Research Article

Prevalence and disease severity of multiple respiratory pathogens among children with severe lower respiratory tract infection

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ABSTRACT

Objectives: To determine the prevalence of multiple respiratory pathogens in children with severe lower respiratory tract infection (LRTI), and compare disease severity, duration of hospitalization and ventilation between those with and without multiple respiratory pathogens.

Methods: This was a cross-sectional, case record review of children aged between one month and 12 years, with severe LRTI in 2019. Case records were reviewed, and data extracted manually using a standard case report form.

Statistical Analysis: Prevalence were expressed as numbers and percentages. Mean (or median) were compared using independent t-test (or Mann-Whitney test). Proportions were compared with chi-square test or Fisher exact test.

Results: Sixty-four children were recruited, with median age of 9.5 months (IQR 17.25 months) and 37 (57.8%) children were male. Forty-six children (71.9%) had multiple respiratory pathogens, with 41 (89.1%) of them having both virus and bacteria. Respiratory syncytial virus (RSV) [RSV A (n=10, 15.6%) and RSV B (n=14, 21.9%)] being the commonest virus detected and children with RSV were significantly younger (7.0 months vs. 15.0 months, P=0.007). The most prevalent bacteria detected was Haemophilus influenzae (n=38, 59.4%), followed by Streptococcus pneumoniae (n=29, 45.3%). Children with multiple respiratory pathogens required higher FiO2 (mean difference 12.3%, 95% CI: 2.5, 25.9, P=0.014). Children with multiple respiratory pathogens were also more likely to require inotropes, have longer ventilation and hospitalization days, although not statistically significant.

Conclusion: Multiple respiratory pathogens was common in children and associated with a higher FiO2 requirement, and a statistically non-significant risk of longer hospitalization, longer duration of ventilation days, and a higher need for inotropes.

Keywords: pediatric lower respiratory tract infection, multiple respiratory pathogens.

INTRODUCTION

Childhood lower respiratory tract infection (LRTI) is one of the most frequent reasons for hospitalization. While the majority of these infections are self-limiting or treatable with antibiotics, some LRTI can be life-threatening. Globally, acute respiratory infections are the second leading cause of death amongst children below five years old.[1] Viruses are the most frequent cause of childhood LRTIs between two to five years old.[2] Concurrent viruses are common; and, up to 48% of hospitalized children with LRTI in Sarawak had molecular evidence of concurrent viruses.[3]

Bacterial pathogens are associated with more severe disease compared to viruses. The commonest bacteria causing LRTIs in children between two to five years old is Streptococcus pneumoniae, while atypical organisms such as Mycoplasma pneumoniae are commonly seen in school-age children.[2] Haemophilus species are the second commonest bacteria causing pneumonia worldwide, but effective vaccination program has reduced the number of Haemophilus influenzae type B LRTI significantly.[4]

Concurrent bacterial and viral pathogens in pediatric LRTIs are also common; as many as 40% of children with severe LRTIs having bacterial coinfection with viruses.[5] Concurrent pathogens are associated with

younger age and more severe illness; and children with Mycoplasma pneumoniae pneumonia associated with viral infection had a longer fever process, higher leukocyte count, higher C-reactive protein, and consolidation on chest radiography.[5,7] Presence of concurrent respiratory pathogens is also associated with a higher risk of complication such as pneumothorax, parapneumonic effusions, intensive care unit admission, need for mechanical ventilation and longer stay.[6,7] Children with comorbidities like asthma or chronic lung disease are more likely to have coinfection with viruses.[7]

While there has been evidence to indicate the significance of concurrent virus and bacteria pathogens, evidence regarding concurrent viruses is contradicting. Asner et al. reported that viral-viral coinfections were generally comparable to single-viral infection.[7] On the other hand, the same study also found that children infected with the combination of rhinovirus and enterovirus were more likely to be admitted to the hospital, and children with respiratory syncytial virus (RSV) infection with another virus were more likely to develop pneumonia compared to RSV infection alone.[7] Thus, it is still unclear how concurrent viruses affects the clinical presentation and outcome in LRTIs in children.

