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Use of polymerase chain reaction to characterize the etiology of culture-negative empyema and parapneumonic effusion among Alaska Native children — 2018-2023

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Abstract

We used polymerase chain reaction (PCR) to identify bacterial infections in culture-negative pleural fluid specimens from Alaska Native children hospitalized with empyema. PCR identified ≥ 1 organism in 11 (79%) of 14 specimens. *Streptococcus pneumoniae* serotype 3 was detected in six specimens; all six participants had received 13-valent pneumococcal conjugate vaccine.

Keywords

empyema; child; American Indian or Alaska Native; bacterial infections; *Streptococcus pneumoniae*; vaccination

Introduction

Empyema and parapneumonic effusion are complications of lung infections involving fluid accumulation in the pleural space [1,2]. Common causative organisms include *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Haemophilus influenzae* [2–4]. Despite decreased incidence following the introduction of pneumococcal conjugate vaccines, these infections continue to cause significant morbidity and mortality among children in the United States [1]. Historically, Alaska Native children have had an increased risk of hospitalization from empyema compared to U.S. children overall [5].

Management of empyema and parapneumonic effusion typically focuses on pleural fluid drainage and antibiotic treatment [2]. Bacterial culture of pleural fluid often is negative due to antibiotic administration before specimen collection [2–4]. Polymerase chain reaction (PCR) has improved the identification of causative organisms [2–4]. We used PCR to

characterize bacterial etiologies in culture-negative pleural fluid specimens from Alaska Native children hospitalized with empyema or parapneumonic effusion.

Methods

Study design and population.

We prospectively enrolled Alaska Native children aged <10 years hospitalized with empyema or parapneumonic effusion at the Alaska Native Medical Center in Anchorage, Alaska during 2018-2023. The Alaska Native Medical Center is the tertiary care center for all Alaska Native people living in Alaska. Children were eligible to participate if they had a negative bacterial culture on a pleural fluid specimen.

Laboratory analysis.

Residual pleural fluid specimens were sent to the CDC Arctic Investigations Program, aliquoted into 0.5 mL, and stored at -70°C until batch-tested during 2024. DNA was extracted using the MagMAX viral/pathogen ultra nucleic acid isolation kit (Applied Biosystems) and tested with the TrueMark Respiratory III panel (Applied Biosystems) multiplex real-time PCR for *S. pneumoniae*, *S. aureus*, *H. influenzae*, *Chlamydia pneumoniae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, and *Mycoplasma pneumoniae*. *S. pyogenes* was detected using species-specific primers and probes to amplify *spy1258* by real-time PCR [6]. Specimens positive for *H. influenzae* were serotyped using two multiplex real-time PCR assays that detected serotypes a through f. Specimens positive for *S. pneumoniae* were serotyped at the CDC *Streptococcus* Laboratory in Atlanta, Georgia using PCR methods previously described [7]. Real-time PCR could not discriminate pneumococcal serotypes 9L and 9N or 15A and 15F, and these are reported as “9L/9N” or “15A/15F.”

Data collection and analysis.

Demographic and clinical data were collected by medical record review. The Alaska immunization registry was used to assess pneumococcal vaccination status. Study personnel administered a questionnaire to parents regarding household composition, environmental exposures, and recent illness.

We compared demographic and clinical characteristics and bacterial etiologies of study participants to those of children aged <10 years hospitalized at the Alaska Native Medical Center during 2018-2023 with empyema or parapneumonic effusion and a positive culture for *S. pneumoniae*, *S. pyogenes*, or *H. influenzae* from pleural fluid. The culture-positive comparison group was identified from Alaska statewide public health surveillance for invasive bacterial disease [8].

Data were analyzed using SAS v9.4. Categorical variables were compared using Fisher's exact test; continuous variables were compared using the Wilcoxon rank-sum test. Urban residence was defined as Anchorage Municipality, Fairbanks North Star Borough, Juneau City/Borough, or Matanuska-Susitna Borough; all other areas were considered rural.

Ethical considerations.

This study was approved by the CDC and Alaska Area Institutional Review Boards, the Alaska Native Tribal Health Consortium, and Southcentral Foundation Board of Directors. Parents provided written informed consent.

Results

Demographic and clinical characteristics.

