



Short communication

Identification of influenza C virus in young South Korean children, from October 2013 to September 2016



Han Sol Lee^{a,1}, Sooyeon Lim^{a,b,1}, Ji Yun Noh^a, Joon Young Song^a, Hee Jin Cheong^{a,c},
Jung Hwa Lee^d, Sung Il Woo^e, Woo Joo Kim^{a,c,*}

^a Division of Infectious Diseases, Department of Internal Medicine, Korea University College of Medicine, Seoul, South Korea

^b Division of Brain Korea 21 Program for Biomedicine Science, College of Medicine, Korea University, Seoul, South Korea

^c Asia Pacific Influenza Institute, Korea University College of Medicine, Seoul, South Korea

^d Department of Pediatrics, Korea University College of Medicine, Seoul, South Korea

^e Department of Pediatrics, Chungbuk National University Hospital and College of Medicine, Cheongju, South Korea

ARTICLE INFO

Keywords:

Influenza C virus

South Korea

Phylogenetic analysis

ABSTRACT

Background: Influenza C virus has been largely neglected, compared to influenza A or B viruses, and is not routinely tested in clinical practices. However, several studies have indicated that influenza C virus causes severe acute respiratory illness and pneumonia in all ages.

Objective: We conducted a study to identify influenza C virus among young children in South Korea. Study design. From October 2013 to September 2016, 973 young children with influenzalike illness (ILI) were enrolled at three university hospitals. We tested nasopharyngeal samples for 16 types of respiratory viruses. Among the tested samples, 564 were positive for one or more respiratory viruses. Except for the samples where 16 types of respiratory viruses were found, 409 negative samples were examined for the presence of influenza C virus, using a matrix gene specific primer set.

Results: Among 409 nasopharyngeal samples, five influenza C viruses were detected. The manifestation of influenza C virus infection in young children was observed acute respiratory illness, such as fever, rhinorrhea, and cough, but no pneumonia or severe respiratory illness. Nucleotide sequencing was conducted and a phylogenetic tree was generated. We found that C/Sao Paulo/387/82-like lineage viruses circulated in South Korea, and the fully sequenced virus (C/Seoul/APD462/2015) was closely related to C/Victoria/2/2012 and C/Tokyo/4/2014 strains.

Conclusions: This study was the first report of influenza C virus detection in South Korea. Although severe illness was not observed in this study, we suggest the necessity for influenza C virus testing in pediatric patients with ILI, considering other reports of severe illnesses caused by influenza C virus infections.

1. Background

Influenza C virus generally causes mild upper respiratory illness in children or young adults [1,2]. However, recent studies have reported that it can cause lower respiratory illness, such as pneumonia and bronchitis in infants [3–5]. Studies of influenza C virus have indicated that the virus is widely distributed throughout the world, and that the majority of the human population acquires immunity by first exposure in childhood [6–18]. Despite a high seropositive rate in children (40–60%) and adults (80–100%), detection and isolation of influenza C viruses have rarely been described [6–11].

The genome of influenza C virus consists of seven RNA segments that encode nine proteins: three polymerase proteins (P3, PB2 and PB1), hemagglutinin-esterase (HE) glycoprotein, nucleoprotein (NP), matrix protein (M), CM2 protein, and two nonstructural proteins (NS1 and NS2). In the antigenic analysis and HE gene phylogenetic analysis of influenza C viruses, the virus separated into six antigenic lineages: C/Taylor/1233/47 (C/Taylor lineage), C/Kanagawa/1/76 (C/Kanagawa lineage), C/Mississippi/80 (C/Mississippi lineage), C/Aichi/1/81 (C/Aichi lineage), C/Yamagata/26/81 (C/Yamagata lineage), and C/Sao Paulo/378/82 (C/Sao Paulo lineage) [11,19]. Influenza C viruses belonging to different lineages that co-circulate in a community can lead

* Corresponding author at: Division of Infectious Diseases, Department of Internal Medicine, Guro Hospital, Korea University College of Medicine, Gurodong-ro 148, Guro-gu, Seoul, 08308, South Korea.