This cross-sectional study aimed to detect the prevailing respiratory pathogens among children with severe LRTI, who required intensive care or ventilatory support in Sibu Hospital, located in the Borneo part of Malaysia, and also to investigate the clinical significance of the presence of multiple pathogens in this cohort of children.

SUBJECTS AND METHODS:

Ethics

This study was registered with the Malaysian National Medical Research Register (NMRR-19-2711-50992) and received approval from the Medical and Research Ethics Committee, Ministry of Health, Malaysia, on 11th October 2019. All procedures followed were in accordance with the Declaration of Helsinki 1975, as revised in the year 2013.

Study Design

This was a cross-sectional study by reviewing the case records of children admitted to Sibu Hospital for severe LRTI from January till December 2019. The particulars of the children who had nasopharyngeal swab taken in the year 2019 were identified from the laboratory. The case records of these children were traced and screened for eligibility to be included into the analysis. Cases that fulfilled the inclusion criteria and none of the exclusion criteria were included. Data extracted included the gender, date of birth, types and level ventilatory support required (including FiO2 requirement), inotropic support, as well as durations of ventilation and hospitalization, using a case report form.

We included children between the age of one month to 12 years. We defined children with severe LRTI as someone who 1) had an acute lower respiratory illness (new cough, sputum production, and/or chest pain), and 2) required higher form of respiratory support (40% or more of FiO2 given via Ventimask, or require humidified high flow nasal cannula, or mechanical ventilation) and/or inotropic support. The World Health Organization (WHO) 2013 defined severe pneumonia requiring hospital admission as the presence of cough or difficulty in breathing and tachypnoea, plus one or more of the general danger signs, but not lower chest indrawing. Our definition of severe LRTI slightly differs from the WHO definition because in this study, we intend to include only cases of severe LRTI which require higher form of respiratory support, which we defined as above. We excluded children who had an alternative diagnosis of a respiratory disorder, for example acute exacerbation of bronchial asthma, reactive airway disease or chronic lung disease, pulmonary tuberculosis, as well as those having life-limiting conditions or immunosuppressed.

In Sibu Hospital, children admitted with severe lower respiratory illness, and required ventilatory support, would have a diagnostic nasopharyngeal swab taken within 48 hours of admission for the detection of respiratory pathogens in addition to other routine laboratory investigations. The specimens were kept in a 4 oC fridge for up to 72 hours in a transport media (Universal Transport Medium, Healthlink®) before being transferred and stored in a -80 oC freezer to be processed in batches. A real-time polymerase chain reaction

(BioRad CFX96 Real Time System) using Allplex™ Respiratory Panel by Seegene® were performed on the specimens to detect viral respiratory pathogens (Influenza A and B, RSV A and B, Adenovirus, Enterovirus, Parainfluenza Virus 1, 2, 3 and 4) and bacterial pathogens (Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila, Haemophilus influenzae, Streptococcus pneumoniae, Bordetella pertussis and Bordetella parapertussis).

STATISTICAL ANALYSIS

Data were analyzed using R software version 3.6.0. For descriptive statistics, qualitative data such as the age of the children were expressed as a mean (or median) with its standard deviation (or interquartile range). We expressed the prevalence for each respiratory pathogen in frequency and percentage. Outcome measurement of severity (FiO2 requirements, inotrope requirements, durations of ventilation and hospitalization) of children with multiple respiratory pathogens (two or more organisms in nasal swab), viral-viral concurrence (at least two different viruses from nasopharyngeal swab) and viral-bacterial concurrence (at least one virus and one bacterium from nasal swab) were compared. For normally distributed data, the mean was compared using independent t-test. We used the median as a measure of central tendency for skewed data, and the comparison made using the Mann-Whitney test. For categorical variable (inotropic requirement), we compared the proportion between the groups using the chi-square test or Fisher exact test. A P value of less than 0.05 was considered statistically significant.

