

Systematic PCR Detection on Culture-negative Osteoarticular Infections

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Background / Introduction

- Correct identification of bone and joint infections is crucial for appropriate antibiotic therapy.
- Culture fails when antimicrobials are applied before sampling or fastidious microorganisms are involved.
- Broad-range PCR is currently used routinely but this technique may lack specificity and sensitivity
- Species-specific real-time PCR tests are alternatives for bone and joint infections such as for *Kingella kingae*, *Mycoplasma pneumonia* or *Mycobacterium* sp.
- In Basel we applied successfully a novel commercial multiplex PCR system Bone&Joint from Mobidiag, Finland.
- In this report a large series of culture-negative bone and joint samples were analyzed with broad-range PCR as well as *S. aureus* and *K. kingae* specific PCRs.

Patients and Methods

A total of 3840 bone and joint samples from 2308 patients were collected prospectively at the Marseille University Hospital from November 2007 to October 2009.

The patients had a suspected osteoarticular infection based on clinical diagnosis.

Each specimen was cultured **15 days**. If negative at day 6, PCR assay was performed (Figure 1)

Specimens were taken by surgical biopsy or by needle aspiration. Specimens were divided in 3 parts for culture, for PCR, and storage -80°C

Conventional methods included direct Gram stain and culture. The specimens also were tested for antimicrobial activity.

Isolates were identified by Vitek 2 (bioMérieux), 16S rRNA gene sequencing or MALDI-TOF MS (Bruker Daltonics).

Molecular Detection

DNA was extracted with MagNAPure LC from Roche after an overnight lysis. *AluI* endonuclease was used to cleave contaminating bacterial DNA. 35-cycle conventional broad-range bacterial PCR was performed with universal primers covering approx. region 540 to 1460 (920bp) of the 16S rRNA gene. In addition a panfungal PCR was run targeting part of the 18S rRNA gene. Post PCR methods included PCR product purification, cycle sequencing, sequencing of ABI Prism 3130 and identified by database search using BLAST analysis.

Strict controls were applied to rule out contamination and PCR inhibition.

Results

A total of 3840 osteoarticular specimens from 2308 patients were analyzed. Patients with prostheses included 1154 (50%). 1089 patients with infections after osteosynthesis. And 38 children <5 had a hematogenous osteomyelitis.

The results of identified microorganisms are summarized in Figure 2.

List of cultured bacteria (703; 30.5%):

Staphylococcus aureus (248)
Coagulase-negative staphylococci (159)
Enterobacteriaceae (128)
Streptococcus sp. or *Enterococcus* sp. (94)
Pseudomonas aeruginosa (52)
Corynebacterium striatum (17)
Miscellaneous bacteria (43)

Systematic use of panfungal PCR (18S rRNA gene) resulted in 3 cases with *Candida albicans*. In 6 cases identified fungal DNA was considered as contaminants.

Culture-negative PCR-positive patients (141, 6.1%)

Group 1: fastidious bacteria (35): 14 anaerobes, 11 *Kingella kingae*; confirmed by species-specific PCR, 10 miscellaneous (1 *Brucella melitensis*, 1 *Neisseria gonorrhoeae*)

Group 2: non-fastidious bacteria (106): 35 *S. aureus*, 30 coagulase-neg. staphylococci, 19 streptococci, 9 *P. aeruginosa*, 7 *Enterobacteriaceae*, 4 enterococci, 2 miscellaneous.

882 (38.2%) patients were diagnosed with culture or PCR. In 289 (32.7%) *S. aureus* was detected (Figure 3). 15 children were PCR-positive: *K. kingae* (11), *S. aureus* (2), *S. sanguis* (1), *S. agalactiae* (1).

Discussion

- Previous studies showed that broad-range PCR is a useful adjunct to culture to diagnose osteoarticular infections.
- The present study confirmed the usefulness of broad-range PCR and allowed diagnosis in 141 culture-negative cases.
- The systematic use of panfungal PCR resulted in only 3 cases confirmed by culture.
- Strict criteria for diagnosis of periprosthetic infection was not applied because corresponding parameters were not available for all patients.
- The study highlights the relevance of PCR assays for the detection of anaerobes and *K. kingae*. *K. kingae* represents approx. 8% of microorganisms detected in PCR tests and is found exclusively in children.
- *Staphylococcus* species represents the majority of bacteria detected by PCR. However, exclusion of contamination in coagulase-negative bacteria is difficult.
- For *S. aureus* previous antibiotic therapy explains one third of cases with a culture-negative, PCR-positive result.
- One third of culture-negative *S. aureus* infections was established with broad-range PCR and specific PCR, 2/3 were found by specific *S. aureus* PCR only, confirming the limits of broad-range PCR.

Conclusion

- Use of broad-range bacterial PCR has been a significant advance in the diagnosis of infectious diseases. Limitations: (1) potential for false-positive results; (2) antibiotic susceptibility testing not possible
- To improve the diagnosis of bone and joint infections we propose systematically use of broad-range PCR and *S. aureus*-specific PCR in culture-negative samples and applying *K. kingae* PCR to samples from children. Panfungal PCR should not be performed systematically.