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Molecular monitoring of causative viruses in child acute respiratory infection in endemo-epidemic situations in Shanghai

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Abstract

Background: Numerous viruses are responsible for respiratory infections; however, both their distribution and genetic diversity, in a limited area and a population subgroup, have been studied only rarely during a sustained period of time.

Methods: A 2-year surveillance program of children presenting with acute respiratory infections (ARIs) was carried out to characterize the viral etiology and to assess whether using gene amplification and sequencing could be a reliable approach to monitor virus introduction and spread in a population subgroup.

Results: Using multiplex RT-PCR, 15 different respiratory viruses were detected within the 486 nasopharyngeal positive samples collected among 817 children aged <9-year old who presented with ARI during October 2006 to September 2008. A single virus was detected in 373 patients (45.7%), and two to four viruses in 113 patients (13.8%). The most frequent causative viruses were respiratory syncytial virus (RSV) (24.7%), human bocavirus (24.5%), and human rhinovirus (HRV) (15%). RSV was more prevalent in winter and among young infants. Cases of seasonal influenza A and B viruses were reported mainly in January and August. An increase in adenovirus infection was observed during the spring of the second year of the study. Sequence analyses showed multiple introductions of different virus subtypes and identified a high prevalence of the newly defined HRV-C species. A higher viral incidence was observed during the winter of 2008, which was unusually cold.

Conclusions: This study supports the usefulness of multiplex RT-PCR for virus detection and co-infection, and for implementation of a molecular monitoring system for endemic and epidemic viral respiratory infections.

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1. Background

Acute respiratory infections (ARIs) are the leading cause of pediatric morbidity worldwide. Many viruses are associated with ARIs: influenza viruses A, B and C (IAV, IBV and ICV); respiratory syncytial virus (RSV); human metapneumovirus (HMPV); human coronaviruses (HCoV) NL63, 229E, OC43, and HKU1; parainfluenza viruses (PIV) 1–4; human rhinovirus (HRV); human enterovirus (HEV); and adenovirus (ADV). A new pathogen, human bocavirus (HBoV) has been shown to be associated with respiratory illnesses, mainly when it is present at a high viral load.

Since the epidemic of severe acute respiratory syndrome (SARS) in 2003, and the recent attention on possible influenza pandemics, sustained surveillance project was required to detect endemic, epidemic and newly emerging respiratory pathogens.

The diagnosis of respiratory viruses mainly relies on molecular techniques. Multiplex RT-PCR (mRT-PCR) techniques allow identification of a majority of respiratory viruses as well as co-infections.

2. Objectives

In the present 2-year study, we used a five-tube mRT-PCR assay we implemented in the Pasteur Institute network in the Asian region, which covered 17 common respiratory viruses, to identify viruses in nasopharyngeal specimens in 817 children with
ARI. Sequencing primers specific to other fragments of viral genes were employed to amplify the positive samples for sequencing and phylogenetic analysis. The sequences showed the genetic variation of viruses circulating in the region, and identified the virus evolution and introduction of new variants. This study allowed cartography of viral etiology of a large panel of viruses and variation of viruses circulating in the region, and identified the

