



UNIVERSITI PUTRA MALAYSIA

***DETECTION OF VIRUSES FROM PATIENTS HOSPITALIZED WITH
LOWER RESPIRATORY TRACT INFECTIONS IN A PUBLIC HOSPITAL,
MALAYSIA***

SITI NOORHIDAYAH BINTI MUSA

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

March 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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**Chair: Professor Zamberi Sekawi, MD, MPath
Faculty: Medicine and Health Sciences**

Lower respiratory tract infections (LRTI) are among the most common human infectious diseases worldwide and responsible for a considerable number of deaths among children, particularly in developing countries. Respiratory tract infections are caused by broad spectrum microbial agents, mostly viruses and some bacteria. The common respiratory viruses includes influenza virus A and B, parainfluenza viruses, adenoviruses, respiratory syncytial virus (RSV), rhinoviruses, and coronaviruses. In Malaysia, studies on the prevalence of these viruses are still lacking of data presentation of the virus prevalence associated with LRTI. The purpose of this study was to detect the respiratory virus aetiology from patients in Sungai Buloh Hospital, Selangor who were hospitalized with lower respiratory tract infections. A total of 210 specimens and patient's data were collected from patients hospitalized with LRTI. The specimens were collected from April 2013 until early January 2014 after routine immunofluorescence (IF) test in hospital. Samples subjected to viral nucleic acid extraction and PCR for adenovirus, bocavirus and RSV detection. Positive PCR samples were sequenced and phylogenetic trees were constructed. Sample then analyzed based on demographic and clinical data. PCR result revealed 18 samples that were positive with adenovirus which were 11 of HAdV-7, two HAdV-1, HAdV-2 and HAdV-4 respectively and one was HAdV-5 while two positive PCR samples were HBoV1. Hospital IF test reported presence of RSV, influenza A, adenovirus, parainfluenza 3 and influenza B. Virus incidence was observed higher in children compared to adult with noticeable symptoms observed were cough, fever and difficulty of breathing, while asthma was the common underlying disease presented. Main diagnoses of the LRTI were pneumonia and bronchiolitis. In summary, viruses contribute to the etiology of LRTI among hospitalized patients in Hospital Sungai Buloh, particularly RSV followed by HAdV, influenza A, parainfluenza 3, HBoV and influenza B.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN VIRUS DARIPADA PESAKIT HOSPITAL DENGAN INFEKSI SALURAN PERNAFASAN BAWAH DI SEBUAH HOSPITAL AWAM, MALAYSIA.

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Infeksi saluran nafas bawah adalah di antara penyakit berjangkit utama di kalangan manusia. Penyakit ini memainkan peranan utama dalam kadar kematian di kalangan kanak-kanak terutama di dalam negara membangun. Infeksi saluran pernafasan adalah disebabkan dari pelbagai mikroorganisma terutamanya virus dan sebahagian bakteria. Punca jangkitan klasikal di kalangan virus pernafasan termasuklah virus influenza A dan B, virus parainfluenza, rinovirus, adenovirus, virus sinsitium pernafasan dan koronavirus. Di Malaysia, penyelidikan tentang kelaziman pengesanan virus ini masih di tahap rendah dan data-data yang menunjukkan kelaziman hubungan virus terhadap jangkitan saluran pernafasan masih kurang. Tujuan penyelidikan ini diadakan adalah untuk mengesan virus pernafasan dari pesakit yang dimasukkan ke Hospital Sungai Buloh, Selangor dengan gejala infeksi saluran pernafasan bawah. Genotip, demografi data dan gejala klinikal positif sampel juga dianalisis. Sebanyak 210 sampel pesakit infeksi salur pernafasan bawah yang dimasukkan ke Hospital Sungai Buloh diambil dan data pesakit juga dicatatkan. Sampel di ambil bermula dari April 2013 sehingga awal Januari 2014 selepas menjalani ujian imunopendafluor di makmal hospital. Sampel yang diambil kemudiannya melalui pengekstrakkan virus nukleik asid dan reaksi rantai polimerase bagi mengesan kehadiran virus boca dan adeno. Sampel juga dipilih untuk ujian pengesanan RSV melalui transcriptase berbalik reaksi rantai polimerase. Sampel yang diuji positif dengan virus dihantar untuk analisis jujukan bagi mengetahui susunan nukleik asid virus itu sebagai kegunaan membentuk pokok filogenetik. Data klinikal dan demografi untuk sampel yang positif juga dianalisis. Daripada 210 sampel, ujian molekular menunjukkan 18 sampel adalah positif untuk virus adeno dengan 11 sampel adalah HAdV7, dua sampel masing-masing adalah HAdV1, HAdV2, dan HAdV4, manakala satu positif sampel adalah HAdV5. Dua positif sampel adalah HBoV1. Ujian imunopendafluor menunjukkan kehadiran sampel positif virus sinsitium pernafasan, influenza A, adenovirus, parainfluenza 3 dan influenza B. Jangkitan virus adalah tinggi di kalangan kanak-kanak berbanding orang dewasa dan pesakit lelaki adalah lebih ramai berbanding wanita. Simptom utama yang dapat dilihat adalah batuk, demam dan kesukaran bernafas. Penyakit asma merupakan