E-mail address: wjkim@korea.ac.kr (W.J. Kim).

¹ These authors contributed equally to this work.

to emergence of virus reassortment [19,20].

2. Objective

Although it has almost 70 years since the first isolation of influenza C virus, there is no information on influenza C virus in South Korea. In this study, we identified influenza C virus infection in children (< 2 years of age) with influenza-like illness (ILI) in South Korea.

3. Study design

From October 2013 to September 2016, 973 young children (< 2 years of age) with ILI were enrolled at the emergency rooms of three university hospitals through a Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance system [21]. ILI was defined as an acute respiratory illness with fever (measured body temperature of ≥ 38 °C) and at least one of the following symptoms, cough, sore throat, or rhinorrhea/nasal congestion, and an onset within the previous seven days. After written informed consent was obtained from the legal representatives of enrolled subjects, we obtained nasopharyngeal swabs from children, and placed them into viral transport media (VTM). Clinical information of subjects, including symptoms and underlying diseases were recorded on the clinical report form. We tested the nasopharyngeal samples for 16 types of respiratory viruses using Anyplex™ II RV16 detection kit (Seegene, Seoul, Korea). Among the tested samples, 564 were positive for one or more respiratory viruses and which 383 were positive for influenza A and / or B virus (Table 1). Excluding the RV16 positive samples, 409 negative samples were examined for the presence of influenza C virus using M gene specific primer set containing TaqMan™ probes [22]. Using influenza C virus positive samples, Madin-Darby canine kidney (MDCK) cells were inoculated with VTM samples and cultured for 72 h.

Viral RNA was extracted and transcribed into complementary DNA (cDNA) with a 3' end of influenza C viral RNAs (5'-AGCAGAAGC AGG-3'), using the Primescript 1st strand cDNA synthesis kit (Takara, Shiga, Japan). Individual segments of influenza C virus were amplified by gene-specific primer sets [16]. After amplification, sequencing was performed using the ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and 3730xl DNA analyzer. The sequence readouts of the PCR products were analyzed with BioEdit and MEGA software [23]. The nucleotide sequences obtained from this study were submitted to GenBank under the accession numbers MK050092-MK050104.

4. Results

Collected samples were tested for influenza A and B, respiratory syncytial virus A and B, adenovirus, parainfluenza virus 1, 2, 3, and 4, bocavirus, metapneumovirus, rhinovirus, coronavirus 229E, NL63 and OC43, and enterovirus, using the Anyplex™ II RV16 detection kit (Seegene, Seoul, Korea). A total of 409 samples negative for 16

Table 1
Number of nasopharyngeal specimens collected from young children with influenza-like illness and number of positive for RV16 or Flu C.

Flu season	Pediatric sample (< 2 years age)	Detection of respiratory viruses (RV16)					Flu C Positive
		Flu A	Flu B	Flu A and B	Other respiratory viruses	Negative	
2013-2014	279	99	34	0	77	69	0
2014-2015	413	105	23	2	71	212	4
2015-2016	281	89	31	0	33	128	1
Total	973	293	88	2	181	409	5

respiratory viruses were examined for detection of influenza C virus. Five samples were positive for influenza C virus, including four samples from 2015 and one sample from 2016 (Table 1). Unfortunately, we only isolated C/Seoul/APD462/2015 virus. Sequence of viral genes showed only the C/Seoul/APD462/2015 virus was fully sequenced for seven segments. The phylogenetic analysis of individual genes was performed using the reference sequences from the influenza research database (IRD). As shown in the HE phylogenetic tree, C/Seoul/APD401/2015, C/Seoul/APD462/2015 and C/Cheongju/GPD805/2015 fell into the C/Sao Paulo lineage (Fig. 1). Interestingly, phylogenetic analyses of individual genes revealed a close proximity of C/Seoul/APD462/2015 to C/Tokyo/4/2014 and C/Victoria/2/2012 strains.