| Criteria                                | Inclusion                                                                 | Exclusion                                                                 |
|-----------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Children younger than 9-year old, first onset within 48 h, AND Fever (T ≥ 38 °C) plus cough and/or sore throat AND/OR Dyspnoea or tachypnoea, cyanosis, cough, pleuritic chest pain, hypoxemia AND Signature of the patient consent agreement | Subjects already under antiviral treatment for any prophylactic or curative purpose |
Virus | Primer name | Sequence 5' – 3' | Region | Position | Reference |
|-----|-------------|-----------------|--------|----------|-----------|
| 229E | 229E/SPKIE-F | CTACAAATGGCGTGAACACTAGYACTC | Spike | 53–80 | This study |
| 229E | 229E/SPKIE-R | TACGCGTGTAACAGCAATTATA | | 1679–1702 | This study |
| NL63 | NL63/SPKIE-F | GAGTGGTTTAAACAGCTGTTTC | Spike | 20391–20415 | AY567487 |
| NL63 | NL63/SPKIE-R | AACACGCCGAGAACACTATCG | | 21049–21073 | This study |
| HKU-1 | HKU1-1-F | CTACACCCCTCAATTGGGAAG | | 23920–23939 | DQ415914 |
| HKU1-1-R | CAACCGCTAAGGAACACACTAT | | | 25067–25045 | This study |
| PIV1 | PIV1-HNP | CAACCGCTAAGGAACACACTAT | | 1–24 | M91648 |
| PIV1 | PIV1-HNR | TCTATTTCTGATATAATATACCTTATCGTC | | 1835–1864 | This study |
| PIV3 | PIV3-HNF | AAATCGGAGATCCTCCTATAT | | 7522–7545 | EU424062 |
| PIV3 | PIV3-HNR | GCCTGGTTCAGACAATATR | | 7946–7956 | This study |
| PIV4 | PIV4-HS | GAAGACGTCCTGAC | | 65–81 | M34033-4A |
| PIV4 | PIV4-H-ANTI | GAAGACTGTTATGTGCATATGAC | | 1417–1439 | This study |
| ADV | ADHEX1F | CAACGCTATGACATAGCAATCA | | 19002–19022 | FJ169625 |
| ADV | ADHEX2R | ACATCCTTCAACTTTG | | 19225–19574 | This study |
| HMPV | F698 | ACATGCCCCAACCTTCGACACAAATACAC | Fusion | 698–727 | EU857610 |
| | F1285 | ACATGCTGCACCTCAGGTAAC | | 1282–1307 | This study |
| | B/Ha98 | ATAACATTGCAACACATTAC | HA | 64–83 | EU852039 |
| | B/Ha936 | GACACATTGAACTGACACAA | | 780–799 | This study |
| | B/NA1 | GCTACCTTCAACATACATACAA | NA | 3–24 | EU852040 |
| | B/NA2 | AACAGGGAGTTCACCTCACTCC | | 233–235 | This study |
| | 43f | GCTGGTGTGGCTGTTTACAAAATCTTCTC | | 35–59 | EU716524 |
| | 1129r | GAAATTTTCATGCCCTAAACGTC | | 1097–1121 | This study |
| | 32f | GATTGCGTCTGCTCTTCTCC | NA | 27–48 | CY031565 |
| | 984r | CTGGGCTGGTCTCCACACACTTG | | 956–980 | This study |
| HRV | P1-1 F | CAAGCTCTTCACTTGCTGAC | 5'UTR | 163–1681 | L24917 |
| | P1-1 R | ACACAGACCAAAAATGAC | | 536–552 | This study |
| | VP4/2 F | GGGACCAACTCTGGTCTTCCTC | VP2/4 | 528–554 | This study |
| | VP4/2 R | GCAGTGGYRYYTCCACACCCANCC | | 1061–1086 | This study |
| RSV | RSV-GLYCO-AF | ATATCATATGACATCGGACCAACAA | Glycoprotein | 4833–4856 | NC_001803 |
| | RSV-GLYCO-AR | ACTCTCAAGGTCCTGAACACTATAT | | 5186–5132 | This study |
| | RSV-GLYCO-BF | TATTCATATGACATCGGACCAACAA | | 4868–4890 | NC_001781 |
| | RSV-GLYCO-BR | ACTCTCAAGGTCCTGAACACTATAT | | 5176–5150 | This study |

4. Results

A viral etiology could be determined in 486 of the 817 (59.5%) patients. Multiple viral infections were detected in 113 (13.8%) patients (94 with two pathogens, 18 with three pathogens, and one with four pathogens) (Table 3). Among 486 virus-positive cases, 346 (71.2%) were diagnosed by clinicians as bronchitis, 135 (27.8%) as pneumonia, and 5 (1.0%) were diagnosed with upper respiratory tract infection.

