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Prevalence and clinical characteristics of human CoV-HKU1 in children with acute respiratory tract infections in China

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ABSTRACT

Background: Human CoV-HKU1 (HCoV-HKU1) has been isolated from a 71-year-old man with pneumonia; however, the impact and role of emerging HCoV-HKU1 have not been defined in children with acute respiratory tract infection (ARTI).
Objective: To investigate the Prevalence and clinical characteristics of HCoV-HKU1 in children with ARTI in Lanzhou, China.
Study design: The reverse transcription polymerase chain reaction (RT-PCR) or PCR was employed to screen HCoV-HKU1 and other common respiratory viruses in 645 nasopharyngeal aspirate (NPA) specimens collected from children with ARTI from November 2006 to October 2008. All PCR positive products were sequenced. And the demographic and clinical data were collected for all patients.
Results: Nineteen of 645 (2.95%) specimens tested positive for HCoV-HKU1, and all HCoV-HKU1 positive specimens were distributed in the winter and spring season. The HCoV-HKU1 co-infection rate with other respiratory viruses was 47.37% (9/19). There was no statistically significant difference in the detection rate between groups by age or gender, except between patients with and without underlying diseases. The phylogenetic analysis indicated that HCoV-HKU1 genotype B was circulating in the years 2007 and 2008 in children with ARTI in Lanzhou, China.
Conclusions: HCoV-HKU1 is an uncommon virus existing among Chinese children with ARTI. Children with underlying diseases are more vulnerable to viral infection. Only HCoV-HKU1 genotype B circulated locally.

1. Background

Human coronavirus (HCoV) are enveloped viruses with a single-stranded RNA genome. The HCoV 229E and OC43 subtypes were initially identified as causes of common colds in the 1960s, and occasionally pneumonia in young children, elderly individuals, and immunocompromised adults.[1–4] Recently three novel human coronavirus were identified, including severe acute respiratory syndrome coronavirus (SARS-CoV), human coronavirus Netherlands (HCoV-NL63), and human coronavirus Hong Kong (HCoV-HKU1).[5–8]

HCoV-HKU1 was first isolated in 2005 from a 71-year-old man with pneumonia.[6] Several successive reports confirmed retrospectively that HCoV-HKU1 was circulating worldwide.[9–12] However, the impact and the role of the emerging HCoV-HKU1 were not defined in children with ARTI. In China, there is limited epidemiological data about HCoV-HKU1 infection, especially concerning the prevalence and clinical characterization of HCoV-HKU1 in children with ARTI. In the present study, 645 NPA specimens were collected. The presence of HCoV-HKU1 and other common respiratory viruses was screened, and the epidemiological and clinical characteristics of HCoV-HKU1 were analyzed.

2. Objectives

The objective of this study was to investigate the prevalence and clinical characteristics of HCoV-HKU1 in Chinese children with ARTI.

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3. Study design

3.1. Patients and specimens

NPA samples were collected from 645 children with ARTI at the First Hospital of Lanzhou University, Gansu Province, China, between November 2006 and October 2008. All patients were 15 years of age or younger, informed consent was obtained from their parents. All NPA samples were collected 1–3 days after the onset of ARTI. Demographic data and clinical findings of these patients were collected on a standard collection form. The study protocol was approved by the hospital ethics committee.

3.2. Collection and processing of NPA samples

NPA samples were collected from patients by instilling 2 ml of virus preservation solution (200 U/ml penicillin, 200 U/ml streptomycin, 200 U/ml amphotericin B, and 0.25% BSA) through a nasopharyngeal mucous extractor. The NPAs were frozen at −80 °C until further processing. Viral DNA and RNA were extracted from 140 μl of each NPA specimen using the QIAamp viral DNA and the QIAamp viral RNA mini kits (Qiagen, Shanghai, China). cDNA was synthesized with Superscript II RH⁻ reverse transcriptase (Invitrogen, Carlsbad, CA, USA) using random hexamer primers.

