Eur J Pediatr (2014) 173:291–301
DOI 10.1007/s00431-013-2127-z

ORIGINAL ARTICLE

Adenovirus respiratory infection in hospitalized children in Hong Kong: serotype–clinical syndrome association and risk factors for lower respiratory tract infection

Shuk-Kuen Chau · So-lun Lee · Malik J. S. Peiris · Kwok-Hung Chan · Eunice Chan · Wilfred Wong · Susan S. Chiu

Received: 4 January 2013 / Revised: 27 July 2013 / Accepted: 30 July 2013 / Published online: 31 August 2013

Abstract Lower respiratory tract infections (LRTI) caused by adenovirus can be severe with resultant chronic pulmonary sequelae. More than 50 serotypes have been recognized; however, the exact association of serotype with clinical phenotype is still unclear. There have been no reports on the adenovirus serotype pattern in Hong Kong, and their relationships with disease manifestations and complications are not known. Clinical and epidemiological data on 287 children (<6 years old) admitted with adenovirus respiratory infections from 2001 to 2004 were reviewed. Common presenting symptoms included fever (97.9 %) and cough and rhinitis (74 %). Extra-pulmonary manifestations were present in 37.3 %. The clinical picture mimicked bacterial infection for its prolonged high fever and neutrophilic blood picture. Forty-two patients (14.6 %) had LRTI, either pneumonia or acute bronchiolitis, but none had severe acute respiratory compromise. Children aged 1 to 2 years old were most at risk for adenovirus LRTI (adjusted $p=0.0165$). Serotypes 1 to 7 could be identified in 93.7 % of the nasopharyngeal specimens, with serotypes 2 and 3 being the most prevalent. Different serotypes showed predilection for different age groups and with different respiratory illness association. The majority of acute bronchiolitis (71.4 %) were associated with serotype 2 infection, and this association was statistically significant ($p<0.0001$). Serotype 3 infection accounted for over half of the pneumonia cases (57–75 %) in those aged 3–5 years old. Only one patient developed mild bronchiectasis after serotype 7 pneumonia. Children aged 1 to 2 years old were the at-risk group for adenovirus LRTI, but respiratory morbidity was relatively mild in our locality. There was an apparent serotype–respiratory illness association.

Keywords Adenovirus · Children · Hong Kong · Respiratory tract infection · Risk factors · Serotypes

S.-K. Chau · S.-l. Lee · E. Chan · W. Wong · S. S. Chiu (✉)
Department of Pediatrics and Adolescent Medicine,
The University of Hong Kong, Pokfulam, Hong Kong SAR
e-mail: ssschiu@hkucc.hku.hk

M. J. S. Peiris
The School of Public Health, The University of Hong Kong, Sassoon Road, Pokfulam, Hong Kong SAR

K.-H. Chan
Department of Microbiology, Li Ka Shing Faculty of Medicine,
The University of Hong Kong, Sassoon Road, Pokfulam,
Hong Kong SAR

Abbreviations

URTI	Upper respiratory tract infection
LRTI	Lower respiratory tract infection
IF	Immunofluorescence
WBC	White cell count
ANC	Absolute neutrophil count
RSV	Respiratory syncytial virus

Background

Adenovirus (Ad) infections account for about 4–10 % of viral pneumonia and bronchiolitis in children [6], and an alarming number of fatal illnesses and long-term pulmonary complications have been reported worldwide. Some particular serotypes, especially 3, 7, and 21, have been reported to cause either epidemics of respiratory illnesses or fulminant events, including lethal pneumonia [3, 15, 18]. Infection by these serotypes can also result in long-term pulmonary complications, e.g., bronchiolitis obliterans, bronchiectasis, and the Swyer James syndrome [8, 25, 29, 31, 36]. Apart from specific serotypes, some host factors have also been found to be associated with development of severe Ad infection, such as

young age (less than 7 years old), underlying chronic disease, post-transplant or immunocompromised state [13, 24, 30].

In the past decade, there were increasing numbers of reports on Ad serotype analysis, in part because of the more readily available polymerase chain reaction sequencing approach for typing Ad as compared to the traditional neutralization method [5, 11, 20, 21]. However, the exact association of serotype with clinical phenotype is still unclear. So far, there have been no reports on the Ad serotype pattern in Hong Kong, and their relationships with disease manifestations and complications are unknown. The few local studies that related to Ad infection only focused on the overall epidemiology of viral pneumonia or acute respiratory tract infections in hospitalized children [7, 32, 33].

Hence, we conducted a retrospective review to study the epidemiology, clinical features, and outcome of Ad respiratory tract infections in children admitted to our hospital. We focused on those pre-school children under the age of 6 years old as nearly 90 % of Ad infections occur in this age group [5, 6, 17]. We also aimed to explore for any serotype–clinical syndrome association and to identify the at-risk group for "lower" versus "upper" respiratory tract infections. The study was approved by the Institutional Review Board of our Hospital Authority. The need for informed consent was waived.

Patients/methods

Case definition and identification

The study was carried out in Queen Mary Hospital, a university affiliated hospital, and one of the two public hospitals in Hong Kong Island serving a population of more than 100,000 children under 18 years old. All preschool children under 6 years of age admitted with acute respiratory symptoms and with a virologic diagnosis of Ad from January 2001 to December 2004 were included in the study. These cases were identified retrospectively from laboratory surveillance database, and their clinical records were reviewed for demographic and clinical details. Based on our admission policy, nasopharyngeal aspirates were obtained routinely in all patients who admitted with respiratory symptoms for isolation purposes and tested for five common viruses—Ad, influenza A and B, parainfluenza virus, and respiratory syncytial virus (RSV)—by direct antigen detection by immunofluorescence (IF) and viral culture.

