Clinical and Epidemiological Characteristics of Respiratory Syncytial Virus and Influenza Virus Associated Hospitalization in Urban Thai Infants

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Background: Respiratory syncytial virus (RSV) and influenza infections are among the leading cause of hospitalized lower respiratory tract infections (LRTI) in children especially among those younger than 1 year of age. Few descriptions of these 2 important viruses in Thai children less than 1 year of age have been published.

Material and Method: The authors conducted a prospective study of children 1-12 months old hospitalized at a pediatric tertiary-care hospital in Bangkok with LRTI during the period December 2007 to August 2009. Respiratory specimens were tested for influenza A/B virus and RSV using a reverse-transcriptase polymerase chain reaction (*RT-PCR*).

Results: Twenty-six (7.3%) had RT-PCR positive for influenza and 104 (29.4%) for RSV from 354 infants. Clinical diagnoses included pneumonia (73.4%), bronchiolitis (17.5%), croup (6.5%) and bronchitis (2.5%) and were similar among groups except the proportion of croup was significantly lower in RSV (p = .018). The proportion of RSV infection was highest between July and October (42-76%). RSV patients were more likely to present with higher temperature than the negative RT-PCR patients (p = .031). Oseltamivir was prescribed in 7.7% of influenza infections. Intravenous antibiotics were prescribed in 69.2%, 56.7% and 60.7% of the influenza, RSV and negative group respectively (p = .736). Percentages of patients requiring mechanical ventilation were 3.8, 6.7 and 6.3% among the influenza, RSV, and negative group respectively (p = .861). Three patients died: 2 from RSV and 1 from the negative group. All three fatality cases had existing co-morbidity.

Conclusion: A high proportion of RSV was detected in infants hospitalized with LRTI especially during July to October. High proportion of antibiotic prescription and relatively low rate of oseltamivir treatment were identified. Surveillance data and the availability of a rapid and reliable viral diagnostic test may help guide treatment, thereby improve outcome of this vulnerable population.

Keywords: Influenza, Respiratory syncytial virus, Hospitalized, Lower respiratory tract infection, Infant, Thailand

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Respiratory syncytial virus (RSV) is a leading cause of hospitalized lower respiratory tract infections (LRTI) in children^(1,2). Studies of epidemiological and clinical features are available from industrialized countries. Surveillance from the United States (US) indicated the prevalence of RSV was highest during winter especially among infants younger than one year of age⁽³⁾. A population-based surveillance indicated that RSV seasonality in Thailand was rather different from those reported from a temperate climate. Nevertheless, the rate of hospitalized RSV infections in Thailand was also highest among infants younger than 1 year of age *i.e.*, up to 1,067: 100,000 population⁽⁴⁾. Earlier reports from Thailand concurred that RSV was the most common respiratory viral pathogen among Thai children under 5 years of age^(5,6).

Influenza is also an important respiratory virus in children that has been studied in great details and evidences which show the highest hospitalization rate

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in young children and the elderly^(7,8). Influenza infections can lead to substantial morbidity and mortality especially among the high risk group as well as significant negative socioeconomic impact⁽⁹⁻¹²⁾. However, little is known about the clinical and epidemiological aspects of hospitalized RSV and influenza infection especially among young Thai infants who are at the highest risk of severe respiratory tract infections from these two pathogens. Therefore, the aim of the present study was to investigate clinical and epidemiological characteristics of infants less than 1 year of age hospitalized with lower respiratory tract infections (LRTI) with confirmed RSV and/or influenza infection.

Material and Method

The authors conducted a prospective study at Queen Sirikit National Institute of Child Health (QSNICH), a 426-bed pediatric hospital located in the center of Bangkok, Thailand. Patients, aged between 1 and 12 months, hospitalized with any acute lower respiratory tract infections *e.g.*, pneumonia, acute bronchiolitis, acute bronchitis and croup were enrolled from December 2007 to August 2009. Patients reporting symptom onset of more than 7 days before enrollment, patients who were diagnosed with tuberculosis, respiratory involvement of a systemic disease or malignancy, and foreign body aspiration were excluded.