penyakit dasar yang tertinggi direkodkan. Diagnosis utama untuk infeksi saluran pernafasan bawah ialah pneumonia dan bronkiolitis. Kesimpulannya, penemuan kajian menyatakan virus adalah penyumbang yang ketara kepada punca infeksi saluran pernafasan bawah di kalangan pesakit yang dimasukkan ke Hospital Sungai Buloh, terutamanya virus sinsitium pernafasan dan diikuti oleh adenovirus, influenza A, parainfluenza 3, bocavirus dan influenza B.



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I certify that a Thesis Examination Committee has met on 17 March 2017 to conduct the final examination of Siti Noorhidayah Musa on her thesis entitled “Detection Of Viruses From Patients Hospitalized With Lower Respiratory Tract Infections In A Public Hospital, Malaysia” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AEBA	Acute Exacerbation of Asthma
bp	Base pair
CAP	Community Acquired Pneumonia
CDC	Centre for Disease Control and Prevention
cDNA	Complementary DNA
dNTP	Deoxyribose nucleoside triphosphates
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FITC	Fluorescein isothiocyanate
HAdV	Human Adenovirus
HBoV	Human Bocavirus
ICU	Intensive Care Unit
IF	Immunofluorescence
ORF	Open reading frame
LRTI	Lower respiratory tract infection
MgCl ₂	Magnesium Chloride
NP	Nucleoprotein
NPA	Nasopharyngeal aspirate
NS	Non-structural
PCR	Polymerase chain reaction
PIV	Parainfluenza virus
RNA	Ribonucleic acid
RNase	Ribonuclease
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse transcription-polymerase chain reaction
RTI	Respiratory tract infection
TA	Tracheal aspirate
TAE	Tris-Acetate EDTA
UK	United Kingdom
USA	United State of America
VP	Viral protein
VTM	Viral transport medium
WHO	World Health Organisation



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CHAPTER 1

INTRODUCTION

Lower respiratory tract infections are among the most common human infectious diseases worldwide. It accounts for the second leading causes of years of life loss among all age groups worldwide in 2012 (WHO, 2014). Globally, childhood respiratory infections are a leading cause of disease, contributing to absenteeism and economic strain through the use of healthcare resources (Monto & Sullivan, 1993). According to a report, pneumonia causes 18% of total death in children below 5 years old in 2010 (WHO, 2013). Lower respiratory tract infection (LRTI) is generally self-limiting, but may cause significant morbidity and result in economic costs to the individual and society (Thomas, 2000).

