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Molecular Diagnosis of Atypical Pneumonia Infection in Children Presenting with Acute Respiratory Tract Infection Attending Yangon Children's Hospital

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Acute respiratory tract infection (ARI) is a clinical condition which causes high morbidity and mortality, especially in infants and young children. Pneumonia is a common complication of respiratory tract infection. Atypical pneumonia, which is commonly caused by *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*, is difficult to be detected because the causal bacteria cannot be diagnosed by routine culture method and presenting with non-specific clinical symptoms. This study aimed to diagnose the atypical pneumonia infection in children presenting with ARI attending Yangon Children's Hospital during 2014-15 by using multiplex polymerase chain reaction (M-PCR). The bacterial DNA was extracted from nasopharyngeal swab samples by using Qiagen DNA minikit and detected by M-PCR. Of 245 patients with ARI, 140(57%) were males and 105(43%) were females. Eleven samples (4.4%) were positive for atypical pneumonia infection, among which 4(1.6%) were *Mycoplasma pneumoniae*, 5(2%) were *Chlamydophila pneumoniae* and 2(0.8%) were *Legionella pneumophila*. The atypical pneumonia cases were mostly seen among the age of 1 to 5 years and sex distribution was nearly equal. The infected cases were detected from pneumonia (36.4%), severe pneumonia (27.3%), viral-induced wheeze (18.2%), severe bronchiolitis (9.1%) and bronchiolitis (9.1%). This study highlights the role of atypical pneumonia infection in ARI cases among children.

Keywords: Atypical pneumonia infection in children

INTRODUCTION

Pneumonia is a common complication of respiratory tract infection. For younger children, elders, and immunocompromised individuals, pneumonia can lead to death.[1] Pneumonia accounts for 15% of all deaths of children under 5 years old, killing an estimated 922,000 children in 2015 globally.[2] Pneumonia kills an estimated 1.1 million children under the age of five years every year - more than AIDS, malaria and tuberculosis combined. Pneumonia can be caused by viruses, bacteria or fungi.[3]

According to Yangon Children's Hospital Statistics Report 2017, pneumonia/pneumonitis is the fourth leading cause of morbidity and third leading cause of mortality (Hospital Statistics, 2017).[4] In 2018, pneumonia/pneumonitis is the fifth leading cause of morbidity and first leading cause of mortality (Hospital Statistics, 2018).[5] Atypical pneumonia refers to pneumonia caused by certain bacteria, including

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Legionella pneumophila, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*. It is called "atypical" because the symptoms differ from those of pneumonia due to other common bacteria. Pneumonia is an infection of the lung. Pneumonia due to *Mycoplasma* and *Chlamydophila* bacteria is usually mild. The most common symptoms of pneumonia are cough, fever, chill and mild or severe shortness of breath.[6]

The incidence of atypical pneumonia in the community-acquired pneumonia was 22 % in the United States and 91% of those had been treated. In Europe, the incidence of atypical pneumonia was 28%, the rate of treatment was 74%. In Latin America, the incidence of atypical pneumonia was 21% and the rate of treatment was 57%. In Asia and Africa, the incidence was 20%, the rate of treatment was 10 %.[7]

Diagnosis can be done by microscopy and culture, serology, polymerase chain reaction and other diagnosis tests. Culture techniques require specialized laboratories and are expensive, time-consuming and labor intensive. Serology usually requires documentation of a rise in antibody concentration from acute phase to convalescent phase blood sample. Currently available nucleic acid amplification techniques such as PCR are highly sensitive method. These amplification techniques are particularly advantageous for detection of fastidious or difficult-to-culture organisms.[8]

Among the respiratory tract infections, atypical pneumonia is frequent in developing countries.[8] There was limited number of studies done for these 3 pathogens from children with acute respiratory tract infection (ARI) in Myanmar. Thus, the present study was conducted to provide the evidence-based information regarding atypical pneumonia infection, also aid in this management of the disease in Yangon Children's Hospital and also reduce the mortality rate of children suffering from ARI in Myanmar.