3.3. Detection of HCoV-HKU1 and other common respiratory viruses

HCoV-HKU1 forward (5′-GGT TGG GAC GAT ATG TTA CGT CA-3′) and reverse (5′-CCATCA TCA CTC AAA ATC ATC ATA-3′) primers, which target the polymerase gene (pol) to amplify a 490 bp product, were used for HCoV-HKU1 screening as described previously.[13] PCR was performed under the following conditions: 95 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. A standard reverse transcription-PCR technique was used to screen for Respiratory syncytial virus (RSV), Human rhinovirus (HRV), Influenza viruses (IFV), Parainfluenza viruses (PIV), Human coronaviruses NL63 (HCoV-NL63), and PCR for Adenovirus (ADV).[14–16]

3.4. Sequencing and phylogenetic analysis

All positive PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced by SinoGenoMax Co. Ltd. (Beijing, China). Sequences were determined and analyzed using the DNAman software package. The pol gene partial sequences were deposited in GenBank under accession numbers (FJ937641–FJ937659). A neighbor-joining tree, which included the HCoV-HKU1 (NC-006577, DQ415914, DQ415911, DQ415912), HCoV-OC43 (NC-005147), HCoV-229E (NC-002645), HCoV-NL63 (AY567487), TGEV (NC-002306), FIPV (NC-007025), MHV (AF201929), BCoV (NC-003045), and SARS-CoV (AY274119) sequences, was constructed using the MEGA ver.3.1 program (www.megasoftware.net), and PEDV (NC-003436) was used as the outgroup.

3.5. Statistical analysis

The univariate associations were evaluated using Pearson's χ^2 for binary variables (with Fisher's exact method, when appropriate), the Mann–Whitney *U*-rank sum test for comparing continuous variables between subgroups, and the Kruskal–Wallace test for comparing continuous variables between subgroups.

4. Results

4.1. Patient characteristics

Approximately 53% (343/645) of the patients were male. The age of the children ranged from 1 day to 173 months, with a median age of 16 months. The majority of patients (569, 88.2%) were 5-years or younger. 75 (11.63%) patients had some underlying diseases. The ratio of outpatients to inpatients was 1:2.7 (173:472).

4.2. Detection and co-infections of viral agents

405 children (62.79%) tested positive for at least one pathogen. HCoV-HKU1 was detected in 19 of 645 (2.95%) specimens. 4 of 344 (1.16%) specimens tested positive from November 2006 to October 2007 and 15 of 301 (4.98%) specimens tested positive from November 2007 to October 2008.The difference in the positive rates between the two time periods was statistically significant ($\chi^2 = 8.196$, $P = 0.004$).

114 of 405 (28.15%) NPAs tested positive for two or more viral agents, and 74 were infected with two potential pathogens, 36 with three, and 4 with four potential pathogens. Nine HCoV-HKU1 co-infections (47.37%) were detected, six with RSV, one with HRV, one with RSV and IFVA, and one with HRV and ADV. RSV was the most common co-infection virus with HCoV-HKU1.

4.3. Epidemiology of HCoV-HKU1

HCoV-HKU1 was only detected from November to April during 2006 to 2008. Positive specimens peaked in November and January, with four and six positive specimens, respectively (Fig. 1). Children with HCoV-HKU1 varied in age from 1 day to 12-years (median age, 24 months) and 78.9% (15/19) of the patients were ≤5-years-of-age, but no statistically significant ($\chi^2 = 0.830$, $P = 0.362$) was revealed in the incidence rate between these two age groups (>5-years-of-age and ≤5). The incidence rate of boys is 2.91% (10/343) and the incidence rate of girls is 2.98 (9/302), No statistically significant ($\chi^2 = 0.002$, $P = 0.961$) was revealed between them.19 positive cases include 4 outpatients (2.3%) and 15 inpatients (3.2%). The difference in rates between them was not statistically significant ($\chi^2 = 0.332$, $P = 0.565$).

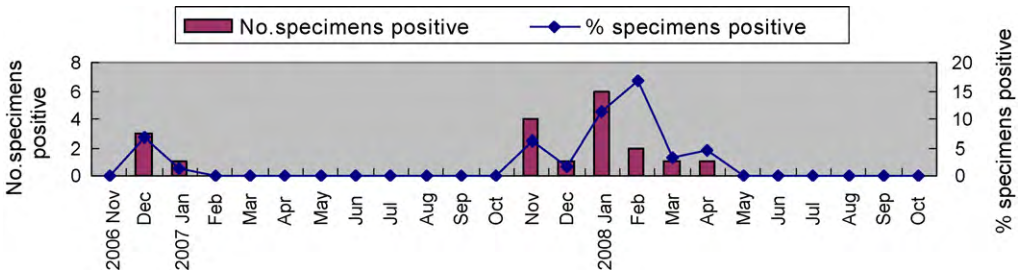

Fig. 1. Seasonal distribution of HKU1 in children with acute respiratory tract infection; November 2006–October 2008.