Children with acute respiratory illnesses were categorized into those with upper respiratory tract infection (URTI) and those with lower respiratory tract infection (LRTI). LRTI included pneumonia and acute bronchiolitis. The medical records were reviewed carefully and the clinical diagnosis of each case was classified cautiously: Acute bronchiolitis was defined as the presence of viral upper respiratory prodrome including rhinorrhea and cough with any signs of increased respiratory effort and wheezing in young children [9]. The diagnosis of pneumonia was based on the presence of cough with tachypnea [28] or positive auscultative findings (bronchial breath sound or crackles) in addition to abnormal radiological features (consolidation/interstitial infiltrates) that were consistent with pneumonia, as interpreted by the attending pediatrician or by the radiologist. Patients with co-infection of other known respiratory viruses or bacteria in the same course of hospital stay were excluded.

Adenovirus isolation and serotyping

Virus isolation was performed by inoculating HEp-2C cell monolayers in tissue culture tubes with 150 μl of the nasopharyngeal aspirate in viral transport medium and incubated at 37 °C for up to 7 days. The culture maintenance fluid, minimum essential medium supplemented with 2 % fetal bovine serum (GibcoBRL, Grand Island, NY, USA), L-glutamine (2 mM), and antimicrobials (final antimicrobial concentration per milliliter of medium: vancomycin, 50 μg; amikacin, 20 μg; nystatin, 20 units), was changed every 2 to 3 days or twice during the incubation period. Ad was identified initially by characteristic cytopathic effect and direct immunofluorescence staining (Adenovirus DFA Reagent, EMD Millipore Corporation, USA) of the infected cells. Definitive typing of the viral isolate was performed by micro-neutralization assay with HEp-2C cells for serotyping as described previously [16]. The isolate was passed to a new HEp2-C tube until ≥75 % cytopathic effect was evident and diluted the culture at 1:10 with 2 % minimum essential medium after freezing and thawing of the culture once before carrying out the neutralization test. Briefly, 0.05 ml of each Ad typing serum type 1 to 7 (Denka Seiken, Japan) at 10–20 antibody unit per 0.05 ml was added to 0.05 ml of each isolate at a viral concentration of 100 $TCID_{50}$ (50 % tissue culture infective dose) per 0.05 ml and incubated at 37 °C in 2.5 % CO_2 for 1 h, followed by incubation with 0.1 ml HEp-2C cells (150,000 cells per ml) for up to 7 days. The serotypes were determined by inhibition of cytopathic effect by corresponding type-specific antisera. End results were read when the virus control showed 100 % cytopathic effect. Back-titration of the input virus dose and cells without virus inoculation as negative control was run in parallel. In case where the virus could not be neutralized by the antisera used, the test was repeated at 1:20, 1:50, or higher viral dilution if necessary. Isolates that did not type after repeated tests were considered as untypeable.

Statistical analysis

Descriptive statistics were used to describe the epidemiological data of the disease and clinical characteristics of patients.

Chi-square test was used to test if there was any significant relationship between individual serotype and each disease category (pneumonia versus acute bronchiolitis versus URTI). Demographic characteristics were compared between the groups of children with LRTI and URTI using Pearson's chi-square test (with Yates' correction) for categorical variables and unpaired *t*-test for continuous variables for any risk factors that might predispose a child to lower respiratory tract involvement. Significant risk factors identified in the univariate analysis were then adjusted using multi-variate logistic regression to determine their independent effects. All analyses were done by using SAS software (version 9.12). A *p*-value of <0.05 was considered as statistically significant.

Results

Demographic characteristics and clinical presentation

A total of 287 children fulfilled the study criteria during the specified period, and their demographic characteristics were shown (Table 1). There was a slight male predominance. The mean age was 2.82±1.61 years old. The majority (85.4 %) of them were previously healthy. Thirty children were admitted from institutions (i.e., residential facilities for children with social problems). Twenty-nine cases were excluded from the study due to co-infection with other pathogens: nine cases with influenza virus A or B, two cases with RSV, 14 cases with parainfluenza virus 1, 2, or 3, one case with Coxsackie virus A, one case with human metapneumovirus, one case with rotavirus, and one case with both *Stapylococcus aureus* and *Moraxella catarrhalis*.

Common presenting symptoms included fever (97.9 %), cough (74.9 %), and rhinitis (73.9 %). A total of 107 (37.3 %) patients had one or more associated extra-pulmonary manifestations: gastrointestinal symptoms (*n*=39), febrile seizure (*n*=36), conjunctivitis (*n*=26), and skin rash (*n*=14). URTI was the most common presentation (245 cases, 85.4 %). Among them, four cases presented as croup-like illness and 14 cases were complicated with otitis media. LRTI was diagnosed in 42 patients (14.6 %) with 28 cases of pneumonia and 14 cases of acute bronchiolitis.