RESULTS

We identified 68 children with the diagnosis of severe LRTI who had nasopharyngeal swab taken, in the year 2019. We managed to retrieve 67 case records for review. Three subjects (one neonate, one outpatient and one asymptomatic patient) were excluded from the study. Table 1 summarizes the characteristics of the 64 included subjects. We compared the median age between subjects with and without multiple pathogens, and found no statistically significant difference (P=0.794). The racial composition of our subjects reflecting racial distribution in the Sarawak population, where the Ibans form the majority.

Nasopharyngeal swabs of six (9.4%) children were negative for organisms tested, while the maximum number of species of organisms detected in a single child were five (n=1, 1.5%). Forty-six (71.9%) children had multiple pathogens from their nasopharyngeal swab. The majority of children (n=41, 89.1%) with multiple pathogens had both virus and bacteria. Figure 1 shows the distribution and prevalence of each respiratory pathogens.

The commonest bacteria detected were Haemophilus influenzae (n=38, 59.4%) and Streptococcus pneumoniae (n=29, 45.3%). RSV type B (n=14, 21.9%) and influenza A (n=11, 17.2%) were the commonest viruses detected. Children with RSV were significantly younger compared to children with no RSV (median age 7.0 months versus 15.0 months, P=0.007). The majority of children with RSV also had concurrent respiratory pathogen with another virus or bacteria (n=21, 80.7%). The commonest organism present concurrently with RSV was Haemophilus influenza (n=16, 66.7%).

The FiO2 requirement of children who had multiple respiratory pathogens was significantly higher than those without (Table 2). Those with multiple virus and bacteria seemed to require higher FiO2 compared to children with single infection or other combination of pathogens, although statistically there was no significant difference (P=0.05). Further analysis showed that children with two or more viruses had higher FiO2 requirement compared to those who had single or no viruses. The results are summarized in Table 2.

Additionally, children with multiple respiratory pathogens (n=46) had an increased need for inotropes compared to children without multiple respiratory pathogens (n=18), although it was statistically not significant (37.0% versus 27.8%, P=0.487).

Table 3 and Table 4 summarize the effect of multiple respiratory pathogens on the durations of ventilation and hospitalization. We excluded the five children who died within 48 hours of admission from this analysis.

Those with multiple respiratory pathogens and those with multiple viruses had a longer duration of ventilation days, but statistically it was not significant. Those with multiple viruses seemed to have longer hospitalization days, but this difference was also not statistically significant.

Five children deteriorated rapidly and succumbed within 48 hours of admission. The median age of children who died was 18.0 months, with a mean FiO2 requirement of 65% while on mechanical ventilation. We detected more than one pathogen in three out of the five children. Three fatal cases had Influenza A pdm09: one had Influenza A pdm09 alone, one had Influenza A pdm09 and Haemophilus influenzae, and one had Influenza A pdm09 with Adenovirus, Streptococcus pneumoniae, and Haemophilus influenzae. The fourth fatal case had RSV B with concurrent Streptococcus pneumoniae and Bordetella pertussis, and had Streptococcus pneumoniae isolated from the blood culture. The last fatal case did not have any respiratory pathogen detected from nasopharyngeal swab but had Salmonella species isolated from blood culture. Table 5 summarizes the clinical characteristics of these children.

We studied the clinical characteristic of children with Streptococcus pneumonia (n=29, 41.2%) and Haemophilus influenzae (n=38, 59.4%) detected from their nasopharyngeal swabs. All children in these groups received at least one dose of Haemophilus influenzae type B (HiB) conjugate vaccine through the Malaysian National Immunization Program. Nineteen (50%) of those children with Haemophilus influenzae infection received three doses of HiB vaccine, 11 (28.9%) received four doses, six (15.8%) received two doses and two (5.3%) received one dose. None of these children received pneumococcal vaccination. Among the 29 children with Streptococcus pneumoniae detected from their nasopharyngeal swabs, three (10.3%) had positive blood culture for Streptococcus pneumoniae. One child died of severe Streptococcus pneumoniae septicemia, one developed pleural empyema requiring drainage, and one had an uncomplicated pneumococcal disease. Out of the 38 children who had Haemophilus influenzae detected from the nasopharyngeal swab, only one (2.6%) had positive blood culture positive for Haemophilus influenzae. This child developed meningitis complicated by a communicating hydrocephalus.