The sequence identity score for each pair was calculated by the Sequence Demarcation Tool (SDTv1.2) program. All encoded protein sequence identity of C/Seoul/APD462/2015 strain with C/Tokyo/4/2014 or C/Victoria/2/2012 strains showed high identity of 99.6% (Fig. 2). Comparison of the amino acid sequences of each segment of C/Seoul/APD462/2015 with C/Victoria/2/2012 revealed several changes in amino acids (Table 2). In the HE gene, alanine was in position 355, instead of threonine found in C/Victoria/2/2012. Alanine at position 291 in the M gene changed to valine in C/Seoul/APD462/2015. Threonine at position 410 in the NP gene was substituted with alanine. Substitutions (V15L, L77P) at two different positions in NS were observed. Also, substitutions were observed in polymerase genes (P3; I330V, PB2; S538N, PB1; I202V).

5. Discussion

Influenza A and B viruses are well studied in the world, including South Korea [24,25]. In contrast, influenza C virus is not well known. In this study, we identified influenza C virus infection in five (1.22%) of 409 respiratory samples negative for sixteen respiratory viruses from young children with ILI. According to several reports, influenza C virus commonly causes mild acute respiratory illness and/or pneumonia in pediatric populations [3–5]. In this study, young children with influenza C virus infections had acute respiratory illnesses, such as fever, rhinorrhea, and cough, but no pneumonia or severe respiratory illness was observed (Supplementary data 1). Thielen BK, et al. reported that influenza C virus was detected among 0.58% of hospitalized patients with severe acute respiratory infection, and 0.48% of outpatients, in Minnesota from 2013 to 2016 [18]. They found that influenza C viruses were most frequent among young children, but occurred in all ages. Influenza C viruses in our study were detected from March to May (Supplementary data 2). This result is consistent with Japanese studies that reported influenza C viruses are mostly isolated between January and June, and usually occur after peaks of influenza A epidemics [11].

Although there is antigenic variation among influenza C viruses, the C/Kanagawa and C/Sao Paulo – related lineage strains have been dominant strains in Japan, the Philippines, the United States, and France since 2005 [13,17–19]. In this study, we confirmed that influenza C viruses belonging to the C/Sao Paulo antigenic group were detected in South Korea. In addition, a re-assortment event was observed in the C/Seoul/APD462/2015 strain, which contains HE, PB1 and PB2 genes belonging to the C/Sao Paulo related lineage, and NP and P3 genes belonging to the C/Mississippi related lineage. Particularly, all segments of C/Seoul/APD462/2015 strain showed high homology to C/Tokyo/4/2014 and C/Victoria/2/2102 (Figs. 1 and 2). Interestingly, Odagiri et al. reported that viruses isolated from the Philippines in 2011 to 2013 were related to C/Victoria/2/2012 [17]. Therefore, these strains are considered to be derived from a common ancestor and spread to South Korea.

In conclusion, this study reports the first identification of influenza C virus among young children and phylogenetic characteristics of a C/Seoul/APD462/2015 strain isolated in South Korea. Approximately 1.22% of young children with ILI negative for sixteen respiratory viruses during the influenza season were identified with influenza C

