCR positive. 3 samples were positive by PCR, but yielded no growth in the culture. This fact could be due to antibiotic effect or other non cultivable or fastidious organisms. 2 samples of synovial fluid with the growth of *Escherichia coli* and *Staphylococcus aureus* in culture were negative by PCR. This could be attributed to inhibition in the PCR reaction. According to our study, the most commonly associated organism, *S. aureus*, showed MRSA and clindamycin resistance (37% and 38% respectively). ESBL production was seen in 38% of *E. coli* and *Proteus mirabilis*.

**Conclusion:** In this study, 17 samples were culture positive, with the most common organism being *Staphylococcus aureus* (41%) followed by *Enterobacter cloacae* (18%), *Enterococcus fecalis* (12%), *Pseudomonas aeruginosa* (12%) and *Escherichia coli* (12%). Out of the 33 culture negative samples, 3 samples were PCR positive. 2 out of the 17 culture positive samples were negative on PCR. *S. aureus*, showed MRSA and clindamycin resistance (37% and 38% respectively). ESBL production was seen in 38% of *E. coli* and *Proteus mirabilis*. High rates of culture negativity may be due to empirical antibiotics given prior to sample collection. But since PCR detects DNA of the micro organism, it doesn't differentiate between dead and living cells hence it usually picks up organisms that are missed on culture.

**Keywords:** septic arthritis, osteomyelitis, PCR, 16sRNA

## INTRODUCTION

Osteomyelitis is pathologically defined as inflammation of bone and bone marrow due to infection with a microbial pathogen. Based on the duration of symptoms, it can be classified as Acute (< 2weeks), Subacute (2 weeks – 3months) and Chronic (>3 months). Septic arthritis is pyogenic infection of the joint either due to direct extension from local tissue infection or more commonly as a result of bacteremia.

Osteomyelitis and septic arthritis commonly result from haematogenous seeding of bacteria into bones and joint. The common organisms involved, i.e. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Kingellakingae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, are frequent colonisers of the respiratory tract, suggesting that the respiratory tract is the most probable portal of entry. *Kingellakingae* has now increasingly been recognised as an important pathogen in septic arthritis among the age group of 6months – 4years, with organisms such as Group B Streptococcus and *Candida* spp. being the common causative agents among neonates. <sup>[1,2]</sup>

Osteoarticular infections can cause significant mortality and morbidity in both children and adults. In children, they have long term effects on the remaining bone growth. Progression to chronic stage, irreversible bone damage and altered bone growth due to incomplete or improper management results in deformity and compromises mobility and function. Early diagnosis and appropriate treatment of bone and joint infections is therefore crucial to prevent any permanent damage. <sup>[3,4]</sup>

Septic arthritis and acute osteomyelitis may occur concurrently in certain situations. Osteomyelitis of sites like proximal humerus, proximal tibia, distal fibula, are more prone to spread sub-periosteally into joint space since the bony metaphysis is intracapsular. In children lesser than 18 months, the epiphysis is vascularised by transphyseal vessels and thus potentially transmits bone infections to the joint space.<sup>[3]</sup>

Acute bone and joint infections are treated from the early stages with broad spectrum antibiotics; hence aspirates from the joint or biopsy samples do not yield any growth on routine culture media. Intravenous antibiotics administered preoperatively also result in negative culture results of biopsy samples. Microbiological diagnosis in these cases can be made by culture on enriched media and PCR. As PCR detects bacterial DNA, it does not rely on the presence of viable bacteria in the sample for identification. The rapidity of PCR along with its higher sensitivity and specificity enables faster and more accurate diagnosis and treatment. It would facilitate accurate diagnosis of the causative agent and may also yield pathogens not covered under empiric therapy. Subjecting culture negative samples to Real time PCR increases the yield of *Kingella kingae*.<sup>[4,5,6]</sup>

Microbial characteristics of acute osteoarticular infections in children <sup>[4]</sup>Cases of children between 0-15 years with SA/OM, were identified through a retrospective search of hospital discharge codes over a 6 year period. The most frequently cultured organisms in both conditions were Gram positive cocci, primarily *Staphylococcus aureus* (44.4%). The study found low rates of culture positivity in the clinically confirmed samples. In SA, 42.29% of the articular fluid was culture positive, in OM intraoperative samples were culture positive in 52.65% with bacteremia present in 23.91% of SA and 21.48% of patients with OM.

*Kingella kingae* as a frequent causative agent of septic arthritis in pediatrics:<sup>[5]</sup> Retrospective analysis of the AS with identification of *K. kingae* in joint fluid, in the pediatric population of two tertiary hospitals of the Community of Madrid, from January 2010 to December 2016. During the period analyzed, five cases of AS were identified by *K. kingae*, all of them in children less than or equal to 6 years old (median age, 24 months). The joint most frequently affected was the knee (4 patients and 1 hip) and all but one of the patients had a fever, with a median duration of 2 days (range: 0-4). The local inflammatory signs had a variable duration (median 9 days, range: 3-12). The patients presented a median of 8 days of hospital admission. *Kingella kingae* Osteoarticular Infections in Young Children: Clinical Features and Contribution of a New Specific Real-time PCR Assay to the Diagnosis: Prospective study of 43 cases of children aged less than 4 years admitted between January 2007 and November 2009 for suspected OAI were enrolled. Identification of the microorganism was possible for 28 cases (65.1%) yielding *K. kingae* in 23 cases (82.1%). Mean age of children with *K. kingae* OAI was 19.6 months. Thirty-nine percent of the children with *K. kingae* OAI had normal C-reactive protein; Direct Gram staining and classical isolation methods were negative for all cases subsequently detected as *K. kingae* OAI by specific real-time PCR.<sup>[6]</sup>

In adults, the etiological agents of joint infections include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella spp.*, *Pseudomonas spp.* and *Neisseria gonorrhoeae*.

