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RESEARCH ARTICLE

Antibiotic use in a co-infection of respiratory syncytial virus and pathogenic bacteria in children in a resource-limited setting in northeast Peninsular Malaysia

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ABSTRACT

To investigate co-infection of bacterial isolates associated with respiratory syncytial virus (RSV) in children aged less than two years who were admitted to hospital with confirmed lower respiratory tract infection (LRTI) in Kelantan, Malaysia. The demographic data, clinical history, case management, haematological as well as infectious parameters (white blood cell differential and count, plus C-reactive protein, CRP) of the patients were systematically recorded. Less than one-third of cases were RSV-positive (21.03% and 26.23% were diagnosed as acute bronchiolitis or pneumonia, respectively). Blood cultures from approximately 10% of patients demonstrated growth of *Haemophilus influenzae*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus, Pseudomonas stutzeri*, haemolytic *Streptococcus* group A, and *Bacillus subtilis*. Further analysis indicated that children with positive bacterial growth had an insignificant predictive value of CRP (2.32–7.16 mg/dl). The total white cell counts were 2.97-7.33 x 10^9/L despite increased lymphocyte values in the bacteria-positive blood culture. Platelet counts were also within normal limits except for a single case of *H. influenzae* infection (685.50 x 10^9/L). Interestingly, 95.01% of patients were treated with antibiotics; 66.23% of RSV infection cases were administered with a combination of antibiotics and 33.77% with only a single antibiotic. The data indicate that the use of antibiotics, either singly or in combination, is not always effective in treating LRTI in infants. Alternative therapeutic regimens should be considered, especially in Asian countries that may have limited resources.

Keywords: Antibiotic; co-infection; lower respiratory tract infection (LRTI); respiratory syncytial virus (RSV); Kelantan Malaysia.

INTRODUCTION

Due to the immaturity of the juvenile respiratory system, respiratory illness is among the principal causes of death and hospitalisation of children in Malaysia (Lim *et al*., 2018) and elsewhere (Glatman-Freedman *et al*., 2020; Taylor *et al*., 2020; Yen *et al*., 2019). According to a recent systematic analysis for the Global Burden of Disease Study (GBD, 2016), 241 neonatal deaths were recorded as due to respiratory illness in Malaysia in 2015. Physiologically, any parts of the respiratory tract can be the site of acute respiratory infection (ARI), yet more severe clinical consequences are typically observed with lower respiratory tract infection (LRTI) (Ritchie *et al*., 2017).

Several risk factors are associated with ARI, including malnutrition, low birth weight, passive smoking, no breastfeeding, low socio-economic status, immunodeficiency, and residential crowding (Shi *et al*., 2015a). Therefore, it is not surprising that ARI is more commonly reported in countries of lower gross national income per capita. Viruses cause the highest proportion of infections, contributed mainly by respiratory syncytial virus (RSV) (O'Grady *et al*., 2017; Teck *et al*., 2019), human metapneumovirus, rhinoviruses and parainfluenza viruses (Shi *et al*., 2015b). As for bacteria (Assane *et al*., 2018), the species commonly associated with ARI are *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pyogenes, Pneumococcus* spp. and *Klebsiella pneumoniae*. However, it should be noted that ARI is most frequently attributed to co-infection by pathogens of both viral and bacterial origin (Liu *et al*., 2018).

The development of rapid diagnostic tests for RSV such as the Abbott™ ID NOW Test Kit offers a highly sensitive and specific identification of the virus. However, laboratory identification of bacterial concomitant infections is still required because viral agents cannot be differentiated based on clinical features alone (Wilmott *et al*., 2018) and tend to occur at late stages of ARI. Furthermore, the confirmatory test is pivotal to detect disease for early and active treatment, to reduce unnecessary antibiotic prescription and to limit the spread of nosocomial infection.

The need for antibiotics in effective treatment of ARI has so far proved inconclusive. A systematic review and meta-analysis of antibiotic therapy in controlled clinical trials revealed a lack of evidence to support their clinical efficacy (Mathur *et al*., 2018). Moreover, the use of rapid molecular testing positively impacts patient management of ARI by reducing turn-around time of decision-making and shortening treatment duration of empiric broad-spectrum antibiotics (6.4 h vs. 32.9 h; $p < 0.001$) (Lee *et al*., 2019). Nonetheless, although numerous published reports have indicated increasing cases of ARI in developing countries, to date there is still a paucity of studies on ARI incidence and treatment in Malaysia. This study aimed to determine the prevalence of bacterial pathogens in RSV-associated ARI infection in a cohort of Malaysian children below two years of age using conventional blood culture techniques in a resource-limited clinicals setting in Kelantan, Malaysia. In addition, the predictive factor of bacteraemia in these children admitted to hospital due to confirmed RSV ARI was investigated using conventional blood culture techniques.

