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Research on major respiratory viruses in South Korea

국내 주요 호흡기바이러스의 최신 분자역학적 연구

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Research on major respiratory viruses in South Korea

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ABSTRACT

Research on major respiratory viruses in South Korea

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Respiratory tract infections (RTIs) are caused by bacteria or viruses and are divided into upper and lower RTIs. Acute RTIs, such as pneumonia and flu, are the third leading cause of death worldwide. Viral RTIs are divided into influenza virus (IFV) and non-IFV (human adenovirus [ADV], human bocavirus, human coronavirus [HCoV], human respiratory syncytial virus [RSV], human rhinovirus [hRV], parainfluenza virus, and human metapneumovirus). RTIs caused by these viruses are accompanied by various clinical symptoms, *e.g.*, fever, severe cough, hoarseness, wheeze, tachypnea, breathlessness, respiratory distress, nasal flaring, and jugular, intercostal, and thoracic indrawings. The increased rate of emergent viral RTIs in the past 15 years is due to various factors, including the increasing human population, urbanization, changes in the interactions between human and animal populations, climate change, and increased international travel and trade. The characteristics of outbreaks of viral RTIs differ

geographically. Given their importance, we analyzed three major respiratory viruses—RSV, IFV, and HCoV.

First, we evaluated the molecular and clinical characteristics of RSV in South Korea from 2009 to 2014 using 4028 respiratory specimens from local hospitals in Gyeonggi Province for 6 consecutive years by real-time one-step reverse transcriptase-polymerase chain reaction and partial sequencing of the RSV glycoprotein gene. A total of 183 patients were positive for RSV. Of the 131 RSV-A specimens sequenced, 61 (43.3%) were genotype ON1, 66 (46.8%) were NA1, three (2.1%) were GA5, and one (0.7%) was genotype GA1. Of the 31 RSV-B specimens sequenced, 29 (87.9%) were genotype BA9 and two (6.1%) were BA10. The most common clinical symptoms were fever, cough, nasal discharge, and phlegm. A multiple logistic regression analysis showed that RSV infection of pediatric patients was strongly associated with cough and wheezing. The majority of respiratory viruses coinfecting with RSV was hRV. The findings enhance our understanding of the molecular and epidemiological characteristics of RSV, which will enable the development of an RSV vaccine.

Second, we characterized the epidemiology of IFV in South Korea from 2009 to 2014. We compared the demographic and clinical characteristics, associated factors, and disease severity of IFV A(H1N1)pdm09, A(H3N2), and IFV B and the characteristics of the first wave in 2009 and the subsequent wave in 2010. A total of 4028 outpatients attended local hospitals with respiratory symptoms and were enrolled in KINRESS in Gyeonggi Province. Of them, 920 (22.8%) were positive for IFV, comprising 305 (33.1%) A(H3N2), 271 (29.5%), A(H1N1)pdm09, and 343

(37.3%) IFV B. IFV epidemics occur annually from November and March and at a far lower frequency during the rest of the year. According to univariate analyses using A(H3N2) as a reference, the risk ratios of 5–24 years and years 2009 and 2010 were > 1.0. A multiple logistic regression analysis showed that cough, chill, headache, and muscular pain were associated with IFV infection, and human rhinovirus comprised the majority of respiratory viruses coinfecting with IFV. These results will facilitate prediction of influenza outbreaks.

Third, we assessed the epidemiological and clinical characteristics of HCoV in South Korea from 2009 to 2014 using 4028 throat and nasal swabs from children and adults with fever and various clinical symptoms. Among the 4028 cases, 112 (2.8%) were positive for HCoV, including 45 (40.2%) males and 54 (48.2%) females. Of them, 70 (62.5%) were HCoV-OC43, 14 (12.5%) were HCoV-229E, and 28 (25.0%) were HCoV-NL63. HCoV epidemics occurred mainly in winter. According to univariate analyses using HCoV-OC43 as a reference, the risk was > 1.0 in only 2009. A multiple logistic regression analysis showed that nasal obstruction was associated with HCoV infection, and age and headache with HCoV-229E infection. Moreover, the majority of respiratory viruses coinfecting with HCoV were hRV and ADV. These studies will enable prediction of HCoV outbreaks; also, further molecular analysis of HCoV is needed.

In conclusion, RSV, IFV, and HCoV have different molecular and epidemiological characteristics. Moreover, RSV, IFV, and HCoV infections cause unique clinical symptoms such as fever and cough. Therefore, identification of the factors that influence respiratory viruses in South Korea is required to prevent pandemics and will assist vaccine development.

Keyword: Respiratory viral infection, respiratory viruses, human respiratory syncytial virus, influenza virus, human coronavirus, clinical symptom

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LIST OF ABBREVIATIONS

RTI : Respiratory tract infection

KINRESS : Korea Influenza and Respiratory Viruses Surveillance System

KISS : Korea Influenza Surveillance Scheme

ARI-net : Acute Respiratory Infections Surveillance Network

IFV : Influenza virus

ADV : Human adenovirus

hBoV : Human bocavirus

PIV : Parainfluenza virus

RSV : Human Respiratory syncytial virus

HCoV : Human coronavirus

hMPV : Human metapneumovirus

hRV : Human rhinovirus

ILI : Influenza-like illness

KCDC : Korea Centers for Disease Control and Prevention

GIHE : The Gyeonggi Province Institute of Health and Environment

RSV : Human respiratory syncytial virus

G protein : G glycoprotein

KNIH : Korea National Institute of Health

VTM : viral transport medium

RNA : ribonucleic acid

mL : milliliter

cDNA : complementary deoxyribonucleic acid

DTT : dithiothreitol

OR : odds ratio

CI : confidence interval

mM : millimolar

dNTPs : deoxynucleotide Triphosphates

µM : micromolar

µL : microliter

min : minute

S : second

PCR : polymerase chain reaction

RT-PCR : Realtime-polymerase chain reaction

COPD : chronic obstructive pulmonary

WHO : World Health Organization

URT : upper respiratory tract

URTI : upper respiratory tract infection

LRT : lower respiratory tract

LRTI : lower respiratory tract infection

BAL : bronchoalveolar lavage

HA : hemagglutinin

NA : neuraminidase

CDC : Centers for Disease Control and Prevention

SARS : severe acute respiratory syndrome

MERS : Middle East respiratory syndrome

CHAPTER I.

BACKGROUNDS

The Korea Influenza and Respiratory Viruses Surveillance System

Background and Necessity

Acute respiratory infections, including by influenza virus, are the most common complaints of outpatients at primary and secondary hospitals. Furthermore, they are important causes of death among children. Although respiratory viruses are the major cause of acute RTI, bacteria and fungi are also important agents. Most therapies target clinical symptoms without laboratory diagnosis; alternatively, antibiotics are prescribed to relieve symptoms and prevent secondary infection.

The influenza surveillance system of South Korea was initiated in October 1997 with a small number of volunteer specialists in internal medicine, pediatrics, and family medicine (Choi, Lee et al. 2012). This surveillance system in 2000 became the Korea Influenza Surveillance Scheme (KISS) and, subsequently, the Korea Influenza and Respiratory Viruses Surveillance System (KINRESS) in May 2009. KINRESS incorporates the Acute Respiratory Infections Surveillance Network (ARI-net) and KISS. When the surveillance was initiated, most respiratory specimens were from young children but from January 2014 patients of any age were included.

Aim of the Surveillance System

The goal of the Korea Influenza and Respiratory Viruses Surveillance System (KINRESS) is rapid identification and control measures, thus

preventing the spread of pathogens (Al-Tawfiq, Zumla et al. 2014). Generally, surveillance monitors when the IFV and other respiratory virus seasons begin and end, characterizes the types and subtypes of circulating strains, monitors the clinical severity of illness, and detects the emergence of novel or reassorted viruses (Al-Tawfiq, Zumla et al. 2014). The goals of the surveillance system can be summarized as follows:

1. Reinforce the influenza and respiratory virus surveillance system and increase the detection rate of acute respiratory viruses.

2. Establish a rapid and accurate laboratory diagnosis system and strengthen diagnostic accuracy via method development.

3. Analyze domestic respiratory virus outbreaks and facilitate prevention of their spread.

Outline and Scheme

KINRESS operates at 52 sites (as of August 2019) that participate in clinical sentinel surveillance. The participating sites collect respiratory specimens from patients with influenza-like illness (ILI) or acute respiratory illness and send them to the regional Research Institutes of Public Health and Environment. These institutes conduct genetic testing and send the results and residual samples to the KCDC. The KCDC conducts virus identification and reexamines any unidentified specimens. Based on the results, the circulation patterns and characteristics of IFV and other respiratory viruses are evaluated (Choi 2019).

The Gyeonggi Province Institute of Health and Environment (GIHE) have participated in KISS and KINRESS since their establishment. Four local hospitals specializing in pediatrics or internal medicine in Gyeonggi

Province were selected for KINRESS. To ensure the representativeness of the local hospitals, objective criteria (such as regional population, gender and age) were considered, and specific incentives were provided. Surveillance is conducted annually, and respiratory specimens are collected and laboratory examinations performed weekly; the participation rate, performance, and results are analyzed annually. The targets are outpatients with clinical symptoms suggestive of influenza-like illness (ILI) or acute respiratory diseases and surveillance is performed by four local hospitals, GIHE, and the Seegene Medical Foundation. Although the participating local hospitals tend to change, typically two specialize in pediatrics and two in internal medicine. For example, Kwon Internal Medicine, Kim Youngsoon Internal Medicine, Bom Pediatrics, and Sangdong Family Medicine participated in 2018.

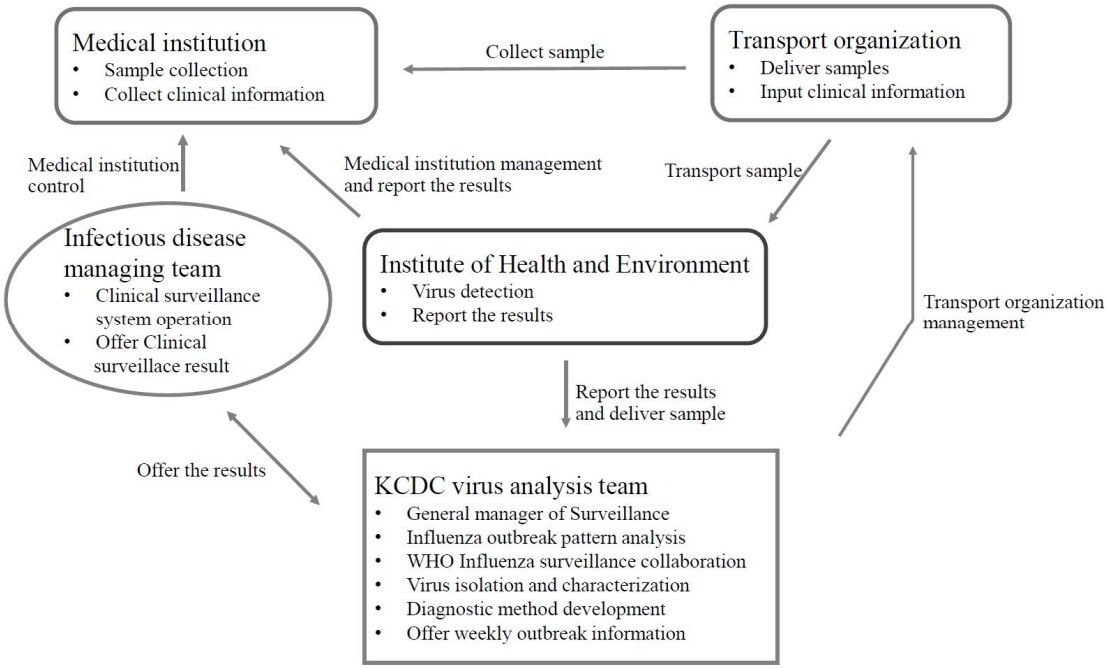


Figure 1.1. Schematic diagram of KINRESS

Contents and Methods

Table 1.1. Viruses targeted by KINRESS.

Influenza virus (IFV)	Respiratory viruses
	Adenovirus (ADV)
	Bocavirus (hBoV)
	Parainfluenza virus (PIV) : PIV 1, PIV 2,
Influenza virus A(H1N1)pdm09	PIV 3
Influenza virus A(H3N2)	Human Respiratory syncytial virus
Influenza virus A(H5N1)	(RSV) : RSVA, RSVB
Influenza virus B	Coronavirus (HCoV) : OC43, 229E,
	NL63
	Metapneumovirus (hMPV)
	Rhinovirus (hRV)

Methods

Specimen collection. Eight respiratory specimens (throat swabs, nasopharyngeal swabs of nasopharyngeal aspirates) from patients attending surveillance medical institutions who had clinical symptoms suggestive of ILI or acute respiratory diseases were collected weekly on Monday or Tuesday. An influenza and acute respiratory disease laboratory examination request form and written informed consent from the patient (name, age, gender, onset of illness, date of specimen collection, putative diagnosis, clinical symptoms, influenza vaccination, and antibiotics used) were obtained.

Specimen delivery and storage. The Seegene Medical Foundation, which is responsible for delivering and managing respiratory specimens, collects

specimens each Wednesday and transports them to GIHE. The respiratory specimens are maintained at 4°C during transport using an icebox and the laboratory examination request and written informed consent are enclosed.

Identification of respiratory viruses. Multiplex real-time PCR/RT-PCR was used to screen for 16 respiratory viruses using the Powerchek™ Real-Time PCR Kit (KogeneBiotech, South Korea). IFV A-positive, but not IFV B-positive, specimens were subjected to subtyping (Tables 1.2, 1.3). Residual respiratory specimens were transported to the KCDC monthly. The sensitivity and specificity of the IFV and respiratory multiplex kits were evaluated by Choi *et al.* (Choi, Kim et al. 2013) and KogeneBiotech, South Korea (Table 1.4).

Reporting results and sharing data. Results of respiratory virus detection are provided on Wednesday (specimen collection date) on the KCDC homepage, and notices are sent to local hospitals directly. Finally, we can search the results through disease integrated management system (http://is.cdc.go.kr).

Expected Effects

Strengthening preparedness for respiratory virus outbreaks. Surveillance enables detection and the mounting of a response to novel respiratory viruses year-round, irrespective of the epidemic status.

Establish fundamental information essential to control and treat RTI.

Surveillance can identify causative agents of outpatients with acute viral RTIs, including IFV. It can also provide information necessary to control and treat viral infection by analyzing viral characteristics.

Table 1.2. Diagnostic kit for IFV.

Multiplex Kit (One-step Real-time RT-PCR premixture)	**Target gene**	**Reporter**	**Target virus**
Influenza A/B Real-time PCR kit	Influenza A-MP Influenza B-NP IPC-GAPDH	FAM VIC CY5	Influenza A Influenza B Human GAPDH
Pandemic H1N1/H3N2/H5N1 Real-time PCR kit	A(H1)pdm09-HA Human_H3 Avian H5-HA	FAM VIC NED	H1N1 (2009) H3N2 Avian influenza H5N1

Table 1.3. Diagnostic kit for respiratory viruses and diagnostic criteria.

Multiplex Kit (PowerChek™ Real-time PCR kit)	**Target gene**	**Reporter**	**Criterion (Ct value)**	**comments**
Parainfluenza virus type I, II, III/IPC (internal positive control)	PIV I PIV II PIV III IPC	FAM VIC NED Cy5	Below 37 Below 38	Threshold : 0.2
Human Coronavirus 229E & OC43 & NL63	OC43 NL63 229E	FAM VIC NED	Below 37	
Rhinovirus	Rhinovirus	FAM	Below 35	
Respiratory syncytial virus A&B/Human Metapneumovirus (HMPV)	RSVA RSVB HMPV	FAM VIC NED	Below 37	
Adenovirus & Bocavirus	Adenovirus Bocavirus	FAM VIC	Below 37	

Table 1.4. The performance of multiplex Real-time RT-PCR.

Multiplex Kit (One-step Real-time RT-PCR premixture)	**Virus**	**Sensitivity**	**Specificity**
Influenza A/B Real-time PCR kit	Influenza A	92.2%	100%
	Influenza B	100%	100%
Pandemic H1N1/H3N2/H5N1 Real-time PCR kit	A(H1)pdm09	95.2%	100%
	Human_H3	85.1%	100%
Parainfluenza virus type I, II, III/IPC (internal positive control)	PIV I	97.8%	99.4%
	PIV II	97.9%	99.8%
	PIV III	97.3%	99.5%
Human Coronavirus 229E & OC43 & NL63	OC43	92.3%	99.8%
	NL63	75.0%	99.8%
	229E	100%	100%
Rhinovirus	Rhinovirus	95.6%	97.1%
Respiratory syncytial virus A&B/Human Metapneumovirus (HMPV)	RSVA	100%	100%
	RSVB	100%	100%
	HMPV	100%	100%
Adenovirus & Bocavirus	Adenovirus	96%	99.5%
	Bocavirus	100%	100%

General objectives

Acute respiratory infections such as pneumonia, flu and respiratory syncytial virus are third largest cause of deaths in the world (after heart and stroke) and the leading killer in low and middle income countries. But despite the death toll and morbidity, respiratory infection gets only a fraction of the support from governments, donor agencies, and charities that other illness receive, says the foundation (Mayor 2010).

In South Korea, recent study reported that tuberculosis was the leading cause of infectious disease death in 1983 (7,853 deaths; 23.7/100,000 population), but in 2015, respiratory infections were the most common causes of deaths from infectious diseases (15,030 deaths; 19.5/100,000 population) (Choe, Choe et al. 2018). Therefore, deaths from respiratory infections are crucial problem not only in South Korea but also other countries.

Furthemore, a number of recent virus outbreaks have resulted in rapid virus spread, placing demands on affected health infrastructures and sparking global concern (Wong, Liu et al. 2015) and over the past decades, the outbreaks of respiratory viruses have been continuously reported in various countries such as Spain, Hong Kong, China and it can be summarized in two representative respiratory viruses such as influenza virus and human coronavirus which greatly affects worldwide.

In this study, we will discuss about major respiratory viruses such as RSV, IFV, HCoV which was selected for the consideration of importance among respiratory viruses throughout recent studies and situations. Until now, there are so many respiratory virus outbreaks that makes phobia and

fearness. To cope with unexpected outbreaks efficiently, first, we should know molecular characteristics about respiratory virus itself and second, epidemiologic, demographic and clinical characteristics about host, in other words, human exactly. From these, we can find unique domestic characteristics and identify epidemiologic major factors and identifiy the correlation between virus and host. We expect that these results help to develop vaccine development and apply to prevent desease and make a good preventive measure.

Objectives and Hypotheses

Objectives:

The objectives of this study were to investigate the molecular and clinical characteristics and the epidemiology of RSV, IFV, and HCoV in South Korea.

Hypothesis 1

The major respiratory viruses have different outbreak trends and epidemiologic characteristics, such as age and gender.

Hypothesis 2

The three major respiratory viruses are associated with different clinical symptoms.

Hypothesis 3

Molecular biologic analysis of the three major respiratory viruses enables assessment of their level of molecular genetic similarity according to geographic location.

CHAPTER II.

MOLECULAR AND CLINICAL CHARACTERIZATION OF HUMAN RESPIRATORY SYNCYTIAL VIRUS IN SOUTH KOREA, 2009–2014

Introduction

Human respiratory syncytial virus (RSV) is the second most frequent cause of respiratory infections, and the main cause of bronchiolitis and pneumonia in infants and young children (Borchers, Chang et al. 2013). The incidence of RSV is known to be age-dependent, with an estimated 60% of children infected with RSV in their first year of life (Glezen, Taber et al. 1986). Almost all children are infected by the time they turn 2 years of age (Sorce 2009), whereas fewer infections are detected in older children and adults (Hogan, Glass et al. 2016). Immunity to RSV following recovery from infection is partial and temporary, thus reinfection throughout early childhood is common (Meng, Stobart et al. 2014). Due to the lack of an effective vaccine, substantial health and economic costs are associated with RSV epidemics due to hospitalizations and treatment of RSV infection-related illnesses (Slovic, Ivancic-Jelecki et al. 2016).

RSV is a non-segmented, negative-sense single-strand RNA enveloped virus belonging to the family *Paramyxoviridae* (Johnson, Spriggs et al. 1987). There are two major RSV subgroups (A and B) categorized on the basis of antigenic and genetic variability. Each subgroup is further categorized into genotypes based on the nucleotide sequence variation within the second hypervariable region of heavily glycosylated G glycoprotein (G protein). There are 12 genotypes for RSV-A (GA1–7, SAA1, NA1–2, and ON1–2) and 20 genotypes for RSV-B (GB1–4, SAB1–4, URU1–2, and BA1–10) (Cui, Zhu et al. 2013, Hirano, Kobayashi et al. 2014). Depending on the genotypes, there are differences in the attachment G glycoprotein which interacts with host cell receptors (Johnson, Spriggs et al. 1987). The RSV attachment G glycoprotein is responsible for virus

binding to the host cell surface receptor and is a target of human neutralizing antibodies, together with the fusion (F) glycoprotein (Sorce 2009). The G protein shows the highest genetic and antigenic variability among all the RSV structural proteins. There is abundant evidence of accumulating amino acid changes in its hypervariable regions over time (Johnson, Spriggs et al. 1987, Cane and Pringle 1995), making its study relevant in vaccine development strategies (Otieno, Agoti et al. 2016).

The dynamics of RSV circulation are further demonstrated with the emergence of new genotypes that spread rapidly worldwide and replace previously circulating genotypes (Slovic, Ivancic-Jelecki et al. 2016). Such changes were shown after the emergence of the BA genotype (Trento, Galiano et al. 2003). Among the RSV subgroup B, almost all strains detected after 2005 belong to the BA genotype (Trento, Casas et al. 2010). Coincidently, a similar duplication event in RSV subgroup A led to the emergence of the ON1 genotype (Eshaghi, Duvvuri et al. 2012), and the importance of epidemiological prevalence has also been reported for the ON1 genotype (Kim, Kim et al. 2014, Pierangeli, Trotta et al. 2014). It is assumed that these specific genetic characteristics provide a selective evolutionary advantage for these viruses (Prifert, Streng et al. 2013).

RSV coinfection with other respiratory viruses has been previously studied, in addition to its simple genetic characterization and annual distribution. The relevance to clinical severity in itself and coinfection with other respiratory viruses have also been investigated. For example, several studies revealed that the human rhinovirus (hRV) is the most common virus that coinfects with RSV. A greater number of viruses is not necessarily synonymous with greater disease severity (Martinez-Roig, Salvado et al.