Overall, 618 viral pathogens were detected: RSV was the most frequent pathogen (n = 120, 19.4%). The second to the fifth most frequent pathogens were HBoV (n = 119, 19.3%); IV (IAV and IBV) (n = 108, 17.5%); PIV1, 3 or 4 (n = 77, 12.5%), and HRV (n = 73, 11.8%). HMPV (n = 44, 7.1%), ADV (n = 43, 7.0%), HCoV-OC43, 229E, NL63 or HCoV-NL63 was detected during the summer of 2007. HRV, PIV and HMPV were detected continuously throughout the year.

4.1. Seasonality of viruses

The monthly distribution of viruses is presented in Figs. 1 and 2. Viruses were detected significantly more often during fall or winter than during other seasons (71% and 49%, respectively, p < 0.01). RSV and HBoV identifications were most frequent in the fall and winter. IV showed a biannual distribution, one peak in winter and another in the summer. One important increase in ADV identification from March to June in 2008 was recorded. Twenty-two ADV strains among 118 samples were detected compared to five strains in 52 samples in the same period of 2007. A small number of HCoV-NL63 was detected during the summer of 2007. HRV, PIV and HMPV were detected continuously throughout the year.

4.2. Impact of age distribution

Patients included in this study were aged from 1 month to 9 years. The median age was 3 years. RSV was more frequent in younger children (p = 10−7) with 12 out of 23 virus-positive cases from 6 months to 2 years age group. No IAV or IBV infection was detected in children younger than 1-year old (Table 4). Only 8 patients from 1 to 6 months age group were enrolled in the study.

4.3. Prevalent types/subtypes of viruses

To identify the prevalent subtype of different viruses and the similarity of the virus strains, virus-positive samples were sequenced for target genes, when the amount of genetic material amplified was sufficient (Table 5), and phylogenetic trees were constructed (data not shown; dendrograms are available on request to W. Wang et al. / Journal of Clinical Virology 49 (2010) 211–218).
the authors). Identity among isolates of each virus type or subtype was calculated by pairwise algorithm and their nearest reference strains are shown in Table 4.

In 40 sequenced ADV strains, 37 were species B (92.5%) and three were species C (7.5%). Most of them were similar to serotype ADV-2 (data not shown). In 65 HRV strains, 25 were species A (38.5%), five were species B (7.7%), and 35 were species C (53.8%). HRV showed high variation in nucleotide identity (73–100% in HRV-A, 75–82% in HRV-B, and 68–100% in HRV-C) as previously described (Huang et al.).26 Among 37 IAV strains, 16 were seasonal H1N1 (95–99% nucleotide identity in the HA gene) and 21 were seasonal H3N2 (92–99% identity) strains. In 36 out of 55 IBV strains, HA and NA gene fragments were sequenced, and comparison showed that the IBV strains were more conserved in the NA gene (96–100% nucleotide identity) than HA gene (87–99% identity). Only 17 out of 119 HBoVs were sequenced but showed high nucleotide identity in the ST2 gene as previously described.7 The sequences of HCoVs were highly conserved (94–100% nucleotide identity in HCoV-NL63, 99% in HCoV-229E). In 39 HMPV strains, 16 were genotype B1 (41%), 16 were genotype B2 (41%), and seven were genotype A1 (17.9%) with high nucleotide identity (98–99% in A1 and 97–100% in B1 and B2). Only 27 out of 120 RSVs were sequenced and nucleotide sequence analysis of a glycoprotein gene fragment showed all of the strains were classified into RSV A subtype (data not shown). The results indicated a low variability of these viruses that circulated in the region during the 2-year period.

### 4.4. Clinical features

Among 486 virus-positive cases, 483 (99.4%) patients presented with high fever (>38°C) and cough, 25 (5.1%) patients with dys-

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**Table 3**

Viral etiology of ARI in 817 outpatient children, Shanghai Nanxiang Hospital, October 2006 to September 2008.