Table 1. Characteristics of children included in this study

Variable	n (%)	Mean (SD)	Median (IQR)
Age (months)			9.5 (17.25) *
With concurrent pathogens			9.5 (15.75) *
Without concurrent pathogens			9.0 (27.25) *
Gender			
Male	37 (57.8)		
Female	27 (42.2)		
Ethnicity			
Iban	34 (53.1)		
Melanau	15 (23.4)		
Malay	6 (9.4)		
Chinese	5 (7.8)		
Others	4 (6.3)		
Highest FiO_2 requirement (%)		47.2 (18.17)	
Duration of ventilation (days)			5.0 (7.00)
Duration of hospitalization (days)			10.0 (13.00)
VENTILATION			
- Invasive	41 (64.1)		
- Non-Invasive			
Humidified high flow nasal cannula	20 (31.2)		
Continuous positive airway pressure	1 (1.6)		
Bilevel positive airway pressure	2 (3.1)		
SD = standard deviation, IQR = interquartile range			
*** distribution skewed to the right**			

Figure 1. Prevalence of respiratory pathogens among children with severe LRTI

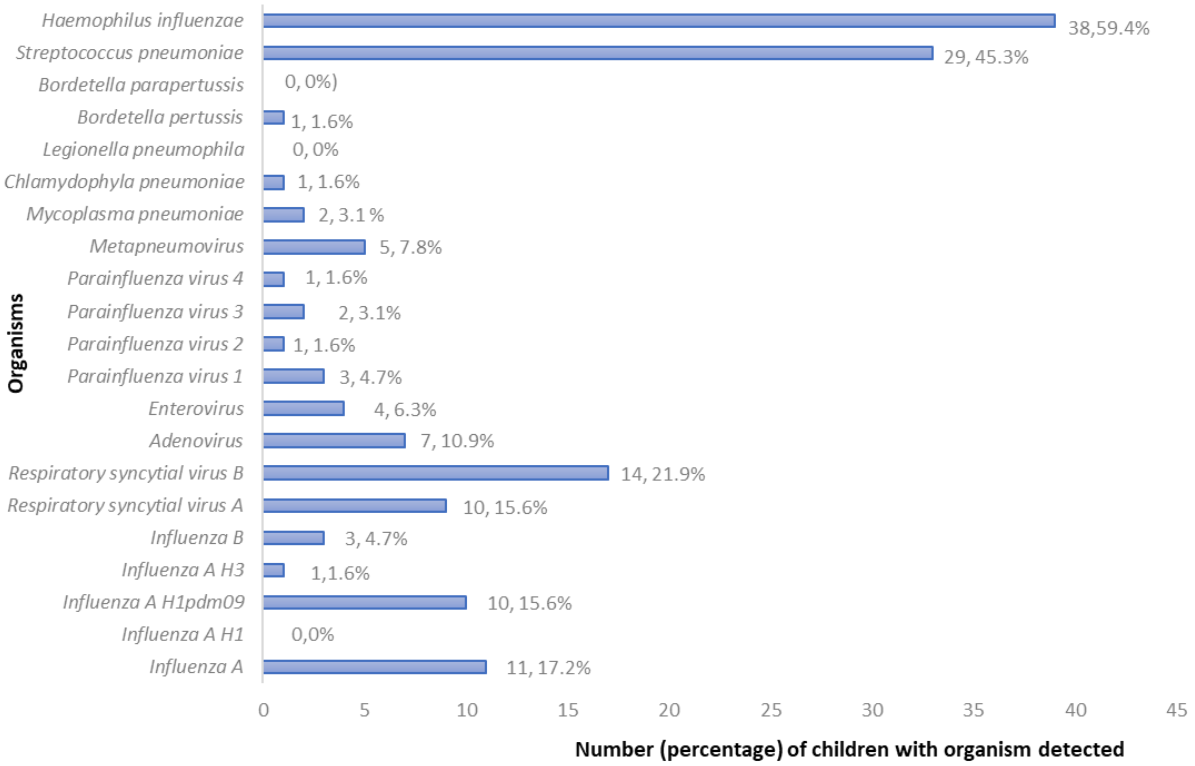

Table 2. Comparison of FiO_2 requirement between different combination of pathogens