The present study was explained to caregivers and written informed consents were obtained. One of four investigators (PS, KS, PS and AP) then obtained a medical history, conducted a physical examination and collected respiratory specimens. The authors used a flexible sterile polyester tipped applicator (Puritan Medical Product Company LLC, Guilford, Maine USA) for nasopharyngeal swabbing then placed in viral transport media (VTM). The VTM was transported at 4°C to the Thai National Influenza Center, National Institute of Health, Ministry of Public Health, to detect influenza A/B and RSV by reverse transcriptase polymerase chain reaction (RT-PCR).

The viral RNA was extracted using NucleoSpin[®] RNA extraction kit and divided into 2 sample sets. The first set was tested with multiplex RT-PCR for influenza A and B, using 2 pairs of primers: M-F52 and M-R253 that are specific for matrix gene of all subtypes of influenza A, B/HA-F54 and B/HA-R874 that are specific for hemagglutinin of influenza B. The second set was tested with single RT-PCR using RSVAB-F and RSVAB-R, specific primers of N (Nucleocapsid) gene of RSV. The result of PCR product

bands on agarose gel were interpreted in comparison with DNA marker and positive control.

The patients were managed by the hospital pediatric staff as per standard of care. Physicians were notified of any positive RT-PCR results. The present study investigators followed patients at their discharge and completed case record forms. The authors reported descriptive statistics and examined statistical association between those with and without positive RSV and/or influenza PCR using Chi-square test and one way analysis of variants (ANOVA) as appropriate. Post hoc analysis (multiple comparisons) using the least significant difference (LSD) procedure was employed to explore which group was significantly different from each other. All statistic analysis was performed using SPSS software version 15.0 (SPSS Inc., USA). The present study protocol was approved by the Research Ethics Committee of Queen Sirikit National Institute of Child Health, Bangkok, Thailand.

Results

A total of 354 episodes of hospitalized LRTI from 349 patients were included. Three patients were hospitalized twice and one patient thrice during the present study period. Sixty-four percent were male. The mean age was 7 months old. Fifty-seven patients (16.1%) were born prematurely and 82 (23.2%) had underlying conditions. The two most common underlying conditions were pulmonary diseases and congenital heart diseases. Pulmonary diseases included 14 bronchopulmonary dysplasia (BPD), 7 recurrent wheezing, 6 laryngotracheomalacia and 4 others. The patient's demographics and underlying conditions are shown in Table 1.

Influenza was detected in 26 patients (7.3%); 22 (84.6%) of them were influenza A. RSV was detected in 104 patients (29.4%). Two-hundred and twenty-four patients (63.3%) were tested negative for both influenza and RSV by RT-PCR. There was no significant difference in the mean age of patients among the three groups. The proportion of patients under 6 months of age, gender, and history of prematurity were not significantly different among the present study groups. The proportion of patients infected with RSV who had an underlying condition was significantly less than the group with no documented RSV or influenza infection by RT-PCR (Table 2).

The most common clinical diagnosis was pneumonia (73.4%), followed by bronchiolitis (17.5%), croup (6.5%) and bronchitis (2.5%). The proportion of pneumonia, bronchiolitis and bronchitis were not

significantly different among infants infected with influenza, RSV or non-documented for both viruses. However, the proportion of croup was significantly lower in infants infected with RSV compared to infants with no documented RSV or influenza infection (Table 3).

The percentage of RSV infection among hospitalized LRTI were higher than 15% in January 2008, June to November 2008 and July to August 2009 (the study ended August 2009). The highest peak was during July-October 2008 when the proportion of RSV positive ranged from 42-76% of all infants hospitalized with LRTI. Influenza was detected in much lower proportion than RSV. Although seasonal pattern was

Table 1. Sample Characteristics

Characteristics	Number (%) n = 354
Mean age (mo)	7 ± 3.1
1-3	53 (15)
> 3-6	73 (20.6)
> 6-9	119 (33.6)
> 9-12	109 (30.8)
Male gender	226 (63.8)
History of prematurity	57 (16.1)
Underlying conditions	82 (23.2)
Pulmonary disorders	31 (8.8)
Congenital heart diseases	19 (5.4)
Anomalies/Syndromes	11 (3.1)
Gastrointestinal diseases	10 (2.8)
Neurologic abnormalities	7 (2)
Others (HIV, malignancy,	4 (1.1)
hematologic disease)	

not clearly demonstrated, there were 2 periods during February and March 2008 and 2009 that influenza virus was detected at > 15% (Fig. 1).