4.4. Clinical characteristics of HCoV-HKU1 infection

The main clinical diagnoses of HCoV-HKU1 positive patients included AURI (5, 26.3%), bronchitis (1, 5.2%), bronchopneumonia (5, 26.3%), acute asthmatic bronchopneumonia (6, 31.6%), and pneumonia (2, 10.5%). The clinical presentations of HCoV-HKU1 positive children included cough (74.7%), fever (68.4%), sputum production (73.7%), crackles (78.9%), wheezing (31.6%), vomiting (5.3%), and diarrhea (10.5%). The hospital stay for the 15 inpatients ranged from 4 to 18 days (mean, 10.1 ± 4.02 days). None of these patients died or required intensive care.

There were no statistically significant differences in the clinical symptoms between HCoV-HKU1 positive and all common respiratory viruses negative groups (subgroup 1), between HCoV-HKU1 mono-infection and co-infection groups (subgroup 3), and between HCoV-HKU1 mono-infection and co-infection with RSV groups (subgroup 4) (Table 1).

Six of 19 (31.58%) HCoV-HKU1 positive patients had an underlying illness, and they were all inpatients (hospital stay, 4–13 days; mean, 11.2 days). Nine inpatients (hospital stay, 5–13 days; mean, 9.2 days) and four outpatients had no underlying illness. There was a statistically significant difference in the detection rate between the two groups with and without underlying illnesses (subgroup 2; $P = 0.042$). However, no statistically significant difference was found for the hospital stay duration or other clinical symptoms (Table 1). Among the six HCoV-HKU1 positive children with underlying illnesses, three had one underlying disease, which included pyelonephritis, persisting enteritis, or endocardial fibroelastosis. The others had two underlying diseases, including acute benign lymphoblastosis, sensitization dermatitis, interventricular septal defect, nephrotic syndrome, patent ductus arteriosus and allergic renopathy.

4.5. Phylogenetic analysis of HCoV-HKU1

The HCoV-HKU1 pol gene sequences shared 95.8–99.6% nucleotide identity and 90.7–99.3% amino acid identity with HCoV-HKU1 strain N15 (No. DQ415911). Moreover, the nucleotide and amino acid sequence identities were 98.4 and 96.3%, respectively, among the 19 HCoV-HKU1 positive strains. The phylogenetic analysis revealed that they all clustered with the HCoV-HKU1 strain N15 (Fig. 2).

5. Discussion

In the present study, a 2.95% incidence rate for HCoV-HKU1 infection was found over 2 years from 645 children with ARTIs. A similar incidence rate has been reported in Hong Kong,[17] Italy,[18] and Australia,[9] but it was different from other studies.[10–12] Furthermore, the incidence rate was statistically significant between 2006–2007 and 2007–2008 (1.16% vs 4.98%), indicating probable different prevalence strength for HCoV-HKU1 during each year. The periodicity of coronavirus infection is also described in other reports.[4,8] The majority of HCoV-HKU1 positive patients (78.9%, 15/19) were ≤5-years-of-age, which indicated that young children are at high risk of HCoV-HKU1 infection. HCoV-HKU1 was only detected from November to April in this study, with a peak in January and February, which agrees with USA, Hong Kong, and Italian reports.[12,17,18] However, HCoV-HKU1 was detected during all four seasons in other reports.[9,11,12,18,19] This indicates the epidemiological patterns of HCoV-HKU1 may be different in different regions.

Approximately 47% of HCoV-HKU1 positive patients were co-infected with other respiratory viruses in this study, which is higher than that in previous studies.[9,18] RSV was the most common additional virus (77.78%, 7/9). Co-infections leading to more

Table 1
Clinical characteristics of the infants infected with respiratory viruses detected in this study.