Combination	Highest FiO_2 requirement (%) Mean (SD)	Mean Difference	95% CI	*t* statistics (df)*	*P* value *
With multiple pathogens (n=46)	50.7 (16.84)	12.3	2.5, 21.9	-2.52 (62)	0.014
Without multiple pathogens (n=18)	38.4 (18.94)				
With concurrent virus and bacteria (n=41)	50.5 (17.53)	9.2	-18.48, 0.01	-2.00 (62)	0.050
Without concurrent virus and bacteria (n=23)	41.3 (18.14)				
	Highest FiO_2 requirement Median (IQR)				***P* value †**
With two or more viruses (n=10)	52.5 (25.25) ‡				0.046
With less than two viruses (n=54)	44.5 (17.50) ‡				

*** Independent t test, equal variance assumed**
† Mann-Whitney test
‡ Skewed to the right

Table 3. Comparison of duration of ventilation between children with and without multiple respiratory pathogens.

Variable	Duration in days Median (IQR)	*P* value *
Effect of multiple respiratory pathogens		
With multiple respiratory pathogens (n=43)	6.0 (5.00) †	0.262
Without concurrent respiratory pathogens (n=16)	4.5 (6.25) †	
Effect of concurrent virus and bacteria		
With concurrent virus and bacteria (n=38)	6.0 (5.00) †	0.176
Without concurrent virus and bacteria (n=21)	4.0 (6.00) †	
Effect of multiple viruses		
Two or more viruses (n=10)	9.0 (8.00) †	0.088
Single virus or no virus (n=54)	5.0 (5.50) †	
* **Mann-Whitney test**	† skewed to the right	

Table 4. Comparison of duration of hospitalization between children with and without multiple respiratory pathogens.

Variable	Duration in days Median (IQR)	*P* value *
Effect of multiple respiratory pathogens		
With multiple respiratory pathogens (n=43)	12.0 (11.00) †	0.726
Without multiple respiratory pathogens (n=16)	10.0 (20.50) †	
Effect of concurrent virus and bacteria		
With concurrent virus and bacteria (n=38)	12.0 (14.75) †	0.924
Without concurrent virus and bacteria (n=21)	10.0 (17.00) †	
Effect of multiple viruses		
Single virus or no virus (n=9)	10.5 (10.75) †	0.254
Two or more viruses (n=50)	19.0 (17.00) †	
* **Mann-Whitney test**	† skewed to the right	

Table 5. Characteristics of fatal cases

Case	Presenting illness	Hematological Parameters	Other investigations	Nasopharyngeal swab	Events leading to death	Cause of death
49	Fever for two days, cough with post tussive vomiting, and rhinorrhea	WBC: 13.8 x 10^3/µL Plt: 376 x 10^3/µL	Brain CT: Generalized cerebral edema Blood culture: No growth CXR: Perihilar haziness	Influenza A H1 pdm-09, Adenovirus, *Haemophilus influenzae*, *Streptococcus pneumoniae*	Acute cerebral edema	Acute encephalitis
50	Fever for two days, cough and rhinorrhea, vomiting and diarrhea	WBC : 6.3 x 10^3/µL Plt : 235 x 10^3/µL	Brain CT: Generalized cerebral edema Blood culture: No growth CXR: Bilateral lung patchy infiltrates	Influenza A H1 pdm-09, *Haemophilus influenzae*	Acute cerebral edema	Acute encephalitis
51	Fever, cough and rhinorrhea for 9 days, squint and bilateral abnormal posture	WBC : 94.2 x 10^3/µL Plt : 600 x 10^3/µL	Nasopharyngeal aspirate for pertussis PCR: positive Blood culture: *Streptococcus pneumoniae* CXR: Bilateral patchy consolidations	RSV B, *Streptococcus pneumoniae* and *Bordetella pertussis*	Acute kidney injury requiring dialysis	Pneumococcal septicemia and pertussis
53	Fever, diarrhea, mild cough and fitting for 3 days	WBC : 10.2 x 10^3/µL Plt : 69 x 10^3/µL	Blood culture: *Salmonella* species CXR: Bilateral perihilar haziness	Negative for all	Acute kidney injury requiring dialysis	Salmonella sepsis
67	Fever, cough, diarrhea and vomiting for 1 day	WBC : 16.3 x 10^3/µL Plt : 275 x 10^3/µL	Brain CT: Severe cerebral edema Blood culture: No growth CXR: Bilateral hilar haziness	Influenza A H1 pdm-09	Acute cerebral edema	Acute encephalitis