MATERIALS AND METHODS

Ethics approval and consent to participate

Study approval was obtained from the Ethics and Research Committee of the School of Medical Sciences, Universiti Sains Malaysia. The procedure for obtaining verbal consent from parents, guardians and caretakers for their child's participation was approved by the Ethics and Research Committee. All methods were performed in accordance with the relevant guidelines and regulation of the Declaration of Helsinki.

Study site description

The study site was Kota Bharu, the capital city of Kelantan, which is a state in the northeast of Peninsular Malaysia. The study was conducted for one year at the Hospital Raja Perempuan Zainab II. Strategically located in the centre of Kota Bharu, this is the largest government hospital in Kelantan with a capacity of 920 beds available for in-patients.

Study design

This was a cross-sectional study involving children aged less than two years of age who were admitted to hospital with confirmed LRTI. Those children diagnosed with any systemic respiratory infection (e.g., pulmonary tuberculosis) or any non-infectious respiratory condition (e.g., bronchial asthma) were excluded from the study, as were those for whom data were incomplete or samples were inadequate. Case-specific information relating to the type of antibiotics administered, use of oxygen therapy, performance of chest radiography, presence of fever and whether the child was given any ventilation support during hospitalisation were systematically recorded using standardised written questionnaires. Additionally, haematological and infectious parameters, including white blood cell (WBC) differential and count, as well as C-reactive protein (CRP), were obtained from the child's clinical case notes. Elevation of WBC was defined as a value above 15,000/µl (Abers & Musher, 2018), while raised CRP was defined as a value above 5 mg/dl (Ocakli *et al*., 2018).

Definition of clinical terminologies

A clinical diagnosis of LRTI was given by the physician in charge of each case based on clinical presentation, diagnostic laboratory identification and radiological findings, in accordance with World Health Organization guidelines (WHO, 1995). Acute LRTI was defined as having a cough with fever (temperature > 38.0°C) for a duration of less than two weeks (Sonawane *et al*., 2019), and the diagnosis was confirmed by a paediatrician (with or without reference to a chest x-ray at the discretion of the attending physician). Pneumonia was defined as having symptoms of a cough, fever, tachypnoea, dyspnoea, one or more signs of respiratory distress and bilateral crepitation and rhonchi on auscultation, or pulmonary infiltration or consolidation on chest radiograph (Abbott & Vlasses, 2011). Detection of acute bronchiolitis was based on having at least two signs, including chest retractions, tachypnoea, and wheezing or rales on auscultation for the first time (Lieberthal *et al*., 2006). Acute laryngotracheobronchitis (croup) was defined as having fever, hoarseness of voice, a barking or brassy cough and inspiratory stridor (with or without sonorous rhonchi) (Armstrong & Bell, 2019). Finally, for this study cohort, chronic lung disease was defined as LRTI presented by an ex-premature child that needed continuous oxygen supplement (Baker, 2019).

Specimen processing

Each child involved in the study had nasopharyngeal secretions. Following nebulization with a solution of either standard saline or 3% sodium chloride in order to enhance virus recovery, a specimen swab sample was collected by a trained staff nurse. As a diagnostic marker of RSV infection, the presence or absence of RSV antigen was tested by rapid immunoassay (RSV Rapid Diagnostic Kit; Abbott, Orlando, USA) on specimens that were either fresh, stored at 2–8°C for 24 hours or stored at -70°C for more than 24 hours.

As per hospital protocols for best practice in blood collection, blood was drawn by a phlebotomist into separate tubes for aerobic and anaerobic *in vitro* culture in BACTEC™ PLUS aerobic medium (Becton Dickinson, Temse, Belgium) in order to determine suspected bacterial aetiologies. If a positive blood culture was detected, microscopical examination of a Gram-stained blood film

was performed for rapid preliminary identification of the bacteria. Subsequently, the blood was cultured onto agar in order to isolate colonies of the pathogenic organism. These were subjected to full microbiological identification by subculture onto specific culture media and antibiotic susceptibility testing.