Characters	Group1	Group2	Group3	Group4	Group5	Group6	Group7	*P*-value				
	HCoV-HKU1 positive	NPA-negative[a]	With underlying illness	Without underlying illness	Mono-infection	Co-infection	HKU1 +RSV	1	2	3	4	
								Group1 vs. Group2	Group3 vs. Group4	Group5 vs. Group6	Group5 vs. Group7	
No. of children	19	240	6	13	10	9	7		0.042[b]			
Male/female	10/9	149/91	5/1	5/8	5/5	5/4	4/3	0.415[b]	0.185[b]	1[c]		0.772[c]
Age ≤ 3 years	11	172	2	9	3	7	5	0.203[b]	0.330[b]	0.105[c]		0.234[c]
Outpatients/inpatients	4/15	73/167	0/6	4/9	7/13	8/1	7/1	0.390[b]		0.656[c]		0.751[c]
Days in hospital (median)	4–13 (11.2)	5–17 (8.2)	4–13 (11.2)	5–13 (9.2)	6–18 (11)	4–16 (9)	4–16 (9.8)	0.234[d]	0.478[d]	0.270[d]		0.566[d]
Clinical diagnosis												
URI	5	84	0	5	3	2	1	0.443[b]		1		0.864[c]
LRI	14	156	6	8	7	7	6			1		0.452[c]
Clinical manifestations												
Fever	13	198	5	8	8	5	3	0.225[c]	0.675[c]	0.515[c]		0.288[c]
Cough	18	214	6	12	10	8	7	0.708[c]				
Wheeze	6	68	1	5	1	5	4	0.763[b]	0.675[c]	0.101[c]		0.119[c]
Rhinorrhea	7	79	3	4	4	3	2	0.727[b]	0.767[c]	1[c]		1[c]
Sputum production	14	146	5	9	10	4	3	0.153[b]	0.929[c]			
Crackles	13	203	5	8	6	7	6	0.133[c]	0.675[c]	0.735[c]		0.546[c]
Diarrhea	2	14	2	0	0	2	2	0.747[c]				

[a] Cases negative for all viruses tested including Respiratory syncytial virus(RSV), Human rhinovirus, Influenza viruses, Parainfluenza viruses, Adenovirus Human coronaviruses NL63, and Human coronaviruses HKU1; URI upper respiratory tract infection LRI lower respiratory infection.
[b] Pearson's χ^2.
[c] χ^2 test (continuity correction).
[d] Mann–Whitney test.