During April 2018-September 2023, 14 participants were enrolled in the study; 9 (64%) were male and 1 (7%) was an infant [Table 1]. Thirteen (93%) participants resided in a rural area, and the median household size was 6 persons (range, 2-14). Six (43%) participants' households had woodstove heating, 3 (21%) had a smoker, 2 (14%) lacked piped water, and 1 (7%) lacked a flush toilet. Two (14%) participants had asthma, 6 (43%) reported a viral illness in the 90 days prior to hospitalization, and 13 (93%) were receiving antibiotics at the time of pleural fluid collection. The median time from antibiotic initiation to specimen collection was 4 days (range, 0-10). One (7%) participant had ≥ 1 positive culture: a blood culture with *Staphylococcus hominis* and *Staphylococcus epidermidis* collected from one lumen of a central venous catheter 13 days after admission. Twelve (86%) participants were hospitalized for ≥ 7 days; none died.

Identification of bacterial organisms.

Bacterial DNA was identified in 11 (79%) of 14 pleural fluid specimens [Table 2]. Among these, 9 (64%) had *S. pneumoniae*, 3 (21%) had *H. influenzae*, and 2 (14%) had *S. pyogenes*. Three (21%) specimens had ≥ 1 organism detected.

Of the nine specimens with *S. pneumoniae* detected, six were a serotype contained in 13-valent pneumococcal conjugate vaccine (PCV13); all six were serotype 3. Five of the children with serotype 3 pneumococcal disease received four doses of PCV13 prior to specimen collection and one child received two doses. The median time between the last PCV13 dose and specimen collection was 1.9 years (range, 0.7-4.4).

Comparison with statewide surveillance data.

During 2018-2023, there were 11 cases of culture-positive invasive disease due to *S. pneumoniae*, *H. influenzae*, or *S. pyogenes* among children aged <10 years hospitalized at the Alaska Native Medical Center with empyema or parapneumonic effusion [Table 1]. Culture-negative study participants were older (median age, 2.7 years; range, 0.6-6.7) than children with culture-positive disease (median age, 1.2 years; range, 0.6-3.0) ($p=0.03$). Study participants had similar lengths of hospitalization (median, 11 days; range, 2-29) compared to children with culture-positive disease (median, 9 days; range, 4-21) ($p=0.12$). One (9%) child with culture-positive disease died.

Among the 11 surveillance cases, 4 (36%) were caused by *S. pneumoniae*, 3 (27%) by *H. influenzae*, and 4 (36%) by *S. pyogenes* [Table 2]. Of the four pneumococcal disease cases, two were caused by serotypes contained in PCV13. One child with serotype 3 disease received three doses of PCV13, with the last dose given 50 days prior to specimen

collection; one child with serotype 4 disease received four doses of PCV13, with the last dose given 13 months prior. Six (55%) of the 11 culture-negative, PCR-positive study participants had *S. pneumoniae* serotype 3 detected compared to 1 (9%) of the 11 children with culture-positive disease identified through statewide surveillance ($p=0.06$).

Discussion

In this prospective study among Alaska Native children hospitalized with empyema or parapneumonic effusion, PCR identified a bacterial organism in 79% of culture-negative pleural fluid specimens. *S. pneumoniae* was the most frequently detected organism, with serotype 3 the most common serotype; other bacteria identified included *H. influenzae* and *S. pyogenes*.

Our findings are consistent with previous studies where PCR detected bacterial DNA in 80-90% of culture-negative pleural fluid specimens and *S. pneumoniae* was the predominant organism identified [2–4]. In those studies, only 25% of children with empyema had bacteria cultured from pleural fluid [2,4]. *S. pneumoniae* may be especially difficult to culture due to autolysis during growth, with positive pleural fluid cultures in approximately 10% of cases [2,4]. These factors might have contributed to its predominance among our culture-negative specimens. Given the high diagnostic yield of PCR, there could be clinical and public health benefit to using molecular methods to identify a bacterial etiology in children with empyema (e.g., by reducing untargeted antimicrobial therapy) [2,9].