Data analysis

All data were analysed using SPSS Statistics for Windows version 20.0 software (IBM, Armonk, USA). Data were described using frequency and percentage. Either the Chi-squared test or Fisher's exact test was used, as appropriate, to perform univariate analysis between clinical factors and outcome. Factors included the type of diagnosis, such as pneumonia or acute bronchiolitis, as well as the type of treatment given to patients, such as antibiotics, ventilation, or salbutamol inhalation. A p-value of < 0.05 was considered statistically significant. Association was presented as odds ratios (OR) with 95% confidence intervals (CI).

RESULTS

Participant socio-demographic characteristics

A total of 412 samples were collected during the 12-month study period, more than half of which were from patients between July to December. Males comprised almost two-thirds (62.62%) of the study participants (Table 1). The ethnicity of participants reflected the demographic distribution of the local population, the vast majority identifying as Malay (98.30%) followed by Chinese and Indian. Four in every five (80.1%) children admitted to hospital with acute LRTI were aged ≤ 12 months. The average age was 7.78 (± 5.39 SD) months, while the mode and median were one year and seven months, respectively.

Bacterial culture results

Blood cultures were performed on 368 (89.32%) of samples. Amongst a total of 93 RSV-positive cases, 10 (10.75%) showed bacteria growth (Table 2). Similarly, 31 (9.72%) of 319 RSV-negative cases showed bacterial growth. The types of bacteria identified were *H. influenzae* (n = 3), *S. aureus* (n = 10), *Pseudomonas stutzeri* (n = 2), haemolytic *Streptococcus* group A (n = 1), *Bacillus subtilis* (n = 6), coagulase-negative *Staphylococcus* (n = 14), *Salmonella* sp. (n = 2), *Escherichia coli* (n = 1), *Micrococcus* (n = 1), and *S. pneumoniae* (n = 1) (Table 3).

RSV cases with co-infection of bacteria isolated from blood culture were further analysed in terms of their relationship with haematological and infectious parameters (Table 3). The CRP findings did not provide any strong indication of infection (CRP values ranging between 2.32–7.16 mg/dl), except for *H. influenzae* (CRP of 14.78 mg/dl) and *P. stutzeri* (CRP of 15.28 mg/dl). WBC differential counts showed a lymphocytosis with positive blood culture, but the total WBC count ranged only between 2.97–7.33 x 10^9/L. Platelet counts were within normal limits except in a single case of *H. influenzae* (685.50 x 10^9/L) (Table 3).

Table 1. Socio-demographic data

Demographic Data	n, % (n = 412)
Gender	
Male	258 (62.62)
Female	154 (37.38)
Ethnicity	
Malay	405 (98.30)
Chinese and Indian	7 (1.70)
Age	
≤ 12 months	330 (80.10)
13–24 months	82 (19.90)
Family income (per month)	
≤ MYR500	165 (40.05)
MYR 501–1999	163 (39.56)
≥ MYR 2000	84 (20.39)
Family size	
One child	62 (15.05)
2–5 children	144 (34.95)
> 5 children	106 (25.73)
Breastfeeding history	
Infant formula	51 (12.38)
Mother's breastmilk	361 (87.62)
Breastfed for first two months	141 (39.10)
Breastfed for first six months	157 (43.49)
Breastfed for first year of life	57 (15.79)
History of hospitalisation	
Previous history	171 (41.50)
No history	241 (58.50)
Patient history	
Ventilated due to pneumonia	11 (2.67)
Respiratory distress syndrome	11 (2.67)
Chronic lung disease	2 (0.49)
Perinatal asphyxia	2 (0.49)
Acute gastroenteritis	1 (0.24)
Chronic pertussis	1 (0.24)
Chronic heart disease	1 (0.24)
Meconium aspiration syndrome	1 (0.24)

Note: MYR = Malaysia Ringgit (1 USD = 4.40 MYR).

Table 2. Blood culture analysis

Blood culture	RSV-negative (n = 319)	RSV-positive (n = 93)
	n, %	n, %
Not performed	35 (10.97)	9 (9.68)
No growth	253 (79.31)	74 (79.57)
Bacteria isolated	31 (9.72)	10 (10.75)

Table 3. Blood culture, full blood count and CRP analysis

Blood culture	No. of cases	CRP (mg/dl)	Total WBC (x 10^9/l)	Neutrophils (%)	Lymphocytes (%)	Platelets (x 10^9/l)
H. influenzae	3	14.78	21.65	65.10	32.00	685.50
Haemolytic *Streptococcus* group A	1	2.32	9.20	54.20	34.80	297.00
S. aureus	10	7.16	15.28	59.91	33.30	354.20
P. stutzeri	2	15.28	13.20	55.85	34.70	248.50
B. subtilis	6	0.61	13.67	53.30	36.33	391.33
Coagulase-negative *Staphylococcus*	14	4.77	13.26	46.45	43.37	327.29

Note: WBC, white blood cells; CRP, C-reactive protein.

