# Performance of TEM-PCR versus Culture for Bacterial Identification in Pediatric Musculoskeletal Infections

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## INTRODUCTION

- Musculoskeletal infections (MSKI) in children require prompt diagnosis and treatment due to risk of local tissue damage and metastatic bacterial spread.
- Despite blood and tissue sampling, cultures remain negative in approximately half of all cases.
- There is a need for improved rapid diagnostics in children with MSKI.
- We compared the detection of pathogens by culture vs. target-enriched multiplex PCR (TEM-PCR™) in children with acute MSKI.

## METHODS

- Children 6 months to 18 years of age admitted to Vanderbilt Children's Hospital with MSKI were prospectively enrolled.
- Synovial fluid and bone samples were collected.
- Bacterial cultures and antibiotic susceptibility testing were performed by the Vanderbilt University Medical Center Clinical Laboratory.
- Samples were evaluated by TEM-PCR (*Fig. 1*) for detection of common pathogens:
  - *Staphylococcus aureus* [including methicillin (*mecA*) and clindamycin/erythromycin (*ermA* or *ermC*) resistance genes and the Panton-Valentine leukocidin (PVL) locus]
  - *Kingella kingae*
  - *Haemophilus influenzae*
  - *Streptococcus pyogenes*
  - *Streptococcus pneumoniae*

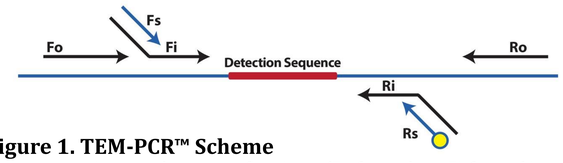



**Figure 1. TEM-PCR™ Scheme**
Low concentration nested gene-specific primers (Fo–forward out; Fi–forward in; Ri–reverse in; and Ro–reverse out) are designed to enrich the targets during the initial PCR cycles. A pair of universal SuperPrimers (Fs and Rs) is subsequently used to amplify all targets. The Rs primer is labeled with biotin for amplicon detection.

## RESULTS

**Table 1. Baseline Characteristics of Patient Population**

| Characteristic | N=25 |
|---|---|
| **Sex** | |
| Male | 14 (56) |
| Female | 11 (44) |
| **Race** | |
| Non-Hispanic White | 17 (68) |
| Non-Hispanic Black | 6 (24) |
| Other | 2(8) |
| **Age, yr (median [IQR])** | **8 (3-12)** |

Data are presented as number (percent) unless otherwise indicated, IQR, interquartile range

## RESULTS

**Table 2. Comparison of Pathogen Recovery and Antibiotic Resistance Testing For Each Sample**

| Specimen | Culture Result | Antibiotic Resistance (Culture-based method) | | | TEM-PCR Result | Antibiotic Resistance Gene Detection (PCR method) | | | PVL |
|---|---|---|---|---|---|---|---|---|---|
| | | Methicillin | Clindamycin | Erythromycin | | Methicillin | Clindamycin | Erythromycin | |
| 1 | *S. aureus* | - | + | + | *S. aureus* | - | + | + | - |
| 2 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |
| 3 | No growth | | | | Negative | | | | |
| 4 | *S. aureus* | + | - | - | *S. aureus* | + | - | - | + |
| 5 | *S. aureus* | + | - | + | *S. aureus* | + | - | - | + |
| 6 | No growth | | | | Negative | | | | |
| 7 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | + |
| 8 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |
| 9 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |
| 10 | *S. aureus* | - | - | + | *S. aureus* | - | - | - | - |
| 11 | *S. aureus* | + | - | + | *S. aureus* | + | - | - | + |
| 12 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | + |
| 13 | *S. aureus* | - | + | + | *S. aureus* | - | + | + | - |
| 14 | *S. aureus* | - | - | + | *S. aureus* | - | - | - | + |
| 15 | No growth | | | | Negative | | | | + |
| 16 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |
| 17 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |
| 18 | No growth | | | | *K. kingae* | | | | |
| 19 | No growth[a] | | | | *K. kingae* | | | | |
| 20 | No growth[b] | | | | *S. aureus* | - | - | - | + |
| 21 | *S. aureus* | + | - | + | *S. aureus* | + | - | - | - |
| 22 | *S. aureus* | + | - | + | *S. aureus* | + | - | - | + |
| 23 | No growth[c] | | | | Negative | | | | |
| 24 | No growth[c] | | | | Negative | | | | |
| 25 | *S. aureus* | - | - | - | *S. aureus* | - | - | - | - |