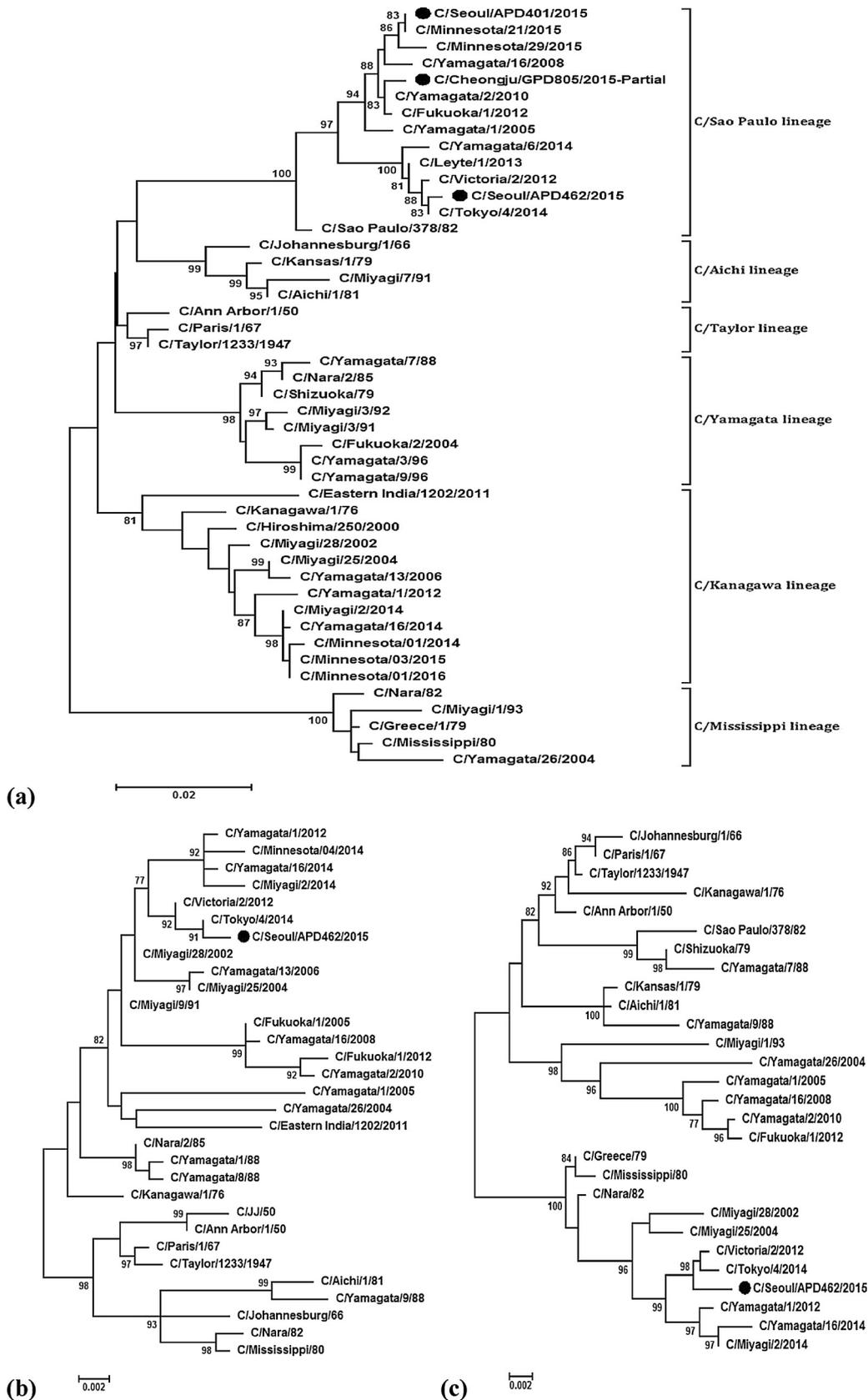


Fig. 1. Phylogenetic trees for seven segments of the influenza C virus. The nucleotide sequences of (a) HE, (b) M, (c) NP, (d) NS, (e) P3, (f) PB1, and (g) PB2 genes of influenza C virus were used for analysis. Phylogenetic trees were constructed using the maximum likelihood method with 1000 bootstrap replicates. The numbers above or below the branches are the bootstrap values (%) of each branch and values of < 75% are hidden. The South Korean strains are marked with black circles.

