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Rapid Detection of *Streptococcus pyogenes* in Pleural Fluid Samples from Pediatric Patients with Empyema

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A total of 120 pleural fluid specimens from 113 pediatric patients were tested using two rapid antigen detection assays for *Streptococcus pyogenes*. Results were compared to culture, Gram stain, and PCR results. Each rapid antigen assay detected 9 out of 10 (90%) PCR-positive samples, with 100% specificity. These antigen detection assays are useful to provide microbiological diagnosis of empyema caused by *S. pyogenes*.

Empyema is a common complication of bacterial pneumonia in children. Based on culture, *Streptococcus pneumoniae* is the leading cause of empyema, followed by other Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, with the latter often reported to be the second leading bacterium (2, 5, 7).

Since bacterial culture positivity rates for pediatric pleural fluid samples are low (1, 7, 10), probably because of antibiotic therapy prior to thoracentesis, non-culture-based rapid laboratory methods may assist in the establishment of an etiological diagnosis to help direct patient management. Rapid antigen assays are widely used for detection of *S. pyogenes* in throat specimens. We have tested pleural fluid specimens from children with complicated pneumonia with two rapid antigen tests, culture, Gram stain, and PCR for *S. pyogenes*.

A total of 120 pleural fluid specimens from 113 pediatric patients with empyema that were submitted for routine bacterial culture were included in the study. Gram stain, bacterial culture, and organism identification were performed using routine methods in the clinical microbiology laboratory. Specimens were stored at −70°C for further testing.

S. pyogenes antigen detection was performed using two rapid antigen assays: the QuickVue+ streptococcus A test (Quidel Corporation, San Diego, CA) and the Directigen EZ group A streptococcus test (Becton, Dickinson and Company, Sparks, MD). To perform the assay with liquid specimens, swabs (included in each test kit) were dipped in pleural fluid. Swabs were then processed the same way as recommended for throat swabs.

Nucleic acids were extracted with the MagNA Pure instrument (Roche Applied Science, Indianapolis, IN). Real-time PCR was performed using analyte-specific reagents (ASRs) from Roche Diagnostics (Indianapolis, IN) targeting *S. pyogenes ptsI* (encoding phosphotransferase system enzyme I) and the Roche LightCycler instrument (11). The analytical sensitivity is 20 copies per reaction.

Overall, 18 of the 120 specimens (15%) tested were positive by culture for a likely pathogen. Organisms identified included *S. pneumoniae* (7), *S. aureus* (2), *S. aureus* and *Haemophilus influenzae* (1), *H. influenzae* (1), *S. pyogenes* (1), *Moraxella catarrhalis* (1), and *Pseudomonas aeruginosa* (1). In addition, *Staphylococcus epidermidis* (1), *Streptococcus mitis* (1), *Propionibacterium acnes* (1), and *Peptostreptococcus* species (1) were isolated.

Gram stain was positive for bacteria in 11 specimens, including 9 that were culture positive: *Streptococcus pneumoniae* (6), *Haemophilus influenzae* (1), *M. catarrhalis* (1), *Peptostreptococcus* species (1). Two specimens that showed Gram-positive cocci by Gram stain were sterile; one of these was positive for *S. pyogenes* by both antigen and PCR assays.

Ten of the 120 specimens from 10 different patients were positive for *S. pyogenes* by PCR. The threshold cycle (C_T) values of these positive PCRs were 22.7 to 31.9 (mean C_T, 26.3). Of these 10 PCR-positive samples, each rapid antigen assay detected the same 9 samples, yielding 90% assay sensitivity (Table 1). There was no sample that was positive by a rapid antigen assay but negative by PCR or culture. Therefore, the specificity of the rapid antigen assays is 100% compared to PCR. Only the specimen that was culture positive for *S. pyogenes* of the 18 culture-positive specimens was positive by rapid strep antigen test.

Of the 10 patients with positive PCR results for *S. pyogenes*, 7 were male and 3 were female. Their ages range from 8 months to 9 years 1 month (mean, 3.8 years; median, 4 years 4 months). All of them required hospitalization for 5 to 12 days (mean, 8.5 days). Among the 9 patients for whom antibiotic treatment records prior to sample collection were available, 8 patients had received at least one of the following antibiotics prior to thoracentesis: ampicillin, cefdinir, cefuroxime, cefotaxime, ceftriaxone, azithromycin, and clindamycin. The 9th patient received only nystatin. Only one of these 10 PCR-positive specimens was culture positive for *S. pyogenes*. Seven of the 10 PCR-positive specimens had white blood cells detectable by direct Gram stain, and only one of the 10 had organisms detectable by Gram stain (Gram-positive cocci).