Eighty-two percent of infants reported fever, the proportion was higher, albeit not statistically significant, in influenza and RSV (92.3 and 87.5%) compared to that of the negative RT-PCR group (78.6%) (p = 0.054). The mean body temperature was higher in infants who had influenza and/or RSV compared to that of the negative RT-PCR group. The RSV group was more likely to have higher temperature and concomitant diarrhea as compared to the negative RT-PCR group (p = 0.031, p = 0.004, respectively). In addition, the RSV group was more likely to have crepitation on chest auscultation compared to influenza and the negative RT-PCR group; (p = 0.016, p = 0.001, p = 0.001)respectively). All other clinical features such as dyspnea, cough and abnormal breath sounds were rather comparable among the present study groups (Table 4).

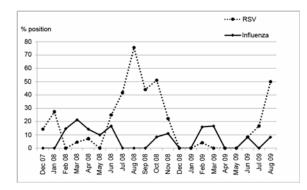


Fig. 1 Monthly distribution of influenza and RSV proportion among infants hospitalized with LRTI

 Table 2. Comparison of demographic characteristics and underlying conditions of infants with positive influenza, positive RSV, and negative RT-PCR test

Characteristic	Influenza n = 26	RSV n = 104	Negative PCR n = 224	p-value
Mean age (mo)	7.3 <u>+</u> 2.8	7 <u>+</u> 3.4	7 <u>+</u> 3	NS
$Age \le 6 \text{ mo}$	10 (38.5)	40 (38.5)	76 (33.9)	NS
Male gender	16 (61.5)	61 (58.7)	149 (66.5)	NS
History of prematurity	5 (19.2)	14 (13.5)	38 (17)	NS
With underlying conditions	6 (23.1)	15 (14.4)	61 (27.2)	0.038 0.011; RSV vs. negative

Number in parenthesis; percent

NS; not significant

Clinical diagnosis	Total n = 354	Influenza n = 26	RSV n = 104	Negative PCR n = 224	p-value
Pneumonia	260 (73.4)	19 (73.1)	80 (76.9)	161 (71.9)	NS
Bronchiolitis	62 (17.5)	5 (19.2)	20 (19.2)	37 (16.5)	NS
Viral croup	23 (6.5)	1 (3.8)	2 (1.9)	20 (8.9)	0.048 0.018; RSV vs. negative
Bronchitis	9 (2.5)	1 (3.8)	2 (1.9)	6 (2.7)	NS

Table 3. Comparison of clinical diagnosis of infants with positive influenza, positive RSV, and negative RT-PCR test

Number in parenthesis; percent

NS; not significant

Clinical presentation	Influenza n = 26	RSV n = 104	Negative $n = 224$	p-value
Mean temperature (°C)	38.0	37.9	37.7	0.043
(95% CI)	(37.7-38.4)	(37.7-38.1)	(37.6-37.8)	0.031; RSV vs. negative
History of fever	24 (92.3)	91 (87.5)	176 (78.6)	0.054
Dyspnea	21 (80.8)	93 (89.4)	197 (87.9)	0.481
Cough	26 (100)	102 (98.1)	218 (97.3)	0.660
Diarrhea	6 (23.1)	33 (31.7)	39 (17.4)	0.014
Abnormal breath sounds Rhonchi Wheezing Crepitation	13 (50.0) 5 (19.2) 12 (46.2)	61 (58.7) 12 (11.5) 74 (71.2)	134 (59.8) 33 (14.7) 115 (51.3)	0.004; RSV vs. negative 0.629 0.549 0.002 0.016; RSV vs. influenza 0.001; RSV vs. negative