Table 1 Demographic characteristics and clinical features among hospitalized children with adenovirus respiratory infections

	URTI (*N*=245)	LRTI (*N*=42)	Total (*N*=287)
Mean age (years)	2.87±1.62	2.47±1.49	2.82±1.61
Age group			
<1 year	39 (15.9 %)	7 (16.7 %)	46 (16.0 %)
1 to 2 years	44 (18.0 %)	15 (35.7 %)	59 (20.6 %)
2 to 3 years	47 (19.2 %)	3 (7.1 %)	50 (17.4 %)
3 to 4 years	50 (20.4 %)	8 (19.0 %)	58 (20.2 %)
4 to 5 years	35 (14.3 %)	7 (16.7 %)	42 (14.6 %)
5 to 6 years	30 (12.2 %)	2 (4.8 %)	32 (11.1 %)
Male/female	138:107 (56.3:43.7 %)	25:17 (59.5:40.5 %)	163: 124 (56.8:43.2 %)
Children living in institutions	19 (7.8 %)	11 (26.2 %)	30 (10.4 %)
History of prematurity (gestational age <37 weeks)	24 (9.8 %)	12 (28.6 %)	36 (12.5 %)
History of ventilator use at neonatal period	10 (4.1 %)	6 (14.3 %)	16 (5.6 %)
Children attending nursery	92 (37.6 %)	13 (31.0 %)	105 (36.6 %)
Presence of underlying chronic illness[a]	33 (13.5 %)	9 (21.4 %)	42 (14.6 %)
Mean fever duration prior to AED admission (days)	3.3±2.4	3.8±3.2	3.4±2.5
Mean peak temperature (°C)	39.6±0.6	39.5±0.7	39.6±0.6
Mean total fever duration (days)	5.0±2.5	6.0±3.4	5.2±2.7
Presence of extra-pulmonary manifestations (gastrointestinal symptoms, febrile seizure, conjunctivitis, skin rash)	97 (39.6 %)	10 (23.8 %)	107 (37.3 %)
Total WBC count ($\times 10^9$/L)	14.5±6	13.3±5.5	14.3±5.9
Neutrophil (ANC) count	9.7±5.1	8.3±4.5	9.4±5.0
Lymphocyte count	3.3±2.3	3.7±2.2	3.4±2.3
Mean length of hospitalization (days)	2.9±1.9	4.6±2.9	3.1±2.1

[a] Forty-two patients had underlying chronic illness which included asthma in 25 patients, congenital heart disease in seven patients, cerebral palsy, mental retardation, or epilepsy in four patients, an underlying immunodeficiency in two patients, Down syndrome in three patients, and inborn error of metabolism in one patient

Laboratory and radiological findings

Blood tests with differential white cell counts (WCC) were available in 255 patients. Among them, 52.2 % had neutrophilia as defined by absolute neutrophil count (ANC) of $\geq 8.5 \times 10^9$/L according to the laboratory reference. The mean total WCC, ANC, and lymphocyte count did not differ significantly between the URTI and LRTI groups (Table 1). C-reactive protein level was not routinely performed in our setting. Among those with LRTI, interstitial pulmonary infiltrates were the most common abnormal radiological findings (73.3 %), while the remaining ones showed lobar-type consolidation.

Clinical course and outcome

Both URTI and LRTI patients suffered from high fever (mean peak temperature of 39.6±0.6 and 39.5±0.7 °C, respectively), and the overall mean duration of fever prior to attendance of the Accident and Emergency Department was 3.4±2.5 days. Those with LRTI had a slightly longer hospital stay than those with URTI (4.6±2.9 versus 2.9±1.9 days). The whole febrile illness usually lasted 5 to 6 days. While awaiting for the rapid IF result, 30.3 % of the patients were empirically treated with antibiotics in view of the high-grade fever together with the finding of neutrophilia. Indeed 43.9 % of the admitted children had received antibiotics from their primary care physicians before hospital admissions.

Four out of 42 patients (9.5 %) with LRTI had hypoxia, defined as oxygen saturation below 92 % on admission, and required oxygen supplement for 1 to 5 days. Two patients needed intensive care support due to associated febrile status epilepticus which were not directly related to the severity of respiratory illness. One had acute bronchiolitis and the other had URTI. There was no mortality.

Among those 42 patients with LRTI, about 60 % of them were offered follow-up as they still had considerable symptoms or significant chest X-ray abnormalities at the time of discharge. Most of them had symptoms or radiological improvement within a few weeks' time and did not require long-term follow-up. Only one patient developed chronic pulmonary sequelae. He was a 3-year-old child with good past health who suffered from right lower lobe pneumonia caused by Ad serotype 7. Serial follow-up chest X-rays showed persistent right lower lobe collapse consolidation even after several months. Initial computer tomography scan of the thorax revealed lobar collapse, but repeated scan after 1 year showed bronchiectatic changes in the corresponding lobe. Workup for other common causes of bronchiectasis was negative.

Risk factors for LRTI

Among the risk factors selected for analysis, age between 1 and 2 years (p=0.0153), history of prematurity (p=0.0018), history of ventilator assistance at birth (p=0.0215), and institutionalization (p=value 0.0009) were found to be associated with LRTI in univariate analysis (Table 2). After multiple logistic regression, only age between 1 and 2 years remained as the statistically significant risk factor for LRTI (adjusted p=0.0165, odds ratio 2.48, 95 % confidence interval 1.18 to 5.22).

Serotype pattern analysis

In our study, rapid detection of Ad by IF had a sensitivity of 73.8 % (212/287) in comparison to cell culture. Serotyping by microneutralization assay was achieved for 93.7 % of the culture samples. Among the seven serotypes (type 1–7) that were isolated during the 4-year period, serotypes 2, 3, and 7 accounted for most of the respiratory tract infections. The prevalent serotype and the peak season varied in each year (Table 3; Fig. 1). Serotype 7 was the predominant type (40.4 %) in 2001. However, it was no longer detected in the years 2003 and 2004. Instead serotype 3 was found in the majority in these 2 years, accounting for 43.5 and 64.1 % of the admissions, respectively.