| Virus          | Virus detection | Total virus strains |
|----------------|-----------------|---------------------|
|                | Single infection| Co-infection        |
| Human RSV      | 71              | 49                  | 120                 |
| IV             | 80              | 26                  | 108                 |
| IAV            | 44              | 9                   | 53                  |
| IBV            | 36              | 19                  | 55                  |
| HMPV           | 33              | 11                  | 44                  |
| PIV            | 52              | 25                  | 77                  |
| PIV1           | 27              | 18                  | 45                  |
| PIV3           | 23              | 6                   | 29                  |
| PIV4           | 2               | 1                   | 3                   |
| HRV            | 43              | 30                  | 73                  |
| HEV            | 5               | 6                   | 11                  |
| HCoV           | 17              | 7                   | 24                  |
| HCoV-OC43      | 1               | 0                   | 1                   |
| HCoV-229E      | 1               | 4                   | 5                   |
| HCoV-NL63      | 14              | 3                   | 17                  |
| HCoV-HKU1      | 1               | 0                   | 1                   |
| ADV            | 18              | 25                  | 43                  |
| HBoV           | 54              | 65                  | 119                 |

a Case number.

**Table 4**

Frequencies of viral pathogens (per age group).

|                     | ≤6 months | 6 months–1 year | 1–2 years | 2–4 years | 5–9 years | All ages |
|---------------------|-----------|-----------------|-----------|-----------|-----------|----------|
|                     | n=8       | n=31            | n=121     | n=449     | n=208     | n=817    |
| Single infection    | 4a 50b    |                 | 37 30.6   | 236 52.8  | 85 40.9   | 373 45.7 |
| Co-infection        | 0         | 12 38.7         | 36 29.8   | 38 8.5    | 27 13 113 | 113 13.8 |
| PCR negative        | 4 50      | 8 25.8          | 48 39.7   | 175 39    | 96 46.2   | 331 40.5 |
| RSV                 | 0         | 12 52.2         | 17 23.3   | 71 25.9   | 20 17.9   | 120 24.7 |
| IV (any)            | 0         | 3 13            | 11 15.1   | 56 20.4   | 38 33.9   | 108 22.2 |
| IAV                 | 0         | 5 6.8           | 31 11.3   | 17 15.2   | 17 5.9    | 53 10.9  |
| IBV                 | 0         | 3 13            | 6 8.2     | 25 9.1    | 21 18.8   | 55 11.3  |
| HMPV                | 1c 12.5d  | 2 8.7           | 6 8.2     | 28 10.2   | 7 6.3     | 44 9.1   |
| PIV (any)           | 0         | 5 21.7          | 10 13.7   | 49 17.9   | 13 11.6   | 77 15.8  |
| Type 1              | 0         | 1 4.3           | 7 9.6     | 29 10.6   | 8 7.1     | 45 9.3   |
| Type 3              | 0         | 2 8.7           | 3 4.1     | 19 6.9    | 5 4.5     | 29 6     |
| Type 4              | 0         | 2 8.7           | 0 1.0     | 0 0.0     | 3 0.6     |
| HRV                 | 2 25      | 4 17.4          | 13 17.8   | 37 13.5   | 17 15.2   | 73 13.3  |
| HCoV (any)          | 0         | 2 2.7           | 7 2.6     | 2 1.7     | 11 2.3    |
| OC43                | 0         | 3 4.1           | 17 6.2    | 4 3.6     | 24 4.9    |
| 229E                | 0         | 0 1             | 0 0.4     | 0 1       | 3 0.2     |
| NL63                | 0         | 0 1             | 4 1.5     | 1 0.9     | 5 1       |
| HKU1                | 0         | 2 2.7           | 12 4.4    | 3 2.7     | 17 3.5    |
| ADV                 | 0         | 1 4.3           | 0 0.0     | 0 1       | 1 0.2     |
| HBoV                | 1 12.5    | 4 17.4          | 27 37     | 61 22.3   | 26 23.2   | 119 24.5 |

a Case number.
b Case percentage in group.
c Detected virus.
d Percentage of detected virus in virus infected cases of each group.
Table 5
Genetic variation of circulating viruses.