**AIM:**To identify the cultivable and non-cultivable organisms causing intraarticular and bone infections using broad range PCR

**OBJECTIVES:**To identify the cultivable pathogens by aerobic culture and to detect cultivable and non-cultivable organisms causing intraarticular and bone infections using broad range PCR

**METHODOLOGY:**

**Study design:** Descriptive study

**Study setting:** Government Wenlock Hospital, KMCH Attavar, KMC Hospital Ambedkar circle and Microbiology Department KMC Mangalore.

**Inclusion criteria:**

All patients with signs and symptoms consistent with acute or chronic osteomyelitis or septic arthritis during the study period.

**Tools for data collection:** Proforma consisting of:

- [A] General information
- [B] Examination findings
- [C] Investigations
- [D] Management

**Methodology:**

Synovial fluid and bone tissue biopsy were processed, Gram stain was performed. Culture of synovial fluid was done using BacTec system and samples were subcultured on chocolate agar, blood agar.

The growth was identified by standard tests and antibiotic susceptibility pattern was assessed by modified Kirby-Bauer method and Vitek 2 compact system

All samples were started at -20°C for PCR.

16s rRNA PCR :

DNA was extracted using QIAamp DNA mini Kit. And the sequence

16s RNA FP AGTTTGATCCTGGCTCAG

16s RNA RP AGGCCCGGGAACGTATTCAC

Cycling conditions- 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C-1 minute annealing 62°C for 1 minute and final extension 72°C for 2 minutes. PCR products were subjected to gel electrophoresis on 1-5% agarose with ethidium bromide [7]

The patient characteristics were studied from the case files.

Results of the PCR were compared with the culture in terms of sensitivity, specificity, PPV, NPV. The study has obtained clearance from the Institutional Ethics Committee.

## Results

A total of 50 clinical non duplicate samples of synovial fluid and biopsies from the osteomyelitic lesions from suspected cases of infection were included in the study during the period July 2019 – September 2019.

Of the 50 samples, 15 samples (30%) were both culture and PCR positive .3 samples were positive by PCR ,but yielded no growth in the culture . This fact could be due to antibiotic effect or other non cultivable or fastidious organisms .2 samples of synovial fluid with the growth of *Escherichia coli* and *Staphylococcus aureus* in culture were negative by PCR. This could be attributed to inhibition in the PCR reaction.

The distribution of the 17 bacterial isolates is shown in Table 1..Rates of MRSA and Clindamycin resistance in *Staphylococcus aureus* were 37% and 38% respectively. ESBL production is seen in 38% of *Escherichia coli* and *Proteus mirabilis* .The rates of antibiotic resistance in Gram negative bacilli are Amikacin (32%) ,Ciprofloxacin(35%), third generation cephalosporins ( 37%) , Carbapenems (12%)

Comparison of PCR with culture is shown in Table 2 and Gel picture is depicted in Fig 1 Among the 50 samples, patient details were collected from 25 patients. Mean age of the patients was 63.56years, with 9 females (36%) and 16 males (64%).

Most common co morbidities associated were Diabetes mellitus Type 2 seen in 18 patients (72%) and Hypertension in 14 patients (56%).Clinical presentation is shown in Table 3.

Out of the 12 patients who presented with symptoms suggestive of septic arthritis, i.e, acute onset of pain in the joint with associated fever with chills, swelling of the joint and reduced range of motion, 2 cases underwent Total knee replacement prior to surgery for management of osteoarthritis.

Patients who presented with features of osteomyelitis or gangrene involving deeper tissues and underlying bone were all managed surgically, with either amputation of the involved toe under regional anaesthesia or bone curettage in 9 patients(69.23%) or wound debridement and bone curettage in 4 patients (30.76%)

In patients presenting with septic arthritis, therapeutic joint aspiration was performed in 6 patients (50%) and arthrotomy with synovectomy was done in 6 (50%).

Antibiotics used for management included Clindamycin in 8 patients, 3<sup>rd</sup> generation Cephalosporins in 5, Fluoroquinolones in 2, Piperacillin-Tazobactam in 2, Vancomycin and gentamicin in 1 patient.

## DISCUSSION:

This study analysed the epidemiology, clinical presentation and treatment of septic arthritis and osteomyelitis based on data obtained from case files among patients presenting to tertiary

care centres. We also aimed to determine the efficacy of Polymerase chain reaction over routine culture for accurate diagnosis of causative pathogens.

