Comparison of Culture and Molecular Techniques to Identify the Microbiological Etiology of Severe Pneumonia in Children: Impact on New Vaccine Development

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INTRODUCTION

Pneumonia is the most common cause of mortality in children < 5 years (non neonatal). *Streptococcus pneumoniae* is the most common etiology of severe bacterial pneumonia in children and the most common cause of vaccine preventable pneumonia. New conjugate vaccines (PCV) were developed in response to the emergence of non-pcv-7 serotypes. Early reports suggest introduction of pcv-13 had significantly decrease invasive pneumococcal disease (IPD). Microbiological diagnosis of pneumonia is uncommon as cultures are often negative. Empyema (R-IPD) often complicates severe pneumonia, microbiological diagnosis will enhance understanding of the changes PCV 13 and provide information for vaccine development.

OBJECTIVES

Determine the changes over time in incidence of *S pneumoniae* empyema by culture and molecular techniques (PCR)

METHODS

Prospective surveillance study of IPD in Orange county, California in a collaboration between our institution (CHOC) and the Orange County Healthcare Agency (OCHA) All *S pneumoniae* isolates recovered from children < 18 years old sent to the OCHA where demographic, and clinical data was obtained. Isolates were sent to CHOC for susceptibility and serotyping by Quellung. To evaluate R-IPD we collected pleural fluid from children at CHOC with community acquired empyema (received a video assisted thoracoscopy). After consent, an aliquot of pleural fluid was collected for culture. All patients receive blood culture also. An aliquot of culture negative pleural fluid was be sent to the University of Utah for PCR testing by Film Array

RESULTS

During the study period we identified 60 patients with empyema

Microbiology:

By culture:

19 (31,7%) subjects had positive cultures (including one for *M tuberculosis* which was excluded from further analysis)

	<i>S pneumoniae</i>	<i>S aureus</i>	<i>S pyogenes</i>	<i>S intermedius</i>	Total
Blood	4	0	0	0	4
Pleural Fluid	4	1 (MRSA)	3	1	9
Both	3	2 (MRSA 1)	1	0	6
	11 (18.6%)	3 (5.1%)	4 (6.8%)	1 (1.7%)	19 (32.2%)*

* These are proportion from bacterial positive cultures

By PCR:

testing was added, 38 pathogens were identified.

	<i>S pneumoniae</i>	<i>S aureus</i>	<i>S pyogenes</i>	<i>S intermedius</i>	<i>H influenzae</i>	Total
Cx + / PCR +	9	3	4	1	0	17
Cx - / PCR +	18	0	2	0	1	21
Cx + / PCR -	0	0	0	0	0	0
Total	27	3	6	1	1	38

	Bacterial Empyema	Non-Bacterial Empyema	
Cx +	17	0	17
Cx -	21	13	34
	38	13	51

	Pneumococcal Empyema	Non-Pneumococcal Empyema	
Cx +	9	0	9
Cx -	18	24	42
	27	24	51

The pleural fluid of 51/59 subjects were available for PCR. Two subjects with positive cultures for *S pneumoniae* did not have fluid available for PCR. Eight pleural fluid specimens not available for PCR were culture negative. Twenty-one children with culture negative empyema had a pathogen identified by PCR only. All children with a positive culture tested positive by PCR for the same organism. There were a total of 40 pathogens identified; 2 were not available for PCR so there were only 38 bacterial empyema when tested with the “gold standard” (PCR). No pathogen was identified by culture only (PCR 100% sensitive); no positive PCR had a different pathogen identified by culture (specificity 100%). *Pneumococcus* was identified in 27/51 specimens available for PCR; 18 by PCR only. The sensitivity of cultures to identify bacterial pathogen is 44.7%. The sensitivity of culture to identify pneumococcal empyema is 33.3%. During study period 31 non-respiratory IPD were identified; 5 (16.1%) were PCV13 serotype; 5/9 R-IPD (55.6%) were PCV13 serotypes.

CONCLUSION

S pneumoniae remains the most common pathogen responsible of severe pneumonia identified by culture (31.7%) or PCR (52.9%). The sensitivity of culture to identify any bacterial pathogens is low (44.7%) and even lower (33.3%) for *S pneumoniae* when using PCR as the gold standard. The larger proportion of PCV13 serotypes identified in respiratory IPD compared to non-respiratory IPD may be misleading. Use of PCR or other molecular techniques will be necessary if new pneumococcal conjugate vaccines are to be developed to target prevention or severe pneumonia in children.