The serotype distribution among different respiratory illnesses is shown in Table 4. Being more common, serotypes 2, 3, and 7 proportionally accounted for most of the pneumonia cases. Serotype 3 infection accounted for over one third of the pneumonia cases. Interestingly, the majority of acute bronchiolitis (71.4 %) were associated with serotype 2 infection, and this association was statistically significant (p<0.0001). Infection with serotype 5 was only associated with URTI in our series.

On stratifying patients according to their age groups and types of respiratory illness (Table 5), we found that serotype 2 accounted for the majority of acute bronchiolitis and half of the pneumonia cases in those aged below 2 years, while it did not cause any LRTI in those aged above 3 years old. The prevalence of serotype 2 in causing URTI also significantly decreased after the first 3 years of age. Serotype 3, despite being the most prevalent serotype, did not play any significant role for LRTI in those aged below 3 years. It was, however, the predominant etiological agent of pneumonia in those aged between 3–4 and 4–5 years old, accounting for 75 and 57.1 % of pneumonia cases, respectively.

Serotype 7, on the contrary, did not seem to have a particular age group predilection. Comparison between serotypes 3 and 7 revealed no significant difference between their clinical and laboratory characteristics, but those patients infected with serotype 7 required a slightly longer mean hospital stay (3.7±3.3 versus 2.8±1.2 days, p=0.0207) and were more likely to have a history of prematurity (p=0.0407) or were living in institutions (p=0.0025) (Table 6). The latter was likely contributed by the fact that an outbreak of serotype 7 occurred between April and May in 2001 which resulted in nine consecutive admissions from an institution.

Table 2 Identification of risk factor(s) for lower versus upper respiratory tract infection by adenovirus

	LRTI group (n=42)	URTI group (n=245)	p-value (univariate analysis)	Adjusted p-value (after multi-variate logistic regression)
Age				
1 to 2 years	15 (35.7)	44 (18.0 %)	0.0153	0.0165
Other age groups	27 (64.3 %)	201 (82.0 %)		
Sex				
Male	25 (59.5 %)	138 (56.3 %)	0.828	
Female	17 (40.5 %)	107 (43.7 %)		
History of ventilator assistance at birth				
Yes	6 (14.3 %)	10 (4.1 %)	0.0215	0.5690
No	36 (85.7 %)	235 (95.9 %)		
History of prematurity				
Yes	12 (28.6 %)	24 (9.8 %)	0.0018	0.1068
No	30 (71.4 %)	221 (90.2 %)		
Attendance of nursery				
Yes	13 (30.9 %)	92 (37.6 %)	0.1085	
No	18 (42.9 %)	66 (26.9 %)		
Unknown	11 (26.2 %)	87 (35.5 %)		
Institutionalized				
Yes	11 (26.2 %)	19 (7.8 %)	0.0009	0.0520
No	31 (73.8 %)	226 (92.2 %)		
Number of siblings				
Yes (1–3)	16 (38.1 %)	113 (46.1 %)	0.3514	
No	19 (45.2 %)	108 (44.1 %)		
Unknown	7 (16.7 %)	24 (9.8 %)		
Pre-existing asthma				
Yes	6 (14.3 %)	19 (7.8 %)	0.2118	
No	36 (85.7 %)	226 (92.2 %)		
Presence of underlying chronic illness				
Yes	9 (21.4 %)	33 (13.5 %)	0.2661	
No	33 (78.6 %)	212 (86.5 %)		

Discussion

Adenovirus has been known as a common cause of childhood respiratory illness since it was first described in 1950s. By now, more than 50 serotypes have been recognized, but the exact association of serotype with clinical phenotype is still unclear. It is likely that different serotypes display different tissue tropisms and clinical manifestations of infection. As the predominant serotypes differ among countries and change over time, the epidemiological and clinical pattern of Ad infection will vary accordingly. However, there is paucity of local data on the epidemiological characteristics, serotype pattern, and clinical outcome of Ad infection in Hong Kong children.

We showed that serotypes 2 and 3 were the most prevalent serotypes among hospitalized children with Ad respiratory infections during this 4-year study period. There was a small epidemic due to serotype 7 in 2001. The number of admissions due to Ad was markedly reduced in 2003, during the SARS outbreak. Reduced infection of other respiratory viruses in Hong Kong during that time had also been documented and might be partly explained by increased social distancing and improved community hygiene during that period [22]. Unlike influenza virus and RSV, no consistent seasonal pattern of Ad respiratory infection was observed, which was similarly seen in some other areas [1, 19].

The clinical picture of Ad respiratory infection was characterized by coryzal symptoms with prolonged and high fever. Our children suffered from a mean peak temperature of 39.6±0.6 °C, and the mean duration of this febrile illness lasted 5.2±2.7 days. The presence of extra-pulmonary manifestations (gastrointestinal symptoms, febrile seizure, conjunctivitis, and skin rash) might potentially serve as a hint for Ad infection as these symptoms were seen in over one third of cases.