A high proportion of patients across all groups received intravenous antibiotics, whereas antiinfluenza drug; oseltamivir was prescribed in only 7.7% of laboratory-confirmed influenza infection. The RSV and the negative RT-PCR group required oxygen therapy more often than the influenza group (p = 0.001,p = 0.018, respectively). Percentages of patients requiring ventilator support were 3.8, 6.7 and 6.3% among influenza, RSV and negative RT-PCR groups respectively (p = 0.861) (Table 5). Three patients died; 2 from RSV group and 1 from negative RT-PCR group. Therefore, the case fatality rates of hospitalized influenza, RSV and LRTI with no documented RSV or influenza infections were 0, 1.9, and 0.4%, respectively. The 2 fatality cases in the RSV group had underlying BPD and another fatality case had complex congenital heart disease.

Discussion

The present findings added to existing evidence that RSV infection is the major respiratory pathogen among hospitalized LRTI especially in young infants. The findings in an urban setting were rather similar with the epidemiological study from the rural areas of Thailand⁽¹³⁾. In contrast, influenza infection was documented in a much lower prevalence than that of the RSV, particularly during July to August 2008 when RSV prevalence was remarkably high. This finding might be explained partly by the year-to-year variation of influenza and RSV activity^(7,13,14). Monthly distributions of RSV-confirmed cases among hospitalized infants with LRTI demonstrated a seasonal pattern similar to earlier studies in children in Thailand^(4,5,14). From the present study the RSV activity began in June, peaked in August and ended in

Clinical course and treatment	Influenza n $= 26$	RSV n = 104	Negative $n = 224$	p-value
Oseltamivir	2 (7.7)	3 (2.9)	3 (1.3)	0.105
IV antibiotics	18 (69.2)	59 (56.7)	136 (60.7)	0.736
O2 therapy	12 (46.2)	81 (77.9)	155 (69.2)	0.006
				0.001; RSV vs. influenza
				0.018; negative vs. influenza
Ventilator support	1 (3.8)	7 (6.7)	14 (6.3)	0.861
Death	0 (0)	2 (1.9)	1 (0.4)	0.341
LOS, Median/ Mean in days	6.5/12.15	6.0/8.41	6.5/8.9	0.163
(Range)	(3-50)	(2-100)	(2-78)	

Table 5. Clinical course, medications and respiratory management of influenza and RSV infants hospitalized with LRTI

LOS; length of stay in the hospital

November-the early month of winter in Thailand.

The mean age and proportion of infants aged 6 months or younger with RSV, influenza and no documented of the two viruses were similar. Nearly forty percents of infants with confirmed influenza LRTI were 6 months or younger. It was well recognized that infants 6-23 months old were having a high risk of influenza related hospitalization^(8,15). Nevertheless, influenza studies in very young infants are limited. Results from a large cohort study conducted in the US indicated that the incidence of influenza infections in less than 3-month-old infants were rather comparable with infants 3-24 months of age. However, they were more likely to be hospitalized⁽¹⁶⁾.

Like earlier studies, pneumonia was the most common admission diagnosis which accounted for approximately three-quarters of hospitalized LRTI in infants 1-12 months of age. The admission diagnoses was proportionately comparable between influenza and RSV, almost all cases were diagnosed with pneumonia or bronchiolitis. The fact that infants in the RSV group were significantly less likely to be diagnosed with croup compared to the negative RT-PCR group implies that RSV is unlikely to be a major cause of hospitalized croup in infants. Recent studies from Korea and Hong Kong showed that parainfluenza virus type 1 and Human coronavirus NL63 were among the most common pathogen associated with viral croup^(17,18).

The proportion of children with fever was higher in those with influenza infection than infections caused by other respiratory viruses in 2 studies in the US^(3,19,20). In the present study, although influenza-confirmed patients presented with highest mean body temperature, it was not significant statistically. This was possibly due to the small numbers of influenza-

confirmed cases. In contrast, the mean body temperature was significantly higher in RSV than negative RT-PCR patients. Generally, fever is an inconstant sign in RSV infection⁽²¹⁾, the finding of significantly higher mean body temperature of RSV group in the present study may in part reflect bacterial co-infection causing high fever in some patients.

