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Species-Specific PCR Improves Detection of Bacterial Pathogens in Parapneumonic Empyema Compared with 16S PCR and Culture

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To the Editors

Parapneumonic empyema (PPE) is a serious complication of bacterial pneumonia in children that is increasingly reported in the U.S.¹. Identification of pathogens causing PPE has generally relied on culture, however in up to 70% of cases pleural fluid cultures are negative²⁻⁴. The high rate of culture-negative disease complicates clinical care and selection of appropriate antibiotics.

Culture-independent techniques such as 16S polymerase chain reaction (PCR) have been used to determine the etiology of PPE²⁻⁵. However, some studies have shown limitations to its sensitivity^{3,5}. Our previously published studies demonstrated the efficacy of genus and

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Conflict of Interest: CH was an employee of Idaho Technology, Inc. (now BioFire Diagnostics, Inc.) at the time of the study. AJB, CLB, ATP and KA collaborate with BioFire Diagnostics, Inc., on several NIH and CDC-funded projects (see sources of support above).

species-specific assays to detect pathogens causing PPE⁴. Here we directly compared the sensitivity of pathogen detection using genus and species-specific PCR to that obtained with broad-range 16S PCR.

This study was approved by the Children's Hospital of Philadelphia (CHOP) Committees for the Protection of Human Subjects and was exempt from IRB oversight at the University of Utah. Samples were collected at CHOP to assess the diagnostic accuracy of broad-range 16S PCR⁵. Patient selection, sample collection methods and 16S PCR methodology have been previously described⁵. Residual frozen pleural fluid samples were transferred to the University of Utah and Idaho Technology, Inc. (now BioFire Diagnostics, Inc.) for analysis by genus and species-specific PCR as previously described⁴. Results of conventional culture were compared to results of 16S and species-specific PCR using McNemar's test.

Twenty-five pleural fluid samples underwent testing with both 16S and genus/species-specific PCR. Culture of pleural fluid and/or blood identified a pathogen in 6 (24%) children and 16S PCR of pleural fluid identified a pathogen in 3 (12%). Genus or species-specific PCR identified at least one bacterial pathogen in pleural fluid samples from 22 of 25 children (88%; $p < 0.001$ for comparison to both culture and 16S PCR). Species-specific assays most commonly detected and identified *S. pneumoniae* (56% of cases). MRSA was detected in one pleural fluid specimen (4%) that was also culture positive. In two cases polymicrobial infection was detected by species-specific PCR. Detailed results are shown in the Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B385>. This table shows the detailed comparison, by organism, of species-specific PCR vs. 16S PCR and conventional culture of pleural fluid and blood.

This study directly compares pathogen detection using 16S PCR to PCR specifically targeting pneumonia-causing bacteria in pleural fluid from children with complicated pneumonia. 16S PCR did not improve detection of bacteria, however species-specific PCR significantly increased pathogen detection with a positivity rate of 88%. *S. pneumoniae* was the most common pathogen identified. MRSA was detected only once, in a culture-positive specimen.

The significantly improved yield of species-specific PCR indicates that PCR methodology is an important consideration for molecular detection of pathogens in pneumonia. While outside the scope of this study, further analysis of results at the patient-level could inform testing algorithms regarding the utility of broad range vs. specific PCR in certain populations. As testing costs decrease and multiplex testing is more often available, the feasibility and cost of performing multiple specific PCR tests on a single sample will improve.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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