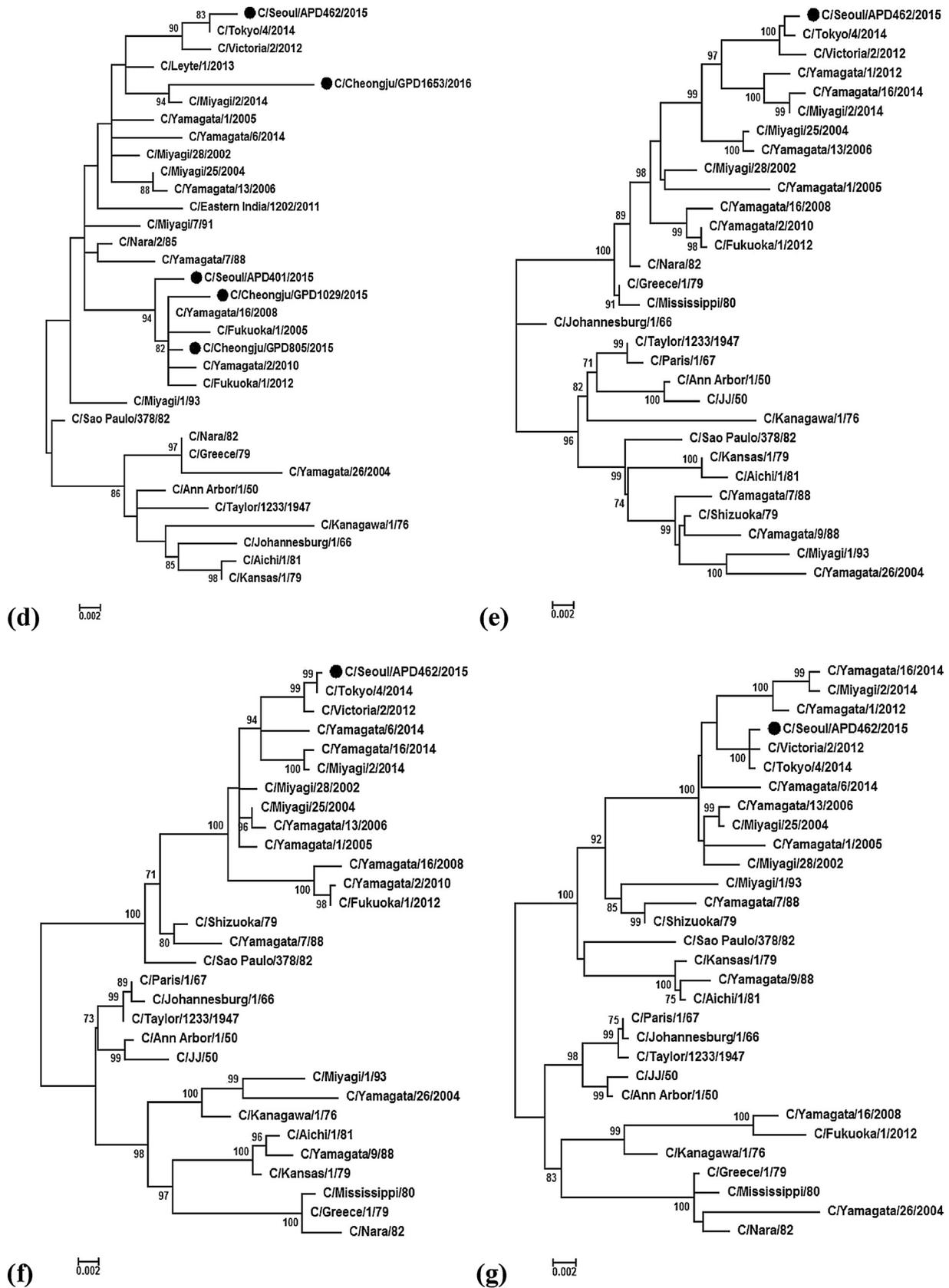


Fig. 1. (continued)