2015), and coinfection occurs frequently with RSV and hRV, as well as RSV and human bocavirus (hBoV). Overall, viral coinfection does not present greater severity, but can present mixed clinical features (Calvo, García-García et al. 2015). However, some studies showed contradictory results. Children coinfected with RSV and other viruses presented more frequently with pneumonia than those with single RSV infection (Asner, Rose et al. 2015). A previous study showed that during coinfections, one virus could block another simply by being the first to infect the available host cells (Pinky and Dobrovolny 2016).

In this study, we investigated the molecular epidemiologic characteristics of RSV and its coinfection with other respiratory viruses in Gyeonggi Province in South Korea.

Materials and Methods

Subjects and data collection. This study was conducted by the Korea National Institute of Health (KNIH) as part of the Korea Influenza and Respiratory Viruses Surveillance System (KINRESS). As part of the KINRESS, we collected a total of 4028 respiratory specimens such as throat swabs or nasal aspirate specimens (nasopharyngeal swab, nasopharyngeal aspirate) from local hospitals which are primary hospitals that first level of health system in Gyeonggi Province, South Korea from 2009 to 2014. Epidemiologic data and respiratory specimens from all patients with influenza-like illnesses were sent from local hospitals to the Gyeonggi Province Institute of Health and Environment (GIHE). Swab specimens were immediately placed at 4°C in 2-mL cryovials containing 1.5 mL of cold viral transport medium (VTM; Difco, USA), and sent to the laboratory. Upon receipt, the specimens were processed immediately for virus detection, identification, and characterization. Aliquots of samples were also stored at −80°C for additional analysis. Case reports and laboratory analysis data were entered into a KINRESS database (the Korea National Institute of Health, South Korea). The samples were collected for respiratory virus diagnosis, and written informed consent was obtained from the patients, their parents, or legal guardians (Kim, Kim et al. 2014). This study was approved by the Korea National Institute of Health Institutional Review Boards (Approval Nos. 2010-03EXP-1-R, 2011-06EXP-01-C, 2012-08EXP-06-3C, 2013-08EXP-03-5C, and 2014-08EXP-08-6C-A).

Virus detection and subsequent molecular analysis. Viral RNA was extracted from 140 μL of each respiratory specimen using QIAamp Viral

RNA Mini Kits (Qiagen GmbH, Hilden, Germany). Seven Multiplex-PCR assays were performed for screening 16 respiratory viral pathogens including RSV, influenza (IFV)-A and B, adenovirus (ADV), hBoV, parainfluenza virus (PIV) 1–3, human metapneumovirus (hMPV), human coronaviruses (HCoV) (229E, OC43, NL63), and hRV (Kim, Kim et al. 2014). Confirmed RSV-positive samples were further screened for subgroup (A/B) (Kim, Kim et al. 2014) and genotyped by amplifying and sequencing the second hypervariable region of the G protein. The G gene-specific primer set for sequence analysis was as follows: forward primer G(151–173) F: CTGGCAATGATAATCTCAACTTC and reverse primer F(3–22) R: CAACTCCATTGTTATTTGCC (Antoniassi da Silva, Spilki et al. 2008). The cDNA was prepared using the viral RNA extraction method employed by the routine respiratory virus test. The reaction mixture contained 5 μL of RNA, which was mixed with a final concentration of 10 mM dNTPs, 20 μM random primer, 1× RT buffer, 200 U of Superscript III reverse transcriptase (Invitrogen, CA, USA), 40 U of RNase-OUT RNase inhibitor (Invitrogen), 25 mM $MgCl_2$, 0.1 mM dithiothreitol (DTT), and RNase-free water in a final volume of 20 μL. The mixture was then incubated at 25°C for 5 min, 50°C for 60 min, and 72°C for 5 min to terminate cDNA synthesis. Next, 5 μL of cDNA were added to a PCR mixture containing 1 μL of SP-Taq DNA polymerase (2.5 U/μL) (Cosmo Genetech, Seoul, South Korea), 36 μL of distilled water, 5 μL of 10× PCR buffer, 1 μL of 10 mM dNTPs, and 1 μL each of the forward and reverse primers (both 20 μM) for the G gene. Primary denaturation was conducted at 95°C for 10 min, which was followed by 35 cycles of PCR where each cycle comprised denaturation for 40 s at 95°C, annealing for 40 s at 54°C, and elongation for 1 min at 72°C,

with a final extension cycle of 5 min at 72°C. The PCR products were separated by electrophoresis using 1% agarose gel and visualized using 1× SYBR Safe DNA Gel Stain (Invitrogen) (Kim, Kim et al. 2014). BigDye Terminator ver.3.1 (Applied Biosystem, Foster City, CA, USA) was utilized for the sequencing reaction, and nucleotide sequence analysis was performed using a 3730 DNA Analyzer (Applied Biosystem). Multiple nucleotide sequences were aligned and edited with ClustalW ver.1.8 software. Phylogenetic analysis was executed using the neighbor-joining method with a bootstrap value of 1000 replicates for testing statistical significance of the tree topology using MEGA ver.6.06 software (Yoshihara, Le et al. 2016). The representative 14 sequences were submitted to Genbank and assigned accession numbers of KY773693-KY773706. Network analysis was visualized using Cytoscape ver.2.6.1 software.

Statistical analysis. The categorical variables were compared using the two-tailed Chi-squared test and multivariate analysis, and multiple logistic regression analysis was applied to estimate the odds ratio (OR) and 95% confidence interval (CI). Statistical analyses were performed using the R.3.0.1 tool. *P*-values <0.05 were considered to indicate statistical significant.

Results

Prevalence of respiratory viral cases

Among 4028 analyzed samples, 1635 samples were positive for respiratory viral infections of various types (Table 2.1). The annual percentage of cases that were positive for respiratory infections were 18.0% (75/416) in 2009, 26.9% (179/665) in 2010, 48.8% (335/686) in 2011, 51.7% (474/917) in 2012, 47.1% (345/732) in 2013, and 37.1% (227/612) in 2014. Most of these infections were due to IFV (overall: 22.8%, type A: 14.3%, type B: 8.5%), hRV (14.2%), and ADV (10.0%). RSV viral infection was detected in ~4.5% (n = 183/4028) of the patients enrolled.

Epidemiological and clinical characteristics of patients with viral respiratory infections

The epidemiological and clinical characteristics of patients with viral respiratory infections are summarized in Table 2.2, Table 2.3 and Table 2.4; 50.4% of the patients with viral infections were female [PIV (50.5%), hCoV (54.5%), hMPV (57.5%), and IFV (52.9%)]. Among the respiratory infections, hBoV and RSV infected the youngest patients (median age = 2 years), whereas IFV was more prevalent in older children (median age = 7 years) than any other viruses. Clinical symptoms such as fever (overall = 81.3%: pediatric patients, 72.7% vs adult, 8.5%) and (overall = 67.0%: ILI, 29.5% vs acute respiratory illness, 37.5%), cough (overall = 66.5%: pediatric patients, 58.5% vs adult, 8.0%) and (overall = 54.7%: ILI, 21.8% vs acute respiratory illness, 32.9%), nasal discharge (overall = 59.1%: pediatric patients, 52.2% vs adult, 6.8%) and (overall = 49.5%: ILI, 19.6% vs acute respiratory illness, 29.9%), and phlegm (overall = 47.8%: pediatric

Table 2.1 Annual incidence of all respiratory infection cases in Gyeonggi Province from 2009 to 2014.

	Overall (n,%)	2009	2010	2011	2012	2013	2014
Total enrolled patients	4028	416	665	686	917	732	612
Positive respiratory infections	1635(40.6)	75(18.0)	179(26.9)	335(48.8)	474(51.7)	345(47.1)	227(37.1)
ADV infection	402(10.0)	5(1.2)	54(8.1)	53(7.7)	102(11.1)	157(21.4)	31(5.1)
hBoV infection	106(2.6)	1(0.2)	5(0.8)	34(5.0)	28(3.1)	26(3.6)	12(2.0)
PIV infection	194(4.8)	1(0.2)	1(0.2)	28(4.1)	69(7.5)	50(6.8)	45(7.4)
RSV infection	**183(4.5)**	**10(2.4)**	**16(2.4)**	**57(8.3)**	**65(7.1)**	**21(2.9)**	**14(2.3)**
RSV subgroup(A/B)							
RSV-A	141(3.5)	9(2.2)	1(0.2)	52(7.6)	60(6.5)	9(1.2)	10(1.6)
RSV-B	33(0.8)	0	8(1.2)	5(0.7)	4(0.4)	12(1.6)	4(0.7)
RSV-A and B	1(0.0)	0	0	0	1(0.1)	0	0
HCoV infection	112(2.8)	12(2.9)	13(2.0)	19(2.8)	25(2.7)	21(2.9)	22(3.6)
hMPV infection	40(1.0)	-	6(0.9)	7(1.0)	3(0.3)	7(1.0)	16(2.6)
hRV infection	573(14.2)	45(10.8)	60(9.0)	137(20.0)	181(19.7)	63(8.6)	87(14.2)
IFV infection	920(22.8)	91(21.9)	217(32.6)	62(9.0)	333(36.3)	86(11.7)	131(21.4)

patients, 43.8% vs adult, 4.0%) and (overall = 41.6%: ILI, 12.6% vs acute respiratory illness, 29.0%) were frequently identified among the infected patients. However, several patients might have had conditions such as febrile seizure, and stomachache that were not listed as clinicalymptoms. The most common pre-existing conditions among the patients were asthma (overall = 0.7%: pediatric patients, 0.6% vs adult, 0.1%) and hypertension (overall = 0.9%: pediatric patients, 0% vs adult, 0.9%). For example, patients infected with IFV had a high prevalence of asthma (n = 8, 30.8%) and hypertension (n = 8, 25.8%); additionally, these patients showed a high prevalence of antibiotic usage (140/210, 66.7%). Pediatric patients means below 19 years old and adult means over 20 years old.

Annual incidence of RSV and RSV subgroups in patients with respiratory viruses

Among 4028 samples, 1635 samples had positive results for respiratory infections; of these 183 (4.5%) were RSV-positive, as confirmed by multiplex-PCR assays. Using real-time one-step RT-PCR to distinguish between RSV subgroups A and B, we categorized 141 RSV-positive samples as subgroup A (80.6%), 33 as subgroup B (18.8%), and 1 as RSV-A/B coinfection (0.6%). RSV-A was the major subgroup between January 2009 and November 2014, with January 2010 to December 2010 as the only exception. RSV-B (n = 8, 88.9%) was found in the majority of RSV cases from January 2013 to December 2013, while it was detected at only a slightly higher rate (n = 12, 57.1%) than RSV-A infections (n = 9, 42.9%). Most cases of RSV-A (n = 112, 79.4%) were detected from January 2011 to

Table 2.2 Demographic and clinical characteristics of all patients with respiratory infections from 2009 to 2014.

	Clinical information(n=3478)								
				Age group					
	Female/ Male	Female (%)	Median age(years)	0-2 years	3-6 years	7-19 years	20-49 years	50-64 years	Over 65 years
All patients (n=4028,%)	1997/1967	50.4	5	980(24.3)	1412(35.1)	913(22.7)	432(10.7)	163(4.0)	83(2.1)
ADV(n=402)	172/227	43.1	3	120(29.9)	229(57.0)	48(11.9)	1(0.2)	2(0.5)	0
hBoV(n=106)	48/58	45.3	2	60(56.6)	36(34.0)	8(7.5)	1(0.9)	1(0.9)	0
PIV(n=194)	97/95	50.5	3	80(41.2)	96(49.5)	10(5.2)	2(1.0)	3(1.5)	1(0.5)
RSV(n=183)	81/102	44.3	2	116(63.4)	58(31.7)	6(3.3)	1(0.5)	1(0.5)	1(0.5)
HCoV(n=112)	54/45	54.5	4	38(33.9)	36(32.1)	20(17.9)	10(8.9)	3(2.7)	5(4.5)
hMPV(n=40)	23/17	57.5	3	13(32.5)	22(55.0)	4(10.0)	0	0	1(2.5)
hRV(n=573)	273/289	48.6	3	211(36.8)	211(36.8)	82(14.3)	52(9.1)	10(1.7)	5(0.9)
IFV(n=920)	474/422	52.9	7	59(6.4)	59(6.4)	359(39.0)	108(11.7)	25(2.7)	15(1.6)

	Clinical information(n=3478)							
	Clinical symptoms							
	Fever	Cough	Sore throat	Chill	Headache	Muscular pain	Nasal discharge	Nasal obstruction
All patients (n=4028,%)	2827(81.3)	2314(66.5)	1403(40.3)	1086(31.2)	1013(29.1)	786(22.6)	2056(59.1)	1398(40.2)
ADV(n=402)	337(83.8)	272(67.7)	148(40.3)	106(26.4)	72(17.9)	66(16.4)	267(66.4)	204(50.7)
hBoV(n=106)	90(84.9)	76(71.7)	27(25.5)	23(21.7)	13(12.3)	11(10.4)	73(68.9)	47(44.3)
PIV(n=194)	172(88.7)	152(78.4)	56(28.9)	26(13.4)	14(7.2)	18(9.3)	135(69.6)	87(44.8)
RSV(n=183)	159(86.9)	154(84.2)	37(20.2)	32(17.5)	17(9.3)	7(3.8)	131(71.6)	63(34.4)
HCoV(n=112)	85(75.9)	73(65.2)	38(33.9)	30(26.8)	22(19.6)	20(17.9)	74(66.1)	63(56.3)
hMPV(n=40)	31(77.5)	29(72.5)	13(32.5)	11(27.5)	3(7.5)	5(12.5)	26(65.0)	20(50.0)
hRV(n=573)	388(67.7)	345(60.2)	161(28.1)	109(19.0)	82(14.3)	69(12.0)	336(58.6)	204(35.6)
IFV(n=920)	681(74.0)	566(61.5)	362(39.3)	351(38.2)	36139.2)	268(29.1)	491(53.4)	283(30.8)

	Clinical information(n=3478)								
	Clinical symptoms								
	Hoarseness	Wheezing	Laboring breath	Phlegm	Constricted chest	Vomiting	Diarrhoea	Loss of appetite	The others[#]
All patients (n=4028,%)	473(13.6)	183(5.3)	12(0.3)	1664(47.8)	5(0.1)	183(5.3)	38(1.1)	907(26.1)	3(0.1)
ADV(n=402)	58(14.4)	20(5.0)	0	223(55.5)	0	18(4.5)	3(0.7)	169(42.0)	1(0.2)
hBoV(n=106)	22(20.8)	15(14.2)	0	63(59.4)	0	5(4.7)	5(4.7)	31(29.2)	0
PIV(n=194)	48(24.7)	21(10.8)	0	135(69.6)	0	8(4.1)	0	72(37.1)	0
RSV(n=183)	30(16.4)	40(21.9)	1(0.5)	120(65.6)	1(0.5)	16(8.7)	1(0.5)	46(25.1)	0
HCoV(n=112)	18(16.1)	7(6.3)	0	63(56.3)	0	2(1.8)	1(0.9)	28(25.0)	0
hMPV(n=40)	11(27.5)	2(5.0)	0	23(57.5)	0	3(7.5)	0	14(35.0)	0
hRV(n=573)	65(11.3)	29(5.1)	1(0.2)	245(42.8)	2(0.3)	24(4.2)	4(0.7)	134(23.4)	1(0.2)
IFV(n=920)	70(7.6)	15(1.6)	2(0.2)	344(37.4)	0	46(5.0)	10(1.1)	124(13.5)	0

	Clinical information(n=3478)						
	Putative diagnosis		History or pre-existing condition				
	Influenza like illness[§]	Acute respiratory illness[□]	asthma	COPD	Hypertension	Diabetes	Immune disease
All patients (n=4028,%)	1046(30.1)	1383(39.8)	26(0.7)	0	31(0.9)	10(0.3)	1(0.0)
ADV(n=402)	94(23.4)	218(54.2)	1(0.2)	0	0	0	0
hBoV(n=106)	18(17.0)	54(50.9)	0	0	0	0	0
PIV(n=194)	20(10.3)	127(65.5)	0	0	0	0	0
RSV(n=183)	19(10.4)	102(55.7)	0	0	0	0	0
HCoV(n=112)	27(24.1)	49(43.8)	1(0.9)	0	1(0.9)	0	0
hMPV(n=40)	11(27.5)	14(35.0)	0	0	0	0	0
hRV(n=573)	107(18.7)	215(37.5)	2(0.3)	0	2(0.3)	1(0.2)	1(0.2)
IFV(n=920)	424(46.1)	111(12.1)	8(0.9)	0	8(0.9)	1(0.1)	0

Clinical information(n=3478)						
	History or pre-existing condition				Treatment	
All patients (n=4028,%)	Transplantation	Cancer	Tuberculosis	Antibiotic used	H1N1 influenza vaccination	H1N1 & H3N2 Influenza and influenza B vaccination
ADV(n=402)	0	0	0	210(6.0)	1289(37.1)	125(3.6)
hBoV(n=106)	0	0	0	7(1.7)	157(39.1)	7(1.7)
PIV(n=194)	0	0	0	1(0.9)	44(41.5)	0
RSV(n=183)	0	0	0	4(2.1)	77(39.7)	0
HCoV(n=112)	0	0	0	5(2.7)	76(41.5)	1(0.5)
hMPV(n=40)	0	0	0	6(5.4)	46(41.1)	2(1.8)
hRV(n=573)	0	0	0	0	14(35.0)	2(5.0)
IFV(n=920)	0	0	0	10(1.7)	186(32.5)	14(2.4)

[#]Clinical conditions including febrile seizure and stomachache., [§] Case was defined based WHO surveillance (Organization 2014), [₱]Case with acute respiratory illness except influenza.

Table 2.3 Clinical characteristics of all patients with stratified by specific age group from 2009 to 2014.

Age group	Clinical information(n=3478)						
	Clinical symptoms						
	Fever	Cough	Sore throat	Chill	Headache	Muscular pain	Nasal discharge
Total(%)	2827(81.3)	2314(66.5)	1403(40.3)	1086(31.2)	1013(29.1)	786(22.6)	2056(59.1)
0-2 years	337(83.8)	272(67.7)	148(40.3)	106(26.4)	72(17.9)	66(16.4)	267(66.4)
3-6 years	90(84.9)	76(71.7)	27(25.5)	23(21.7)	13(12.3)	11(10.4)	73(68.9)
7-19 years	172(88.7)	152(78.4)	56(28.9)	26(13.4)	14(7.2)	18(9.3)	135(69.6)
20-49 years	159(86.9)	154(84.2)	37(20.2)	32(17.5)	17(9.3)	7(3.8)	131(71.6)
50-64 years	85(75.9)	73(65.2)	38(33.9)	30(26.8)	22(19.6)	20(17.9)	74(66.1)
Over 65 years	31(77.5)	29(72.5)	13(32.5)	11(27.5)	3(7.5)	5(12.5)	26(65.0)

Age group	Clinical information(n=3478)						
	Clinical symptoms						
	Nasal obstruction	Hoarseness	Wheezing	Laboring breath	Phlegm	Constricted chest	Vomiting
Total(%)	1398(40.2)	473(13.6)	183(5.3)	12(0.3)	1664(47.8)	5(0.1)	183(5.3)
0-2 years	683(19.6)	242(7.0)	102(2.9)	2(0.1)	840(24.2)	2(0.1)	53(1.5)
3-6 years	386(11.1)	109(3.1)	63(1.8)	4(0.1)	489(14.1)	2(0.1)	68(2.0)
7-19 years	188(5.4)	52(1.5)	11(0.3)	0	193(5.5)	1(0.1)	47(1.4)
20-49 years	103(3.0)	50(1.4)	3(0.1)	1(0.0)	109(3.1)	0	12(0.3)
50-64 years	23(0.7)	14(0.4)	3(0.1)	2(0.1)	18(0.5)	0	3(0.1)
Over 65 years	14(0.4)	6(0.2)	0	3(0.1)	13(0.4)	0	0

Age group	Clinical information(n=3478)						
	Clinical symptoms			History or pre-existing condition			
	Diarrhoea	Loss of appetite	The others	asthma	Hypertension	Diabetes	Immune disease
Total(%)	38(1.1)	907(26.1)	3(0.1)	26(0.7)	31(0.9)	10(0.3)	1(0.0)
0-2 years	13(0.4)	542(15.6)	2(0.1)	3(0.1)	0	0	0
3-6 years	10(0.3)	211(6.1)	0	5(0.1)	0	0	0
7-19 years	9(0.3)	86(2.5)	1(0.0)	14(0.4)	0	0	1(0.0)
20-49 years	1(0.0)	58(1.7)	0	3(0.1)	3(0.1)	0	0
50-64 years	1(0.0)	6(0.2)	0	1(0.0)	9(0.3)	3(0.1)	0
Over 65 years	2(0.1)	3(0.1)	0	0	19(0.5)	7(0.2)	0

Table 2.4 Clinical characteristics of all patients with stratified by putative diagnosis from 2009 to 2014.

Putative diagnosis	Clinical information(n=3478)						
	Clinical symptoms						
	Fever	Cough	Sore throat	Chill	Headache	Muscular pain	Nasal discharge
Total(%)	2329(67.0)	1903(54.7)	1182(40.0)	917(26.4)	800(23.0)	655(18.8)	1721(49.5)
ILI[#]	1026(29.5)	757(21.8)	557(16.0)	551(15.8)	535(15.4)	398(11.4)	682(19.6)
Acute respiratory illness[§]	1303(37.5)	1146(32.9)	625(18.0)	366(10.5)	265(7.6)	257(7.4)	1039(29.9)

Age group	Clinical information(n=3478)						
	Clinical symptoms						
	Nasal obstruction	Hoarseness	Wheezing	Laboring breath	Phlegm	Constricted chest	Vomiting
Total(%)	1214(34.9)	371(10.7)	169(4.9)	5(0.1)	1446(41.6)	5(0.1)	131(3.8)
ILI[#]	431(12.4)	62(1.8)	35(1.0)	4(0.1)	439(12.6)	1(0.0)	75(2.2)
Acute respiratory illness[§]	783(22.5)	309(8.9)	134(3.9)	1(0.0)	1007(29.0)	4(0.1)	56(1.6)

Age group	Clinical information(n=3478)						
	Clinical symptoms			History or pre-existing condition			
	Diarrhoea	Loss of appetite	The others	asthma	Hypertension	Diabetes	Immune disease
Total(%)	29(0.8)	810(23.3)	9(0.3)	18(0.5)	17(0.5)	3(0.1)	1(0.0)
ILI[#]	14(0.4)	189(5.4)	2(0.1)	17(0.5)	5(0.1)	1(0.0)	1(0.0)
Acute respiratory illness[§]	15(0.4)	621(17.9)	7(0.2)	1(0.1)	12(0.4)	2(0.1)	0

[#]Influenza like illness, [§] Acute respiratory illness (=Acute respiratory disease)

December 2012, whereas most cases of RSV-B (n = 12, 36.4%) were detected from January 2013 to December 2013.