In the developing world, respiratory infections are also a major cause of childhood death, although the contribution of viruses to such deaths is unclear (Berman, 1991). An estimated 1.9 million children die from respiratory tract infections every year, with 70% of the mortality occurring in Africa and Southeast Asia (B. G. Williams, Gouws, Boschi-Pinto, Bryce, & Dye, 2002). Most of the respiratory tract infections are caused by viruses (Khor, Sam, Hooi, Quek, & Chan, 2012), but relatively few studies on viral RTIs have been conducted in Southeast Asian countries like (Chan, Chew, Tan, Chua, & Hooi, 2002; Zamberi, Zulkifli, & Ilina, 2003) despite reports from the Ministry of Health that diseases of respiratory system are the third main causes of death in Malaysia for the year 2012 (WHO, 2015).

Adults' cases with LRTI who presented to their respective general practitioner took account of 44 per 1000 person per year (Macfarlane, Macfarlane, Rose, Colville, & Guion, 1993). In elderly patients aged 60 and above, the incidence is 2 to 4 times higher than those who were below 50 years old. Most of LRTI cases are common in those with pre-existing chronic respiratory disease (Thomas, 2000). In Malaysia, studies were mostly done targeting children as respondents (Abd-Jamil, Teoh, Hassan, Roslan, & AbuBakar, 2010; Chan, Goh, Chua, Kharullah, & Hooi, 1999; Ng, Tan, Ng, Nair, & Gan, 2015) that overlooked the incidence of viral LRTI in adults. Thus, this study was done to present the incidence of LRTI virus infections in both children and adults.

The respiratory viruses are usually transmitted via inhalation or direct contact with aerosols or its secretions. The transmission also often associated with geographic and climatic factors such as lower temperature and higher humidity that prolong the survival rate of the virus (Khor et al., 2012). LRTI are defined by the presence of signs and symptoms of an acute respiratory infection (cough, nasal discharge, oropharyngeal hyperemia, with or without fever), and lower respiratory signs such as tachypnea, retractions, prolonged expiratory time, or crackles/wheezing on auscultation (da Silva et al., 2013). Most studies, showed that the most common causes of respiratory viral infections are RSV followed by parainfluenza, rhinovirus, influenza, adenovirus and also new viruses such as metapneumovirus and bocavirus, while the involvement of

bacteria and others are also seen in small amount (Khor et al., 2012; Munywoki et al., 2013; Shafik et al., 2012). Studies also reported that there were many cases with unidentified etiology of disease which was due to not conducting appropriate tests or missed organism (M Woodhead et al., 2011).

Currently, the most available treatments are limited to symptomatic therapies (Turner, 2001) that reduce the symptoms, rather than cure it. In some cases, viral infections had been treated as bacterial infection with unsuitable antibiotics, which might lead to resistance in the future (Berry, Gamielien, & Fielding, 2015). Virus infections cause a high burden of diseases in human, but crucial knowledge of the variety of viruses that infect humans is still lacking (Allander et al., 2005). The disease was accountable for a huge economic burden in terms of medical care needs, pharmacological treatment, hospitalizations as well as work absenteeism (Jaguś, Chorostowska-Wynimko, Radzikowska, & Gawryluk, 2011).

Thus, it is important to investigate the viruses that are responsible for these infections and its common serotype involved in cases of LRTI patients especially in local population, as it will help for the better treatment of LRTI. Result from this study can be used as local authorities' reference if any outbreaks or reoccurrence of the respiratory virus infections and as a preventative measure for viral infection. This study is important in order to increase the efficiency of vaccination and prophylaxis programs and, for future roles in preventing outbreak intervention (Khor et al., 2012). Subsequently, through this study, it will fill the gap of knowledge on virus detection and genetic distribution on different respiratory viruses in Malaysia.

1.1 General objective

To detect and identify viruses and its demographic and clinical presentations from patients hospitalized with lower respiratory tract infections in Hospital Sungai Buloh, Selangor, Malaysia.

1.2 Specific objectives

1. To identify the incidence of respiratory virus infections among hospitalized LRTI patients in Hospital Sungai Buloh, Malaysia.
2. To identify selected viruses of lower respiratory tract infections by using polymerase chain reaction (PCR) and classify its genetic diversity by using phylogenetic analysis.
3. To compare the incidence of LRTI infection between children and adult in current Malaysian situation.
4. To describe demographic and clinical presentation of patients infected with different respiratory viruses.

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