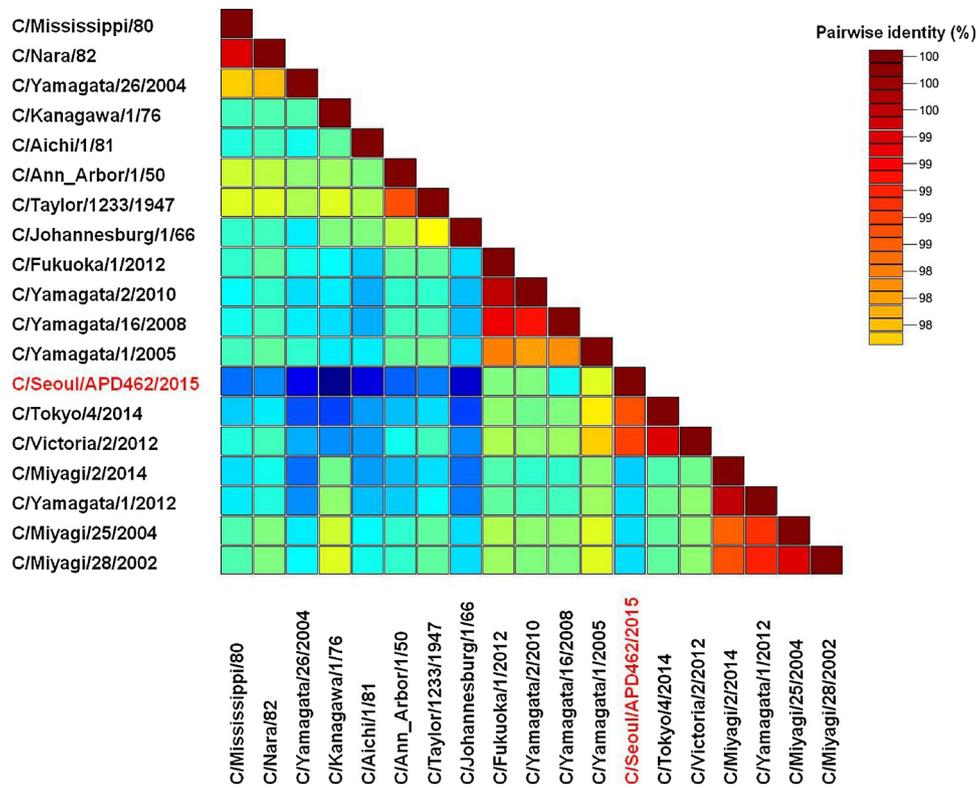


Fig. 2. All encoded protein sequence identity of influenza C viruses. The isolated virus strain was labeled in red color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2

Comparison of amino acid sequences among influenza C virus three strains, C/Victoria/2/2012, C/Tokyo/4/2014 and C/Seoul/APD462/2015.

Strain	HE		M		NP		NS		P3	PB2	PB1
	355	291	410	573	15	77	330	538			
C/Victoria/2/2012	T	A	T	K	V	L	I	S	I		
C/Tokyo/4/2014	A	A	T	T	I	P	I	S	V		
C/Seoul/APD462/2015	A	V	A	K	I	P	V	N	V		

virus infections. Influenza C virus infections in children are known to be capable of co-infections, but this study focused on identifying influenza C virus in samples negative for sixteen respiratory viruses. Considering the case of co-infections that we have not tested, it is assumed that the actual rate of influenza C virus infection among children will be high. Therefore, expanded investigations and serologic analysis of influenza C virus are needed to elucidate disease burden and epidemiology of influenza C virus.

Funding

This research was funded by a grant from Korea University Guro Hospital (Grant no. HI15C1665) and the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP) (NRF-2016R1A5A1010148, NRF-2018R1D1A1B07045711). This research was also supported by the TEPIK (Transgovernmental Enterprise for Pandemic Influenza in Korea), which is part of the Korea Healthcare Technology R&D Project funded by the Ministry of Health & Welfare, Republic of Korea (Grant no. A103001).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

online version, at doi:<https://doi.org/10.1016/j.jcv.2019.03.016>.