Phylogenetic analysis of RSV and annual distribution of RSV subgroup A and B genotypes

We conducted viral genotyping based on the nucleic acid sequence of the second hypervariable region of the G protein. The target region was sequenced and aligned using MEGA ver.6.06. This phylogenetic analysis included 40 representative RSV-A isolates, 14 RSV-A reference sequences, 31 RSV-B isolates, and 26 RSV-B reference sequences. Of the RSV subgroup A (n = 141) and RSV subgroup B (n = 33) samples, 131 RSV-A (92.9%) and 31 RSV-B (93.9%) were sequenced successfully. RSV-A samples clustered in the genotypes of ON1 (n = 61, 43.3%), NA1 (n = 66, 46.8%), GA1 (n = 1, 0.7%), and GA5 (n = 3, 2.1%), whereas RSV-B samples clustered in the genotypes of BA9 (n = 29, 87.9%) and BA10 (n = 2, 6.1%) (Figure 2.1). The ON1 and NA1 genotypes (total: n = 127, 96.9%) constituted the majority of RSV-A genotypes, while the BA9 genotype (n = 29, 87.9%) constituted the majority of RSV-B genotypes. In contrast, GA1 and GA5 genotypes (n = 4, 3.1%) in RSV-A, and BA10 genotype (n = 2, 6.1%) in RSV-B were observed less frequently. From 2009 to 2012, the NA1 genotype was consistently identified in the study subjects. The ON1 genotype was newly detected from 2011 to 2014. Between 2011 and 2012, both ON1 and NA1 genotypes were identified; however, the NA1 genotype

was not identified from 2013 to 2014. These results suggest that the ON1 genotype of RSV newly emerged and was first isolated in December 2011. Then, it gradually dominated and replaced the NA1 genotype. For the RSV-B genotype, BA9 was observed continuously, except in 2009. There were no other notable genetic changes detected in RSV-B genotypes during the study period (Table 2.5, Figure 2.2).

General information and clinical characteristics of patients with RSV subgroups and genotypes

Table 2.6 summarizes the demographic and clinical characteristics of patients associated with RSV subgroups and genotypes. The ON1 genotype (n = 31, 50.8%), RSV subgroup B (n = 19, 55.9%), BA9 genotype (n = 15, 51.7%), and BA10 genotype (n = 2, 100%) had more females than males. All RSV subgroups and genotypes was associated with the young patient group (median age of below 3 years) and the GA5 genotype with the youngest group (median age of 1.0 years). Most of cases were 0–2 years of age (n = 116, 63.4%), however, two older patients (1 in the 50–65 year range and 1 >65 years) were infected with RSV subgroup B (BA9 genotype) (Figure 3a). We performed network analysis to address the correlation between RSV genotype and clinical symptoms (Figure 3b). Fever, cough, nasal discharge, and phlegm were the most common clinical symptoms; however, the ON1 genotype was associated with slightly more nasal discharge and phlegm than the NA1 genotype. Subjects infected with the

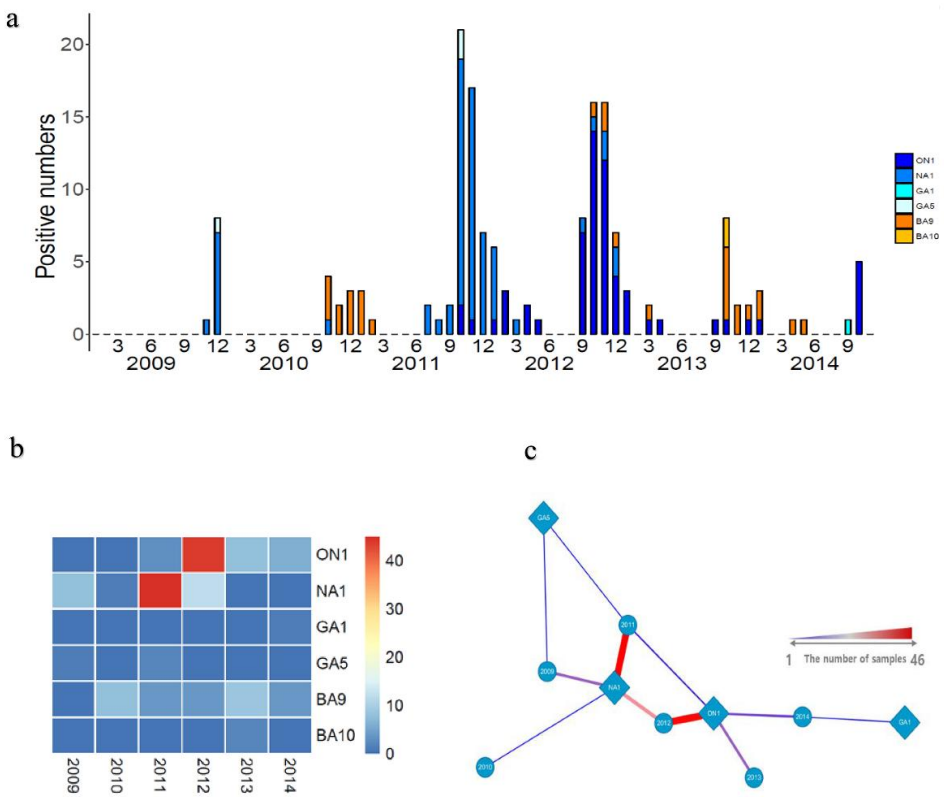


Figure 2.1. Annual distribution of RSV genotypes in Gyeonggi Province from 2009 to 2014. (a) Seasonal distribution of RSV genotypes. (b),(c) Heatmap and network analyses show the relationship between isolation year and RSV genotype.

Table 2.5 Annual incidence of infections with all respiratory viruses, RSV, RSV subgroups(A/B) and genotypes in Gyeonggi Province from 2009 to 2014.

	Overall (n,%)	2009	2010	2011	2012	2013	2014
Overall patients	4028	416	665	686	917	732	612
Positive respiratory infections	1635(40.6)	75(18.0)	179(26.9)	335(48.8)	474(51.7)	345(47.1)	227(37.1)
RSV positive infection	183(11.3)	10(13.3)	16(8.9)	57(17.0)	65(13.9)	21(6.1)	14(6.2)
RSV subgroup(A/B) (n=175)							
RSV-A positive infection	141(80.6)	9(100)	1(11.1)	52(91.2)	60(90.9)	9(42.9)	10(71.4)
RSV-B positive infection	33(18.9)	0	8(88.9)	5(8.8)	4(6.1)	12(57.1)	4(28.6)
RSV-A and B coinfection	1(0.6)	0	0	0	1(1.5)	0	0
Total	175	9	9	57	66	21	14
RSV-A genotype(n=131)							
ON1 genotype	61(43.3)	0	0	3(5.8)	44(73.3)	8(88.9)	6(60.0)
NA1 genotype	66(46.8)	8(88.9)	1(100)	45(86.5)	12(20.0)	0	0
GA1 genotype	1(0.7)	0	0	0	0	0	1(10.0)
GA5 genotype	3(2.1)	1(11.1)	0	2(3.8)	0	0	0
Not classified	10(7.1)	0	0	2(3.8)	4(6.7)	1(11.1)	3(30.0)
Total	141	9	1	52	60	9	10
RSV-B genotype(n=31)							
BA9 genotype	29(87.9)	0	8(100)	4(80.0)	4(100)	9(75.0)	4(100)
BA10 genotype	2(6.1)	0	0	0	0	2(16.7)	0
Not classified	2(6.1)	0	0	1(20.0)	0	1(8.3)	0
Total	33	0	8	5	4	12	4

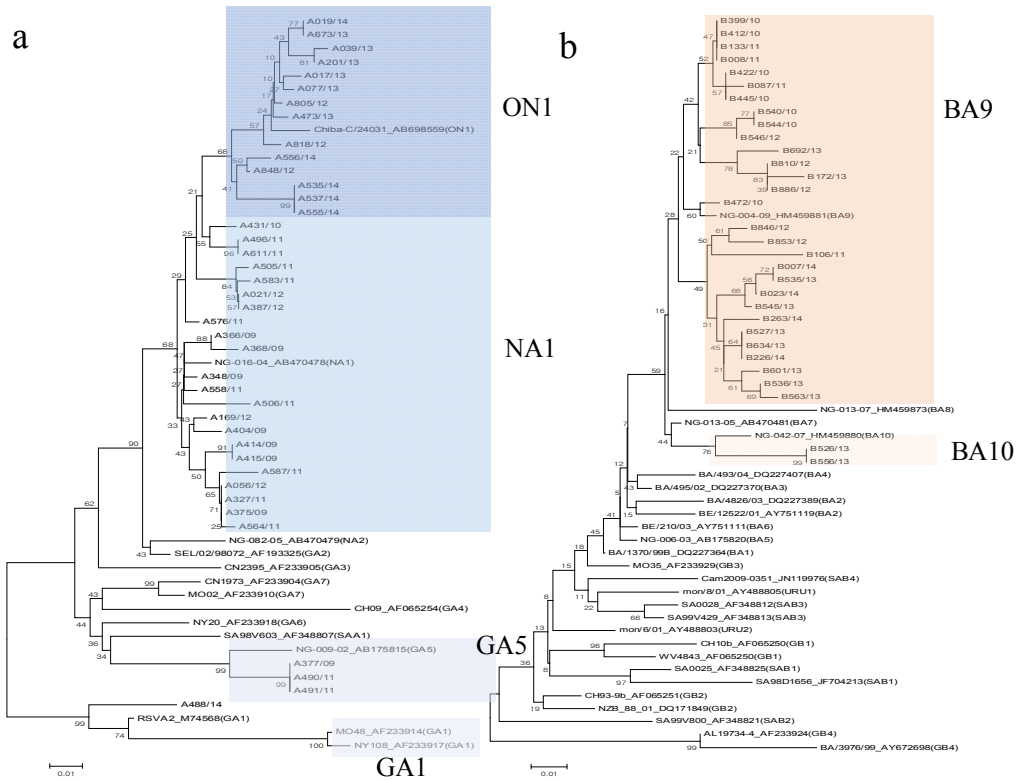


Figure 2.2. Phylogenetic analysis of the RSV-positive samples using partial G protein sequence based on nucleotide sequences of (a) RSV subgroup A (b) RSV subgroup B. (●) indicates HRSV-A genotype, and (●) indicates RSV-B genotype.

Table 2.6 Demographic and clinical characteristics of patients in the RSV, RSV subgroups(A/B), and RSV genotype groups from 2009 to 2014.

	Clinical information(n=3478)								
				Age group					
	Female/ Male	Female (%)	Median age(years)	0-2 years	3-6 years	7-19 years	20-49 years	50-64 years	Over 65 years
RSV(n=183)	1997/1967	50.4	5	980(24.3)	1412(35.1)	913(22.7)	432(10.7)	163(4.0)	83(2.1)
RSV-A(n=142)	172/227	43.1	3	120(29.9)	229(57.0)	48(11.9)	1(0.2)	2(0.5)	0
ON1 genotype(n=61)	48/58	45.3	2	60(56.6)	36(34.0)	8(7.5)	1(0.9)	1(0.9)	0
NA1 genotype(n=66)	97/95	50.5	3	80(41.2)	96(49.5)	10(5.2)	2(1.0)	3(1.5)	1(0.5)
GA1 genotype(n=1)	81/102	44.3	2	116(63.4)	58(31.7)	6(3.3)	1(0.5)	1(0.5)	1(0.5)
GA5 genotype (n=3)	54/45	54.5	4	38(33.9)	36(32.1)	20(17.9)	10(8.9)	3(2.7)	5(4.5)
RSV-B(n=34)	23/17	57.5	3	13(32.5)	22(55.0)	4(10.0)	0	0	1(2.5)
BA9 genotype (n=29)	273/289	48.6	3	211(36.8)	211(36.8)	82(14.3)	52(9.1)	10(1.7)	5(0.9)
BA10 genotype (n=2)	474/422	52.9	7	59(6.4)	59(6.4)	359(39.0)	108(11.7)	25(2.7)	15(1.6)

	Clinical information(n=3478)							
	Clinical symptoms							
	Fever	Cough	Sore throat	Chill	Headache	Muscular pain	Nasal discharge	Nasal obstruction
RSV(n=183)	159(86.9)	154(84.2)	37(20.2)	32(17.5)	17(9.3)	7(3.8)	131(71.6)	63(34.4)
RSV-A(n=142)	129(90.8))	125(88.0)	30(21.1)	24(16.9)	14(9.9)	5(3.5)	106(74.6)	46(32.4)
ON1 genotype(n=61)	60(98.4)	57(93.4)	14(23.0)	7(11.5)	7(11.5)	1(1.6)	52(85.2)	25(41.0)
NA1 genotype(n=66)	57(86.4)	56(84.8)	12(18.2)	13(19.7)	4(6.1)	2(3.0)	44(66.7)	13(19.7)
GA1 genotype(n=1)	1(100.0)	1(100.0)	0	0	1(100.0)	0	1(100.0)	1(100.0)
GA5 genotype (n=3)	1(33.3)	1(33.3)	0	0	0	0	0	0
RSV-B(n=34)	26(76.5)	26(76.5)	9(26.5)	9(26.5)	4(11.8)	2(5.9)	24(70.6)	19(55.9)
BA9 genotype (n=29)	21(72.4)	21(72.4)	7(24.1)	7(24.1)	3(10.3)	2(6.9)	20(69.0)	16(55.2)
BA10 genotype (n=2)	2(100.0)	2(100.0)	1(50.0)	0	0	0	2(100.0)	1(50.0)

	Clinical information(n=3478)								
	Clinical symptoms								
	Hoarseness	Wheezing	Laboring breath	Phlegm	Constricted chest	Vomiting	Diarrhoea	Loss of appetite	The others
RSV(n=183)	30(16.4)	40(21.9)	1(0.5)	120(65.6)	1(0.5)	16(8.7)	1(0.5)	46(25.1)	0
RSV-A(n=142)	18(12.7)	30(21.1)	0	99(69.7)	0	12(8.5)	1(0.7)	33(23.2)	0
ON1 genotype(n=61)	11(18.0)	12(19.7)	0	55(90.2)	0	8(13.1)	0	17(27.9)	0
NA1 genotype(n=66)	3(4.5)	15(22.7)	0	34(51.5)	0	3(4.5)	1(1.5)	10(15.2)	0
GA1 genotype(n=1)	0	0	0	1(100.0)	0	0	0	0	0
GA5 genotype (n=3)	0	0	0	0	0	0	0	0	0
RSV-B(n=34)	11(32.4)	10(29.4)	1(2.9)	24(70.6)	1(2.9)	4(11.8)	0	13(38.2)	0
BA9 genotype (n=29)	10(34.5)	8(27.6)	1(3.4)	19(65.5)	1(3.4)	3(10.3)	0	12(41.4)	0
BA10 genotype (n=2)	0	1(50.0)	0	2(100.0)	0	0	0	0	0

	Clinical information(n=3478)				
	Putative diagnosis			Treatment	
	Influenza like illness	Acute respiratory illness	Antibiotic used	H1N1 influenza vaccination	H1N1 & H3N2 Influenza and influenza B vaccination
RSV(n=183)	26(14.2)	103(56.3)	5(2.7)	76(41.5)	1(0.5)
RSV-A(n=142)	17(12.0)	83(58.5)	1(0.7)	63(44.4)	0
ON1 genotype(n=61)	8(13.1)	37(60.7)	0	32(52.5)	0
NA1 genotype(n=66)	7(10.6)	38(57.6)	1(1.5)	26(39.4)	0
GA1 genotype(n=1)	0	0	0	0	0
GA5 genotype (n=3)	0	0	0	0	0
RSV-B(n=34)	6(17.6)	19(55.9)	0	14(41.2)	1(2.9)
BA9 genotype (n=29)	5(17.2)	15(51.7)	0	9(31.0)	1(3.4)
BA10 genotype (n=2)	0	2(100.0)	0	2(100.0)	0

BA9 genotype (RSV-B) had labored breathing, which is generally regarded as a serious symptom (Table 2.6).

Multiple logistic regression analysis of association of clinical characteristics in patients with RSV and RSV subgroups and genotypes

We performed multiple logistic regression analysis to understand the association between clinical features and RSV by dividing into pediatric patients (below 19 years) and adult (over 20 years) as a separate group. Demographical information (age and gender) and 16 clinical symptoms were included in the statistical analysis. OR values >1 indicate a positive association. Clinical symptoms such as cough (OR = 2.8, 95% CI:1.6-5.1), wheezing (OR = 2.8, 95% CI:1.7-4.4), and vomiting (OR = 2.2, 95% CI:1.2-3.9) were significantly higher in RSV infected groups compared to non-RSV infected groups on pediatric patients ($p < 0.05$). No statistically significant ORs were observed in the RSV subgroup A and BA9 genotype.

In the ON1 genotype, only phlegm (OR = 11.8, 95% CI:3.8-46.7) was statistically significant ($p < 0.05$) and the NA1 genotype showed a significant association with gender (males, OR = 2.4, 95% CI:1.1-5.4) and chills (OR = 5.1, 95% CI:1.1-27.1). RSV subgroup B showed a significant association with nasal obstruction (OR = 4.6, 95% CI:1.2-20.0), but no other symptoms showed a significant association (Table 2.7). However, No statistically significant ORs were observed in the RSV infected groups compared to non-RSV infected groups on adult (data not shown) and there

Table 2.7 Multiple logistic regression analysis for RSV, RSV subgroups, and RSV genotypes with clinical symptoms on pediatric patients.

		Demographic information and clinical information (Odds ratio:95%CI, p-value)					
		Clinical symptoms					
		Age	Gender	Fever	Cough	Sore throat	Chill
RSV	OR	0.7(0.6-0.8)	1.1(0.8-1.5)	0.3(0.2-0.7)	**2.8(1.6-5.1)**	0.8(0.5-1.3)	1.5(0.9-2.4)
	p-value	<0.001*	0.718	<0.01*	<0.001*	0.406	0.092
RSV-A	OR	0.9(0.7-1.1)	1.8(0.7-4.9)	1.3(0.2-7.7)	4.0(0.8-18.3)	0.5(0.1-2.4)	1.0(0.3-4.6)
	p-value	0.177	0.205	0.761	0.076	0.406	0.975
ON1 genotype	OR	1.2(1.0-1.5)	0.5(0.2-1.1)	5.4(0.4-333.3)	1.5(0.3-8.6)	0.7(0.2-2.2)	0.3(0.1-1.3)
	p-value	0.059	0.109	0.297	0.596	0.506	0.114
NA1 genotype	OR	0.8(0.6-1.0)	**2.4(1.1-5.4)**	1.5(0.3-7.5)	2.4(0.6-10.8)	1.7(0.4-6.8)	**5.1(1.1-27.1)**
	p-value	<0.05*	<0.05*	0.623	0.218	0.480	<0.05*
RSV-B	OR	1.2(0.9-1.4)	0.5(0.2-1.4)	0.8(0.1-5.0)	0.3(0.1-1.2)	1.8(0.4-7.7)	1.0(0.2-4.0)
	p-value	0.177	0.205	0.761	0.076	0.406	0.975
BA9 genotype	OR	1.1(0.9-1.4)	0.7(0.3-1.8)	0.8(0.1-5.2)	0.3(0.1-1.3)	1.3(0.3-6.0)	1.2(0.3-5.0)
	p-value	0.232	0.453	0.761	0.083	0.701	0.802

		Demographic information and clinical information (Odds ratio:95%CI, p-value)					
		Clinical symptoms					
		Headache	Muscular pain	Nasal discharge	Nasal obstruction	Hoarseness	Wheezing
RSV	OR	1.1(0.6-2.0)	0.4(0.1-0.8)	1.3(0.9-2.1)	0.5(0.3-0.8)	1.0(0.6-1.6)	**2.8(1.7-4.4)**
	p-value	0.739	<0.05*	0.219	<0.01*	0.923	<0.001*
RSV-A	OR	8.2(0.9-129)	0.2(0.0-2.6)	1.4(0.4-4.7)	0.2(0.0-0.8)	0.4(0.1-1.6)	0.3(0.1-1.0)
	p-value	0.091	0.206	0.558	<0.05*	0.197	<0.05*
ON1 genotype	OR	3.1(0.6-20.5)	0.3(0.0-3.9)	2.2(0.8-6.6)	0.8(0.3-2.6)	1.5(0.5-5.0)	0.4(0.2-1.1)
	p-value	0.208	0.424	0.129	0.771	0.460	0.069
NA1 genotype	OR	0.4(0.0-2.2)	1.0(0.1-10.7)	1.0(0.4-2.5)	0.2(0.1-0.8)	0.3(0.1-1.1)	1.8(0.7-4.9)
	p-value	0.301	0.971	0.917	<0.05*	0.081	0.250
RSV-B	OR	0.1(0.0-1.1)	5.1(0.4-70.4)	0.7(0.2-2.3)	**4.6(1.2-20.0)**	2.3(0.6-8.2)	3.5(1.0-12.7)
	p-value	0.091	0.206	0.558	**<0.05***	0.197	0.083
BA9 genotype	OR	0.2(0.0-1.8)	4.5(0.3-64.1)	0.6(0.2-2.0)	4.0(1.0-18.1)	2.5(0.7-9.0)	3.2(0.9-12.2)
	p-value	0.175	0.254	0.396	0.057	0.160	0.073

		Demographic information and clinical information (Odds ratio:95%CI, p-value)					
		Clinical symptoms					
		Laboring breath	Phlegm	Constricted chest	Vomiting	Diarrhoea	Loss of appetite
RSV	OR	2.0(0.1-16.5)	1.3(0.8-2.0)	1.0(0.0-10.9)	**2.2(1.2-3.9)**	0.3(0.0-1.8)	0.7(0.4-1.0)
	p-value	0.567	0.299	0.999	<0.01*	0.282	0.067
RSV-A	OR	0.0 (ND***)	1.6(0.4-6.2)	ND***	0.8(0.2-4.9)	ND**	0.8(0.2-3.1)
	p-value	0.991	0.522	ND***	0.807	0.993	0.766
ON1 genotype	OR	0.0(ND***)	**11.8(3.8-46.7)**	ND***	2.2(0.6-9.2)	0.0(ND***)	0.9(0.3-2.6)
	p-value	0.992	<0.001*	ND***	0.253	0.993	0.900
NA1 genotype	OR	0.0(ND***)	0.1(0.0-0.3)	ND***	0.3(0.1-1.4)	ND**(ND***)	1.0(0.3-3.3)
	p-value	0.993	<0.001*	ND***	0.154	0.994	0.970
RSV-B	OR	ND**(ND***)	0.6(0.2-2.7)	ND***	1.2(0.2-5.8)	0.0(ND***)	1.2(0.3-4.5)
	p-value	0.991	0.522	ND***	0.807	0.993	0.766
BA9 genotype	OR	ND**(ND***)	0.6(0.1-2.5)	ND***	1.5(0.2-7.1)	0.0(ND***)	1.8(0.5-6.9)
	p-value	0.994	0.419	ND***	0.657	0.996	0.399

* Statistically significant

** ND [not determined]: one data point.

*** ND [not determined]: missing data.

are only 3 data on adult in RSV subgroups and genotypes that is too little to analyze.