Table 4. Distribution of diagnosis and management of ARI infection

Diagnosis and management	RSV infection				Crude OR	95% CI	p
	Negative		Positive				
	n	%	n	%			
Pneumonia	229	78.97	61	21.03	0.74	0.46–1.23	0.25
Acute bronchiolitis	90	73.77	32	26.23	1.34	0.82–2.18	0.25
Antibiotic treatment	264	77.42	77	22.58	1.00	0.54–1.85	0.99

Note: OR, odds ratio; CI, confidence interval; $p < 0.05$ is considered statistically significant.

Diagnosis and antibiotic administration

The most common diagnoses were acute bronchiolitis and pneumonia. Less than one-third of cases (93 out of 402) were RSV-positive, with 21.03% diagnosed as acute bronchiolitis and 26.23% as pneumonia. Nonetheless, children aged one-year-old and below were affected the most with LRTI (Table 4). Based on the clinical presentation, more than 80% of patients were treated with antibiotics either singly or in a combination. Of these, 66.23% (n = 51) of RSV infection cases were given a combination of antibiotics (mostly intravenous benzylpenicillin and gentamicin), 33.77% (n = 26) were administered with a single antibiotic (mostly intravenous benzylpenicillin), while only 20.78% (n = 16) did not receive any antibiotic treatment (data not shown).

DISCUSSION

Our population-based study elucidated the burden of RSV co-infection with bacteria on paediatric ARI hospitalisation with confirmed LRTIs in Kelantan, a resource-limited state in northeast Peninsular Malaysia. The study was based on the detection of viral infection using rapid immunoassay test or bacteria using conventional blood culture techniques. To the best of our knowledge, this is the first investigation of the prevalence of bacteraemia and antibiotic use in RSV-associated ARI infection among children below two years of age in Malaysia. Although just over one-fifth of cases (22.57%) were identified as RSV-positive, almost all patients (95.01%) were given antibiotics intravenously.

RSV diagnosis was performed by a commercially available rapid immunoassay test for which the overall sensitivity and specificity has been reported to be 94.30% and 95.30%, respectively (Hall & Douglas Jr, 1975; McIntosh *et al*., 1982; Swierkosz *et al*., 1989). Suitable microbiological culture facilities (lack of expertise) were not available in the diagnostic virology laboratory at Hospital Raja Perempuan Zainab II to propagate RSV *in vitro*. Among RSV-positive cases, 10 (10.75%) showed bacterial growth, while 31 (9.72%) of those samples that tested as RSV-negative contained bacteria. This finding is consistent with earlier reports of the low occurrence of culture-confirmed concomitant bacterial co-infection in paediatric patients with viral infection (Mahajan *et al*., 2018; Nicholson *et al*., 2019). The recent introduction of RSV rapid diagnostic testing has enabled rapid and accurate viral detection, thereby reducing the possibility of prescribing inappropriate empirical antibiotic treatment (Dale *et al*., 2019; O'Callaghan & Jones, 2019). Further research is required to analyse the effect of rapid diagnostic testing on the occurrence of bacteraemia in a larger sample size of this population.

The bacterial pathogens isolated in this study include *H. influenzae, S. aureus, P. stutzeri*, haemolytic *Streptococcus* group A*, B. subtilis* and coagulase-negative *Staphylococcus*. Although the latter was the predominant type of bacteria to be detected, its role in respiratory infections needs to be evaluated more thoroughly as it is a known possible blood culture contaminant (Morioka *et al*., 2018). Additionally, it is recognised that bacteria can cause secondary infections of patients with existing viral infections, so they should not be excluded from the screening process. In our hospital setting, a blood culture was requested only on those occasions when a bacterial superinfection was suspected by the referring physician, and hence a full blood count and CRP analysis were not conducted systematically. Despite blood culture being considered the gold standard method for bacterial detection, it has a low sensitivity such that some species of bacteria require to be first grown *in vitro* to assist identification. Recently, the detection of fastidious bacteria by a reverse-transcription polymerase chain reaction (RT-PCR) test has been demonstrated to facilitate the faster instigation of patient therapy (Drews *et al*., 2019). Timely identification of causative agents of ARI can prevent inappropriate or overuse of antibiotic therapy, which is a major factor in the emergence of multidrug-resistant bacteria. Synergism or competition among different bacteria may be involved (Hindupur *et al*., 2019). Moreover, it has also been reported that certain bacteria implicated in chronic lung disease may be associated with a predisposition to coinfection with particular viruses or vice versa (Baker, 2019). However, this relationship was not established in the present study.