Intravenous antibiotics were prescribed in approximately 60-70% of the patients regardless of the RT-PCR results. The high rate of antibiotics prescription was probably due to the difficulty in distinguishing between bacterial and viral infection among young infants who were especially vulnerable to both types of infection. Diagnosis of bacterial superimposed infection and antibiotics prescriptions were reported to be as high as 80% in non-intubated RSV-infected young children hospitalized in intensive care unit⁽²²⁾.

In contrast to antibiotics prescription, oseltamivir prescription rate was relatively low even in the positive influenza PCR group. There were several possible contributing factors for the low rate of oseltamivir prescription. First, oseltamivir was not widely used in Thailand before the 2009 influenza pandemic thus physicians were rather unfamiliar and thus being more cautious with the use of this specific influenza antiviral treatment. Additionally, oseltamivir use in infants less than 1 year of age has just been recommended recently after the 2009 influenza pandemics occurred, with limited pharmacokinetic data in infants⁽²³⁾. Second, in addition to the limited availability or stockpiling of this medication, the RT-PCR result might have been notified later in the course of illness when clinical symptoms had already been improved substantially. The latter issue highlights the importance of the availability of the point-of-care

diagnostic tests for viral pathogens in children in Thailand.

With a small number of patients with unfavorable outcome *e.g.*, respiratory failure requiring mechanical ventilation support and deaths, the authors did not have enough power to determine prognostic factors for hospitalized LRTI in the present study. The rate of complications may also be confounded by an underlying disease as all of those who died had existing co-morbidity. The length of hospital stay was not different statistically among groups; the median was 6.5 days. Extended duration of hospital stay was likely to be a result of participants' underlying co-morbidity which accounted for approximately one-quarter of infants enrolled in the present study.

The authors did not perform RT-PCR to detect other respiratory pathogens that may have clinical importance in infants with LRTI, such as parainfluenza and metapneumovirus. Nonetheless, their prevalence in Thailand was much lower than $RSV^{\scriptscriptstyle (13,14)}$ and the preventable vaccine or treatments have not yet been developed, or in an early preclinical stage. In young infants with influenza, fever may be an admission diagnosis⁽³⁾, thus the authors could not capture those infants with influenza infection without clinical signs and symptoms of LRTI. However, this is a prospective study that provides the distribution of the two common respiratory pathogens causing hospitalized LRTI in Thai infants as well as their clinical information. Similar to existing studies, the authors identified a high proportion of RSV infection among infants hospitalized with LRTI, especially during the months of July to October. Clinical features of RSV or influenza-confirmed LRTI in infants were rather similar to LRTI caused by other pathogens. Provision of surveillance data and introduction of rapid and reliable, point-of-care, viral diagnostic test may be useful for therapeutic guidance and improved treatment outcome of this condition.

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Potential conflicts of interest

None.

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ลักษณะทางคลินิกและระบาดวิทยาของไวรัสอาร์เอสวี และไข้หวัดใหญ่ในเด็กทารกไทยที่นอน โรงพยาบาล

ปียรัชต์ สันตะรัตติวงศ์, กนกวรรณ โศจิศิริกุล, ปราณี สิตะโปสะ, อรุณทิพย์ พรพัฒนนางกูร, มาลินี จิตตกานต์พิชย์, ศรัญญา ศรีจันทร์ทองศิริ, ทวี โชติพิทยสุนนท์

ภูมิหลัง: เชื้อไวรัสอาร์เอสวีไข้หวัดใหญ่เป็นสาเหตุสำคัญของการนอนโรงพยาบาลด้วยโรคติดเชื้อทางเดินหายใจ ส่วนล่างในเด็กโดยเฉพาะเด็กอายุน้อยกว่า 1 ปี ข้อมูลทางด้านลักษณะทางคลินิก และระบาดวิทยาของเชื้อไวรัส ดังกล่าวในเด็กทารกในประเทศไทยยังมีจำกัด