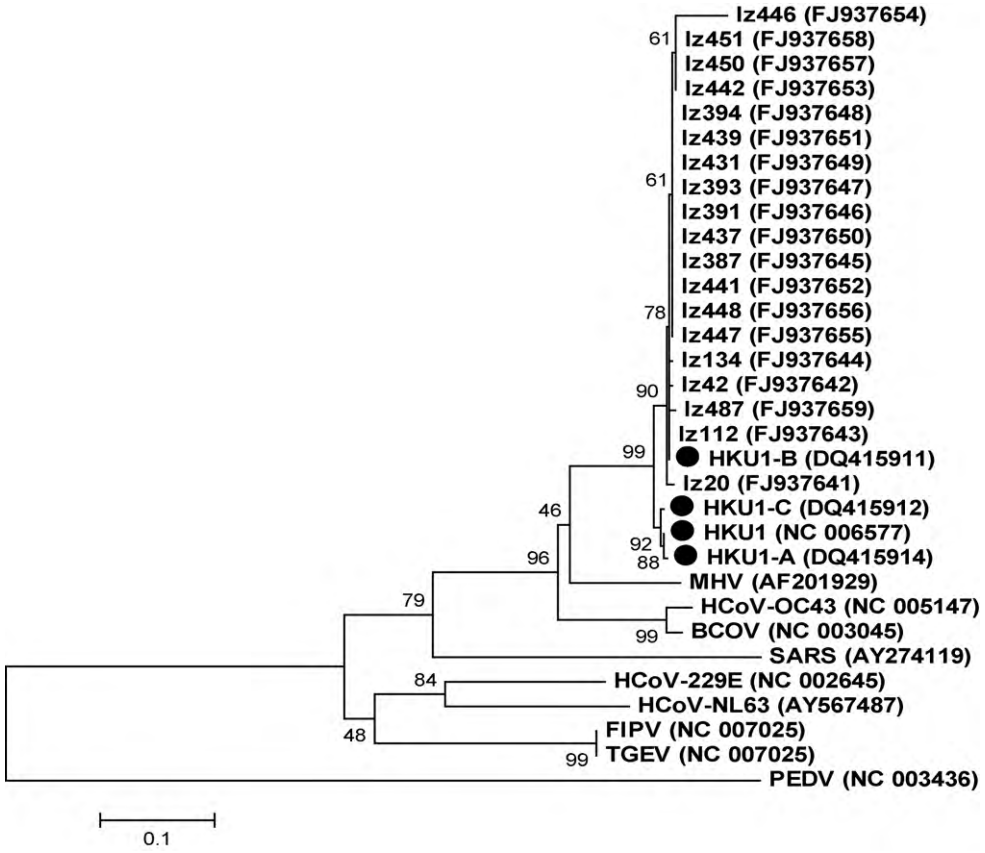

Fig. 2. Phylogenetic analysis of the partial polymerase gene HCoV-HKU1 nucleotide sequences (455 bp). Phylogenetic trees were constructed by the neighbor-joining method using MEGA 3.1. Bootstrap values were determined with 1000 replicates. Viral sequences in bold were generated from the present study, and other reference sequences were obtained from GenBank. Bootstrap values are shown at each branching point. The sequences generated from the present study were deposited in the GenBank under accession numbers.

severe diseases have been described for RSV and coronaviruses.[20] However, in this study no significant difference was revealed in the clinical symptoms, outpatient/inpatient department, and hospital stay duration between the mono-infection group and the co-infection group or the co-infection with RSV group in HCoV-HKU1 infected patients (Table 1).

It was reported that HCoV-HKU1 was less often associated with lower respiratory tract diseases than other HCoV types.[11,18] However, Esper et al. reported that HCoV-HKU1 might contribute to upper and lower respiratory tract infections in children.[12] In the present study, no significant difference in the incidence rate was observed between the upper and lower respiratory tract diseases ($P = 0.928$). Crackles, cough, sputum production, and fever were the most common symptoms of these patients. These symptoms resemble those reported for children in Italy[18] and the USA.[12] Additionally, Lau's study reported that HCoV-HKU1 may play a role in epileptic seizures.[19] However, in this study, no epileptic seizures occurred. The exact clinical association and potential neurotropism of HCoV-HKU1 infection remains to be addressed.

HCoV-HKU1 was first detected in patients with underlying illnesses.[6] A part of positive patients was reported with underlying diseases in the subsequent studies.[11,12,19] In our study, 6 of 19 positive patients had at least one underlying disease (Table 1). Interestingly, a significant difference in the incidence rate ($P = 0.042$) was observed between HCoV-HKU1patients with and without underlying disease. But no significant difference was observed between the two groups for the number of hospital stay days or clinical symptoms. Additionally, the incidence rate for HCoV-HKU1 infection between outpatients and inpatients was not significantly different (Table 1). These results indicated that patients with underlying diseases may be more prone to HCoV-HKU1 infection, but that HCoV-HKU1 infection did not aggravate the clinical symptoms and did not contribute to the hospitalization rate or hospital stay duration. There were three patients with cardiovascular diseases and three with kidney diseases, which ranked first in terms of underlying diseases. Further study is needed to investigate whether cardiovascular and kidney diseases are major risk factors for HCoV-HKU1 infection.

Three HCoV-HKU1 genotypes (A, B and C) have been reported[21] and no correlation was observed between the HCoV-HKU1 genotype and clinical syndromes or symptoms.[18] The partial pol gene in our study displayed minor sequence differences with human coronavirus HCoV-HKU1 strain N15, which belongs to genotype B. The phylogenetic analyses showed that the 19 HCoV-HKU1 strains were all classified into the HCoV-HKU1 genotype B lineage. This result indicated that a single cluster of HCoV-HKU1 genotype B was circulating locally. One previous study from Australian also reported genotype B was dominant locally.[9] But In Italian and Hong Kong genotype A, B, and C were all circulating.[18,21]

In conclusion, we showed the prevalence and clinical characteristics of HCoV-HKU1 in a pediatric population with ARTI in China. HCoV-HKU1 was detected in 19 of 645 (2.95%) children. 47.37% of them were coinfected with other respiratory viruses with RSV the most common coinfection virus. The most common symptom and clinical diagnoses with HCoV-HKU1 infection were crackles and acute asthmatic bronchopneumonia. A single HCoV-HKU1 genotype B was circulating locally in the years 2007 and 2008. Our study indicated that patients with underlying disease may be more prone to HCoV-HKU1 infection with no impact on clinical symptoms, hospitalization rate or hospital stay duration.