Table 3 Serotype distribution among 287 children hospitalized for adenovirus infection between 2001 and 2004

	2001	2002	2003	2004	All
Ad 1	3	9	8	8	28 (9.8 %)
Ad 2	16	32	6	12	66 (23 %)
Ad 3	9	21	20	41	91 (31.7 %)
Ad 4	15	2	1	0	18 (6.3 %)
Ad 5	5	5	1	1	12 (4.2 %)
Ad 6	0	1	1	1	3 (1 %)
Ad 7	36	14	0	0	50 (17.4 %)
Ad 1 and 3	0	1	0	0	1 (0.3 %)
Unknown	5	3	9	1	18 (6.3 %)
Total	89	88	46	64	287

Some studies have reported leukocytosis or elevated C-reactive protein with Ad infections [5, 8]. We have also found neutrophilia in over half of our cases as opposed to lymphopenia or lymphocytosis seen in most other viral infections. Because of the high fever and neutrophilic blood picture, the disease presentation often mimicked bacterial disease and led to unnecessary antibiotic prescription. The fact that Ad infections mimicked bacterial infection and parental anxiety due to persistent high fever might lead to a higher likelihood of hospital admission and hence explained why a considerable number of children were admitted to the hospital for URTI in our locality.

There was a relatively even distribution in age among children who suffered from URTI, but over one third of the children suffered from LRTI aged between 1 and 2 years old.

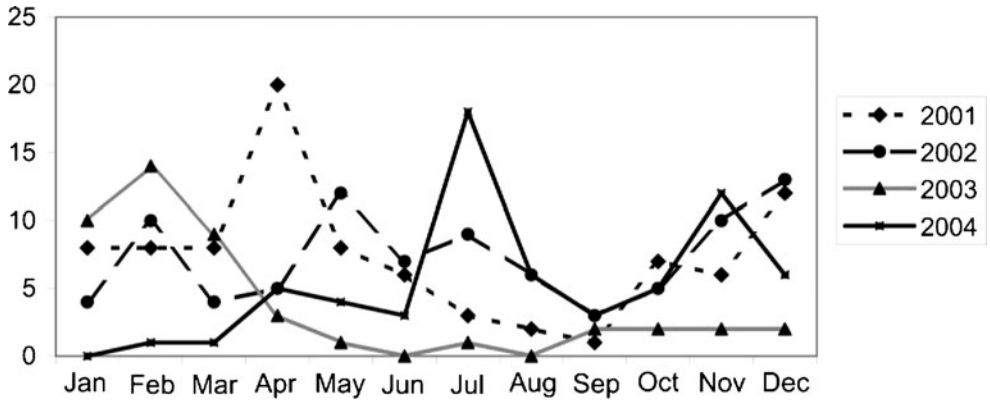

Fig. 1 Monthly distribution of hospitalized cases of adenovirus respiratory infection

Table 4 Serotype distribution in different types of adenovirus respiratory illnesses

	Pneumonia (n=28)	Acute bronchiolitis (n=14)	URTI (n=245)	p-value[a]
Ad 1	2 (7.1 %)	1 (7.1 %)	25 (10.2 %)	0.8263
Ad 2	5 (17.9 %)	10 (71.4 %)	51 (20.8 %)	<0.0001[b]
Ad 3	10 (35.7 %)	1 (7.1 %)	80 (32.7 %)	0.1218
Ad 4	2 (7.1 %)	0 (0 %)	16 (6.5 %)	0.6063
Ad 5	0 (0 %)	0 (0 %)	12 (4.9 %)	0.3418
Ad 6	1 (3.6 %)	0 (0 %)	2 (0.8 %)	0.368
Ad 7	4 (14.3 %)	2 (14.3 %)	44 (18 %)	0.8452
Ad 1 and 3	1 (3.6 %)	0 (0 %)	0 (0.0 %)	Not applicable
Unknown	3 (10.7 %)	0 (0 %)	15 (6.1 %)	Not applicable

[a] Chi-square test was done for serotypes 1 to 7 to test for their statistical association with different disease categories (pneumonia versus acute bronchiolitis and URTI)

[b] Overall p-value<0.0001. Post-test comparison was also significant for acute bronchiolitis versus pneumonia (p-value=0.002) and acute bronchiolitis versus URTI (p-value <0.0001)

Table 5 Serotype distribution stratified according to age groups and types of respiratory illness