Empyema is a complication of bacterial pneumonia in both adult and pediatric patients. Successful management relies in part on early and rapid recognition of the etiology (3, 8, 12). Current microbiological diagnosis is usually based on direct Gram stain and routine bacterial culture even though the large majority of pediatric specimens are culture and Gram stain negative (3, 8), likely related to antibiotic treatment before sample collection, limited organism load in specimens, and/or suboptimal transport

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TABLE 1 Isolation, Gram stain, *S. pyogenes* PCR, and rapid antigen test results for 120 pleural fluids from pediatric patients

Test result	No. of samples				
				Antigen detection by:	
	Bacterial isolation	Gram stain	PCR for *S. pyogenes*	QuickVue+ streptococcus A	Directigen EZ group A streptococcus
Positive					
S. pneumoniae	7	6	0	0	0
S. pyogenes	1	1[a]	10	9	9
Others[b]	10	4[c]	0	0	0
Negative	102	109	110	111	111

[a] Gram-positive cocci. This sample was negative by culture but positive by both antigen and PCR for *S. pyogenes*.
[b] Two *S. aureus* isolates, one each of *H. influenzae*, a mixture of *H. influenzae* and *S. aureus*, *Pseudomonas aeruginosa*, *M. catarrhalis*, *Staphylococcus epidermidis*, *Streptococcus mitis*, *Propionibacterium acnes*, and *Peptostreptococcus* species.
[c] *H. influenzae*, *M. catarrhalis*, and *Peptostreptococcus* species. One was culture negative.

and storage conditions. In a recent report from Canada, only 32% of patients with empyema had a microbiologic diagnosis, even after combining results of culture from pleural fluid, blood, and lung tissue (5). Another study reported similar data in that only 35% of blood and/or pleural fluid specimens of patients with empyema were positive by culture (1). Overall, bacterial culture positivity of patients with empyema ranges from 20 to 60% depending on study settings (6). In the present study, only 15% of specimens were positive by culture.

S. pyogenes is an important cause of empyema. Despite the relatively insensitive nature of culture methods used in most studies, *S. pyogenes* has been isolated as often or more often than *S. aureus*, making it the second leading bacterial pathogen following *S. pneumoniae* (1, 4, 5). In addition to having relatively low recovery rates, culture requires at least 1 to 2 days to achieve results. More-sensitive and more-rapid non-culture-based assays are needed for this sample type. Molecular assays have been shown to be helpful (1, 6). Although there is a lack of standardization for most bacterial targets, the *S. pyogenes* PCR assay used in this study appears to be useful for detecting *S. pyogenes* in pleural fluid. Rapid antigen assays, including latex agglutination and immunochromatographic testing, have been used for detecting *S. pneumoniae* (4, 6, 7, 9). To date, there is no report describing the utility of rapid antigen assays for diagnosis of empyema caused by *S. pyogenes*. In the present study, using PCR as a reference method, the two antigen assays for *S. pyogenes* demonstrated substantially higher assay sensitivity (90% for each) than the 10% by culture and Gram stain. Due to the relatively small sample size of the current study and the fact that most rapid antigen assays for *S. pyogenes* on throat samples have sensitivities of about 80%, a study of a larger number of samples would likely help verify the antigen assay's sensitivity.

Since the antigen assays are rapid and very specific with high positive predictive value and *S. pyogenes* antimicrobial susceptibility is relatively predictable, a positive result can be helpful to assist treating physicians in selection of appropriate antibiotics. Since the assays are easy to perform and results can be obtained in less than 15 min, they are suitable for most laboratories to perform.

In summary, we found that a greater proportion of pediatric empyema cases are caused by *S. pyogenes* than indicated by culture and/or Gram stain; non-culture-based assays, i.e., PCR and rapid antigen tests, are much more sensitive and may provide useful etiological information for diagnosis.

REFERENCES

1. **Blaschke AJ, et al.** 2011. Molecular analysis improves pathogen identification and epidemiologic study of pediatric parapneumonic empyema. Pediatr. Infect. Dis. J. **30**:289–294.
2. **Byington CL, et al.** 2002. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. Clin. Infect. Dis. **34**:434–440.
3. **Clark J.** 2009. Microbiology and management of pleural empyema. *In* Finn A, et al (ed), Hot topics in infection and immunity in children V. Springer Science+Business Media, LLC, New York, NY.
4. **Eastham KM, et al.** 2004. Clinical features, aetiology and outcome of empyema in children in the north east of England. Thorax **59**:522–525.
5. **Langley JM, et al.** 2008. Empyema associated with community-acquired pneumonia: a pediatric investigator's collaborative network in infections in Canada (PICNIC) study. BMC Infect. Dis. **8**:129.
6. **Lee J-H, Kim SH, Lee J, Choi EH, Lee HJ.** 2012. Diagnosis of pneumococcal empyema using immunochromatographic test of pleural fluid and serotype distribution in Korean children. Diagn. Microbiol. Infect. Dis. **72**:119–124.
7. **Le Monnier AL, et al.** 2006. Microbiological diagnosis of empyema in children: comparative evaluations by culture, polymerase chain reaction, and pneumococcal antigen detection in pleural fluids. Clin. Infect. Dis. **42**:1135–1140.
8. **Lewis RA, Feigin RD.** 2002. Current issues in the diagnosis and management of pediatric empyema. Semin. Pediatr. Infect. Dis. **13**:280–288.
9. **Martinon-Torres F, et al.** 2011. Pleural antigen assay in the diagnosis of pediatric pneumococcal empyema. J. Crit. Care. **27**:321.e1–321.e4. doi: 10.1016/j.jcrc.2011.05.004.
10. **Nathavitharana KA, Watkinson M.** 1994. Neonatal pleural empyema caused by group A Streptococcus. Pediatr. Infect. Dis. J. **13**:671–672.
11. **Uhl JR, et al.** 2003. Comparison of LightCycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. J. Clin. Microbiol. **41**:1 242–249.
12. **Vuori-Holopainen E, et al.** 2002. Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. Clin. Infect. Dis. **34**:583–590.