[a] Positive for *K. kingae* by PCR, [b] Subject 14 and 20 are the same, samples from different dates, [c] Diagnosed with non-infectious arthritis, PVL- Pantone Valentine Leukocidin

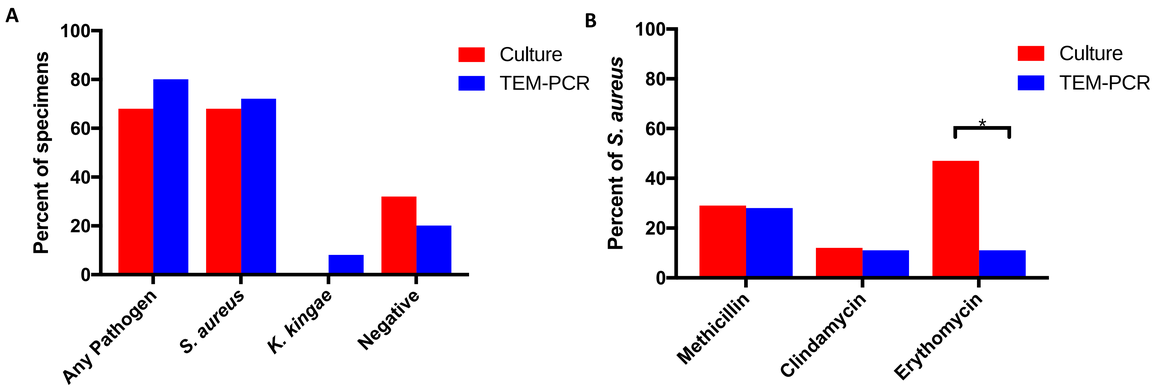
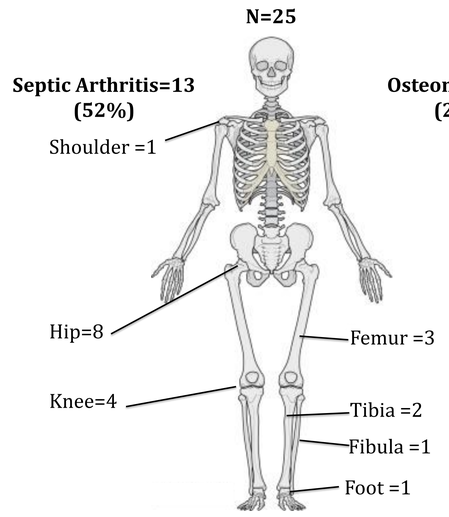



**Figure 2. Comparison of Culture vs. TEM-PCR by Specific Pathogen (A) and Antibiotic Resistance (B)**




**Osteomyelitis + Septic Arthritis=4 (16%)**
Upper extremity=2
Lower extremity=2

**Pyomyositis=1 (4%)**
Gluteus =1

**Figure 3. Site of Infection In Children with MSKI**

## CONCLUSIONS

- TEM-PCR showed 100% concordance with conventional, culture-based testing (gold standard) in pathogen identification.
- TEM-PCR increased pathogen identification to 80%, compared to 68% by culture alone. This effect was seen for both *K. kingae* and *S. aureus.*
- TEM-PCR detection for erythromycin resistance is not inclusive of all known resistance genes, resulting in 11% detection compared to 47% by culture for *S. aureus* specimens.
- TEM-PCR provided rapid antibiotic resistance results that were concordant with culture-based results for methicillin and clindamycin resistance.

## IMPLICATIONS

- TEM-PCR has the potential to inform antibiotic selection early in the disease course, decreasing the use of broad-spectrum, empiric antibiotic regimens, and promoting antimicrobial stewardship.
- TEM-PCR, may be a useful adjunct to conventional, culture-based testing for children with acute MSKI.

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