CT = computed tomography
PCR= polymerase chain reaction
CXR = chest

DISCUSSION

In this study, we observed that multiple respiratory pathogens were very common among children with severe LRTIs (71.9%), higher than other studies, which ranged from 17% to 40%. [6,7,8] The difference was probably related to the patient selection criteria; we select only those requiring higher respiratory support, as reflected by oxygen requirement of at least 40% or requiring ventilation, rather than hospitalized children with mild or moderate LRTI who did not require much respiratory support. Our findings were consistent with the previous studies, which showed that more severe diseases were associated with the presence of multiple pathogens. [6]

We found that RSV A (n=10, 15.6%) and RSV B (n=14, 21.9%) were the most prevalent viral pathogens, while Haemophilus influenzae (n=38, 59.4%) and Streptococcus pneumoniae (n=29, 45.3%) were the most common bacterial pathogens detected. This finding is similar to previous reports in Sibu, where RSV was reported to be the most common virus detected among hospitalized pneumonia patient in Sibu, Sarawak. [4] However, our rate of Haemophilus influenzae and Streptococcus pneumoniae detection was higher than other

studies. Pan et al reported nasal carriage rate of 2.3% for Haemophilus influenzae and 26.6% for Streptococcus pneumoniae.[14] In Malaysia, Yatim et al reported that 35.4% of healthy Malaysian children were nasopharyngeal carriers of Streptococcus pneumoniae.[15] It must be noted, however, that the previously reported figures were from healthy children, while our figures reflected the nasopharyngeal carriage of two organisms in hospitalized children with severe disease. The association between nasopharyngeal carriage of these organisms and rate of hospitalization need further study.

We also noticed that the presence of multiple respiratory pathogens, especially those with multiple viruses, were associated with more severe disease in terms of higher FiO2 requirement. As the marker of clinical severity (as opposed to using the need for oxygen supplementation), FiO2 enabled quantitative classification of disease severity and analysis of the spectrum of disease severity, i.e. children with a more severe lung infection required higher FiO2. This finding is similar to the previous studies. Nolan et al showed that the presence of virus and bacteria caused a more severe disease compared to virus alone, and multiple viruses produced a similar disease severity (except for the rate of supplemental oxygen use, which was higher in the first 24 hours in the virus-virus group). [6]

Previous studies showed that the presence of multiple pathogens increases the length of hospital stay, need for intensive care admission and ventilation. [6] In support of previous findings, we found that children with multiple respiratory pathogens had a higher risk for prolonged ventilation and hospitalization days, as well as an increased need for inotropic support, although these associations did not reach statistical significance in our study.