References

- [1] M. Homma, S. Ohyama, S. Katagiri, Age distribution of the antibody to type C influenza virus, *Microbiol. Immunol.* 26 (1982) 639–642, <https://doi.org/10.1111/mim.1982.26.7.639>.
- [2] H. Nishimura, K. Sugawara, F. Kitame, K. Nakamura, H. Sasaki, Prevalence of the antibody to influenza C virus in a northern Luzon Highland Village, Philippines, *Microbiol. Immunol.* 31 (1987) 1137–1143, <https://doi.org/10.1111/j.1348-0421.1987.tb01348.x>.
- [3] Y. Matsuzaki, N. Katsushima, Y. Nagai, M. Shoji, T. Itagaki, M. Sakamoto, et al., Clinical features of influenza C virus infection in children, *J. Infect. Dis.* 193 (2006) 1229–1235, <https://doi.org/10.1086/502973>.
- [4] C. Calvo, M.L. García-García, B. Borrell, F. Pozo, I. Casas, Prospective study of influenza C in hospitalized children, *Pediatr. Infect. Dis. J.* 32 (2013) 916–919, <https://doi.org/10.1097/INF.0b013e31828fca10>.
- [5] N. Principi, A. Scala, C. Daleno, S. Esposito, Influenza C virus-associated community-acquired pneumonia in children, *Influenza Other Respir. Viruses* 7 (6) (2013) 999–1003, <https://doi.org/10.1111/irv.12062>.
- [6] A.C. Dykes, J.D. Cherry, C.E. Nolan, A clinical, epidemiologic, serologic, and virologic study of influenza C virus infection, *Arch. Intern. Med.* 140 (1980) 1295–1298, <https://doi.org/10.1001/archinte.1980.00330210043021>.
- [7] R.J. O’Callaghan, R.S. Gohd, D.D. Labat, Human antibody to influenza C virus: its age-related distribution and distinction from receptor analogs, *Infect. Immun.* 30 (1980) 500–505.
- [8] M. Homma, S. Ohyama, S. Katagiri, Age distribution of the antibody to type C influenza virus, *Microbiol. Immunol.* 26 (1982) 639–642, <https://doi.org/10.1111/mim.1982.26.7.639>.
- [9] J.C. Manuguerra, C. Hannoun, M. Aymard, Influenza C virus infection in France, *J. Infect.* 24 (1992) 91–99, [https://doi.org/10.1016/0163-4453\(92\)91150-A](https://doi.org/10.1016/0163-4453(92)91150-A).
- [10] J.C. Manuguerra, C. Hannoun, C. Sáenz Mdel, E. Villar, J.A. Cabezas, Seroepidemiological survey of influenza C virus infection in Spain, *Eur. J. Epidemiol.* 10 (1994) 91–94, <https://doi.org/10.1007/BF01717459>.
- [11] Y. Matsuzaki, K. Sugawara, C. Abiko, T. Ikeda, Y. Aoki, K. Mizuta, et al., Epidemiological information regarding the periodic epidemics of influenza C virus in Japan (1996–2013) and the seroprevalence of antibodies to different antigenic groups, *J. Clin. Virol.* 61 (2014) 87–93, <https://doi.org/10.1016/j.jcv.2014.06.017>.
- [12] K.V. Atkinson, L.A. Bishop, G. Rhodes, N. Salez, N.R. McEwan, M.J. Hegarty, et al., Influenza C in Lancaster, UK, in the winter of 2014–2015, *Sci. Rep.* 7 (2017) 46578, <https://doi.org/10.1038/srep46578>.
- [13] S. Gouarin, A. Vabret, J. Dina, J. Petitjean, J. Brouard, D. Cuvillon-Nimal, et al., Study of influenza C virus infection in France, *J. Med. Virol.* 80 (2008) 1441–1446,