| Virus          | Detected | Sequenced | Gene   | Species | Genotype/type | Number | Nucleotide identity % among strains | Reference strain         |
|----------------|----------|-----------|--------|---------|---------------|--------|-------------------------------------|--------------------------|
| HMPV           | 44       | 39        | F      | A1      | 7             | 98–99  | EU698012, EU179277                  |
|                |          |           |        | B1      | 16            | 97–100 | EU698017                            |
|                |          |           |        | B2      | 16            | 97–100 | EF694069                            |
|                |          |           |        | H1      | 16            | 95–99  | CY031379                            |
|                |          |           |        | H3      | 21            | 92–99  | CY040098, CY044788                  |
| IAV            | 53       | 37        | HA     | A1      | 16            | 97–100 | EU698017                            |
|                |          |           |        | B1      | 16            | 97–100 | CY031546, EU982188                  |
|                |          |           |        | B2      | 16            | 97–100 | CY040451, GQ423424                  |
| IBV            | 55       | 36        | NA     | A1      | 25            | 95–99  | CY031379                            |
|                |          |           |        | B1      | 5             | 75–82  | CY031379                            |
|                |          |           |        | B2      | 35            | 68–100 | DFJ473480, E1973424                 |
| HRV            | 73       | 65        | VP4    | A       | 25            | 73–100 | EU948071, EU948040                  |
|                |          |           |        | B       | 5             | 75–82  | CY040098, CY044788                  |
|                |          |           |        | C       | 35            | 68–100 | DFJ473480, E1973424                 |
| ADV            | 43       | 40        | Hexon  | B       | 37            | 94–100 | AY819918, AY819919                  |
|                |          |           |        | C       | 3             | 86–98  | AY819936                            |
|                |          |           |        | Type 2  |               | 94–100 | DQ445912                            |
| HCoV-NL63      | 17       | 13        | Spike  |         |               | 99     | AF304460                            |
| HCoV-229E      | 5        | 5         | Spike  |         |               | 99     | AF304460                            |
| HBoV           | 119      | 17        | VP2    | Type 1  | 17            | 99.3   | DQ000496                            |
| RSV            | 120      | 27        | Glycoprotein | 27     | 94–100     | AFS12538                           |

pneoa or tachypnea and 46 (9.4%) patients with lymphopenia. Although 346 (71.2%) were clinically diagnosed as bronchitis, 135 (27.8%) were pneumonia and 5 (1.0%) were diagnosed with upper respiratory tract infection by the clinician, we considered that only 25 patients met the severe respiratory infection as showing dyspnoea or tachypnea symptoms. One hundred twenty patients (22.6%) were suggested to be hospitalized after their first consulting but none became inpatients. No correlation was observed between infection with any specific virus (single or co-infection) and clinical severity (Table 6A and B). In addition, the repartition of the different symptoms was statistically insignificant when compared with virus-negative diagnosed patients (Table 6A and B). Interestingly, 30 patients had polynucleosis associated to an inflammatory response may be linked to a bacterial infection. No specific virus was associated to polynucleosis.

5. Discussion

From October 2006 to September 2008, 817 outpatients aged from 1 month to 9 years were included in a surveillance program of viral etiology in ARI. Less than 1% of the children enrolled in this outpatient study were aged of less than 6 months, suggesting that very young children may show more severe symptoms and hospitalized. Fifteen different viruses were detected in 486 samples (59.5%). Thus, respiratory viruses were the major pathogens responsible for ARI in children and multi-infections of different viruses (13.8%) were frequently observed. Although serotype identification is critical for epidemiological surveillance, the serotyping is time consuming and costly, and limited due to cross-reactivity of the tests. We sequenced the fragments of genes coding for virus antigenic proteins and analyzed by phylogenetic analysis the sequence diversity to monitor the molecular evolution of circulating virus.

During the outbreak of ADV from March to June in 2008, the majority of strains (37 out of 40) was ADV-B species and of serotype ADV-2. In ADV-B species, only serotype ADV-14 was reported to cause severe infection, which may partly explained that in our study ADV-infected patients showed only mild symptoms.

The IAV strains detected during the period from January 2007 to April 2008 were mainly H3N2 but the strains detected from July 2008 to September 2008 were mainly H1N1. This suggests that subtype H1N1 replaced the H3N2 subtype and predominated during the next year in the region. As vaccination for seasonal influenza (IAV H1N1, H3N2 and IBV) was not included in the children's routine vaccination program in Shanghai, and was usually based on the strains circulating worldwide in the precedent summer, the local population was not protected against the new subtype that emerged in the summer of 2008.