The association between clinical severity and presence of virus-bacteria could be explained by virus-bacteria synergy. [5,6,7] The most common viruses implicated in this context were influenza, parainfluenza, adenovirus, rhinovirus and measles virus.[9] Animal models have also shown that specific pairings of organism better complement each other than other potential pairings.[10] The presence of the virus in the respiratory trees can alter the mucosal surface, leading to the decreased muco-ciliary function of the epithelium loss of integrity, which can enhance bacterial colonization and translocation, besides alteration of innate immune response.[9,11]

We had five children who deteriorated and succumbed within 48 hours of admission. While we could not make any concrete conclusion due to the small number, there seemed to be an association between the presence of concurrent virus-bacteria and mortality. The synergism between viruses such as influenza and RSV with common respiratory bacteria like Streptococcus pneumoniae has been documented, where their simultaneity can increase the virulence of both pathogens. [9,11,12]

Despite a higher nasal carriage detection rate of Haemophilus influenzae, the rate of invasive disease for Haemophilus influenzae was lower than Streptococcus pneumoniae in our cohort. Prior to the development of the Haemophilus influenzae type B (HiB) conjugate vaccine, HiB was the most common cause of invasive bacterial infection and meningitis in children. [4] The HiB vaccine was included into the Malaysian National Immunization Program schedule since the year 2002, with vaccine coverage as high as 94%.[15] With this successful vaccination program, the incidence of Hemophilus influenzae meningitis has reduced; and Streptococcus pneumoniae has replaced Hemophilus influenzae to become the most common cause of childhood bacterial meningitis.[16]As with other vaccine-preventable disease, other non-vaccine serotypes of Haemophilus influenzae have emerged as the cause for invasive disease in the post-vaccine era.[17] The serotype of the Haemophilus influenzae from the blood culture in our study was not known. It is possible that this organism was one of the non-vaccine types described above because the child had received the HiB conjugate vaccine, which was incorporated into the national immunization program in Malaysia.

The pneumococcal vaccine has not been part of the national immunization program in Malaysia during this study period. We observed that the rate of invasive disease caused by Streptococcus pneumoniae in our cohort was three times higher than that caused by Haemophilus influenzae, consistent with earlier studies. The mortality and morbidity associated with invasive disease caused by these organisms are high. The introduction of pneumococcal vaccine (PCV) into the national immunization program can potentially reduce the rate of invasive pneumococcal disease, as reported in other countries who have adapted the vaccine. The effectiveness of PCV in reducing invasive pneumococcal disease range between 26% to 83%. [18] In order to have a maximum impact

from the vaccine to reduce invasive pneumococcal disease, the knowledge of circulating pneumococcal serotypes in the community is important prior to introduction of the PCV into the national immunization program. [19] This require further research, especially on the serotypes circulating among healthy carriers because nasal carriage is often the precursor to invasive disease. [14]

Finally, we noted that children with RSV related severe LRTIs were significantly younger, consistent with the literature. [13] Our results showed that children with severe RSV-related LRTIs were generally below one year of age. It is worth noting that out of 24 subjects with RSV, only four (16.6%) were single virus infections, RSV was present concurrently with at least one other virus or bacteria in the rest of the subjects. Our findings are consistent with previous studies, where RSV was frequently present concurrently with other organisms. [12,13] Thus, even if RSV was suspected as a cause of severe LRTI, it is important to consider concurrent pathogens, as the severity may be contributed by the presence of concurrent organisms which might act synergistically with RSV. [11]

Our study has several limitations. The organism detected from the nasopharyngeal swab might be a colonizer rather than the actual infection. The ideal sample to establish a temporal causality of respiratory pathogens should be samples from a sterile site, such as bronco-alveolar lavage sample, which was not available at our hospital, or not justifiable given the relatively milder clinical illness. While we looked at the simultaneous presence of respiratory pathogens, we did not measure the bacterial and viral load, which might be a factor contributing to the clinical severity.

In conclusion, the presence of multiple respiratory pathogens in children with severe LRTI was common and it was associated with higher FiO2 requirement. Children with multiple respiratory pathogens seemed to require a longer hospital stay, longer duration of ventilation and more inotropic use.

IMPLICATIONS OF THE STUDY RESULTS

The results of our study indicated that in severe LRTIs, multiple respiratory pathogens were common, and besides considering isolated viral or bacterial infection, the clinicians should consider the presence of multiple respiratory pathogens contributing to the severity of the disease, and managed them accordingly.

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