Coinfection with RSV and other respiratory viruses

We investigated simultaneous infections with RSV and other respiratory viruses using a multiplex-PCR assay. Among all tested respiratory viruses, hRV (n = 25, 47.2%) and ADV (n = 12, 22.6%) were detected most frequently with RSV. Most coinfections were detected from January 2011 to December 2012 (n = 45, 84.9%) proportionate with RSV-positive cases. Among all RSV- positive cases (n = 183), RSV single-infection was observed with the most frequency (n = 137, 74.5%), followed by mono-coinfections (n = 39, 21.2%) and dual-coinfections (n = 7, 3.8%) with RSV. Due to the number of cases in our study, only hRV coinfection with RSV (n = 21) could be evaluated with RSV single-infection (n = 138) in demographic and clinical comparisons between patients with RSV single- and coinfections. In RSV single-infections, fever (n = 117, 85.4%), cough (n = 115, 83.9%), nasal discharge (n = 100, 73.0%), and phlegm (n = 88, 64.2%) were the major clinical symptoms. In cases with hRV and RSV coinfection, fever (n = 20, 95.2%), cough (n = 18, 85.7%), nasal discharge (n = 17, 81.0%), and phlegm (n = 17, 81.0%) were the major clinical symptoms (Table 2.8). We performed a two-tailed Chi-squared test to test the statistical difference between two independent groups (Yoshihara, Le et al. 2016). No significant association was detected between single (n = 137)

and overall coinfection (n = 46) or between single (n = 137) and RSV and hRV coinfection (n = 21) ($p > 0.05$) (data not shown).

Table 2.8 Demographic and clinical characteristics of patients with RSV coinfection.

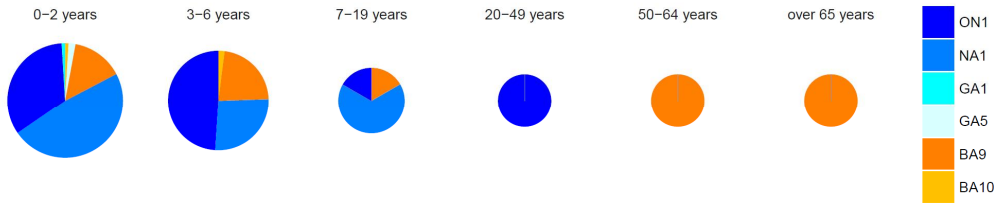
	Clinical information(n=3478)								
				Age group					
	Female/ Male	Female (%)	Median age(years)	0-2 years	3-6 years	7-19 years	20-49 years	50-64 years	Over 65 years
RSV single infection(n=137)	60/77	43.8	3.5	85(62.0)	44(32.1)	5(3.6)	1(0.7)	1(0.7)	1(0.7)
RSV+ ADV(n=7)	5/2	71.4	1.7	6(85.7)	1(14.3)	0	0	0	0
RSV+PIV1(n=3)	2/1	66.7	2.3	2(66.7)	1(33.3)	0	0	0	0
RSV+hRV(n=21)	11/10	52.4	2.0	15(71.4)	6(28.6)	0	0	0	0
RSV+IFV(n=2)	0/2	0	6.5	0	1(50.0)	1(50.0)	0	0	0
RSV+HCoV(n=1)	0/1	0	4.0	0	1(100.0)	0	0	0	0
RSV+hBoV(n=5)	1/4	20.0	1.8	4(80.0)	1(20.0)	0	0	0	0
RSV+ ADV+hBoV(n=2)	1/1	50.0	1.5	2(100.0)	0	0	0	0	0
RSV+ ADV+HCoV(n=1)	0/1	0	4.0	0	1(100.0)	0	0	0	0
RSV+ ADV+hRV(n=2)	0/2	0	1.5	2(100.0)	0	0	0	0	0
RSV+ HCoV+hRV(n=1)	-	-	3.0	0	1(100.0)	0	0	0	0
RSV+ hMPV+hRV(n=1)	1/0	100.0	3.0	0	1(100.0)	0	0	0	0

	Clinical information(n=3478)							
	Clinical symptoms							
	Fever	Cough	Sore throat	Chill	Headache	Muscular pain	Nasal discharge	Nasal obstruction
RSV single infection(n=137)	117(85.4)	115(83.9)	30(21.9)	23(16.8)	14(10.2)	7(5.1)	100(73.0)	46(33.6)
RSV+ ADV(n=7)	6(85.7)	6(85.7)	1(14.3)	2(28.6)	0	0	4(57.1)	2(28.6)
RSV+PIV1(n=3)	3(100.0)	3(100.0)	1(33.3)	0	0	0	2(66.7)	1(33.3)
RSV+hRV(n=21)	20(95.2)	18(85.7)	3(14.3)	2(9.5)	1(4.8)	0	17(81.0)	6(28.6)
RSV+IFV(n=2)	2((100.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0	1(50.0)	1(50.0)
RSV+HCoV(n=1)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0	1(100.0)	1(100.0)
RSV+hBoV(n=5)	3(60.0)	3(60.0)	0	1(20.0)	0	0	3(60.0)	2(40.0)
RSV+ ADV+hBoV(n=2)	2(100.0)	2(100.0)	0	1(50.0)	0	0	0	1(50.0)
RSV+ ADV+HCoV(n=1)	1(100.0)	1(100.0)	0	0	0	0	1(100.0)	0
RSV+ ADV+hRV(n=2)	2(100.0)	2(100.0)	0	0	0	0	1(50.0)	1(50.0)
RSV+ HCoV+hRV(n=1)	1(100.0)	1(100.0)	0	0	0	0	0	1(100.0)
RSV+ hMPV+hRV(n=1)	1(100.0)	1(100.0)	0	0	0	0	1(100.0)	1(100.0)

	Clinical information(n=3478)							
	Clinical symptoms							
	Hoarseness	Wheezing	Laborin g breath	Phlegm	Constricted chest	Vomiting	Diarrhoea	Loss of appetite
RSV single infection(n=137)	21(15.3)	29(21.2)	1(0.7)	88(64.2)	1(0.7)	11(8.0)	1(0.7)	35(25.5)
RSV+ ADV(n=7)	1(14.3)	0	0	4(57.1)	0	0	0	4(57.1)
RSV+PIV1(n=3)	0	1(33.3)	0	2(66.7)	0	0	0	1(33.3)
RSV+hRV(n=21)	4(19.0)	6(28.6)	0	17(81.0)	0	4(9.0)	0	3(14.3)
RSV+IFV(n=2)	0	0	0	1(50.0)	0	0	0	0

RSV+HCoV(n=1)	0	1(100.0)	0	0	0	0	0	0
RSV+hBoV(n=5)	1(20.0)	1(20.0)	0	3(60.0)	0	0	0	1(20.0)
RSV+ ADV+hBoV(n=2)	2(100.0)	2(100.0)	0	2(100.0)	0	0	0	0
RSV+ ADV+HCoV(n=1)	0	0	0	1(100.0)	0	0	0	0
RSV+ ADV+hRV(n=2)	0	0	0	2(100.0)	0	0	0	1(50.0)
RSV+ HCoV+hRV(n=1)	0	0	0	1(100.0)	0	0	0	0
RSV+ hMPV+hRV(n=1)	1(100.0)	0	0	1(100.0)	0	0	0	1(100.0)

a



b

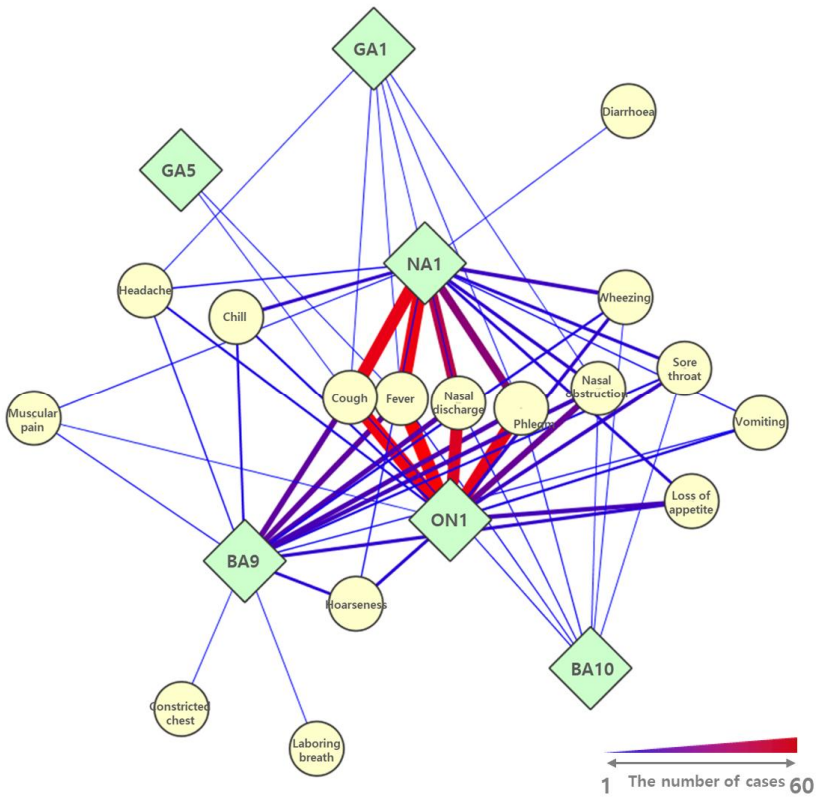


Figure 2.3. Demographic and clinical characteristics of patients with RSV genotypes. (a) RSV genotype incidence rates stratified by patient age. (b) Associations between RSV genotypes and clinical symptoms in patients. Thicker red lines indicate stronger relationships.

Discussion

In this study, we investigated the distributions of all respiratory viruses, the RSV subgroups, and the coinfections of RSV with other respiratory viruses using real-time one-step RT-PCR. The RSV genotypes were determined by sequencing the second hypervariable region of the G protein, and the results were correlated with the demographic and clinical characteristics from consented clinical information from patients.

RSV evolved and changed continuously after an RSV presumed outbreak reported in 1941 (Adams 1941). For six consecutive years in Gyeonggi Province located in South Korea, RSV-A and B cocirculated; RSV-A predominated during most seasons, but RSV-B predominated in 2010 and 2013 (Table 2.5). This tendency was consistent with the results from previous studies in northern Italy (Esposito, Piralla et al. 2015) and Japan (Dapat, Shobugawa et al. 2010), but differed somewhat from the results from other countries in East Asia (Cui, Zhu et al. 2013, Cui, Zhu et al. 2015, Yu, Kou et al. 2015). The difference appears to be due to various geographic regions and periods. Consistent with previous reports, higher proportions of infected patients were male (Cui, Zhu et al. 2013, Esposito, Piralla et al. 2015, Yu, Kou et al. 2015), and most RSV infections were detected in patients <2 years of age (Gimferrer, Campins et al. 2015). In terms of seasonality, RSV epidemics commonly span the months from October to March, peaking between November and January (Thorburn 2016), and our study showed an analogous pattern. However, different seasonal patterns

were noted in tropical regions such as Vietnam, with peaks in the hot and dry season (July through September), suggesting a role of climate parameters such as temperature and relative humidity (Yoshihara, Le et al. 2016).

After the first discovery of the ON1 genotype in Ontario, Canada in 2010 (Eshaghi, Duvvuri et al. 2012), the ON1 genotype was reported in many other countries including Japan (Tsukagoshi, Yokoi et al. 2013), China (Cui, Qian et al. 2013) , Germany (Prifert, Streng et al. 2013), Italy (Pierangeli, Trotta et al. 2014), India (Choudhary, Anand et al. 2013), and South Africa (Valley-Omar, Muloiwa et al. 2013). In our study, the ON1 genotype emerged in October 2011. In South Korea, the first ON1 genotype was discovered in August 2011 (Lee, Kim et al. 2012), which was the next season after Eshagi et al. first discovered the novel ON1 genotype in Canada (Eshaghi, Duvvuri et al. 2012, Kim, Kim et al. 2014). Other previous studies in South Korea (Lee, Kim et al. 2012, Kim, Kim et al. 2014) used clinical samples collected from the Korean Influenza and Respiratory Viruses Surveillance System, which was incorporated nationwide with the Institute of Health and Environment. Therefore, the first ON1 genotype discovered in August 2011 may be from clinical specimens collected from another Province in South Korea. From 2009 to 2010, most RSV subgroup A infections were clustered in the NA1 genotype; from 2011 to 2012, the NA1 genotype and ON1 genotypes coexisted but demonstrated opposite predominance: 2011 showed genotype distributions of 92% NA1 and 6%

ON1, whereas 2012 showed distributions of 21% NA1 and 79% ON1. Furthermore, no cases of NA1 genotype (subgroup A) were detected from January 2013 to November 2014. Based on the pattern of disease epidemics, we surmise that the NA1 genotype (ancestor of ON1) was replaced with the ON1 genotype during 2011 to 2012. Such substitutions were observed in studies conducted in other countries (Esposito, Piralla et al. 2015, Yoshihara, Le et al. 2016).

The results of many studies (Eshaghi, Duvvuri et al. 2012, Fall, Dia et al. 2016) demonstrate that the main characteristic of the ON1 genotype is the presence of a 72-nucleotide duplication insertion in the G protein, and this characteristic may have a positive evolutionary effect on RSV. For example, genetic variations may play a crucial biological role for enhancing the efficiency of viral attachment to the cell receptors or for faster viral replication during pathogenesis (Yoshihara, Le et al. 2016), which may elicit strong resistance to herd immunity (Cui, Zhu et al. 2015). The BA genotype of RSV-B gained a 60-nucleotide duplication insertion in the G protein, a change that occurred since the BA genotype was first isolated in 1999 (Buenos Aires, Argentina) (Trento, Galiano et al. 2003). The GA1 genotype was newly isolated in September 2014, but reports showed that it was isolated previously in 2009. In addition, isolation of the GA1 genotype was reported in Senegal, Africa in 2015 (Fall, Dia et al. 2016) (Table 2.5).

According to the recent RSV study which was conducted by Lee *et al* (Lee, Choi et al, 2018) from June 2015 to May 2018 in Gyeonggi Province,

among 2331 samples, 88 (3.8%) had positive results for RSV and 36 (39.2%) were RSV-A and 52 (60.8%) were RSV-B. In contrast to our study, RSV-B was predominant subgroup and of these samples, RSV subgroup A (n=18) and RSV subgroup B (n=36) samples were successfully sequenced. All RSV-A samples clustered in the genotypes of ON1 (n=18, 100%) and all RSV-B samples clustered in the genotypes of BA9 (n=36, 100%). From these results, we could find that NA1 genotype of RSV-A was gradually replaced to ON1 genotype at the point of the ON1 genotype of RSV newly emerged and was first isolated in December 2011 and completely displaced in 2013 as our study suggested. For the RSV-B genotype, BA9 was predominantly observed continuously from in our study's period.

Demographic and clinical information is important to complement the limitations of advanced molecular technology, as it can take several days to identify a respiratory virus genotype in an infected patient. Therefore, the relevance of clinical information and RSV-positive infections has been investigated continuously through various studies (Pierangeli, Trotta et al. 2014, Esposito, Piralla et al. 2015, Yu, Kou et al. 2015, Fall, Dia et al. 2016, Yoshihara, Le et al. 2016). We performed two analyses with respect to the clinical relevance of RSV-positive infections. We first performed a network analysis using simple frequencies, and then conducted a multiple logistic regression analysis. In RSV-infected patients, clinical symptoms such as fever, cough, nasal discharge, and phlegm were major signs. Serious symptoms such as labored breathing (n = 1) and constricted chest (n = 1)

were reported in patients with the BA9 genotype in RSV-B, however, the low frequency prevented a robust analysis (Table 2.6, Figure 2.3b). On the other hand, multiple logistic regression analysis on paediatric patients showed that RSV-positive infections were associated with a 2.8-fold increased risk of cough and wheezing. Furthermore, the ON1 genotype was associated with a 11.8-fold increased risk of phlegm, and the NA1 genotype was associated with a 5.1-fold increased risk of chills and RSV subgroup B was associated with a 4.6-fold increased risk of nasal obstruction (Table 2.7).

However, these results had limitations because the demographic and clinical information was not fully recorded in some patients and our study was performed through local hospital in Gyeonggi Province and these hospitals were all primary hospital. Therefore, most of clinical symptoms were mild and so few patients had serious symptoms. Further studies will be necessary to overcome these limitation and understand the exact associations of clinical symptoms and respiratory virus genotypes.

With respect to respiratory infection, RSV is known to infect both upper respiratory tract infection and lower respiratory tract infection (Viegas, Barrero et al. 2004, Nair, Nokes et al. 2010). In this study, respiratory specimen such as throat swabs or nasal aspirate specimens are used because the KINRESS was supported by local hospitals which is primary hospital and these are representative upper respiratory tract (URT) samples and are used commonly because these are easy to take and acceptable to the patients (Heikkinen, Marttila et al. 2002, Gruteke, Glas et al. 2004, Abu-Diab,

Azzeh et al. 2008, Chan, Peiris et al. 2008, Lambert, Whiley et al. 2008, Sung, Chan et al. 2008, de la Tabla, Masiá et al. 2010, Meerhoff, Houben et al. 2010, DeByle, Bulkow et al. 2012). On the other hands, lower respiratory tract samples such as sputum and bronchoalveolar lavage (BAL) are useful for dectection of LRT infection but difficult to collect and need to require pre-treatment (Falsey, Formica et al. 2012, Branche, Walsh et al. 2014, Jeong, Kim et al. 2014). Even though these samples were collected from upper respiratory tract, we couldn't regard it as not infected on lower respiratory tract and actually wheezing which is one of the lower respiratory tract's clinical symptoms were founded in 40 (21.9%) outpatients and also laboring breath (n=1, 0.5%) and constricted chest (n=1, 0.5%) among 183 RSV infected patients (Table 2.3). Furthermore, when we compared it at the point of time trend, there won't any evidences to prove changes the decrease or increase of lower respiratory tract infection in the process of replacing NA1 genotype with ON1 genotype, because wheezing was found similar portion both NA1 (n=15, 22.7%) and ON1 genotype (n=12, 19.7%). This result wasn't consistent with Esposito *et al* (Esposito, Piralla et al. 2015) and Prifert *et al* (Pierangeli, Trotta et al. 2014) reports which describes ON1 genotype was significantly less virulent than NA1 genotype, as shown by the lower incidence of LRTIs and less frequent hospitalization of ON1 genotype outpatients and a considerable number of children with ON1 genotype infection were admitted to an intensive care unit (Table 2.6).

Even though our study mainly focused on patients who dropped by primary hospitals, various studies were performed to investigate RSV infection in community and hospital (Pilie, Werbel et al. 2015, Tuan, Thanh et al. 2015, Oladokun, Muloiwa et al. 2016, Yu, Xie et al. 2018, Pham, Thompson et al. 2019, Yu, Liu et al. 2019). Especially nosocomial RSV infection occurred below 10 percent during hospitalization and increased when younger and had a more duration of hospitalization (Tuan, Thanh et al. 2015, Oladokun, Muloiwa et al. 2016).

Many studies have reported contradictory results about coinfection and clinical severity between RSV subgroup and genotype (Tran, Pham et al. 2013, Calvo, García-García et al. 2015, Martinez-Roig, Salvado et al. 2015, Yu, Kou et al. 2015, Cebey-López, Herberg et al. 2016, Míguez, Iftimi et al. 2016, Pinky and Dobrovolny 2016, Skjerven, Megremis et al. 2016). hRV was the most commonly detected virus (47.2%) with RSV among all respiratory viruses in Gyeoggi Province (Table 2.8). These results were consistent with other studies in China, Spain, and Norway (Calvo, García-García et al. 2015, Martinez-Roig, Salvado et al. 2015, Yu, Kou et al. 2015, Skjerven, Megremis et al. 2016). No significant associations were detected between coinfection and the severity of clinical symptoms between RSV single-infection and overall coinfection, and between RSV single-infection and hRV and RSV coinfection (data not shown); these results were consistent with those reported in studies conducted in Vietnam (Tran, Pham et al. 2013), United Kingdom (Cebey-López, Herberg et al. 2016), and

Spain (Calvo, García-García et al. 2015, Martinez-Roig, Salvado et al. 2015). However, other studies have shown that coinfections with RSV and respiratory viruses such as hRV, hMPV, and PIV type 3 increase clinical severity (Míguez, Iftimi et al. 2016). Recently, experimental studies were performed to determine the mechanism underlying coinfection and clinical severity. According to Pinky et al. (Pinky and Dobrovolny 2016), one virus can block another during coinfection by being the first to infect the available host cells. hRV, which is a fast-growing virus, reduces the replication of the remaining viruses. The slowest growing virus is suppressed in the presence of another virus, and the effect of viral coinfections on clinical outcomes is no more severe than a single-virus infection, and can even result in a less severe clinical impact. Skjerven et al. (Skjerven, Megremis et al. 2016) reported that viral genomic load shows a positive correlation with disease severity. In other words, clinical severity depends on the viral genomic load rather than coinfection. However, other studies reported no correlation between viral load and clinical symptoms (Franz, Adams et al. 2010, Lee, Chan et al. 2015). Further study is warranted to investigate this clinical aspect in future.

We investigated the molecular epidemiological characteristics of RSV in patients with viral respiratory infections. The association between clinical symptoms in patients and the molecular epidemiology of RSV infections will be useful for developing treatment therapies and vaccines against RSV.

CHAPTER III.