MATERIALS AND METHODS

It was a cross-sectional, laboratory-based descriptive study conducted at Yangon Children's Hospital and Bacteriology Research Division, Department of Medical Research, Myanmar. The study period was from January 2014 to October 2015. A total of 245 children from Yangon Children's Hospital presenting with signs and symptoms of acute respiratory tract infection were studied. Children with known chronic respiratory problem (congenital malformation of respiratory tract) and patient with congenital heart disease who cannot withstand the procedures were excluded from this study.

Specimen collection and transport

Written informed consent was obtained followed by relevant history taking from the guardian. Nasopharyngeal swab was collected by inserting sterile Copan Flocked swab through a nostril into the nasopharynx. The swab was placed in collection tube containing normal saline. Each sample was labeled and sent to the laboratory of Bacteriology Research Division of the Department of Medical Research in a cool box within 4 hours.

Extraction of bacterial DNA

Bacterial DNA was extracted from nasopharyngeal swab specimens by using Qiagen DNA minikit according to the manufacturer's instruction. The preparation of PCR components were distilled water, 10X buffer, 25mM $MgCl_2$, dNTP concentration of 10 mM, the primer concentration of MP 03F (10 μM), MP TM2R (10 μM), CP C1 (10 μM), CP TM2R (10 μM), LP F3 (10 μM), PL TM2R (10 μM) and Taq Polymerase. Multiplex PCR was carried out by using 3 sets of primer specific; 5′ AAC TAT GTT GGT GTA TGA CCA GTA C 3′(forward) and 5′ ACC TTG ACT GGA GGC CGT TA 3′ (reverse), 5′ GTT GTT CAT GAA GGC CTA C 3′ (forward) and 5′ CGT GTC GTC CAG CCA TTT TA 3′(reverse), 5′ ATA AGT TGT CTT ATA GCA TTG GTG 3′(forward) and 5′ TGT TAA GAA CGT CTT TCA TTT GCT G 3′(reverse) which were specific for *Mycoplasma pneumoniae*, *Chlamadophila pneumoniae* and *Legionella pneumophila*, respectively.

The amplification was done on thermal cycler with initiation temperature of 94ºC for 5 minutes, the denaturation temperature of 94ºC for 30 seconds, annealing temperature of 51ºC for 30 seconds and extension temperature of 72ºC for 45 seconds. The final extension temperature was 72ºC for 10 minutes. Finally, the PCR products which are *Legionella pneumophila* (150 bp), *Mycoplasma pneumoniae* (230 bp) and *Chlamydophila pneumoniae* (290 bp) were run by gel electrophoresis and visualized directly upon illumination with UV light.

Data analysis

Data were collected by using Pro-forma. After collection of data, data entry, data editing, data cleansing, data compilation, data processing and data analysis were done by using SPSS software. Analysis was also done on frequency distribution and detection of pathogen causing atypical pneumonia infection.

Ethical consideration

Informed consents were taken from the parents or guardian of the children with ARI. Ethical issues concerning voluntary participation, description of process of taking specimen, compensation and confidentiality were considered in the study. Ethical approval was obtained from Ethical Review Committee, Department of Medical Research to conduct the study.

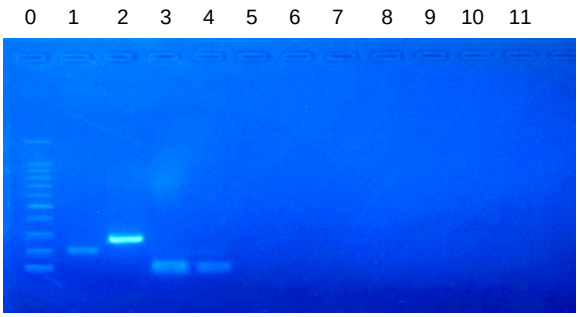
RESULTS

The demographic and clinical profiles of the ARI patients in this study and PCR positive atypical pneumonia infection cases are shown in Table 1. The clinical presentation of atypical pneumonia infected patients in this study population were more or less the same with other ARI patients.