In clinical practice, a child hospitalised with a confirmed viral ARI and presenting with a fever will most commonly be prescribed antibiotic treatment empirically (McDonagh *et al*., 2018). Similarly, herein over 80% of patients were administered antibiotics intravenously, mostly with a combination of benzylpenicillin and gentamicin. Although blood cultures were obtained from 368 of 412 patients (89.32%), only 10.75% of those yielded a positive result in RSV-infected children. More than half of the blood culture-positive findings were likely due to contamination, as shown by the non-significant increase in inflammatory markers for bacterial infection; CRP values were not significantly raised, except for a single case of *H. influenzae* infection. Moreover, the total WBC and platelet counts were within normal limits. However, RSV NS1 and NS2 proteins may lower the levels of blood infection markers, such as through inhibiting interferon induction by human macrophages and epithelial cells (Thwaites *et al*., 2018). Also, it has been proposed that in typical presentations of RSV infection an abnormal WBC count is of limited use to detect concurrent bacterial infection (Zhong *et al*., 2018). Nonetheless, in the present study each decision to continue a course of antibiotics despite no indication of bacterial co-infection was made on clinical grounds based on the recommendation of the treating physicians. On the other hand, it has been argued that RSV-infected children with complications should not receive antibiotics (Papan *et al*., 2020). This is because viral factors may be responsible for differences in RSV subgroup-related disease severity, as antigenically distinct strains exhibit a varied capacity to trigger a pathogenic inflammatory host response.

Co-detection of viral infection with bacteria has been reported to increase the severity of respiratory illnesses, as indicated by an elevated number of patients having severe pneumonia, hospital stays of longer duration, and even higher rates of admission to

intensive care units (Grunwell *et al*., 2019; Heppe-Montero *et al*., 2022). In comparison with no bacterial co-infection, an *in vitro* airway model using cellular exudate from children with viral LRTI co-infected with bacteria demonstrated raised levels of neutrophil activation markers, but a defective respiratory burst and thus an impaired ability to kill bacteria (Deng *et al*., 2018). It was not the intention of the research presented here to predict the association of disease severity with a particular combination of pathogens. Similarly, severe disease was common in children infected with unidentified pathogens. For these patients, one might speculate the possibility of co-infection with 'typical' bacteria, including from *Streptococcus* and *Haemophilus* groups, although unknown viruses may have caused the LRTI symptoms. Mortality from RSV infection occurred only in a single case, which was not attributed to RSV infection *per se*.

There are some limitations to the current study. There is a possibility of under-enumeration because episodes of co-infection that might have occurred between monthly visits may not have been reported by each child's parent, guardian or caretaker. The blood culture isolates were probably contaminants and hence the infective markers were not considered useful. This is a plausible conclusion to draw since a similar proportion of children, around 10%, were blood culture-positive in RSV-positive and RSV-negative cases. Future investigation to compare outcomes of mixed and non-mixed infection would be interesting to conduct on a larger cohort of children presenting with LTRI.

A greater emphasis should be placed on accurate diagnosis tools for RSV co-infection with bacteria to ensure that antibiotics are not prescribed indiscriminately. This includes the introduction of a more accurate diagnostic tool such as RT-PCR testing of nasopharyngeal secretions for RSV infection due to its higher sensitivity and specificity. At present, most hospital microbiology laboratories in low to middle-income countries, including Malaysia, do not perform RT-PCR routinely for RSV detection and rely on rapid antigen detection tests. Of note, other associated factors may influence the ability of rapid diagnostic tests to detect RSV infection.

In conclusion, our results suggest that in young children RSV and bacterial co-infection does not imply causation, although further large-scale investigations are needed to confirm this finding. In the resource-limited Malaysian state of Kelantan, there is a pressing need for a better clinical microbiological service to provide diagnostic reference standards. This facility will enable more accurate determination of paediatric viral-bacterial co-infection rates in Peninsular Malaysia and thereby inform improved patient treatment.

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Conflict of Interest

There are no potential conflicts of interest to declare.

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