The Diagnostic Yield of 16/18S rRNA PCR of Sterile Site Samples in Pediatric Patients

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**BACKGROUND**
- Microbiologic work-up of infection in children is largely based on stains, cultures, and phenotypical identification of clinical specimens.
- Molecular genetic techniques for expedient and sensitive pathogen identification have been studied and implemented.
- 16S and 18S rRNA fungal gene polymerase chain reactions (PCR) and amplicon sequencing from sterile site clinical specimens are used to detect and identify bacterial and fungal pathogens, respectively.
- Existing literature reports rRNA PCR in suspected adult infections including bloodstream infections, endocarditis, osteoarticular infections, and meningitis.
- Clinical and performance data for 16S rRNA and 18S rRNA PCR as a molecular pathogen diagnostic in children are limited.

**OBJECTIVE**
To assess patterns of utilization and the diagnostic yield of 16S rRNA and 18S rRNA gene PCR with amplicon sequencing of sterile site samples in pediatric patients with suspected infection.

**METHODS**

16/18S rRNA Gene PCR and Amplicon Sequencing

**Polymerease chain reaction**
- Fungal full nucleic acid extraction
- DNA extraction
- PCR amplification
  - 16S: V1-V4 region primers; 18S: ITS1-ITS4 region primers; 400bp amplicon
- Amplicon purification
- Positive/negative extraction and PCR controls

**Sanger sequencing**
- Gel electrophoresis for positive band
- Sanger sequencing of PCR amplicon
- Species identification: - National Center for Biotechnology Information (NCBI) database BLAST
- Assignment was based on match identity percent and length
- Turnaround time: 1-2 days

**Cost**
16S rRNA PCR or 18S rRNA PCR with Sanger sequencing = $474/sample

**RESULTS**

16S rRNA gene PCR
- 9/163 (5.5%) positive, all with single organism ID
- 79 (48%) positive PCR assigned as mixed
- 1 discordant result: PCR + Curtainhamella, culture = Aspergillus fumigatus
- Of samples with negative 18S PCR, culture was positive in 3 cases:
  - Blood and skin abscesses
  - Brain abscesses

**18S rRNA gene PCR**
- 15/138 (11%) 16S PCR positive; 5/138 (4%) 18S PCR positive
- 40/214 (18.7%) positive
- Of samples with negative 18S PCR, culture was positive in 3 cases:
  - Blood and skin abscesses
  - Brain abscesses

**Sample source**
- Blood
- Skin
- Respiratory/bronchial
- Organ/tissue
- Cerebrospinal fluid
- Pleural fluid

**16S PCR sent**
- 16S PCR = 234 samples
- 16S PCR = 163 samples
- 16S PCR + 18S PCR = 138 samples

**Sample type**
- Blood
- Skin
- Respiratory
- Organ/tissue
- Cerebrospinal fluid

**16S and 18S PCR sent**
- 16S/18S (61%) 16S PCR positive, 5/138 (5%) 18S PCR positive
- 1 sample with both 16 and 18S PCRs positive (debridement abscess p/o intestinal perforation in an immunocompromised patient with 6×10^3 A. baumannii / 9×10^8 S. aureus, positive chest x-ray, parainfluenza, and pneumococcal IgM, with minimal invasive [H] and versus 2.8% [25%] with surgical biopsy

**CONCLUSIONS**
- 16 and 18S rRNA gene PCR can provide pathogen diagnostics.
- Highest yield sample sites include brain, abscess, pleural effusion, respiratory, osteoarticular, and CSF.
- 16S PCR appears especially useful for suspected anaerobic pathogens where culture conditions are typically not optimal for recovery.
- 18S PCR yield is highest in patients at risk for invasive mold disease and provides more timely results than culture growth to affect antimicrobial therapy.

**REFERENCES**
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