วัสดุและวิธีการ: การศึกษาแบบไปข้างหน้าในเด็กอายุ 1-12 เดือน ที่รับไว้ในสถาบันสุขภาพเด็กแห่งชาติมหาราชินี ด้วยโรคติดเชื้อทางเดินหายใจส่วนล่าง ระหว่างเดือนธันวาคม พ.ศ. 2550 ถึงเดือนสิงหาคม พ.ศ. 2552 โดยทำการ เก็บสิ่งส่งตรวจจากทางเดินหายใจของผู้ป่วยเพื่อตรวจหาเชื้อไวรัสไข้หวัดใหญ่ชนิดเอหรือบี และเชื้อไวรัสอาร์เอสวีด้วยวิธี reverse transcriptase polymerase chain reaction (RT-PCR)

ผลการศึกษา: ผู[้]ปวยจำนว[ั]น 26 ราย (ร้อยละ 7.3) ตรวจพบเชื้อไวรัสไข้หวัดใหญ่ และ 104 ราย (ร้อยละ 29.4) ตรวจพบเชื้อไวรัสอาร์เอสวีจากผู้ปวยที่นอนโรงพยาบาล 354 ครั้ง ผู้ป่วยได้รับการวินิจฉัยโรคปอดอักเสบร้อยละ 73.4 โรคหลอดลมฝอยอักเสบร้อยละ 17.5 โรคกล่องเสียงอักเสบร้อยละ 6.5 และหลอดลมอักเสบร้อยละ 2.5 ซึ่งสัดส่วน ของผู้ป่วยที่ได้รับการวินิจฉัยโรคกล่องเสียงอักเสบในกลุ่มทารก ที่มีไวรัสอาร์เอสวีน้อยกว่ากลุ่มที่ไม่พบเชื้อไวรัส อย่างมีนัยสำคัญ (p = .018) เชื้อไวรัสอาร์เอสวีพบสูงสุดระหว่างเดือนกรกฎาคม ถึง ตุลาคม (42-76%) ผู้ป่วยที่ติดเชื้อ ไวรัสอาร์เอสวีพบมีอุณหภูมิกายเฉลี่ยสูงกว่าผู้ป่วยที่ไม่พบเชื้ออย่างมีนัยสำคัญ (p = .031) ในผู้ป่วยที่ติดเชื้อไวรัส ไข้หวัดใหญ่ได้รับยาต้านไวรัสโอเซลตามิเวียร์เพียงร้อยละ 7.7 แต่ผู้ป่วยได้รับยาปฏิชีวนะฉีดเข้าหลอดเลือดดำร้อยละ 69.2, 56.7 และ 60.7 ในกลุ่มไข้หวัดใหญ่ อาร์เอสวี และไม่พบเชื้อตามลำดับ (p = .736) ผู้ป่วยที่ต้องใช้เครื่องช่วยหายใจ มีสัดส่วนร้อยละ 3.8, 6.7 และ 6.3 ในกลุ่มไข้หวัดใหญ่ อาร์เอสวี และไม่พบเชื้อตามลำดับ (p = .861) ผู้ป่วยเสียชีวิต 3 รายโดย 2 รายมาจากกลุ่มไวรัสอาร์เอสวี และอีก 1 รายมาจากกลุ่มที่ตรวจไม่พบไวรัส 2 ชนิดนี้ ผู้ป่วยทั้ง 3 รายที่เสียชีวิตมีโรคประจำตัว

สรุป: ทารกที่นอนโรงพยาบาลด้วยโรคติดเซื้อทางเดินหายใจส่วนล่างมีการติดเซื้อไวรัสอาร์เอสวีในอัตราส่วนที่สูง โดยสูงสุดระหว่างเดือนกรกฎาคมและตุลาคม พบมีการใช้ยาปฏิชีวนะค่อนข้างสูงแต่การใช้ยาต้านไวรัส โอเซลตามิเวียร์ค่อนข้างต่ำ ดังนั้นการที่แพทย์ทราบข้อมูลของการเฝ้าระวังโรคและมีการทดสอบเชื้อไวรัสที่ได้ผลเร็ว และเชื่อถือได้จะช่วยพัฒนาการดูแลรักษาผู้ป่วยทารกที่ติดเชื้อทางเดินหายใจส่วนล่างของประเทศไทย