EPIDEMIOLOGY OF INFLUENZA VIRUS IN SOUTH KOREA, 2009–2014

Introduction

IFV is a major respiratory pathogen that causes annual epidemics (Xu, Chan et al. 2015), imposing a considerable social and economic burden (Sobolev, Kurskaya et al. 2012). IFV A(H1N1), IFV A(H3N2), and IFV B are the most common variants circulating in humans globally (Xu, Chan et al. 2015). In 2009, a novel pandemic strain, IFV A(H1N1)pdm09, became the dominant IFV circulating worldwide (Team 2009). That is, after the pandemic, IFV A(H1N1)pdm09 replaced the pre-existing seasonal IFV A H1N1 (Zhou, Yang et al. 2019).

IFVs are members of the family *Orthomyxoviridae* and are divided into A, B, and C types. IFV A has a wide host range, being able to infect birds, animals, and humans, and is divided into subtypes on the basis of their hemagglutinin (HA) and neuraminidase (NA) (Webster, Bean et al. 1992). Occasional antigenic shifts in IFV A result in significant human disease, leading to influenza pandemics. From 3 to 5 million cases of severe influenza disease occur annually (Nicholson Karl and Wood John 2003).

Influenza is a globally important contagion. About 20% of children and 5% of adults worldwide develop symptomatic influenza A or B annually (Turner, Wailoo et al. 2003). IFV causes a broad range of illness, from symptomless infection to various respiratory syndromes; disorders affecting the lung, heart, brain, liver, kidneys, and muscles to fulminant primary viral and secondary bacterial pneumonia. The course is affected by the patient's age, the degree of pre-existing immunity, properties of the virus, smoking, comorbidities, immunosuppression, and pregnancy (Nicholson Karl and Wood John 2003).

Influenza is a common seasonal respiratory infection, the severity of which

ranges from asymptomatic/subclinical infection and illness not requiring medical care to hospitalization and death. In addition to seasonal epidemics, which can vary widely in severity, IFVs also cause pandemics, such as the 2009 H1N1 pandemic (Garten, Davis et al. 2009), and limited animal-to-human outbreaks, such as IFV A(H3N2) variant infections at agricultural fairs (Jhung, Epperson et al. 2013). In each of these situations, it may be critical to characterize the severity and potential impact of the situation rapidly to inform preparedness and response efforts (Holloway, Rasmussen et al. 2014).

In April 2009, the Centers for Disease Control and Prevention (CDC) identified in Meco two cases of infection with a swine-origin IFV (H1N1), with a novel genetic composition that rapidly spread globally, causing the first influenza pandemic of the 21st century (Control and Prevention 2010). The World Health Organization (WHO) declared in August 2010 the end of the most recent pandemic and the beginning of the post-pandemic phase. During this period, IFV A(H1N1)pdm09 came to resemble a seasonal IFV; the intensity decreased to the level of seasonal influenza, the pattern of spread changed, out-of-season outbreaks were no longer detected, and circulation of mixed IFVs was reported in many countries (Organization 2010). Severe cases were less frequent than during the pandemic, while the at-risk groups were unchanged (young children; pregnant women; and individuals with respiratory or chronic conditions, including asthma, diabetes, and obesity) (Necula, Popovici et al. 2019). The epidemiological characteristics of A(H1N1)pdm09 during the post-pandemic period are unclear (Zhou, Yang et al. 2019).

The clinical and epidemiologic characteristics of IFVs in South Korea are unclear (Cohen, Hellferscee et al. 2014), and the epidemiological characteristics of IFV A(H1N1)pdm09 during the post-pandemic period warrant investigation (Zhou, Yang et al. 2019). We evaluated the demographic and clinical characteristics, associated factors, and disease severity of IFV A(H1N1)pdm09, A(H3N2), and IFV B in South Korea from 2009–2014 and the characteristics of the first wave in 2009 and the subsequent wave in 2010.

Materials and Methods

Setting and time. KINRESS is an active, prospective, and local hospital-based surveillance system that monitors outpatients with ILI in South Korea. In May 2009, KINRESS was implemented by the Korea National Institute of Health (KNIH) and the Institute of Health and Environment in 48 hospitals, in 13 provinces of South Korea. At the same time, the Korea Influenza Surveillance Scheme (KISS), which started in January 2000, was terminated. The surveillance, which encompasses testing for 16 respiratory viruses, including IFV, was conducted with the written informed consent of the patients, their parents, or their legal guardians and was approved by the Institutional Review Board of the Korea National Institute of Health. This study was conducted in Gyeonggi Province during 2009–2014 as part of KINRESS (Cohen, Hellferscee et al. 2014).

Case definition and sample collection. The WHO case definition of ILI was acute RTI with fever of ≥ 38°C and cough, and onset within the last 10 days (Organization 2014). All patients with ILI or non-IFV acute respiratory infection were enrolled. A total of 4028 respiratory specimens were obtained from hospitals in Gyeonggi Province, South Korea, from 2009 to 2014. We collected information on the age, gender, date of onset, clinical symptoms, putative diagnosis, pre-existing conditions, vaccinations, and antibiotic treatment of the patients (Cohen, Hellferscee et al. 2014).

Laboratory methods. Respiratory specimens were placed in viral transport medium (VTM), maintained at 4–8°C, and sent to the GIHE within 72 hours of collection. The respiratory specimens were tested by multiplex real-time

RT-PCR for 16 respiratory viruses (IFV A and B; ADV; human bocavirus; parainfluenza viruses 1, 2 and 3; RSV A and B; human metapneumovirus; HCoV 229E, OC43 and NL63; and human rhinovirus). Confirmed IFV A-positive samples were subtyped into A(H1N1)pdm09, A(H3N2), or A(H5N1).

Statistical analysis. We conducted univariate analyses and a multiple logistic regression analysis of the demographic and clinical characteristics, associated factors, and disease severity of patients infected with IFV and compared those infected during the first and subsequent waves of IFV A(H1N1)pdm09 (Cohen, Hellferscee et al. 2014). Statistical analyses were performed using R 3.5.0 and p-values < 0.05 were considered to indicate statistical significance.

Results

IFV types and subtypes.

From January 2009 to November 2014, 4028 outpatients attended local hospitals with respiratory symptoms and were enrolled in KINRESS. Among them, 920 (22.8%) were positive for IFV. Of the IFV-positive cases, 305 (33.1%) were IFV A(H3N2), 271 (29.5%) were IFV A(H1N1)pdm09, and 343 (37.3%) were IFV B; one (0.1%) IFV A could not be subtyped. IFV epidemics occur annually from November to March in Gyeonggi Province; the incidence of IFV infection is negligible during the rest of the year (Figure 3.1). During 2009, IFV A(H1N1)pdm09 (89/271, 32.8%) predominated and A(H3N2) was detected at a lower frequency (1/305, 0.3%); IFV B was not detected. IFV A(H1N1)pdm09 (113/271, 41.7%) predominated in 2010, A(H1N1)pdm09 (30/271, 11.1%) in 2011, A(H3N2) (164/305, 53.8%) and B (167/343, 48.7%) in 2012, A(H3N2) (59/305, 19.3%) in 2013, and A(H3N2) (49/305, 16.1%) and B (70/343, 20.4%) in 2014 (Table 1). From 2009 to 2011, with the exception of one unsubtyped IFV A(H1N1), which was reported on 18 May 2009, we regarded A(H1N1) as A(H1N1)pdm09 because A(H1N1) was completely replaced by A(H1N1)pdm09 from July 2009 (KCDC, 2010) and the KINRESS program began using a detection kit for A(H1N1)pdm09 in 2012.

Most of the IFV-infected patients were 5–24 years old (551/920, 59.9%), followed by < 5 years old (220/920, 23.9%) and > 65 years old (14/920, 1.5%). Co-infection with other viruses was detected for IFV A(H3N2) (21/305, 6.9%), A(H1N1)pdm09 (14/271, 5.2%), and IFV B (36/343, 10.5%). Asthma was associated with IFV A(H1N1)pdm09 (5/227, 2.2%) and IFV B (3/336, 0.9%) and hypertension with IFV A(H3N2) (5/283,

1.8%), A(H1N1)pdm09 (1/227, 0.4%), and IFV B (2/336, 0.6%). Fever was associated with IFV A(H3N2) (268/283, 94.7%), A(H1N1)pdm09 (143/227, 63.0%), and IFV B (270/336, 80.4%) and cough with IFV A(H3N2) (230/283, 81.3%), A(H1N1)pdm09 (120/227, 52.9%), and IFV B (216/336, 64.3%) (Table 3.1).

In univariate analyses using A(H3N2) as a reference, 5–24 years old and the years 2009 and 2010 had RRs of > 1.0. Patients infected with IFV A(H1N1)pdm09 (RR 1.23, 95% CI 1.06–1.42) and IFV B (RR 1.28, 95% CI 1.12–1.47) were more likely to be 5–24 years old than those infected with IFV A(H3N2). In 2009, IFV A(H1N1)pdm09 (RR 100.17, 95% CI 14.05–714.06) was more prevalent than IFV A(H3N2); in 2010, IFV A(H1N1)pdm09 (RR 25.44, 95% CI 10.54–61.36) and IFV B (RR 17.61, 95% CI 7.27–42.66) were more prevalent than IFV A(H3N2). There were no significant differences among the IFV subtypes (Table 3.1).

Disease severity in the first (2009) and second (2010) waves of A(H1N1)pdm09.

We compared the first and second waves of IFV A(H1N1)pdm09 in 2009 and 2010, respectively (Table 3.2, Figure 3.1). In univariate analyses, age < 5 years, cough, nasal obstruction, and phlegm were more strongly associated with the second than the first wave. Age < 5 years (OR 3.07, 95% CI 1.37–6.89) was more strongly associated with the first than the second wave. Cough (OR 3.28, 95% CI 1.76–6.10), nasal obstruction (OR 6.24, 95% CI 2.04–19.05) and phlegm (OR 7.09, 95% CI 1.55–32.44) were more strongly associated with the second than the first wave (Table 3.2).

Disease severity of A(H1N1)pdm09, H3N2 and B in second wave, post-pandemic wave (2010).

We compared IFV A(H1N1)pdm09 and B in second waves, post-pandemic wave (2010), respectively (Table 3.3) and we couldn't compare with H3N2 because there are only 5 data in H3N2 that is too little to analyaze. In univariate analyses, fever, cough, nasal discharge, nasal obstruction, and phlegm were more strongly associated with A(H1N1)pdm09 than B. Fever (OR 2.09, 95% CI 1.16–3.75), cough (OR 3.45, 95% CI 1.89–6.29), nasal discharge (OR 2.05, 95% CI 1.07–3.91), nasal obstruction (OR 5.72, 95% CI 2.06–15.92) and phlegm (OR 5.40, 95% CI 1.49–19.64) were more strongly associated with A(H1N1)pdm09 than B (Table 3.3).

Associations of clinical characteristics with influenza subtypes.

We performed a multiple logistic regression analysis of the associations of clinical features with IFV. Demographic information (age and gender) and 16 clinical symptoms were included in the analysis. An OR of > 1 indicates a positive association. The frequency of cough (OR=1.4, 95% CI 1.2–1.8), chill (OR=1.4, 95% CI 1.2–1.8), headache (OR=1.9, 95% CI 1.5–2.3), and muscular pain (OR=1.6, 95% CI 1.3–2.0) was significantly higher in the IFV-infected than the non-IFV-infected groups ($p < 0.05$). No significant ORs were detected for IFV A(H1N1)pdm09. Fever (OR=3.7, 95% CI 2.0–7.2), nasal discharge (OR=2.5, 95% CI 1.7–3.7), and phlegm (OR=1.7, 95% CI 1.2–2.4) were significantly associated with IFV A(H3N2). IFV B was associated with hoarseness (OR=2.1, 95% CI 1.2–3.5) and loss of appetite (OR=1.6, 95% CI 1.0–2.5) (Table 3.3).

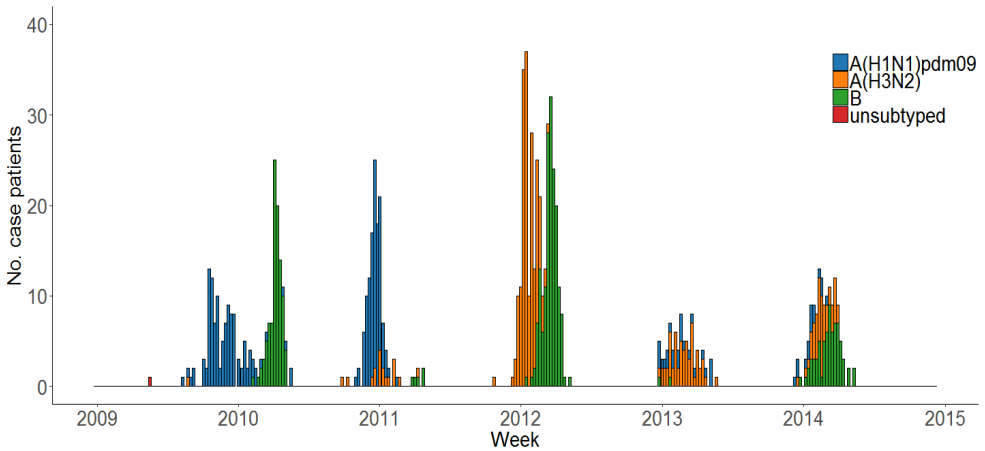


Figure 3.1 Overall trends and number of case patients with influenza-associated acute respiratory illness by week in South Korea between 2009 and 2014.

Table 3.1. Characteristics of outpatients with IFV-associated acute respiratory illness according to IFV type and subtype, Gyeonggi Province, South Korea, 2009–2014.

	Influenza type and subtype				
	A(H3N2)(reference)	A(H1N1), A(H1N1)pdm09		B	
Characteristic	No. pos/no. all pos(% pos)	No. pos/no. all pos (% pos)	Relative Risk (95% CI)	No. pos/no. all pos (% pos)	Relative Risk (95% CI)
Age group, y					
<5	86/305 (28.2)	55/271 (20.3)	0.72 (0.54-0.94)†	79/343 (23.0)	0.82(0.63-1.06)
5-24	156/305 (51.1)	170/271 (62.7)	**1.23 (1.06-1.42)**†	225/343 (65.6)	**1.28 (1.12-1.47)**†
25-44	34/305 (11.1)	32/271 (11.8)	1.06 (0.67-1.67)	13/343 (3.8)	0.34 (0.18-0.63)†
45-64	18/305 (5.9)	7/271 (2.6)	0.44 (0.19-1.03)	13/343 (3.8)	0.64 (0.32-1.29)
≥65	7/305 (2.3)	3/271 (1.1)	0.48 (0.13-1.85)	4/343 (1.2)	0.51 (0.15-1.72)
Male	144/305 (47.2)	118/271 (43.5)	0.92 (0.77-1.10)	160/343 (46.6)	0.99 (0.84-1.16)
Year					
2009	1/305 (0.3)	89/271 (32.8)	**100.17 (14.05-714.06)**†	0	Not calculated
2010	5/305 (1.6)	113/271 (41.7)	**25.44 (10.54-61.36)**†	99/343 (28.9)	**17.61 (7.27-42.66)**†
2011	27/305 (8.9)	30/271 (11.1)	1.25 (0.76-2.05)	5/343 (1.5)	0.16 (0.06-0.42)†
2012	164/305 (53.8)	2/271 (0.7)	0.01 (0.00- 0.05)†	167/343 (48.7)	0.91 (0.78-1.05)
2013	59/305 (19.3)	25/271 (9.2)	0.54 (0.35- 0.83)†	2/343 (0.6)	0.03 (0.01-0.12)†
2014	49/305 (16.1)	12/271 (4.4)	0.28 (0.15-0.51)†	70/343 (20.4)	1.27 (0.91-1.77)
Co-infection and History or pre-exisiting condition					
Other viruses	21/305 (6.9)	14/271 (5.2)	0.75 (0.39-1.45)	36/343 (10.5)	1.52 (0.91-2.55)
Asthma	0/283 (0)	5/227 (2.2)	Not calculated	3/336(0.9)	Not calculated
Hypertension	5/283 (1.8)	1/227 (0.4)	0.25 (0.03-2.12)	2/336(0.6)	0.34 (0.07-1.72)
Clinical presentation					
Fever	268/283 (94.7)	143/227 (63.0)	0.67 (0.60-0.74)†	270/336(80.4)	0.85 (0.80-0.91)†
Cough	230/283 (81.3)	120/227 (52.9)	0.65 (0.57-0.74)†	216/336(64.3)	0.80 (0.72-0.88)†
Sore throat	149/283 (52.7)	74/227 (32.6)	0.62 (0.50-0.77)†	139/336(41.4)	0.79 (0.67-0.94)†
Chill	147/283 (51.9)	85/227 (37.4)	0.72 (0.59-0.88)†	119/336(35.4)	0.69 (0.57-0.82)†
Headache	136/283 (48.1)	86/227 (37.9)	0.79 (0.64-0.97)†	139/336(41.4)	0.87 (0.73-1.03)
Muscular pain	114/283 (40.3)	60/227 (26.4)	0.66 (0.51-0.85)†	94/336(28.0)	0.70 (0.56-0.87)†
Nasal discharge	219/283 (77.4)	84/227 (37.0)	0.48 (0.40-0.57)†	188/336(56.0)	0.73 (0.65-0.81)†
Nasal obstruction	119/283 (42.0)	48/227 (21.1)	0.50 (0.38-0.67)†	116/336(34.5)	0.83 (0.68-1.01)
Hoarseness	26/283 (9.2)	7/227 (3.1)	0.34 (0.15-0.76)†	37/336(11.0)	1.20 (0.74-1.93)
Wheezing	6/283 (2.1)	4/227 (1.8)	0.83 (0.24-2.91)	5/336(1.5)	0.70 (0.22-2.28)
Laboring breath	0/283 (0)	2/227 (0.9)	Not calculated	0/336 (0)	Not calculated
Phlegm	157/283 (55.5)	45/227 (19.8)	0.36 (0.27-0.47)†	142/336(42.3)	0.77 (0.65-0.90)†
Constricted chest	0/283 (0)	0/227 (0)	Not calculated	0/336(0)	Not calculated
Vomiting	21/283 (7.4)	10/227 (4.4)	0.59 (0.29-1.24)	15/336(4.5)	0.60 (0.32-1.14)
Diarrhea	2/283 (0.7)	1/227 (0.4)	0.62 (0.06-6.83)	6/336(1.8)	2.53 (0.51-12.42)
Loss of appetite	56/283 (19.8)	11/227 (4.8)	0.24 (0.13-0.46)†	57/336(17.0)	0.86 (0.62-1.20)

* Pos, positive; CI, Confidence interval

† Significant results at the $p < 0.05$

Coinfecting viruses and associations of IFV subtypes with clinical symptoms.

hRV (n=27, 38.0%) and ADV (n=25, 35.2%) were detected most frequently with IFV. ADV (n=13, 61.9%) was detected most commonly with IFV A(H3N2), and hRV (n=9, 64.3%) with A(H1N1)pdm09. Finally, hRV (n=15, 41.7%) and ADV (n=10, 27.8%) were detected most frequently with IFV B (Figure 3.2a).

We performed a network analysis of the associations between influenza subtypes and clinical symptoms. Fever, cough, nasal discharge, phlegm, and nasal obstruction were the most common symptoms of IFV A(H3N2) and IFV B infection. However, A(H1N1)pdm09 was more strongly associated with fever and cough than the other IFV subtypes (Figure 3.2b).

Table 3.2. Characteristics of outpatients with IFV A(H1N1)pdm09-associated acute respiratory illness in the first and second waves, Gyeonggi Province, South Korea, 2009–2014.