		Pneumonia	Acute bronchiolitis	URTI	Total
<1 year	Ad 1	0 (0 %)	0 (0 %)	4 (10.3 %)	4 (8.7 %)
	Ad 2	0 (0 %)	5 (83.3 %)	11 (28.2 %)	16 (34.8 %)
	Ad 3	0 (0 %)	0 (0 %)	7 (17.9 %)	7 (15.2 %)
	Ad 4	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 5	0 (0 %)	0 (0 %)	6 (15.4 %)	6 (13.0 %)
	Ad 6	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 7	1 (100 %)	1 (16.7 %)	7 (17.9)	9 (19.6 %)
	Ad 1 and 3	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown	0 (0 %)	0 (0 %)	4 (10.3 %)	4 (8.7 %)
	Total	1	6	39	46
1 to 2 years	Ad 1	1 (14.3 %)	1 (12.5 %)	7 (15.9 %)	9 (15.3 %)
	Ad 2	4 (57.1 %)	5 (62.5 %)	15 (34.1 %)	24 (40.7 %)
	Ad 3	0 (0 %)	1 (12.5 %)	8 (18.2 %)	9 (15.3 %)
	Ad 4	0 (0 %)	0 (0 %)	1 (2.3 %)	1 (1.7 %)
	Ad 5	0 (0 %)	0 (0 %)	3 (6.8 %)	3 (5.1 %)
	Ad 6	0 (0 %)	0 (0 %)	1 (2.3 %)	1 (1.7 %)
	Ad 7	0 (0 %)	1 (12.5 %)	5 (11.3 %)	6 (10.2 %)
	Ad 1 and 3	1 (14.3 %)	0 (0 %)	0 (0 %)	1 (1.7 %)
	Unknown	1 (14.3 %)	0 (0 %)	4 (9.1 %)	5 (8.5 %)
	Total	7	8	44	59
2 to 3 years	Ad 1	0 (0 %)	0 (0 %)	7 (14.9 %)	7 (14.0 %)
	Ad 2	1 (33.3 %)	0 (0 %)	12 (25.5 %)	13 (26.0 %)
	Ad 3	0 (0 %)	0 (0 %)	12 (25.5 %)	12 (24.0 %)
	Ad 4	0 (0 %)	0 (0 %)	1 (2.1 %)	1 (2.0 %)
	Ad 5	0 (0 %)	0 (0 %)	1 (2.1 %)	1 (2.0 %)
	Ad 6	0 (0 %)	0 (0 %)	1 (2.1 %)	1 (2.0 %)
	Ad 7	1 (33.3 %)	0 (0 %)	10 (21.3 %)	11 (22.0 %)
	Ad 1 and 3	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown	1 (33.3 %)	0 (0 %)	3 (6.4 %)	4 (8.0 %)
	Total	3	0	47	50
3 to 4 years	Ad 1	0 (0 %)	0 (0 %)	4 (8.0 %)	4 (6.9 %)
	Ad 2	0 (0 %)	0 (0 %)	8 (16.0 %)	8 (13.8 %)
	Ad 3	6 (75.0 %)	0 (0 %)	23 (46.0 %)	29 (50.0 %)
	Ad 4	1 (12.5 %)	0 (0 %)	6 (12.0 %)	7 (12.1 %)
	Ad 5	0 (0 %)	0 (0 %)	2 (4.0 %)	2 (3.4 %)
	Ad 6	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 7	1 (12.5 %)	0 (0 %)	6 (12.0 %)	7 (12.1 %)
	Ad 1 and 3	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown	0 (0 %)	0 (0 %)	1 (2.0 %)	1 (1.7 %)
	Total	8	0	50	58
4 to 5 years	Ad 1	1 (14.3 %)	0 (0 %)	2 (5.7 %)	3 (7.1 %)
	Ad 2	0 (0 %)	0 (0 %)	3 (8.6 %)	3 (7.1 %)
	Ad 3	4 (57.1 %)	0 (0 %)	17 (48.6 %)	21 (50.0 %)
	Ad 4	1 (14.3 %)	0 (0 %)	5 (14.3 %)	6 (14.3 %)
	Ad 5	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 6	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 7	0 (0 %)	0 (0 %)	6 (17.1 %)	6 (14.3 %)
	Ad 1 and 3	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown	1 (14.3 %)	0 (0 %)	2 (5.7 %)	3 (7.1 %)
	Total	7	0	35	42

Table 5 (continued)

		Pneumonia	Acute bronchiolitis	URTI	Total
5 to 6 years	Ad 1	0 (0 %)	0 (0 %)	1 (3.3 %)	1 (3.1 %)
	Ad 2	0 (0 %)	0 (0 %)	2 (6.7 %)	2 (6.3 %)
	Ad 3	0 (0 %)	0 (0 %)	13 (43.3 %)	13 (40.6 %)
	Ad 4	0 (0 %)	0 (0 %)	3 (10.0 %)	3 (9.4 %)
	Ad 5	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Ad 6	1 (50.0 %)	0 (0 %)	0 (0 %)	1 (3.1 %)
	Ad 7	1 (50.0 %)	0 (0 %)	10 (33.3 %)	11 (34.4 %)
	Ad 1 and 3	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Unknown	0 (0 %)	0 (0 %)	1 (3.3 %)	1 (3.1 %)
	Total	2	0	30	32

We have confirmed from the multi-variate analysis that children in this age group were more predisposed for "lower" versus "upper" respiratory tract infection by Ad. Acute bronchiolitis is generally more common in those aged below 2 years old but possible explanation that these children (1 to 2 years old), rather than the even younger age group, were at higher risk for developing LRTI might include waning of protective antibodies at this age.

It is well reported that a subgroup of patients with Ad infection can progress to severe pneumonia with long-term complications. Serotypes 3, 7, and 21 have been previously identified as the culprits [3, 15, 18, 29]. Apart from susceptible host risk factors, severe infection can also occur in previously healthy person [14, 26]. Interestingly, there was a marked variation in the morbidity and mortality rates of adenovirus LRTI in different studied populations. Ad respiratory infection

Table 6 Comparison of demographic and clinical features between adenovirus serotypes 3 and 7 respiratory infections

	Serotype 3 (n=91)	Serotype 7 (n=50)	p-value
URTI	80 (87.9 %)	44 (88.0 %)	0.9878
Acute bronchiolitis	1 (1.1 %)	2 (4.0 %)	0.5947
Pneumonia	10 (11.0 %)	4 (8.0 %)	0.7845
Age groups			
0 to <2 years old	16 (17.6 %)	15 (30.0 %)	0.1360
2–<4 years old	41 (45.0 %)	18 (36.0 %0	0.3874
4–6 years old	34 (37.4 %)	17 (34.0 %)	0.8303
Mean age (years)	3.43±1.42	3.17±1.84	0.3518
Male/female	50:41	28:22	0.9041
History of prematurity	4 (4.4 %)	8 (16.0 %)	0.0407
Presence of siblings at home	42 (46.2 %)	20 (40.0 %)	0.5982
Attendance of nursery	48 (52.7 %)	23 (46.0 %)	0.5548
Institutionalised	2 (2.2 %)	9 (18.0 %)	0.0025
Presence of underlying chronic illness	16 (17.6 %)	7 (14.0 %)	0.7546
Mean fever prior to AED admission (days)	3.7±2.0	3.6±2.4	0.7920
Mean peak temperature (°C)	39.7±0.6	39.6±0.5	0.3179
Mean total fever duration (days)	5.5±1.9	5.4±2.5	0.7902
Presence of extrapulmonary manifestations	30 (33.0 %)	21 (42.0 %)	0.3763
Mean total WBC count	13.8±6.0	11.8±5.5	0.0533
Neutrophil (ANC) count	9.5±5.0	7.8±5.1	0.0572
Lymphocyte count	3.0±2.6	2.5±1.5	0.2137
CXR abnormalities (for pneumonia cases)	4/10 (40 %)	3/4 (75.0 %)	0.5541
Lobar consolidation			
Mean hospital stay (days)	2.8±1.2	3.7±3.3	0.0207
Antibiotics given after admission	26 (28.6 %)	15 (30.0 %)	0.8582