Conflict of interest

None of the authors has a conflict of interest.

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References

1. Kendall EJ, Bynoe ML, Tyrrell DA. Virus isolations from common colds occurring in a residential school. *Br Med J* 1962;**5297**:82–6.
2. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med* 1966;**121**:190–3.
3. Talbot HK, Crowe JE, Edwards KM, Griffin MR, Zhu Y, Weinberg GA, et al. Coronavirus infection and hospitalizations for acute respiratory illness in young children. *J Med Virol* 2009;**81**:853–6.
4. Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974;**129**(3):271–6.
5. Drosten C, Gunther S, Preiser W, Van der Werf S, Brodt HR, Becker S, et al. *Identification of a novel coronavirus in patients with severe acute respiratory syndrome* 2003;**348**:1967–76.
6. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;**79**:884–95.
7. Fouchier RA, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci USA* 2004;**101**:6212–6.
8. Lia van der H, Gabriele I, Klaus S, Astrid V, Ronald D, Michel DV, et al. Burden of disease due to human coronavirus NL63 infections and periodicity of infection. *J Clin Virol* 2010;**48**(2):104–8.
9. Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKUI and human bocavirus in Australian children. *J Clin Virol* 2006;**35**:99–102.
10. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES. Detection of viruses identified recently in children with acute wheezing. *J Med Virol* 2007;**79**:1238–43.
11. Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. Detection of the new human coronavirus HKUI: a report of 6 cases. *Clin Infect Dis* 2006;**42**: 634–9.
12. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Coronavirus HKUI infection in the United States. *Emerg Infec Dis* 2006;**12**:775–9.
13. Duan ZJ, Huang CP, Qu XW, Xie ZP, Qi ZY, Gao HC, et al. Phylogenetic and sequence analyses of the N and S genes of coronavirus HKU1, a novel human coronavirus in Mainland China. *Chin J Virol* 2006;**22**:241–7.
14. Bastien N, Robinson JL, Tse A, Lee BE, Hart L, Li Y. Human coronavirus NL-63 infections in children: a 1-year study. *J Clin Microbiol* 2005;**43**:4567–73.
15. Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, et al. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. *J Virol Methods* 2005;**126**:53–63.
16. Hierholzer JC, Halonen PE, Dahlen PO, Bingham PG, McDonough MM. Detection of adenovirus in clinical specimens by polymerase chain reaction and liquid-phase hybridization quantitated by time-resolved fluorometry. *J Clin Microbiol* 1993;**31**:1886–91.
17. Woo PC, Lau SK, Tsoi HW, Huang Y, Poon RW, Chu CM, et al. Clinical and molecular epidemiological features of coronavirus HKUI-associated community-acquired pneumonia. *J Infect Dis* 2005;**192**:1898–907.
18. Gerna G, Percivalle E, Sarasini A, Campanini G, Piralla A, Rovida F, et al. Human respiratory coronavirus HKU1 versus other coronavirus infections in Italian hospitalised patients. *J ClinVirol* 2007;**38**:244–50.
19. Lau SK, Woo PC, Yip CC, Tse H, Tsoi HW, Cheng VC, et al. Coronavirus HKU1 and other coronavirus infection in Hong Kong. *J Clin Microbiol* 2006;**44**:2063–71.
20. Templeton KE, Scheltinga SA, van den Eeden WC, Graffelman AW, van den Broek PJ, Claas EC. Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. *Clin Infect Dis* 2005;**41**(3):345–51.
21. Woo PC, Lau SK, Yip CC, Huang Y, Tsoi HW, Chan KH, et al. Comparative analysis of 22 coronavirus HKU1 genomes reveals a novel genotype and evidence of natural recombination in coronavirus HKU1. *J Virol* 2006;**80**:7136–45.