Characteristic	A(H1N1)pdm09		Odds Ratio (95% CI)	p-value
	First wave (2009), no. pos/no. all pos (% pos)	Second wave (2010), no. pos/no. all pos (% pos)		
Age group, y				
<5	9/89 (10.1)	29/113 (25.7)	**3.07 (1.37-6.89)**	0.005†
5-24	69/89 (77.5)	66/113 (58.4)	0.41 (0.22-0.76)	0.004†
25-44	6/89 (6.7)	14/113 (12.4)	1.96 (0.72-5.32)	0.182
45-64	1/89 (1.1)	4/113 (3.5)	3.23 (0.35-29.42)	0.237
≥65	1/89 (1.1)	0/113 (0)	Not calculated	
Male	42/89 (47.2)	46/113 (40.7)	0.77 (0.44-1.35)	0.356
Co-infection and history or pre-existing condition				
Asthma	0/86	4/90 (4.4)	Not calculated	
Hypertension	0/86	0/90	Not calculated	
Adenovirus	1/89 (1.1)	1/113 (0.9)	0.79 (0.05-12.74)	0.865
Human bocavirus	0/89	0/113	Not calculated	
Parainfluenza virus 1-3	0/89	0/113	Not calculated	
Human metapneumovirus	0/89	0/113	Not calculated	
Human coronavirus	1/89 (1.1)	1/113 (0.9)	0.72 (0.04-11.69)	0.817
Human rhinovirus	7/89 (7.9)	2/113 (1.8)	0.21 (0.04-1.04)	0.037†
Respiratory syncytial virus	0/89	0/113	Not calculated	
Clinical presentation				
Fever	40/86 (46.5)	58/90 (64.4)	2.08 (1.14-3.82)	0.017†
Cough	27/86 (31.4)	54/90 (60.0)	**3.28 (1.76-6.10)**	<0.001†
Sore throat	21/86 (24.4)	26/90 (28.9)	1.26 (0.64-2.46)	0.503
Chill	23/86 (26.7)	32/90 (35.6)	1.51 (0.79-2.88)	0.207
Headache	25/86 (29.1)	30/90 (33.3)	1.22 (0.64-2.31)	0.542
Muscular pain	11/86 (12.8)	24/90 (26.7)	2.48 (1.13-5.44)	0.021†
Nasal discharge	18/86 (20.9)	32/90 (35.6)	2.08 (1.06-4.10)	0.032†
Nasal obstruction	4/86 (4.7)	21/90 (23.3)	**6.24 (2.04-19.05)**	<0.001†
Hoarseness	0/86	0/90	Not calculated	
Wheezing	0/86	2/90 (2.2)	Not calculated	
Laboring breath	1/86 (1.2)	1/90 (1.1)	0.96 (0.06-15.51)	0.974
Phlegm	2/86 (2.3)	13/90 (14.4)	**7.09 (1.55-32.44)**	0.004†
Constricted chest	0/86	0/90	Not calculated	
Vomiting	2/86	4/90 (4.4)	1.95 (0.35-10.95)	0.439
Diarrhea	0/86	1/90 (1.1)	Not calculated	
Loss of appetite	1/86 (1.2)	1/90 (1.1)	0.96 (0.06-15.51)	0.974

* Pos, positive; CI, Confidence interval

† Significant results at the $p < 0.05$

Table 3.3. Characteristics of outpatients with IFV A(H1N1)pdm09 and A(H3N2) associated acute respiratory illness in the post 2009-pandemic (2010), Gyeonggi Province, South Korea.

	post-pandemic (2010)			
Characteristic	B, no. pos/no. all pos (% pos)	A(H1N1)pdm09, no. pos/no. all pos (% pos)	Odds Ratio (95% CI)	p-value
Age group, y				
<5	25/99 (25.3)	29/113 (25.7)	1.02 (0.55-1.90)	0.945
5-24	64/99 (64.6)	66/113 (58.4)	0.77 (0.44-1.34)	0.352
25-44	5/99 (5.1)	14/113 (12.4)	2.66 (0.92-7.67)	0.062
45-64	0/99	4/113 (3.5)	Not calculated	
≥65	22/99 (22.2)	0/113 (0)	Not calculated	
Male	48/99 (48.5)	46/113 (40.7)	0.73 (0.42-1.26)	0.255
Co-infection and history or pre-existing condition				
Asthma	2/99 (2.0)	4/90 (4.4)	2.26 (0.40-12.62)	0.342
Hypertension	1/99 (1.0)	0/90	Not calculated	
Adenovirus	25/99 (25.3)	1/113 (0.9)	0.03 (0.00, 0.20)	<0.001†
Human bocavirus	0/99	0/113	Not calculated	
Parainfluenza virus 1-3	0/99	0/113	Not calculated	
Human metapneumovirus	0/99	0/113	Not calculated	
Human coronavirus	0/99	1/113 (0.9)	Not calculated	0.817
Human rhinovirus	0/99	2/113 (1.8)	Not calculated	0.037†
Respiratory syncytial virus	0/99	0/113	Not calculated	
Clinical presentation				
Fever	46/99 (46.5)	58/90 (64.4)	**2.09 (1.16-3.75)**	**0.013†**
Cough	30/99 (30.3)	54/90 (60.0)	**3.45 (1.89-6.29)**	**<0.001†**
Sore throat	24/99 (24.2)	26/90 (28.9)	1.27 (0.66-2.43)	0.470
Chill	25/99 (25.3)	32/90 (35.6)	1.63 (0.87-3.05)	0.123
Headache	24/99 (24.2)	30/90 (33.3)	1.56 (0.83-2.95)	0.167
Muscular pain	17/99 (17.2)	24/90 (26.7)	1.75 (0.87-3.53)	0.114
Nasal discharge	21/99 (21.2)	32/90 (35.6)	**2.05 (1.07-3.91)**	**0.028†**
Nasal obstruction	5/99 (5.1)	21/90 (23.3)	**5.72 (2.06-15.92)**	**<0.001†**
Hoarseness	1/99 (1.0)	0/90	Not calculated	
Wheezing	0/99	2/90 (2.2)	Not calculated	
Laboring breath	0/99	1/90 (1.1)	Not calculated	
Phlegm	3/99 (3.0)	13/90 (14.4)	**5.40 (1.49-19.64)**	**0.005†**
Constricted chest	0/99	0/90	Not calculated	
Vomiting	1/99 (1.0)	4/90 (4.4)	4.56 (0.50-41.57)	0.142
Diarrhea	1/99 (1.0)	1/90 (1.1)	1.10 (0.07-17.87)	0.946
Loss of appetite	3/99 (3.0)	1/90 (1.1)	1.10 (0.07-17.87)	0.360

* Pos, positive; CI, Confidence interval

† Significant results at the $p < 0.05$

Table 3.4. Multiple logistic regression analysis of the associations of IFV subtypes with clinical symptoms in Gyeonggi Province, South Korea, 2009–2014.

Characteristics		IFV	A(H1N1)pdm09	A(H3N2)	B
Age	OR[τ]	0.997 (0.991-1.002)	0.997 (0.985-1.009)	**1.021 (1.010-1.033)**	0.983(0.971-0.994)
	p-value	0.254	0.666	0.000[†]	0.005
Gender	OR	0.9 (0.8-1.1)	1.0 (0.7-1.4)	1.1 (0.8-1.5)	0.9 (0.7-1.2)
	p-value	0.214	0.942	0.684	0.599
Fever	OR	0.8 (0.6-1.0)	0.3 (0.2-0.6)	**3.7 (2.0-7.2)**	1.3 (0.8-2.0)
	p-value	0.112	0.000	0.000[†]	0.323
Cough	OR	**1.4 (1.2-1.8)**	1.5 (1.0-2.4)	1.1 (0.7-1.7)	0.7 (0.5-1.1)
	p-value	0.001[†]	0.084	0.626	0.107
Sore throat	OR	0.7 (0.6-0.8)	0.7 (0.4-1.1)	1.1 (0.7-1.7)	1.1 (0.8-1.7)
	p-value	0.000	0.119	0.632	0.582
Chill	OR	**1.4 (1.2-1.8)**	1.5 (1.0-2.4)	1.4 (0.9-2.1)	0.6 (0.4-0.8)
	p-value	0.001[†]	0.084	0.102	0.005
Headache	OR	**1.9 (1.5-2.3)**	1.1 (0.7-1.8)	0.6 (0.4-1.0)	1.6 (1.1-2.4)
	p-value	0.000[†]	0.750	0.032	0.027
Muscular pain	OR	**1.6 (1.3-2.0)**	1.5 (0.9-2.4)	1.0 (0.6-1.5)	0.8 (0.5-1.2)
	p-value	0.000[†]	0.142	0.831	0.244
Nasal discharge	OR	1.2 (1.0-1.5)	0.5 (0.3-0.7)	**2.5 (1.7-3.7)**	0.8 (0.5-1.1)
	p-value	0.033	0.001	0.000[†]	0.152
Nasal obstruction	OR	0.7 (0.6-0.9)	1.2 (0.7-2.0)	0.8 (0.6-1.2)	1.0 (0.7-1.5)
	p-value	0.009	0.456	0.394	0.866
Hoarseness	OR	0.7 (0.5-0.9)	0.4 (0.2-1.0)	0.8 (0.4-1.3)	**2.1 (1.2-3.5)**
	p-value	0.005	0.059	0.313	0.008[†]
Wheezing	OR	0.3 (0.1-0.4)	2.2 (0.6-7.1)	0.9 (0.3-2.7)	0.7 (0.2-2.0)
	p-value	0.000	0.221	0.870	0.518
Laboring breath	OR	0.8 (0.1-3.4)	ND[**]	ND[***]	ND[***]
	p-value	0.782	ND[**]	ND[***]	ND[***]
Phlegm	OR	0.9 (0.8-1.2)	0.4 (0.2-0.6)	**1.7 (1.2-2.4)**	1.2 (0.9-1.8)
	p-value	0.633	0.000	0.005[†]	0.274
Constricted chest	OR	ND[***]	ND[***]	ND[***]	ND[***]
	p-value	0.959	ND[***]	ND[***]	ND[***]
Vomiting	OR	1.0 (0.7-1.4)	1.5 (0.6-3.2)	1.4 (0.8-2.7)	0.5 (0.3-1.0)
	p-value	0.859	0.322	0.254	0.075
Diarrhea	OR	1.0 (0.4-2.1)	0.5 (0.0-3.1)	0.4 (0.1-1.8)	3.2 (0.8-15.8)
	p-value	0.986	0.536	0.263	0.118
Loss of appetite	OR	0.4 (0.3-0.5)	0.3 (0.1-0.6)	1.2 (0.8-1.8)	**1.6 (1.0-2.5)**
	p-value	0.000	0.001	0.477	0.033[†]

[τ] OR; Odds Ratio

[†] Significant results at the $p < 0.05$

[*]ND [not determined]: one data point.

[**]ND [not determined]: two data point

[***]ND [not determined]: missing data.

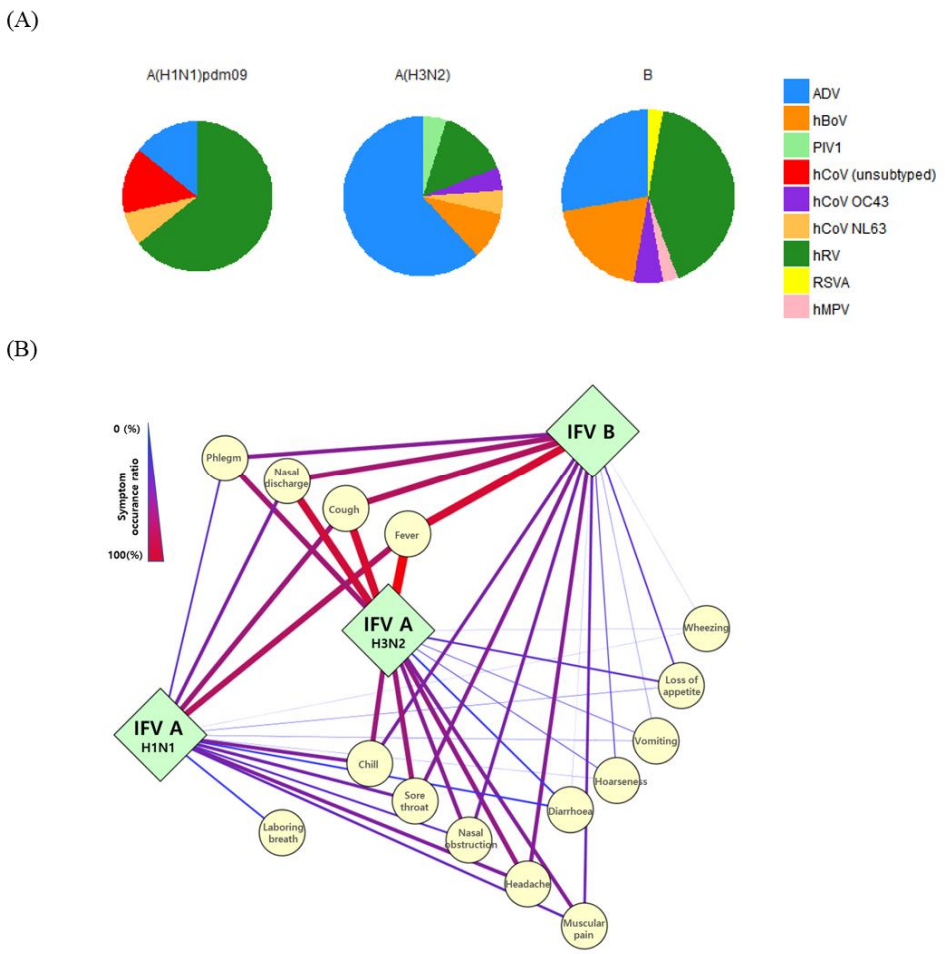


Figure 3.2. Coinfecting viruses and clinical characteristics of IFV subtypes. (a) Viruses coinfecting with IFV according to subtype. (b) Associations between IFV subtypes and the clinical symptoms of patients. Pie chart width are in proportional to sum of coinfection; the thickness of red lines is proportional to the strength of the association.

Discussion

We investigated the tendency of IFV, coinfections with other respiratory viruses, and the associations of demographic and clinical characteristics with IFV types and subtypes. We also analyzed the characteristics of the first and second waves of A(H1N1)pdm09 infection.

The major burden of disease is caused by seasonal epidemics of IFV A and B, with most infections occurring in children, although most severe cases involve very young or elderly individuals (Krammer, Smith et al. 2018). Seasonal IFV outbreaks typically occur in the winter months, when low humidity and low temperatures favor transmission; two influenza seasons occur per year, one each in the Northern and Southern Hemispheres (Yu, Alonso et al. 2013). For six consecutive years in Gyeonggi Province, IFV A and B cocirculated (Figure 3.1). Generally, IFV A infections occur first; subsequently, IFV B has predominated or there is a mix of IFV A and B infections. In this study, A(H1N1)pdm09 IFV was isolated before IFV B in 2009–2010. Specifically, IFV A(H1N1) was first isolated on 18 May, 2009, and was continuously reported from 11 August, 2009; IFV B was subsequently isolated. In 2010–2011 and 2011–2012, although the tendency was similar to that in 2009–2010, IFV A(H1N1)pdm09 predominated in 2010–2011 and A(H3N2) in 2011–2012; IFV B was isolated less frequently in 2010–2011. In 2012–2013 and 2013–2014, both IFV A and B were isolated. In 2012–2013, IFV A(H3N2) predominated while IFV A(H1N1)pdm09 and IFV B were isolated at a lower frequency. In 2013–2014, IFV A(H3N2) predominated and IFV B and A(H1N1)pdm09 were isolated less commonly (Figure 3.1). This tendency is consistent with prior studies based on the weekly reports of IFV activity provided by the KCDC

(Kim, Lee et al. 2017) and the 2011–2012 hospital-based clinical and laboratory IFV surveillance scheme in South Korea (Noh, Song et al. 2013, Wie, So et al. 2013). In South Africa IFV epidemics occur annually from May to September (Cohen, Hellferscee et al. 2014). In South Romania IFV A(H1N1)pdm09 and IFV B co-circulated in 2010–2011 and 2012–2013, and IFV A(H1N1)pdm09 and A(H3N2) (which predominated) co-circulated in 2011–2012 (Necula, Popovici et al. 2019). The characteristics and outbreak patterns of IFV differ geographically, which influences herd immunity and the efficacy of vaccination.

According to the recent IFV study which was conducted by Lee *et al* (Lee, Choi et al, 2017) from September 2012 to August 2016 in Gyeonggi Province, among 2726 samples, 400 (14.7%) were positive for IFV. Of the IFV-positive cases, 233 (58.2%) were positive for IFV A and 167 (41.8%) were positive for IFV B and 81 (2.2%) had positive results for IFV A(H1N1)pdm09 and 152 (38.0%) were IFV A(H3N2) and 167 (41.8%) were IFV B. Depending on the age groups, prevalence was the highest in the school-age and adolescent age group of 7-18 years. Major clinical symptoms were fever (88.0%), cough (83.3%), sputum (69.0%) and rhinorrhea (68.0%).

After the first reports of human infection with IFV A(H1N1) from Mexico in April 2009, the virus began a rapid global spread. As of 4 April, 2010, more than 214 countries have confirmed cases of IFV H1N1 2009, with more than 18,366 deaths (Organization 2009). The first case in South Korea was confirmed on 2 May, 2009, in a patient who had traveled to Mexico in April 2009. During the spring and summer of 2009, the prevalence of IFV A(H1N1) was lower in South Korea than in the United States and United

Kingdom; however, it spread rapidly in South Korea from September to November 2009. As of 30 April, 2010, the KCDC reported that > 3 million persons were suspected to be infected with IFV A(H1N1) and treated with antiviral agents (Jeon, Chung et al. 2011).

We compared the first (2009) and second (2010) post-2009 pandemic waves and IFV A(H1N1)pdm09, H3N2 and B in second waves, post-2009 pandemic wave (2010). The number of infected patients < 5 years old and the frequency of cough, nasal obstruction, and phlegm differed and were significantly greater in the second than in the first wave (Table 3.2) and fever, cough, nasal discharge, nasal obstruction, and phlegm were more strongly associated with A(H1N1)pdm09 than B (Table 3.3). Cohen *et al*. reported that age group (25–44 and 45–64 years), coinfection with RSV, and clinical symptoms (cough, tachypnea, stridor, tachycardia, and vomiting) differed significantly between the first (2009) and second (2011) waves, although the statistical methods used and the factors analyzed differed from our study (Cohen, Hellferscee et al. 2014). Kusznierz *et al*. reported that post-pandemic era of A(H1N1) virus was more increased risk of severe disease in relation to H3N2 virus during 2013 (Kusznierz, Carolina et al. 2017).

From these results, we couldn't exactly measure how disease severity of A(H1N1)pdm09 was changed in second wave, post-2009 pandemic wave and whether disease severity of A(H1N1)pdm09 became be mild or not and finally be equal to other IFV subtype because our study was incorporated with primary hospital that could get simple information such as clinical symptoms. Usuallly, disease severity measured by hospitalization rate, mortality rate and mechanical ventilation an so on, which can get from

tertiary hospitals. Until recently, there are so many studies concerning to disease severity between pandemic and post-pandemic. Prokopeva *et el* reported that A(H1N1)pdm09 virus strain isolated in pandemic period is more virulent and able to cause more severe pathological processes in lung tissue of experimentally infected mice compared to the strain A(H1N1)pdm09 isolated in the post-pandemic period (Prokopeva, Sayfutdinova et al. 2013, Prokop'eva, Kurskaya et al. 2014). Some studies showed that disease severity in post-pandemic was more severe than pandemic (Athanasiou, Baka et al. 2011, Chuang, Huang et al. 2012, Viasus, Cordero et al. 2012) and other showed opposite result (Rao, Torok et al. 2015) and the others showed severity did not differ between pandemic and post-pandemic (Rahamat-Langendoen, Tutuhatunewa et al. 2012, Cohen, Hellferscee et al. 2014, Saad, Hayajneh et al. 2014). Further studies incorporated with tertiary hospitals need to know exact changes in disease severity between pandemic and post-pandemics and IFV subtypes.

It is generally not possible to distinguish infection by different IFV types and subtypes based on the clinical features (Irving, Patel et al. 2012, Paul Glezen, Schmier et al. 2013). IFV A(H1N1)pdm09 infection, as reported, has more severe outcomes than infection with other types and subtypes (Chaves, Aragon et al. 2013, Kawai, Ikematsu et al. 2013).

There was no difference in severity among the IFV subtypes (RR < 1.0) (Table 3.1). In a multiple logistic regression analysis, IFV infection was associated with a 1.4-fold increased risk of cough and chill, a 1.9-fold increased risk of headache, and a 1.6-fold increased risk of muscular pain compared with other respiratory viruses. In addition, IFV A(H3N2) was associated with a 3.7-fold increased risk of fever, a 2.5-fold increased risk of

nasal discharge, and a 1.7-fold increased risk of phlegm. IFV B was associated with a 2.1-fold increased risk of hoarseness and a 1.6-fold increased risk of loss of appetite. IFV A(H1N1)pdm09 was not significantly associated with the risk of clinical symptoms (Table 3.3). However, the results were inconsistent because the objectives, statistical methods, and target analytical values were different.

Furthermore, many community surveillance and nosocomial studies on IFV were conducted on various aspects (Khandaker, Rashid et al. 2012, Nukiwa-Souma, Burmaa et al. 2012, Casalegno, Eibach et al. 2017, Saha, Gupta et al. 2018, Roy, Hartley et al. 2019). Especially community surveillance studies was very activated worldwide because of the importance of IFV and Casalengno et al. study was comparable with our study on period and managing system (Casalegno, Eibach et al. 2017).

This study has several limitations. Based on KCDC reports, all influenza A(H1N1) viruses were A(H1N1)pdm09, except for the first case reported in 2009 (11 May 2009), which was not subtyped because the KINRESS surveillance program first used a A(H1N1)pdm09 detection kit in 2012; However, all analyzed results were cross checked to ensure the accuacy with KCDC influenza reports and papers dealing with influenza virus in South Korea.

We investigated the epidemiologic characteristics of IFV in patients with RTIs. The findings will enable prediction of IFV outbreaks.

CHAPTER IV.

EPIDEMIOLOGIC AND CLINICAL CHARACTERISTICS OF HUMAN CORONAVIRUS IN SOUTH KOREA, 2009–2014

Introduction

HCoVs are commonly detected in nasopharyngeal aspirates from children with RTIs. They were first described in the 1960s as agents of the common cold (Tyrrell and Bynoe 1965, Hamre and Procknow 1966, McIntosh, Dees et al. 1967). The severe acute respiratory syndrome (SARS) outbreak in 2002 renewed interest in HCoV, resulting in the identification of new HCoV subtypes (Drosten, Günther et al. 2003, Ksiazek, Erdman et al. 2003, van der Hoek, Pyrc et al. 2004, Woo, Lau et al. 2005).

Coronaviruses are the largest enveloped single-strand RNA viruses, with a genome of 26 to 31 kb. They belong to the family Coronaviridae in the order Nidovirales, which also encompasses the families Arteriviridae, Roniviridae, and Mesoniviridae (Gorbalenya, Enjuanes et al. 2006, Lauber, Ziebuhr et al. 2012). CoVs are classified into four genera—Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus, and Deltacoronavirus—based on phylogenetics and serology (Fields, Knipe et al. 2013). α-CoV (including HCoV-229E and NL63) and β-CoV (including HCoV-OC43, HKU1, SARS-CoV, and Middle East respiratory syndrome CoV [MERS-CoV]) cause human infections (Fields, Knipe et al. 2013).

Interest in coronaviruses was increased by the emergence of SARS-CoV and MERS-CoV in 2002 and 2012, respectively. These are Betacoronaviruses of clades B and C, respectively, and cause severe respiratory disease in humans. The first SARS-CoV outbreak occurred in 2002–2003, and involved > 8000 cases, with a fatality rate approaching 10% (Peiris, Guan et al. 2004). MERS-CoV emerged in 2012 in the Middle East (Zaki, Van Boheemen et al. 2012). To date, 2428 laboratory-confirmed

cases of MERS-CoV infection have been confirmed, including 838 fatal cases, as reported by the WHO. SARS-CoV and MERS-CoV are of zoonotic origin, and their animal reservoir is in close contact with the human population. The zoonotic potential of CoVs implies the need for surveillance of domesticated animals (Nathalie, Miszczak et al. 2016).

Coronaviruses cause human and veterinary outbreaks due to their ability to recombine, mutate, and infect multiple species and cell types, so they have the propensity to jump between species. There is no antiviral for HCoV, and the options for preventing CoV infection are limited (Tsang, Ho et al. 2003, Consortium 2004, Pyrc, Berkhout et al. 2007, Al-Tawfiq, Zumla et al. 2014, Fehr and Perlman 2015, Mackay and Arden 2015). Therefore, surveillance of HCoV and investigation of its epidemiological characteristics are important for predicting, preventing, and controlling HCoV outbreaks (Zhang, Tuo et al. 2018). The first travel-associated MERS-CoV outbreak outside the Middle East (Hui, Perlman et al. 2015, Kim, Kim et al. 2016) began in South Korea on May 20, 2015; 186 patients were involved and 36 died within 2 months (Kim, Kim et al. 2016). Also, the virus spread rapidly in hospitals (Kim, Tandi et al. 2017). There are many reports on MERS-CoV, but few studies of the epidemiological characteristics of HCoV in South Korea have been conducted (Zhang, Tuo et al. 2018).