HRV is classified into three species: HRV-A, HRV-B and HRV-C by phylogenetic analysis based on sequences of VP4 gene and/or 5′UTR. A predominance of the newly identified species HRV-C (53.8%) and the recombinant strains were observed based on which two new subspecies of HRV-C were proposed as HRV-Cα and HRV-Cc. This suggests the emergence of new variant strains of HRV in future that might cause a new epidemic. Eleven HEV were detected in the study but were not analyzed further.

HMPV is another recently identified respiratory virus and has been found worldwide. It is grouped into four distinct genetic

Fig. 2. Monthly distribution of virus strains detected by mRT-PCR, in 817 outpatient children, Shanghai Nanxiang Hospital, October 2006 to September 2008.
### Table 6
Clinical features in 817 ARI cases.

| A | Single infection | B: All viruses RT-PCR negative |
|---|------------------|--------------------------------|
| | IAV  IBV  RSV  HMPV  PIV11  PIV3  PIV4  RHV  HEV  OC43  229E  HKU-1  NL63  ADV  HBoV | | |
| Total case | 44 36 71 33 27 23 2 43 5 1 1 1 14 18 54 | 373 113 331 |
| Fever (>38 °C) | 44 36 71 33 27 23 2 41 5 1 1 1 14 18 54 | 370 113 329 |
| Cough | 44 36 70 32 27 23 2 42 5 1 1 1 14 18 54 | 370 113 330 |
| Pleuritic pain | 0 2 0 0 0 0 0 1 0 0 0 0 0 0 1 | 4 4 4 |
| Purulent expectoration | 5 8 16 8 5 3 2 11 0 1 0 0 3 3 10 | 75 25 50 |
| Dyspnoea or tachypnea | 1 (2.3) 3 (8.3) 2 (2.8) 1 (3.0) 1 (1.7) 1 (4.3) 1 (50) 3 (7.0) 0 0 0 0 0 | 15 (55.6) 10 (37.0) |
| Hypoxemia | 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 | 25 (56.8) 25 (56.8) |
| Intersitial abnormality at chest X-ray | 25 (69.4) 47 (66.2) 17 (51.5) 15 (55.6) 14 (60.9) 1 (50) 29 (67.4) 2 (40) 1 1 0 | 9 (64.3) 11 (61.1) 34 (63.0) |
| Polynucleosis (>10,000 on absolute count) | 32 8 11 1 1 6 0 0 0 0 1 2 4 | 32 8 11 1 1 6 0 0 0 0 1 2 4 |
| Lymphopenia (<1500 on absolute count) | 12 (27.3) 4 (11.1) 4 (5.6) 4 (12.1) 0 1 (4.3) 0 2 (9.3) 0 0 1 0 | 12 (27.3) 4 (11.1) 4 (5.6) 4 (12.1) 0 1 (4.3) 0 2 (9.3) 0 0 1 0 |
| Bronchitis | 31 (70.5) 29 (80.6) 46 (64.8) 22 (66.7) 20 (74.1) 19 (82.6) 0 35 (81.4) 5 (100) 1 0 1 10 (71.4) 13 (72.2) 38 (70.4) | 0 0 0 |
| Pneumonia | 13 (29.5) 7 (19.4) 24 (33.8) 10 (30.3) 6 (22.2) 4 (17.4) 1 (50) 8 (18.6) 0 0 1 0 | 13 (29.5) 7 (19.4) 24 (33.8) 10 (30.3) 6 (22.2) 4 (17.4) 1 (50) 8 (18.6) 0 0 1 0 |
| Upper respiratory tract infection | 0 0 1 (1.4) 1 (3.0) 1 (3.7) 0 1 (50) 0 0 0 0 0 0 0 0 | 0 0 1 (1.4) 1 (3.0) 1 (3.7) 0 1 (50) 0 0 0 0 0 0 0 0 |
| Suggested to be hospitalized | 8 (18.2) 10 (27.8) 21 (29.6) 10 (30.3) 5 (18.5) 4 (17.4) 0 8 (18.6) 0 1 0 0 | 8 (18.2) 10 (27.8) 21 (29.6) 10 (30.3) 5 (18.5) 4 (17.4) 0 8 (18.6) 0 1 0 0 |
| | | 3 (21.4) 5 (27.8) 17 (31.5) |

**A**: each virus; **B**: all viruses including single infection and co-infection.

* Case number.