had a profound impact in some countries. Mortality up to 16 % and permanent pulmonary sequelae ranging from 14 to 60 % were reported in children in some South American countries following Ad pneumonia [3, 4, 24, 34]. Lower yet still significant mortality and morbidity rates were reported in some neighboring Asian countries. In Korea, Hong et al. reported that Ad LRTI-related mortality in a children hospital was 12 % overall and 19 % among patients who were infected with serotype 7 [17]. Residual sequelae were identified in 50 % of the patients who were infected with serotype 3 and in 25 % of those infected with serotype 7. In a Taiwanese study, two out of 48 patients with Ad pneumonia died and five had permanent lung damage, while there was no mortality nor long-term sequelae found in the non-Ad group [12]. While traditionally serotypes 3 and 7 were well known to cause more severe respiratory infections, there were occasional reports on other serotypes which could be potentially associated with severe infections. For example, in the review by Lee et al. [20], serotype 8 was one risk factor for severe respiratory infection in Korea, while serotype 4 was identified as an important pathogen responsible for the fatal childhood pneumonia in South China [27]. The reason why a certain strain of Ad in some populations was more virulent and damaging to the growing lung was not understood clearly. It was speculated that host variation in immune response and the local virulence of the specific serotype might both play a part [10, 23, 35].

However, the disease severity of Ad respiratory infections in our locality appeared to be less alarming than many of our neighbouring countries. In our study, 14.6 % of those admitted were classified to have lower respiratory tract complications, either acute bronchiolitis or pneumonia, based on rather stringent diagnostic criteria, yet only four patients with LRTI suffered from mild hypoxemia, without severe acute respiratory compromise or mortality. As our study was conducted in a hospital providing both secondary and tertiary care in the region, it should have covered the whole spectrum of patients with severe illness.

Still one of our patients developed bronchiectasis after serotype 7 infection, a subtype notorious in causing long-term pulmonary sequelae. This serotype was indeed well known to be associated with severe pneumonia, but it only ranked third in causing pneumonia in our hospitalized cases, probably because it was only present in two cases out of the 4-year study period. It was no longer detected in 2003–2004 among our hospitalized children after the apparent small epidemic in 2001. We could not ascertain whether this might reflect an increasing trend of herd immunity in the community, resulting in lesser case severity. Lee et al. also observed a decreasing size of subsequent epidemics after a large outbreak of serotype 7 [20]. This could partly account for the milder disease phenotype associated with Ad infection during the study period in our locality.

As shown, the most prominent serotype–clinical syndrome correlation appeared to be serotype 2 with acute bronchiolitis. While Ad has been known to be occasionally associated with acute bronchiolitis, the role of serotype 2 has not been previously reported. One study from Cuba reported that serotype 5 accounted for 71 % of the 14 cases of acute bronchiolitis identified, but serotype 2 was not tested for in that study [2]. Their sample size was smaller (49 cases) and with a lower serotyping rate (63 %). The study was not confined to the pediatric age group and there was no clear case definition of acute bronchiolitis versus pneumonia.

It would be difficult to clearly delineate the complex relationship between serotype virulence, host factor, and resultant disease phenotype. For example, serotype 2 seemed to infect the youngest children predominantly (mean age 1.94±-1.29 years old), but "young age" could also be the risk factor for developing acute bronchiolitis. On the other hand, serotype 3 displayed predilection for the slightly older age groups (mean age 3.43±1.42 years old). It was the leading cause for pneumonia among those aged between 3 and 5 years old, but it did not play any significant role for LRTI in those younger children. Hence, host factor might also be a potential confounding factor for the serotype–respiratory illness association. Further epidemiological studies would be needed to better support our observation.

The potential limitations of our study must be addressed. Firstly, there was no assurance of standardized documentation relating to the history, physical findings, and radiological interpretation because this was a retrospective study. Secondly, our study was confined to a single centre and the result might not be extrapolated beyond our local community. Thirdly, the neutralization test only routinely tested for serotypes 1 to 7 as these serotypes were considered to be most frequently associated with respiratory disease. It meant that other serotypes (constituted 6.3 % of the total cases) were not serotyped, though the majority of them only caused URTI. The neutralization test also might not be able to type virus isolates containing coinfections with multiple Ad serotypes. Such coinfections, even by serotypes 1–7, might appear non-neutralizable by the serotype-specific sera and would be falsely categorized as non-1–7 serotype. Fourthly, although we had tried to exclude the possible impact of co-infections by other pathogens in the analysis of the clinical disease spectrum, viruses such as human metapneumovirus, coronavirus, and rhinovirus and atypical bacteria such as mycoplasma and chlamydia were not tested for. Lastly, it was postulated that Ad might persist and be shed for prolonged periods and thus positive cell cultures for Ad might not represent an etiological link with disease. However, direct immunofluorescence assay from nasopharyngeal aspirate was known to have lower sensitivity than the cell culture method. Currently, there is no good diagnostic test to ascertain acute Ad infection as seroconversion test is not available in most clinical settings.