We collected 4,028 throat and nasal swab specimens from adults and children with fever and various clinical symptoms in Gyeonggi Province, South Korea from 2009 to 2014. We evaluated the epidemiological features of HCoV, and its association with clinical characteristics.

Materials and Methods

Ethics statement. This study involving human participants was approved by the Korea National Institute of Health Institutional Review Board (approval nos. 2010-03EXP-1-R, 2011-06EXP-01-C, 2012-08EXP-06-3C, 2013-08EXP-03-5C, and 2014-08EXP-6C-A). The specimens were collected for the purpose of diagnosing RTIs, and written informed consent was obtained from the patients, their parents, or their legal guardians (Kim, Kim et al. 2014).

Patients and specimens. From January 2009 to November 2014, 4028 throat and nasal swabs were obtained from 3305 pediatric patients (< 19 years old) and 678 adult patients (> 19 years old) attending hospitals in Gyeonggi Province, South Korea. Specimens were obtained from individuals with fever (temperature ≥ 38°C) and cough, sore throat, hoarseness, chill, and/or other symptoms of acute RTI. There were 1967 male (48.8%) and 1997 female (49.6%) patients (male: female ratio 0.98:1). Demographic, epidemiologic, and clinical parameters—including pre-existing conditions, symptoms, and putative diagnosis—were assessed using a standardized questionnaire. The specimens were added to 2 mL of VTM, transported at 4°C to the laboratory, divided into aliquots, and stored at −80°C.

The specimens were tested for HCoV and 15 other common respiratory viruses, including IFV A and B, parainfluenza virus (1–3), RSV, human metapneumovirus, ADV, hRV, and human bocavirus.

Nucleic acid extraction and respiratory virus screening. Viral DNA and

RNA were extracted from 140 μL of respiratory specimen using QIAamp Viral RNA Mini Kits (Qiagen GmbH Hilden, Germany). IFV A and B, parainfluenza virus (1–3), RSV A and B, human metapneumovirus, ADV, hRV, human bocavirus, and HCoV (OC43, 229E, NL63) were detected using the Powerchek™ Real-time PCR Kit (Kogen Biotech, South Korea) and an ABI 7500 Fast Real-time PCR System (Applied Biosystems) (Zhang, Tuo et al. 2018). The reaction conditions were 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Statistical analysis. We conducted univariate analyses and a multiple logistic regression analysis of the demographic and clinical characteristics, associated factors, and disease severity of patients infected with HCoV OC43, 229E, NL63 and compared symptomatic and asymptomatic patients. Statistical analyses were performed using R 3.5.0 and p-values < 0.05 were considered to indicate statistical significance.

Results

Epidemiological distribution of HCoV subtypes and clinical characteristics of HCoV-infected patients.

Among the 4028 specimens, 112 (2.8%) were positive for HCoV, comprising 45 males (40.2%) and 54 females (48.2%). The most common symptoms of HCoV-infected patients were fever (n=85, 75.9%), nasal discharge (n=74, 66.1%), cough (n=73, 65.2%), nasal obstruction (n=63, 56.3%), and phlegm (n=63, 56.3%). Other symptoms included wheezing (n=7, 6.3%), vomiting (n=2, 1.8%), and diarrhea (n=1, 0.9%). Most of the HCoV-positive patients were < 5 years old (n=64, 57.1%) or 5–17 years old (n=28, 25.0%).

Three HCoV subtypes were detected in patients with acute RTIs. Among the 112 HCoV-positive patients, 70 (62.5%) were HCoV-OC43 positive, 14 (12.5%) were HCoV-229E positive, and 28 (25.0%) were HCoV-NL63 positive. The most frequently detected HCoV in Gyeonggi Province from 2009 to 2014 was HCoV-OC43 (Table 4.1).

The annual distribution of all respiratory viruses is shown in Figure 4.1. HCoV infection occurred mainly in winter. HCoV-OC43 was detected throughout the year, and its detection rate was higher in winter (n=34, 48.6%, December to February) and spring (n=19, 27.1%, March to May) and lower in summer (n=7, 10.0%, June to August). HCoV-229E and HCoV-NL63 were detected more frequently in winter (HCoV-229E n=9, 64.3%; HCoV-NL63 n=14, 50.0%) and autumn (HCoV-229E; n=4, 28.6%, HCoV-NL63; n=10, 50.0%) compared to spring and summer (HCoV-229E, HCoV-NL63; n=0). The number of HCoV cases by age group when compared with all positive respiratory viruses detected in Gyeonggi

Province, South Korea, from 2009 to 2014, similar pattern was observed. The majority of patients positive for any respiratory virus or for HCoV were < 5 years old, followed by those > 5 years and < 17 years old (Figure 4.2).

According to univariate analyses using HCoV-OC43 as a reference, the RR of 2009 and 2011 was significant; indeed, 2009 had an RR of > 5.0. In 2009, HCoV-229E (RR 5.0, 95% CI 1.89–13.26) was more prevalent than HCoV-OC43; and in 2011, HCoV-NL63 (RR 4.58, 95% CI 1.88–11.19) was more prevalent than HCoV-OC43. Regarding clinical characteristics, HCoV-229E had an RR for headache of > 2.5. Therefore, compared with HCoV-OC43 and HCoV-229E, the risk of headache was 2.5-fold greater for HCoV-229E (Table 4.1).

Table 4.1. Characteristics of outpatients with HCoV-associated acute RTI subtype in Gyeonggi Province, South Korea, 2009-2014.

	HCoV subtype				
	HCoV-OC43(reference)	HCoV-NL63		HCoV-229E	
Characteristic	No. pos/no. all pos(% pos)	No. pos/no. all pos (% pos)	Relative Risk (95% CI)	No. pos/no. all pos (% pos)	Relative Risk (95% CI)
Age group, y					
<5	42/70 (60.0)	18/28 (64.3)	1.07 (0.77-1.50)	4/14 (28.6)	0.48 (0.20, 1.11)†
5-24	17/70 (24.3)	7/28 (25.0)	1.03 (0.48, 2.21)	4/14 (28.6)	1.18 (0.47, 2.97)
25-44	5/70 (7.1)	3/28 (10.7)	1.50 (0.38, 5.86)	3/14 (21.4)	3.00 (0.81, 11.13)
45-64	3/70 (4.3)	0/28 (0)	-	1/14 (7.1)	1.67 (0.19, 14.88)
≥65	3/70 (4.3)	0/28 (0)	-	2/14 (14.3)	3.33 (0.61, 18.15)
Male	33/70 (47.1)	15/28 (53.6)	1.14 (0.74, 1.74)	6/14 (42.9)	0.91 (0.47, 1.75)
Year					
2009	6/70 (8.6)	0/28 (0)	-	6/14 (42.9)	**5.00 (1.89, 13.26)**†
2010	9/70 (12.9)	0/28 (0)	-	4/14 (28.6)	2.22 (0.79, 6.22)
2011	6/70 (8.6)	11/28 (39.3)	**4.58 (1.88, 11.19)**†	2/14 (14.3)	1.67 (0.37, 7.42)
2012	24/70 (34.3)	1/28 (3.6)	0.10 (0.01, 0.73)†	0/14 (0)	-
2013	13/70 (18.6)	8/28 (28.6)	1.54 (0.72, 3.30)	0/14 (0)	-
2014	12/70 (17.1)	8/28 (28.6)	1.67 (0.76, 3.64)	2/14 (0)	0.83 (0.21, 3.32)
Co-infection and History or pre-exisiting condition					
Other viruses	26/70 (37.1)	6/28 (21.4)	0.58 (0.27, 1.25)	3/14 (21.4)	0.58 (0.20, 1.65)
Asthma	0/65 (0)	0/28 (0)	-	1/13 (7.7)	-
Hypertension	0/65 (0)	0/28 (0)	-	1/13 (7.7)	-
Clinical presentation					
Fever	53/65 (81.5)	24/28 (85.7)	1.05 (0.87, 1.27)	8/13 (61.5)	0.75 (0.48, 1.18)
Cough	46/65 (70.8)	23/28 (82.1)	1.16 (0.92, 1.47)	4/13 (30.8)	0.43 (0.19, 1.00)†
Sore throat	23/65 (35.4)	10/28 (35.7)	1.01 (0.56, 1.83)	5/13 (38.5)	1.09 (0.51, 2.33)
Chill	20/65 (30.8)	7/28 (25.0)	0.81 (0.39, 1.70)	3/13 (23.1)	0.75 (0.26, 2.16)
Headache	12/65 (18.5)	4/28 (14.3)	0.77 (0.27, 2.19)	6/13 (46.2)	**2.50 (1.15, 5.44)**†
Muscular pain	13/65 (20.0)	5/28 (17.9)	0.89 (0.35, 2.27)	2/13 (15.4)	0.77 (0.20, 3.01)
Nasal discharge	44/65 (67.7)	21/28 (75.0)	1.11 (0.84, 1.45)	9/13 (69.2)	1.02 (0.69, 1.52)
Nasal obstruction	40/65 (61.5)	18/28 (64.3)	1.04 (0.75, 1.46)	5/13 (38.5)	0.62 (0.31, 1.28)
Hoarseness	11/65 (16.9)	6/28 (21.4)	1.27 (0.52, 3.09)	1/13 (7.7)	0.45 (0.06, 3.22)
Wheezing	5/65 (7.7)	1/28 (3.6)	0.46 (0.06, 3.79)	1/13 (7.7)	1.00 (0.13, 7.87)
Laboring breath	0/65 (0)	0/28 (0)	-	0/13 (0)	-
Phlegm	39/65 (60.0)	22/28 (78.6)	1.31 (0.99, 1.73)	2/13 (15.4)	0.26 (0.07, 0.93)†
Constricted chest	0/65 (0)	0/28 (0)	-	0/13 (0)	-
Vomiting	1/65 (1.5)	1/28 (3.6)	2.32 (0.15, 35.81)	0/13 (0)	-
Diarrhea	0/65 (0)	0/28 (0)	-	1/13 (7.7)	-
Loss of appetite	20/65 (30.8)	9/28 (32.1)	1.04 (0.55, 2.00)	0/13 (0)	-

* Pos, positive; CI, Confidence interval

† Significant results at the $p < 0.05$

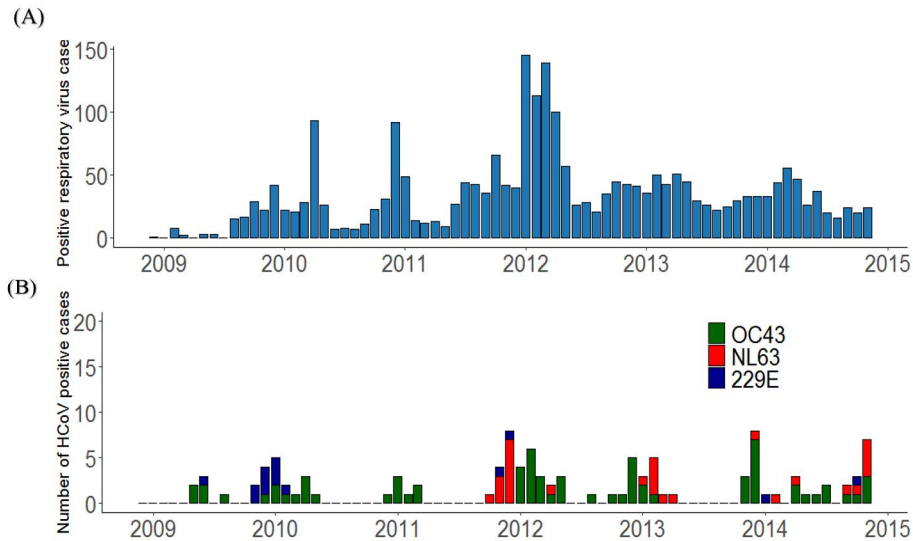


Figure 4.1. Annual distribution of HCoV subtypes in Gyeonggi Province, 2009–2014. Seasonal distribution of cases positive for (a) any respiratory virus and (b) HCoV. Light-blue, green, red, and deep-blue bars, all positive respiratory cases, HCoV-OC43, HCoV-NL63, and HCoV-229E, respectively.

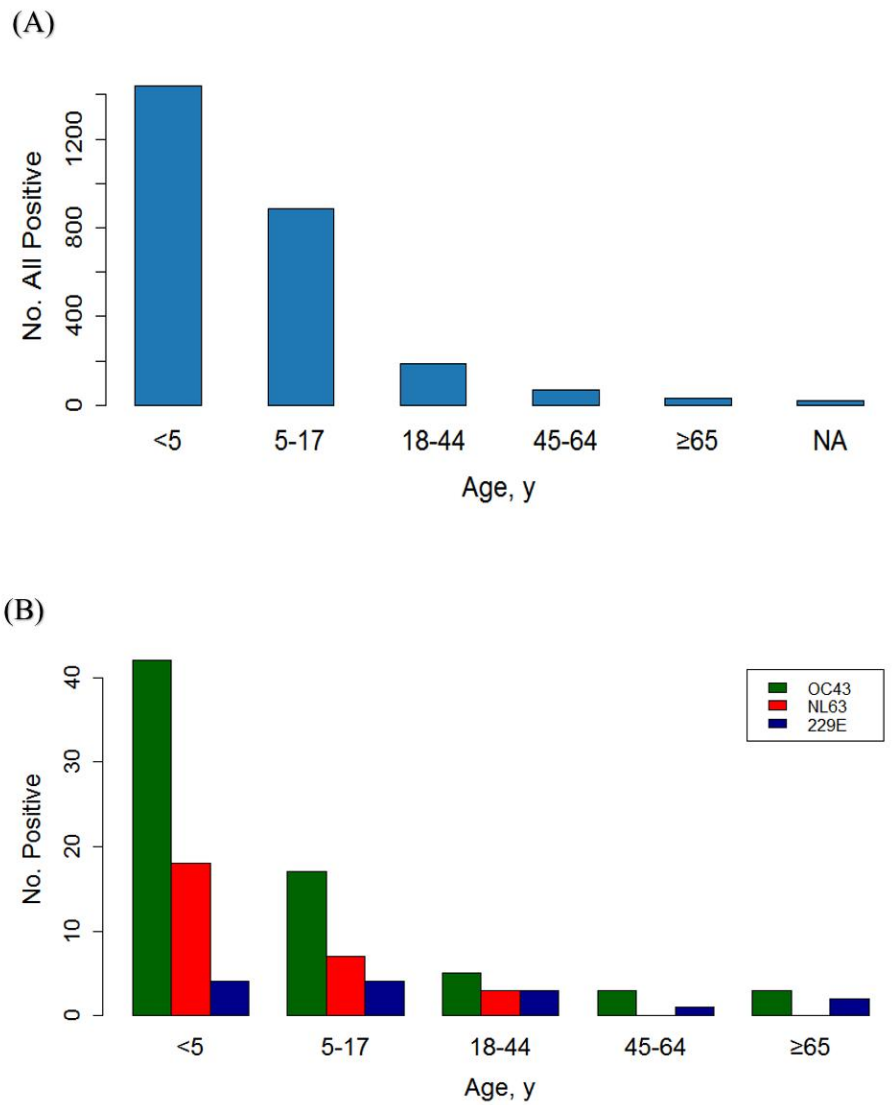


Figure 4.2. Number of cases by age group. (a) respiratory viruses by age group (b) HCoV by age group. Green, red and deep-blue bars, HCoV-OC43, HCoV-NL63, and HCoV-229E, respectively.

Demographic characteristics of symptomatic and asymptomatic HCoV-infected patients.

To explore the relationship between symptomatic (n=99) and asymptomatic (n=7) HCoV-infected patients, univariate analyses of age, gender, coinfection and history or pre-existing condition were performed (Table 4.2). However, no OR of > 1.0 was detected.

Associations of clinical characteristics with HCoV subtypes.

We performed a multiple logistic regression analysis of the association between clinical features and HCoV subtypes. Age, gender, and 16 clinical symptoms were included in the analysis. Nasal obstruction (OR=3.1, 95% CI 1.8–5.3) was significantly more frequent in the HCoV-infected compared with the non-HCoV-infected group ($p < 0.05$). No significant ORs were found for HCoV-OC43 and HCoV-NL63. Age (OR=1.1, 95% CI 1.0–1.3) and headache (OR=94.2, 95% CI 2.4–17,186.3) were strongly associated with HCoV-229E (Table 4.3).

Table 4.2 Characteristics of outpatients with symptomatic (n=99) and asymptomatic (n=7) HCoV infection in Gyeonggi Province, South Korea, 2009–2014.

Characteristic	Univeriate analysis		Odds Ratio (95% CI)	p-value
	Symptomatic	Asymtomatic		
Age group, y				
<5	67/99 (67.7)	4/7 (57.1)	0.64 (0.13, 3.02)	0.567
5-17	17/99 (17.2)	2/7 (28.6)	1.66 (0.48, 5.80)	0.447
18-44	8/99 (8.1)	1/7 (14.3)	1.77 (0.26, 12.21)	0.569
45-64	4/99 (4.0)	0/7 (0)	-	-
≥65	3/99 (3.0)	0/7 (0)	-	-
Male	47/99 (47.5)	0/7 (0)	-	-
Co-infection and history or pre-existing condition				
Asthma	1/99 (1.0)	0/7 (0)	-	-
Hypertension	1/99 (1.0)	0/7 (0)	-	-
Adenovirus	8/99 (8.1)	1/7 (14.3)	1.77 (0.26, 12.21)	0.569
Human bocavirus	1/99 (1.0)	0/7 (0)	-	-
Parainfluenza virus 1-3	2/99 (2.0)	0/7 (0)	-	-
Human metapneumovirus	0/99 (0)	0/7 (0)	-	-
Human rhinovirus	12/99 (12.1)	2/7 (28.6)	2.36 (0.65, 8.53)	0.214
Respiratory syncytial virus	3/99 (3.0)	0/7 (0)	-	-

* Pos, positive; CI, Confidence interval

† Significant results at the $p < 0.05$

Table 4.3 Multiple logistic regression analysis of the association of HCoV subtypes with clinical symptoms in Gyeonggi Province, South Korea, 2009-2014.

Characteristics		HCoV	HCoV-OC43	HCoV- NL63	HCoV-229E
Age	OR[τ]	1.006 (0.989-1.020)[§]	1.006 (0.971-1.044)[§]	0.9 (0.9-1.0)	1.1 (1.0-1.3)
	p-value	0.439	0.725	0.072	0.012[†]
Gender	OR	0.995 (0.670-1.477)[§]	0.8 (0.3-1.8)	1.4 (0.5-3.9)	0.5 (0.1-4.2)
	p-value	0.982	0.523	0.545	0.501
Fever	OR	0.7 (0.4-1.4)	1.1 (0.3-3.6)	0.7 (0.1-3.8)	3.9 (0.3-73.2)
	p-value	0.352	0.891	0.707	0.322
Cough	OR	0.7 (0.4-1.3)	0.9 (0.3-2.8)	2.4 (0.6-10.9)	0.1 (0.0-1.7)
	p-value	0.299	0.825	0.244	0.173
Sore throat	OR	0.8 (0.5-1.2)	0.9 (0.3-2.4)	0.7 (0.2-2.3)	7.3 (0.3-592.3)
	p-value	0.290	0.785	0.515	0.254
Chill	OR	1.0 (0.6-1.7)	1.7 (0.6-5.4)	0.8 (0.2-2.7)	0.1 (0.0-2.4)
	p-value	0.999	0.368	0.731	0.198
Headache	OR	0.6 (0.3-1.1)	0.5 (0.1-1.8)	1.0 (0.2-5.5)	**94.2 (2.4-17186.3)**
	p-value	0.132	0.274	0.972	0.037[†]
Muscular pain	OR	0.9 (0.5-1.6)	1.2 (0.3-5.5)	3.6 (0.7-20.1)	0.0 (0.0-0.1)
	p-value	0.777	0.771	0.135	0.014[†]
Nasal discharge	OR	1.2 (0.7-2.2)	0.7 (0.2-2.0)	0.6 (0.2-2.2)	8.0 (0.8-106.0)
	p-value	0.473	0.460	0.445	0.087
Nasal obstruction	OR	**3.1 (1.8-5.3)**	1.3 (0.5-3.6)	1.2 (0.4-4.2)	0.1 (0.0-1.0)
	p-value	0.000[†]	0.615	0.765	0.094
Hoarseness	OR	0.9 (0.5-1.5)	0.9 (0.3-3.3)	1.0 (0.3-3.9)	2.5 (0.0-102.7)
	p-value	0.635	0.909	0.975	0.639
Wheezing	OR	1.3 (0.5-2.9)	1.9 (0.3-15.5)	0.2 (0.0-1.7)	11.7 (0.1-4258.3)
	p-value	0.496	0.481	0.209	0.313
Laboring breath	OR	ND[‡]	ND[‡]	ND[‡]	ND[‡]
	p-value	ND[‡]	ND[‡]	ND[‡]	ND[‡]
Phlegm	OR	1.4 (0.8-2.4)	1.0 (0.3-3.0)	3.0 (0.8-12.6)	0.0 (0.0-0.4)
	p-value	0.238	0.969	0.116	0.036[†]
Constricted chest	OR	ND[‡]	ND[‡]	ND[‡]	ND[‡]
	p-value	ND[‡]	ND	ND[‡]	ND[‡]
Vomiting	OR	0.3 (0.1-1.1)	0.9 (0.0-26.0)	1.5 (0.1-42.6)	ND[‡]
	p-value	0.126	0.959	0.789	ND[‡]
Diarrhea	OR	1.2 (0.1-6.0)	ND[‡]	ND[‡]	ND[†]
	p-value	0.839	ND[‡]	ND[‡]	ND[†]
Loss of appetite	OR	0.6 (0.4-1.1)	1.6 (0.5-5.6)	0.6 (0.2-2.3)	ND[‡]
	p-value	0.099	0.434	0.505	ND[‡]

[τ] OR; Odds Ratio

[§] Decimal three digits were used to represent exact value

[†] Significant results at the $p < 0.05$

[†] ND [not determined]: one data point.

[‡] ND [not determined]: missing data.

Coinfections and network analysis of HCoV subtypes and clinical symptoms.

We investigated coinfection with HCoV and other respiratory viruses by multiplex-PCR. hRV (n=14, 12.5%) and ADV (n=11, 9.8%) most frequently coinfected with HCoV. hRV (n=9, 12.9%) and ADV (n=9, 12.9%) most frequently coinfected with HCoV-OC43. hRV (n=4, 14.3%) most frequently coinfected with HCoV-NL63, and RSV (n=1, 7.1%), hRV (n=1, 7.1%), and IFV (n=1, 7.1%) with HCoV-229E (Table 4.4).