The mean age of the patients in our study was 63.56 years, with 64% males and 36% females. A retrospective study conducted by Zhang X et al<sup>[8]</sup> analysed records of patients with chronic osteomyelitis who presented between 2003-2014, mean age of the patients was 39.3±16.5 years and 78.2% of the patients were male. In our study, mean age of patients presenting with signs suggestive of osteomyelitis was 59.69 years with 84.61% males.

In a cohort study conducted by Ruangpin C et al<sup>[9]</sup> using hospital database in Thailand, included 450 patients with confirmed cases of septic arthritis, mean age was 53.6±17.8 years with 51% males. In our study, mean age among patients with septic arthritis was 67.75 years, with an equal distribution among males and females.

Associated co morbidities in our study were Type 2 diabetes mellitus (72%) and Hypertension (56%). Al-Mayahi M et al<sup>[10]</sup> reviewed databases of patients admitted for orthopaedic infections at Geneva University Hospitals from 2004 to 2014, diabetes was noted in 24% of the cases, and patients were older with a male preponderance. They presented with predominantly foot infections, and were associated more commonly with polymicrobial infections.

Multiple studies highlight the importance of using molecular diagnostic techniques for more accurate detection of micro organisms. PCR plays a major role in pediatric bone and joint infections. *Kingella kingae* has recently come up as a major cause for osteomyelitis, septic arthritis in children <4 years old, which is not detected by routine culture. Prospective study was conducted by Ferroni A et al<sup>[11]</sup> with osteoarticular samples collected from children <15 years old where samples were subjected to Real Time PCR using 16s rRNA and specific PCR targeted on *Kingella kingae*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *S. pneumoniae*. *Kingella kingae* was identified in 53% of the samples collected from children <4 years, conversely it was not present in older children in whom the predominant agent was *S. aureus* (73%).

In this study, 17 samples were culture positive, with the most common organism being *Staphylococcus aureus* (41%) followed by *Enterobacter cloacae* (18%), *Enterococcus faecalis* (12%), *Pseudomonas aeruginosa* (12%) and *Escherichia coli* (12%). Out of the 33 culture negative samples, 3 samples were PCR positive. 2 out of the 17 culture positive samples were negative on PCR. According to Zhang X et al<sup>[8]</sup>, top five species were *Staphylococcus aureus* (27.9%), followed by *Pseudomonas aeruginosa* (12.1%); *Enterobacter cloacae* (9.5%); *Acinetobacter baumannii* (9.0%) and *Escherichia coli* (7.8%).

According to our study, the most commonly associated organism, *S. aureus*, showed MRSA and clindamycin resistance (37% and 38% respectively). ESBL production was seen in 38% of *E. coli* and *Proteus mirabilis*. In a study conducted by Matthews J et al<sup>[12]</sup> to determine bacteriology of community acquired musculoskeletal infections, the most common organism isolated was *S. aureus* accounting for 69.7% of the cases, MRSA sensitive to vancomycin was

isolated in 12.1% of the cases. Out of the cohort, 67.7% and 61.3% isolates that were tested were sensitive to cefazolin and flucloxacillin, respectively.

Among patients with septic arthritis, surgical drainage was done in 60% patients. Factors that necessitate surgical intervention are pre existing joint disease, abscess formation, positive synovial fluid culture, osteomyelitis. Surgical drainage on one hand reduces mortality but it increases duration of hospital stay and patients are also less likely to recover completely. <sup>[9]</sup>

High rates of culture negativity may be due to empirical antibiotics given prior to sample collection. But since PCR detects DNA of the micro organism, it doesn't differentiate between dead and living cells hence it usually picks up organisms that are missed on culture. According to the British society of rheumatology guidelines for management of septic arthritis<sup>[13]</sup>, the synovial fluid must be aspirated, Gram-stained and cultured prior to starting antibiotics and the absence of organisms on Gram stain or a negative synovial fluid culture does not exclude the diagnosis of septic arthritis.

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Table 1 : Bacterial spectrum in synovial fluid and bone biopsies

Isolate	Number	Percentage
<i>Staphylococcus aureus</i>	7	41%
<i>Enterobacter cloacae</i>	3	18%
<i>Enterococcus faecalis</i>	2	12%
<i>Pseudomonas aeruginosa</i>	2	12%
<i>Escherichia coli</i>	2	12%
<i>Proteus mirabilis</i>	1	6%
Total	17	

Table 2 : Comparison of PCR with culture

	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
PCR POSITIVE	15	3	18
PCR NEGATIVE	2	30	32
TOTAL	17	33	50

- Sensitivity : 89%
- Specificity :92%



- PPV :84%
- NPV :92 %

With culture as gold standard

Table 3: Clinical Presentation in patients

Presentation	Number	Percentage (%)
Acute onset joint pain	12	48
Non healing ulcer/ Sinus	9	36
Diabetic foot with ulcer	4	16
	25	

Table 4: Findings on Clinical Examination

Findings	Present	Percentage
Discharging sinus	5	20
Wound	7	28
Bone thickening	5	20
Swelling	23	92
Tenderness	24	96