Table 1. Demographic and clinical profiles of the ARI patients in this study and PCR-positive atypical pneumonia infection cases

Demographic and clinical picture	All ARI cases (n=245) Frequency (%)	Atypical pneumonia infection cases (n=11) Frequency (%)
Age (month)		
Median	16	15
Age group (year)		
1 to 5 year	241(98.3)	11(4.5)
5 to 12 year	4(1.6)	-
Sex		
Male	140(57)	5(45.5)
Female	105(43)	6(54.5)
Clinical presentation of ARI patients		
Cough	244(99.5)	11(100)
Fever	144(58.7)	10(91)
Difficult breathing	110(44.9)	10(91)
Chest indrawing	84(34.2)	3(27.2)
Wheeze	77(31.4)	7(64)
Tachypnoea	62(25.3)	4(36.4)
Cyanosis	7(2.8)	
Clinical diagnosis		
Severe pneumonia	138(56.3)	3(27.3)
Bronchiolitis	55(22.4)	3(27.3)
Pneumonia	26(10.6)	4(36.4)
Severe bronchiolitis	17(7)	1(9.1)
Very severe pneumonia	5(2)	
Influenza	3(1.2)	
Croup	1(0.4)	

ARI=Acute respiratory tract infection



Lane 0 - 100 base pair (bp) marker
Lane 1 - *Mycoplasma pneumoniae* positive control (230 bp)
Lane 2 - *Chlamydophila pneumoniae* positive control (290 bp)
Lane 3 - *Legionella pneumophila* positive control (150 bp)
Lane 4 to 11 - Samples
Lane 4 - *L. pneumophila* DNA positive sample (150 bp)

Fig. 1. Gel electrophoresis result of *Legionella pneumophila* infections after multiplex polymerase chain reaction (M-PCR)

Among 245 patients, *Mycoplasma pneumoniae* DNA was detected in 4(1.63%) patients, *Chlamydophila pneumoniae* DNA in 5(2%) patients, *Legionella pneumophilla* DNA in 2(0.81%) comprising a total 11(4.48%) atypical pneumonia cases (Fig. 1).

DISCUSSION

Atypical pathogens (*M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*) can cause mild, moderate or severe acute respiratory tract infections in children. These pathogens are increasingly recognized as important causes of pneumonia in many countries.[9] However, the prevalence study has not been well documented in Myanmar.

In this study, the age group was divided into 1 to 5 years age group and 5 to 12 years age group. In this study, 241(98.3%) were in 1 to 5 years age group and 4 patients (1.6%) were in 5 to 12 years age group. The male to female ratio was 1.33:1. Therefore, the acute respiratory infection was most commonly seen in 1 to 5 years age group and more common in male.

The study done by Kumar *et al*. showed that the prevalence of ARI among under five children in urban and rural areas of Puducherry, India was 59.1% (301/509). Higher proportions of boys were reported to have ARI as compared with girls. ARI is an important public health problem among under-five children.[10] The study from Ethiopia also stated that as the children get older their immunity grows stronger and becomes better able to resist infection.[11]

The study from Vietnam reported that the incidence of children with atypical pneumonia infection was higher in the 1 to 5 years age group, that is 73.5%.[7] Another study from Pakistan also proved that the high incidence (68%) of atypical pneumonia was found in hospitalized children less than four years of age.[12] In the present study, all the atypical pneumonia-infected cases were detected from 1 to 5 years age group of ARI patients but the incidence rate was only 4.5%.

All PCR-positive cases were in the age group of 1-5 years. The gender distribution was statistically not significant. The clinical presentation of atypical pneumonia-infected patients in this study population were more or less the same with other ARI patients. The duration of hospital stay was not more than 2 weeks. All cases were well-treated and discharged with full recovery in this study population.

Conclusion

The study highlights the role of atypical pneumonia infection (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*) in children. It also confirmed that multiplex polymerase chain reaction (PCR) method can be a useful diagnostic tool for detection of atypical bacteria but incorporation of different diagnostic tool should be used to increase the detection rate. Large-scale clinical and etiological studies should be carried out to provide more information on disease burden and clinical determinants which are applicable in updating the effective management strategies for these atypical infections.

Competing interests

The authors declare that they have no competing interests.

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