We performed a network analysis of the correlation between HCoV subtypes and clinical symptoms. Fever, cough, nasal discharge, phlegm, and nasal obstruction were the most common clinical symptoms of HCoV-OC43 and HCoV-NL63. HCoV-229E was more closely associated with fever, nasal discharge, and headache compared with other HCoV subtypes (Figure 4.3).

Table 4.4 Virus detected in HCoV-positive respiratory specimens from local hospitals, 2009–2014.

	HCoV detections, Total and Subtypes. No. (%)			
	Total HCoV (n=112)	HCoV-OC43 (n=70)	HCoV- NL63 (n=28)	HCoV-229E (n=14)
Respiratory viruses				
Adenovirus	11 (9.8)	9 (12.9)	2 (7.1)	0 (0)
Human Bovavirus	1 (0.9)	1 (1.4)	0 (0)	0 (0)
Parainfluenza virus 1-3	1 (0.9)	1 (1.4)	0 (0)	0 (0)
Respiratory syncytial virus	4 (3.6)	3 (4.3)	0 (0)	1 (7.1)
Human metapneumovirus	0 (0)	0 (0)	0 (0)	0 (0)
Human rhinovirus	14 (12.5)	9 (12.9)	4 (14.3)	1 (7.1)
Influenza virus	7 (6.3)	4 (5.7)	2 (7.1)	1 (7.1)
No. of detections				
Single HCoV detection	76 (67.9)	45 (64.3)	21 (75.0)	10 (71.4)
HCoV +1 codetection	31 (27.7)	21 (30.0)	6 (21.4)	4 (28.6)
HCoV +2 codetections	5 (4.5)	4 (5.7)	1 (3.6)	0 (0)

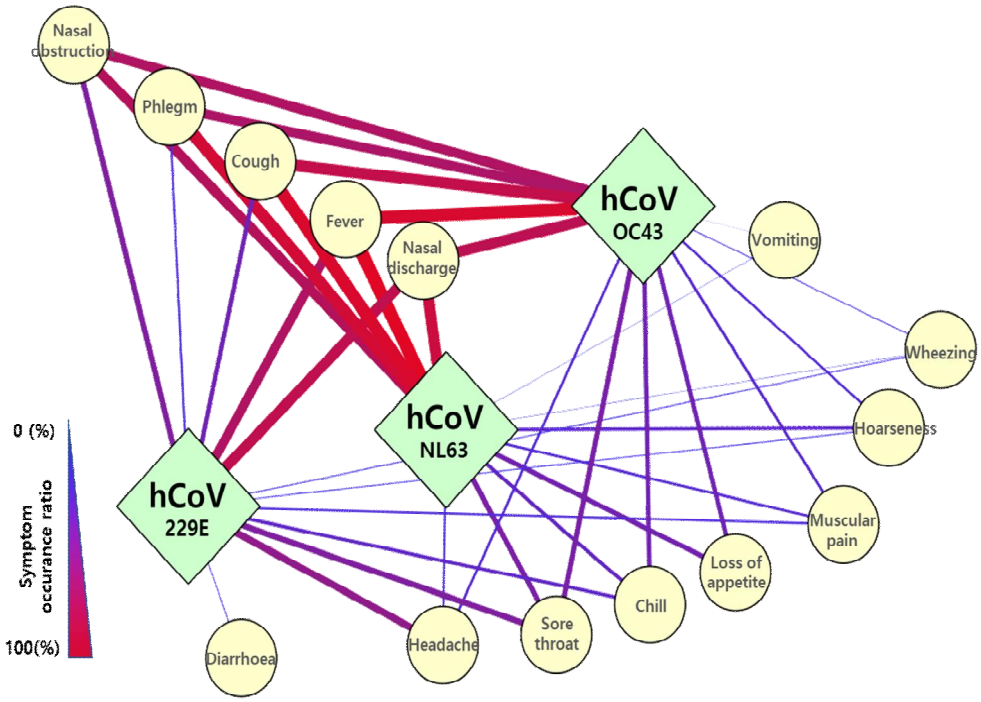


Figure 4.3. Associations between HCoV subtypes and clinical symptoms. The thickness of red lines is proportional to the strength of the relationship.

Discussion

Since the MERS-CoV outbreak in May 2015 in South Korea, the possibility of another such epidemic resulted in the establishment of a laboratory surveillance system reporting to the KCDC. Prior to the MERS-CoV outbreak in South Korea, coronaviruses were thought to cause mild, self-limiting RTI (Gerna, Campanini et al. 2006, Fehr and Perlman 2015) and to be restricted to the Middle East, but the emergence of SARS-CoV and MERS-CoV altered this perception (Zhang, Tuo et al. 2018). Research on the origin of SARS- and MERS-CoV revealed the possibility of coronavirus variation and transmission from animal hosts to human (Consortium 2004, Woo, Huang et al. 2010, Fehr and Perlman 2015, Lu, Wang et al. 2015, de Wit, van Doremalen et al. 2016). Therefore, investigation of HCoV is important for preventing and controlling outbreaks. However, few studies have focused on HCoV epidemiology in South Korea and a number of studies undertaken soon after the MERS-CoV outbreak in May 2015 have not been published yet. Therefore, we assessed the epidemiologic characteristics of patients with acute HCoV infection in Gyeonggi Province, South Korea, from 2009 to 2014.

We collected 4028 throat and nasal swabs from patients with acute RTI in Gyeonggi Province, South Korea, from January 2009 to November 2014. HCoV was detected in 112 cases (2.8%) of RTI, among which 70 (62.5%) were HCoV-OC43, 14 (12.5%) were HCoV-229E, and 28 (25.0%) were HCoV-NL63. The most prevalent HCoV in Gyeonggi Province from 2009 to 2014 was HCoV-OC43 (Table 4.1). Previous studies in South Korea have reported different detection rates. Sung *et al*. reported that the predominant HCoV in South Korea from January 2005 to June 2009 was HCoV-NL63

(n=30, 16.5%) (Sung, Lee et al. 2010), while another study focused on HCoV-NL63 (Han, Chung et al. 2007). The most prevalent HCoV in China was HCoV-OC43, followed by 229E and NL63 (Ren, Gonzalez et al. 2011, Lu, Yu et al. 2012, Zeng, Chen et al. 2018, Zhang, Tuo et al. 2018) except Liu *et al*'s in a 3-year study of children in Ghangzhou (Liu, Liu et al. 2014). In Japan, HCoV-NL63 predominated, followed by OC43 and 229E (Matoba, Abiko et al. 2015, Matoba, Aoki et al. 2018). Studies performed in the United states, United Kingdom, and Kenya (Gaunt, Hardie et al. 2010, Lee and Storch 2014, Biggs, Killerby et al. 2017, Ogimi, Waghmare et al. 2017, Kiyuka, Agoti et al. 2018) showed that HCoV-OC43 predominated, followed by NL63 and 229E.

HCoV epidemics occurred most frequently in winter (December to February). The HCoV-OC43 rate was higher in winter and spring (March to May) and lower in summer (June to August). HCoV-229E and HCoV-NL63 were predominantly detected in winter and autumn, and at a lower frequency in spring and summer (Figure 4.1). Also, HCoV infection peaked at < 5 years old, followed by > 5 years and < 17 years old (Figure 4.2).

These results are inconsistent with the reports of Zhang *et al*., Soonnarong *et al*., and Friedman *et al*. in some respects (such as HCoV subtypes and age groups), but the age groups differed among the studies (Soonnarong, Thongpan et al. 2016, Friedman, Alter et al. 2018, Zhang, Tuo et al. 2018). Therefore, more data are needed to gain a comprehensive understanding of the epidemiology of HCoV.

Univariate analyses showed that in 2009 of HCoV-229E, 2010 of HCoV-NL63 and headache of HCoV-229E, the RR was > 1.0. In other words, compared with HCoV-OC43, HCoV-229E and HCoV-NL63 were

associated with a greater risk than HCoV-OC43 (Table 4.1). Secondly, because HCoV causes more serious clinical symptoms than other respiratory viruses, a univariate analysis of symptomatic and asymptomatic HCoV-infected patients was performed; however, no significant associations were found (Table 4.2). Third, in a multiple logistic regression analysis, the OR for nasal obstruction in the HCoV-infected group and headache in the HCoV-229E infected group was > 1.0 ($p < 0.05$) (Table 4.3). Fourth, a network analysis showed that fever, cough, nasal discharge, phlegm, and nasal obstruction were the most common clinical symptoms of HCoV-OC43 and HCoV-NL63 infection. In contrast, HCoV-229E was associated with fever, nasal discharge, and headache (Figure 4.3).

HCoVs are frequent causes of upper RTI in the elderly, which can lead to acute pneumonia and renal failure (Gorse, O'Connor et al. 2009, Memish, Zumla et al. 2013). HCoVs also cause the common cold, particularly in children (Moës, Vijgen et al. 2005, Lu, Yu et al. 2012). Furthermore, HCoVs are associated with mild to severe upper and lower RTI and can cause more serious respiratory diseases in children, the elderly, and people with underlying conditions (McIntosh, Chao et al. 1974, van Elden, Anton M et al. 2004). The WHO reported that the MERS-CoV case-fatality rate in South Korea was 35.5% from 2012 to 30 June 2018 (Organization 2017). Most prior studies showed that fever was the most frequent symptom (Lu, Yu et al. 2012, Soonnarong, Thongpan et al. 2016), compared to cough in prior works (Lee and Storch 2014, Friedman, Alter et al. 2018, Zeng, Chen et al. 2018). Also, these results were different from HCoV subtypes (Lee and Storch 2014, Al-Khannaq, Ng et al. 2016). In this study, respiratory specimen such as throat swabs or nasal aspirate specimens are used because

the KINRESS was supported by local hospitals which is primary hospital and these are representative upper respiratory tract (URT) samples and are used commonly because these are easy to take and acceptable to the patients (Heikkinen, Marttila et al. 2002, Gruteke, Glas et al. 2004, Abu-Diab, Azzeh et al. 2008, Chan, Peiris et al. 2008, Lambert, Whiley et al. 2008, Sung, Chan et al. 2008, de la Tabla, Masiá et al. 2010, Meerhoff, Houben et al. 2010, DeByle, Bulkow et al. 2012). On the other hands, lower respiratory tract samples such as sputum and bronchoalveolar lavage (BAL) are useful for dectection of LRT infection but difficult to collect and need to require pre-treatment (Falsey, Formica et al. 2012, Branche, Walsh et al. 2014, Jeong, Kim et al. 2014). Even though these samples were collected from upper respiratory tract, we couldn't regard it as not infected on lower respiratory tract and actually wheezing which is one of the lower respiratory tract's clinical symptoms were founded in 7 (6.3%) outpatients and 6 (5.4%) was <5 years old and 1 (0.9%) was > 5 years and < 17 years old (Table 4.1). Few studies have evaluated the association between HCoV and clinical symptoms; therefore, further research is needed. Finally, hRV was the most frequent virus co-infecting with HCoV (Table 4). In contrast, Zhang *et al*. reported that IFV was the most frequent coinfecting respiratory virus (Zhang, Tuo et al. 2018).

Varghese and Uddin et al. investigated community and hospital based studies on HCoV and Gagneur et al. showed nosocomial HCoV infection (Gagneur, Sizun et al. 2002, Varghese, Zachariah et al. 2017, Uddin, Englund et al. 2018). Varghese et al. reported that HCoV detected more in hospitals than in community but the distribution of HCoV subtypes and

seasonal trends was similar in the community and hospital (Varghese, Zachariah et al. 2017).

We investigated the epidemiologic characteristics of HCoV virus in patients with RTIs. The findings will assist prediction of future HCoV outbreaks.

CHAPTER V.

CONCLUSIONS

Summary and Conclusions

Nowadays many studies concerning to respiratory viruses were actively conducted and they encompass various aspects from molecular to epidemiologic and clinical points. Considering the importance of respiratory viruses, KINRESS surveillance program was continuously performed in South Korea in corporate with local hospitals, Institute of Health and Environment and KCDC. From KINRESS surveillance program, we can monitor national outbreak trend of respiratory viruses and the method how to efficiently protect from infectious diseases. This study was performed on major three viruses, RSV, IFV, hCoV which was collected from KINRESS surveillance program and we investigated unique characteristics on molecular, epidemiologic and clinical characteristics about three major viruses in South Korea.

First, we found 183 RSV infections and identified the genotypes. Among 4028 respiratory specimens, 183 patients were positive for RSV infection and from partial sequencing of the glycoprotein gene, we found various genotype such as ON1, NA1, GA5, GA1, BA9, BA10 genotypes in Gyeonggi Province, South Korea. Furthemore, NA1 was completely replaced to ON1 from 2013 because of the presence of 72-nucleotide duplication insertion in the G protein which characteristics may have positive evolutionary effect on RSV. Besides, the most common clinical symptoms were fever, cough, nasal discharge, and phlegm, cough and wheezing showed strong association with RSV and each genotype was

significantly associated with each unique clinical symptoms. The majority of respiratory virus coinfections with RSV were human rhinovirus.

Second, we characterized the epidemiology of 920 IFV infection. Of a total of 4028 outpatients attended local hospitals, 920 were positive for IFV, comprising 305 A(H3N2), 271 A(H1N1)pdm09, and 343 IFV B. Comparison characteristics of outpatients between IFV subtypes using A(H3N2) as a reference was significantly higher RR in 2009 and 2010. In comparing the first and second waves of IFV A(H1N1)pdm09 in 2009 and 2010, age < 5 years, cough, nasal obstruction, and phlegm were more strongly associated with the second than the first wave. Cough, chill, headache, and muscular pain was significantly higher in the IFV-infected than the non-IFV-infected groups and IFV subtypes have associated with each unique clinical symptoms. Human rhinovirus comprised the majority of respiratory viruses coinfecting with IFV.

Third, we assessed the epidemiological and clinical characteristics of 112 HCoV infections in South Korea from 2009 to 2014. Among the 4028 cases, 112 were positive for HCoV, including 45 males and 54 females. Of them, 70 were HCoV-OC43, 14 were HCoV-229E, and 28 were HCoV-NL63. HCoV epidemics occurred mainly in winter. Comparison characteristics of outpatients between HCoV subtypes using HCoV-OC43 as a reference was significantly higher RR in 2009, 2010 and headache. No significant results obserbed in relationship between symptomatic and asymptomatic HCoV-infected patients. Nasal obstruction was significantly associated with HCoV infection, and age and headache were strongly associated with HCoV-229E infection. Moreover, the majority of

respiratory viruses coinfecting with HCoV were hRV and ADV.

The research on major respiratory viruses could provide the information about the molecular, demographic and clinical characteristics of RSV, IFV, and HCoV in Gyeonggi Province in South korea. The major respiratory viruses in South Korea had different seasonal distributions, genetic features, and clinical symptoms; We found out RSV genotype replacement from NA1 to ON1 and observed how the IFV post-pandemic wave was changed in comparison with first pandemic wave. In particular, certain clinical symptoms showed strong association with major respiratory viruses. These will determine the measures needed to prevent future outbreaks. The data will assist prevention of pandemics. In addition, the results enhance our knowledge of these three major respiratory viruses.

Overall, this study indicated that major respiratory viruses had their own molecular and epidemiological characteristics and host, in other words, human had their own unique clinical and epidemiological characteristics. From these, we could find out domestic characteristics of major respiratory viruses and identify epidemiologic major factors and the correlation between virus and host. These data suggest that the research on major respiratory viruses will provide important information to understand the association between respiratory viruses, human and diseases and further studies of other respiratory viruses in South Korea are needed.

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국문초록

국내 주요 호흡기바이러스의 최신 분자역학적 연구

보건학과 환경보건학 전공 박건영

호흡기감염 (Respiratory infections)은 박테리아나 바이러스에 의해 야기되는 감염성 질병을 의미하며, 상기도 감염과 하기도 감염으로 나눌 수 있다. 폐렴이나 독감 그리고 호흡기세포융합바이러스와 같은 급성 호흡기 감염은 세계에서 세번째로 사망률이 높은 질병에 속한다. 이 중에서 바이러스에 의해 야기되는 호흡기 감염 즉, 호흡기바이러스감염 (Respiratory viral infections)은 크게 인플루엔자와 인플루엔자가 아닌 바이러스로 나눌 수 있으며, 인플루엔자가 아닌 바이러스에는 아데노바이러스, 보카바이러스, 코로나바이러스, 호흡기세포융합바이러스, 라이노바이러스, 파라인플루엔자바이러스와 메타뉴모바이러스가 이에 속한다. 이러한 호흡기바이러스 감염은 열, 기침, 호흡곤란 등의 다양한 임상증상을 수반하며, 심한 경우에는 흉곽함몰이 일어나기도 한다. 새로운 호흡기바이러스 감염의 증가는 인구증가, 도시화, 기후변화, 국제적인 여행과 교역 증가를 포함한 다양한 국제적인 수렴 (convergence)에 의해 야기되었

으며, 호흡기바이러스감염에 의한 유행 (outbreak)은 지역과 나라마다 각각의 특정한 형태 (pattern)를 가지고 있다. 최근의 연구와 상황을 통하여 그 중요성을 고려하여 호흡기세포융합바이러스, 인플루엔자바이러스 그리고 코로나바이러스를 선정하여 더욱 깊은 연구를 수행하였다.

첫째, 4028건의 호흡기 검체에 대하여 real-time one-step RT-PCR을 수행하여 호흡기세포융합바이러스의 유행양상을 파악하였으며, 유전자염기서열분석을 통하여 각각의 유전적인 genotype을 확인하였다. 183건의 시료에서 호흡기세포융합바이러스 양성이 확인되었으며, 131건의 RSV-A subgroup에서 61건의 ON1 genotype이 확인되었고, 66건에서 NA1 genotype이, 3건에서 GA5가, 1건이 GA1이 확인되었다. RSV-B subgroup에서는 29건이 BA9 genotype이, 2건이 BA10 genotype이 확인되었다. 호흡기세포융합바이러스에서 가장 많은 임상증상은 열, 기침, 콧물, 가래였으며, 19세 이하의 소아에 대하여 다중로지스틱회귀분석을 통하여 호흡기세포융합바이러스와 임상증상간의 연관성을 알아본 결과, 기침과 쌕쌕거림이 가장 크게 관련이 있는 것으로 나타났다. 또한 호흡기세포융합바이러스와 가장 많이 중복 감염된 바이러스는 라이노바이러스 였다. 이러한 결과들은 호흡기세포융합바이러스의 분자생물학적 역학적 이해를 돕는데 공헌할 것이며, 호흡기세포융합바이러스 백신개발의 잠재 가능성을 촉진하는데도 기여할 것으로 기대된다.

둘째, 2009년과 2014년 사이에 인플루엔자의 형 (type)과 아형 (subtype)에 대하여 인구통계학적 (demographic), 임상적 (clinical) 특성을 비교함으로써 인플루엔자의 역학적인 특성을 파악하고, 2009년에 크게 유행한

A(H1N1)pdm09을 첫번째 유행으로 정하고 그 이후에 크게 발생한 시점 즉, 2010년을 두번째 유행시점으로 정하여 두 시점에 대하여 연령을 포함한 다양한 요인과 임상적인 특성에 대하여 알아보았다. 경기지역에 인플루엔자 및 호흡기바이러스 감시사업에 참여한 지역 병원에 호흡기증상이 있어서 내원한 환자 중 수집에 동의 하에 수집된 4028개의 호흡기 검체 중 920건이 인플루엔자 양성이었다. 이중에서 305건이 A(H3N2)에 속하였고, 291건이 A(H1N1)pdm09, 그리고 343건이 인플루엔자 B 타입에 속하였다. 주로 겨울에 발생하는 양상을 가지고 있었으며, 중증도가 심하다고 알려진 A(H3N2)를 기준으로 하였을 때 5-24의 연령그룹과 2009년과 2010년이 단변량분석 (univariate analysis) 에서 유의한 차이를 보였고, 다중로지스틱분석에서는 기침과 오한, 두통, 근육통과 같은 임상증상이 인플루엔자 감염환자와 강한 연관성을 가졌고 라이노바이러스가 인플루엔자와 가장 중복감염이 많은 바이러스였다. 이러한 결과들은 인플루엔자의 백신예측과 미래에 유행 가능한 인플루엔자 발생을 예측하는데 도움이 될 것으로 보인다.

셋째, 코로나바이러스에 대하여 역학적 임상적 특성에 대해 역시 4028개의 호흡기검체를 대상으로 알아보았다. 112건이 코로나바이러스에 대하여 양성이었으며, 여성의 비율이 54건으로 다소 높았다. 70건이 HCoV-OC43이었으며, 14건은 HCoV-229E에 속하였고, 28건은 HCoV-NL63이었다. 대부분 겨울에 유행하는 양상을 보였으며, HCoV-OC43을 기준으로 하여 HCoV-229E와 HCoV-NL63에 대하여 연령을 포함한 다양한 요인들에 대하여 단변량분석을 수행한 결과 2009년에 해당하는 부분만이 유의한 결

과를 나타냈다. 다중로지스틱회귀분석의 결과에서는 코막힘이 코로나바이러스 감염환자와 연관성이 있는 것으로 나타났고 연령과 두통이 HCoV-229E와 강한 연관성이 있었고 코로나바이러스와 중복감염이 가장 많은 바이러스는 아데노바이러스와 라이노바이러스였다. 이러한 결과들이 향후 발생할 가능성이 있는 코로나바이러스 유행에 대하여 적절한 조치를 취하는데 도움이 될 수 있다고 생각되며, 향후 분자생물학적인 분석을 코로나바이러스에 대해 추가적으로 시행하여 좀더 정확한 추이분석에 필요할 것이다.

결론적으로, 본 연구를 통하여 호흡기세포융합바이러스, 인플루엔자바이러스와 코로나바이러스를 포함한 세가지 주요 호흡기바이러스는 고유한 분자생물학적 역학적 특성을 가지고 있으며, 또한 계절적인 발생 분포와 년간 경향성 역시 바이러스마다 특정 특성을 가지고 있다는 것을 확인하였다. 또한 이러한 다양한 분석을 통하여 주요 호흡기바이러스에 영향을 미치는 주요한 인자를 확인하는 것은 향후 바이러스 발생양상 예측과 예방 그리고 백신개발에 기여할 것으로 기대된다.

표제어: 호흡기 바이러스 감염, 주요 호흡기바이러스, 호흡기세포융합바이러스, 인플루엔자, 코로나바이러스, 임상증상

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