S. pneumoniae serotype 3 was present in 43% of culture-negative pleural fluid specimens and two-thirds of those that were positive for *S. pneumoniae* by PCR. Serotype 3 is a common cause of pediatric empyema, possibly due to its propensity to invade the pleural space [3]. Capsular polysaccharide shedding during bacterial growth also may impede opsonophagocytosis and contribute to empyema development [3]. Serotype 3 is contained in PCV13 and all subsequent higher-valency pneumococcal conjugate vaccines available in the U.S. [8]. All six participants with pneumococcal serotype 3 detected had received PCV13 prior to illness, and five had received four doses. Available estimates of PCV13 vaccine effectiveness against pediatric invasive pneumococcal disease due to serotype 3 are variable but suggest lower effectiveness than other PCV13 serotypes [10]. Reduced vaccine effectiveness may be due to decreased immunogenicity of the serotype 3 capsular polysaccharide, increased antibody concentrations necessary for bacterial killing, or shorter protection following vaccination [3,10]. Reductions in serotype 3 invasive pneumococcal disease incidence were documented in Alaska children and U.S. children overall in the early years following PCV13 introduction [11,12]. Additional research is needed to characterize current trends, including among vulnerable subpopulations. PCR analysis of pleural fluid could be a useful adjunct to bacterial culture in identifying populations at increased risk for invasive pneumococcal disease and evaluating the effectiveness and impact of pneumococcal vaccines.

Our study has several limitations. First, we tested pleural fluid for nine bacterial organisms, and other bacteria or viruses present would not have been detected. Second, the small sample size limited our ability to make comparisons between groups. Third, we do

not have information on eligible children who did not enroll in the study and were unable to further evaluate the demographic and clinical differences between the study and surveillance populations. Finally, we were unable to fully assess the potential contribution of antibiotic therapy or susceptibility to our findings because antibiotic administration data were unavailable for culture-positive patients and susceptibility data were unavailable for culture-negative organisms.

PCR was effective at identifying bacterial organisms in culture-negative pleural fluid specimens from Alaska Native children, a population with an increased risk of empyema hospitalization. PCR analysis of pleural fluid during treatment may help guide antimicrobial selection and potentially further characterize patterns of disease at the population level.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Data Availability

The datasets generated and analyzed during this study are not publicly available as they were collected from children under a research protocol and are considered sensitive. Access to anonymized data will be considered on reasonable request and following approval by the Alaska Area Institutional Review Board, Alaska Native Tribal Health Consortium, Southcentral Foundation, and the Centers for Disease Control and Prevention.

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Table 1.

Children aged <10 years hospitalized at Alaska Native Medical Center with empyema or parapneumonic effusion, by culture positivity — Alaska, 2018-2023

	Culture-negative [*] [N=14]	Culture-positive [†] [N=11]
	No. (%)	No. (%)
Male	9 (64)	5 (45)
Age group in years		
<1	1 (7)	2 (18)
1-4	11 (79)	9 (82)
5-9	2 (14)	0 (0)
Rural residence	13 (93)	9 (82)
Underlying medical condition		
Asthma	2 (14)	0 (0)
Seizure disorder	0 (0)	1 (9)
Number of days hospitalized		
<7	2 (14)	5 (45)
7-13	7 (50)	5 (45)
≥14	5 (36)	1 (9)
Died	0 (0)	1 (9)

^{*} Study participants with culture-negative pleural fluid

[†] Comparison group identified through statewide surveillance for invasive bacterial disease with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Streptococcus pyogenes* isolated from pleural fluid.

Table 2.

Children aged <10 years hospitalized at Alaska Native Medical Center with empyema or parapneumonic effusion and an etiology identified by PCR or culture — Alaska, 2018-2023

	PCR-positive [*] [N=11]	Culture-positive [†] [N=11]
	No. (%)	No. (%)
<i>Streptococcus pneumoniae</i> [¶]	9 (82)	4 (36)
Serotype 3	6 (55)	1 (9)
Serotype 9L/9N	2 (18)	0 (0)
Serotype 15A/15F	1 (9)	0 (0)
Other serotype [§]	0 (0)	3 (27)
<i>Haemophilus influenzae</i> [‡]	3 (27)	3 (27)
Type a	3 (27)	1 (9)
Nontypeable	0 (0)	2 (18)
<i>Streptococcus pyogenes</i> [¶]	2 (18)	4 (36)

^{*} Study participants with culture-negative pleural fluid but an etiology identified by PCR. Three participants were positive for two organisms. No specimens were positive for *Chlamydia pneumoniae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, or *Staphylococcus aureus*.

[†] Comparison group identified through statewide surveillance for invasive bacterial disease with *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Streptococcus pyogenes* isolated from pleural fluid.

[¶] Two PCR-positive study participants had *S. pneumoniae* and *S. pyogenes* detected. The *S. pneumoniae* were serotype 3 (n=1) and serotype 9L/9N (n=1).

[‡] One PCR-positive study participant had *S. pneumoniae* serotype 3 and *H. influenzae* type a detected.

[§] Other *S. pneumoniae* serotypes included one each of serotype 4, serotype 20, and serotype 33F.