- <https://doi.org/10.1002/jmv.21218>.
- [14] A. Antón, M.A. Marcos, F.M. Codoner, P. de Molina, A. Martínez, N. Cardenosa, et al., Influenza C virus surveillance during the first influenza A (H1N1) 2009 pandemic wave in Catalonia, Spain, *Diagn. Microbiol. Infect. Dis.* 69 (2011) 419–427, <https://doi.org/10.1016/j.diagmicrobio.2010.11.006>.
- [15] C. Calvo, M.L. García-García, M. Centeno, P. Pérez-Breña, I. Casas, Influenza C virus infection in children, Spain, *Emerg. Infect. Dis.* 2 (2006) 1621–1622, <https://doi.org/10.3201/eid1210.051170>.
- [16] T. Roy Mukherjee, A. Mukherjee, S. Mullick, M. Chawla-Sarkar, Full genome analysis and characterization of influenza C virus identified in eastern India, *Infect. Genet. Evol.* 16 (2013) 419–425, <https://doi.org/10.1016/j.meegid.2013.02.014>.
- [17] T. Odagiri, Y. Matsuzaki, M. Okamoto, A. Suzuki, M. Saito, R. Tamaki, et al., Isolation and characterization of influenza C viruses in the Philippines and Japan, *J. Clin. Microbiol.* 53 (2015) 847–858, <https://doi.org/10.1128/JCM.02628-14>.
- [18] B.K. Thielen, H. Friedlander, S. Bistodeau, B. Shu, B. Lynch, K. Martin, et al., Detection of influenza C viruses among outpatients and patients hospitalized for severe acute respiratory infection, Minnesota, 2013–2016, *Clin. Infect. Dis.* 66 (2018) 1092–1098, <https://doi.org/10.1093/cid/cix931>.
- [19] Y. Matsuzaki, K. Sugawara, Y. Furuse, Y. Shimotai, S. Hongo, H. Oshitani, et al., Genetic lineage and reassortment of influenza C viruses circulating between 1947 and 2014, *J. Virol.* 90 (2016) 8251–8265, <https://doi.org/10.1128/JVI.00969-16>.
- [20] Y. Furuse, Y. Matsuzaki, H. Nishimura, H. Oshitani, Analyses of evolutionary characteristics of the hemagglutinin-esterase gene of influenza C virus during a period of 68 years reveals evolutionary patterns different from influenza A and B viruses, *Viruses* 8 (2016) 321, <https://doi.org/10.3390/v8120321>.
- [21] J.Y. Song, H.J. Cheong, S.H. Choi, J.H. Baek, S.B. Han, S.H. Wie, et al., Hospital-based influenza surveillance in Korea: hospital-based influenza morbidity and mortality study group, *J. Med. Virol.* 85 (2013) 910–917, <https://doi.org/10.1002/jmv.23548>.
- [22] K. Pabbaraju, S. Wong, A. Wong, J. May-Hadford, R. Tellier, K. Fonseca, Detection of influenza C virus by a real-time RT-PCR assay, *Influenza Other Respir. Viruses* 7 (2013) 954–960, <https://doi.org/10.1111/irv.12099>.
- [23] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, *Mol. Biol. Evol.* 30 (2013) 2725–2729, <https://doi.org/10.1093/molbev/mst197>.
- [24] J.Y. Noh, S. Lim, J.Y. Song, W.S. Choi, H.W. Jeong, J.Y. Heo, et al., Interim estimates of the effectiveness of the influenza vaccine against A(H3N2) influenza in adults in South Korea, 2016–2017 season, *PLoS One* 12 (2017) e0178010, <https://doi.org/10.1371/journal.pone.0178010>.
- [25] J.H. Nam, E.J. Song, D. Song, E. España, S.M. Shim, S.H. Jeong, et al., Molecular and phylogenetic analyses of influenza B viruses isolated from pediatric inpatients in South Korea during the 2011–2012 winter season, *J. Gen. Virol.* 98 (2017) 2950–2954, <https://doi.org/10.1099/jgv.0.000983>.