* Percentage (%).
lines based on the F gene: A1, A2, B1, and B2. In this study, two strains of HMPV-A1, 11 strains of HMPV-B1 and two strains of HMPV-B2 were detected in the first season, whereas one strain of HMPV-A1, three strains of HMPV-B1 and 12 strains of HMPV-B2 were identified in the second season. No HMPV-A2 lineage was found. Hence, a change of predominant lineage in the season was observed, but no association between the severity of infection and genetic drift of HMPV was found, as shown in other previous studies. Besides, studies showed more sequence diversity in G and SH genes but not in F gene, which could explain the constant incidence of HMPV infection in the population.

HCoV-HKU1 (in group I with HCoV-OC43) and HCoV-NL63 (in group I with HCoV-229E) are two novel coronaviruses. During the present 2-year study, HCoV-NL63 and HCoV-229E were the major HCoV circulating in Shanghai, whereas only one strain of HCoV-OC43 and one of HCoV-HKU1 were detected, indicating a sporadic introduction of group II HCoV to the region. In context that the recent emerged HCoV, like HKU-1 and SARS whose sequence is more homologous to group II virus, could cause severe respiratory infection, the surveillance for emergence of new species of HCoV is necessary.

The co-infection of RSV and HBoV was frequently detected among the samples, whereas these two viruses co-dominated in cold season. HBoV was the second most prevalent virus (24.5%), and the co-infection rate of HBoV with other respiratory viruses was 54.6%, compared to 14% in non-HBoV-infected patients. This was lower than the co-infection rate reported previously, which ranged up to 71%. A previous study showed that HBoV increased the severity of bronchiolitis in children less than 1-year-old co-infected with RSV, and that it is not an occasional virus. However, no correlation of HBoV infection with clinical severity was observed in this and its related study.

One study carried out in Wuhan, China analyzed peripheral blood samples by indirect immunofluorescence to detect RSV, IAV, IBV, ADV, PIV1–3, Chlamydia pneumonia and Mycoplasma pneumonia in children ARI inpatients and used viremia as sign of severe infection. It showed that 36% of cases were co-infected by multiple agents and IAV, IBV and PIV1 were associated with coinfection. In addition, studies showed up to 30% of co-infection in hospitalized children and RSV co-infection was associated with clinical severity. However, no such correlation was found in this study. This may be due to differences in the criteria of patient enrollment and in the lower severity of clinical signs observed. Bacteria-virus co-infection was commonly found in inpatients. In our study, only polyenucleosis (>10,000 on absolute count) was considered as a sign of bacterial infection and was observed in 30 patients infected with a respiratory virus. Hence, future studies should focus on severe respiratory infection to identify viral determinants of disease severity and should introduce bacteriological test.

Up to 331 specimens were negative in mRT-PCR, despite all of them matched well with the inclusion criteria for ARI. Negative results could have resulted from the low load of viral material in samples, or to infection with bacteria instead of virus. New sensitive tools such as Mass-Tag or high-throughput sequencing have been developed recently to identify new viruses and bacterial pathogens. Implementation of these new molecular techniques for samples that are negative in mRT-PCR might be considered in the future.

This is believed to be the first study in China to characterize 17 common respiratory viruses in pediatric ARI during a 2-year consecutive period in a limited community with an important immigrant population. Using mRT-PCR followed by sequencing and phylogenetic analysis, we could identify a wide variety of agents and differentiate highly pathogenic viruses from less virulent seasonal respiratory viruses. The sequence analysis result could be useful to improve the primer design for RT-PCR and to identify new subtype virus, for example HRV-C. It monitored sustaining virus circulation in the community, which could serve as a baseline of the annual distribution of viruses for surveillance of unusual prevalence of one specific virus.

Conflict of interest

The authors declare no conflict of interest.

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