Conclusion

The current study was the first comprehensive review of Ad respiratory infections among hospitalized children in Hong Kong with serotype data presented. Apart from revealing the local serotype pattern and epidemiology, we have shown that Ad generally did not cause severe acute respiratory disease in our young children and long-term pulmonary sequelae was comparatively infrequent, but as the clinical manifestations of Ad infections mimicked bacterial infections, it often led to unnecessary hospital admissions and antibiotic use. Children aged between 1 and 2 years were most at risk for LRTI. Serotypes 2 and 3 were the most prevalent serotypes among our hospitalized children, and they seemed to have a predilection for different age groups and with different resultant respiratory illness association. A longer study period over time is needed to monitor the serotype pattern and disease complication for better understanding and prognostication of Ad infections in the region and to better delineate the potential complex relationship between serotype virulence, host factor, and resultant disease phenotype.

Acknowledgments We would like to acknowledge the "Virology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong SAR" for performing the Ad serotyping. This study was also supported in part by the Area of Excellence Scheme of the University Grants Committee (AoE/M-12/06), Hong Kong SAR.

Conflict of interest The author(s) declared no conflicts of interests with respect to the authorship or publication of this article and no personal financial relationship with the University Grants Committee.

References

1. Ampuero JS, Ocana V, Gomez J, Gamero ME, Garcia J, Halsey ES, Laguna-Torres VA (2012) Adenovirus respiratory tract infections in Peru. PLoS One 7(10):e46898. doi:10.1371/journal.pone.0046898
2. Belsy AOV, Alexander P, Clara S, Angel G, Grehete G, Guelsys G, Luis S, Pedro M, Guadalupe GM, Alina L, Pilar PB, Inmaculada C (2009) Molecular characterization of adenoviral infections in Cuba: report of an unusual association of species D adenoviruses with different clinical syndromes. Arch Virol 154(4):619–627
3. Carballal G, Videla C, Misirlian A, Requeijo PV, Aguilar Mdel C (2002) Adenovirus type 7 associated with severe and fatal acute lower respiratory infections in Argentine children. BMC Pediatr 2:6
4. Castro-Rodriguez JA, Daszenies C, Garcia M, Meyer R, Gonzales R (2006) Adenovirus pneumonia in infants and factors for developing bronchiolitis obliterans: a 5-year follow-up. Pediatr Pulmonol 41(10):947–953. doi:10.1002/ppul.20472
5. Cheng CC, Huang LM, Kao CL, Lee PI, Chen JM, Lu CY, Lee CY, Chang SY, Chang LY (2008) Molecular and clinical characteristics of adenoviral infections in Taiwanese children in 2004–2005. Eur J Pediatr 167(6):633–640. doi:10.1007/s00431-007-0562-4
6. Cherry JD (1998) Adenoviruses. In: Feigin RD, Cherry JD (eds) Textbook of pediatrics infectious diseases, 4th edn. Saunders, Philadelphia, pp 1666–1684
7. Chiu SS, Chan KH, Chen H, Young BW, Lim W, Wong WH, Peiris JS (2010) Virologically confirmed population-based burden of hospitalization caused by respiratory syncytial virus, adenovirus, and parainfluenza viruses in children in Hong Kong. Pediatr Infect Dis J 29(12):1088–1092. doi:10.1097/INF.0b013e3181e9de24
8. Chuang YY, Chiu CH, Wong KS, Huang JG, Huang YC, Chang LY, Lin TY (2003) Severe adenovirus infection in children. J Microbiol Immunol Infect 36(1):37–40
9. The American Academy of Pediatrics (2006) Diagnosis and management of bronchiolitis. Pediatrics 118 (4):1774–1793. doi: 10.1542/peds.2006-2223
10. Diaz PV, Calhoun WJ, Hinton KL, Avendano LF, Gaggero A, Simon V, Arredondo SM, Pinto R, Diaz A (1999) Differential effects of respiratory syncytial virus and adenovirus on mononuclear cell cytokine responses. Am J Respir Crit Care Med 160(4):1157–1164
11. Faden H, Wilby M, Hainer ZD, Rush-Wilson K, Ramani R, Lamson D, Boromisa R (2011) Pediatric adenovirus infection: relationship of clinical spectrum, seasonal distribution, and serotype. Clin Pediatr (Phila) 50(6):483–487. doi:10.1177/0009922810392775
12. Farng KT, Wu KG, Lee YS, Lin YH, Hwang BT (2002) Comparison of clinical characteristics of adenovirus and non-adenovirus pneumonia in children. J Microbiol Immunol Infect 35(1):37–41
13. Gray GC, McCarthy T, Lebeck MG, Schnurr DP, Russell KL, Kajon AE, Landry ML, Leland DS, Storch GA, Ginocchio CC, Robinson CC, Demmler GJ, Saubolle MA, Kehl SC, Selvarangan R, Miller MB, Chappell JD, Zerr DM, Kiska DL, Halstead DC, Capuano AW, Setterquist SF, Chorazy ML, Dawson JD, Erdman DD (2007) Genotype prevalence and risk factors for severe clinical adenovirus infection, United States 2004–2006. Clin Infect Dis 45(9):1120–1131. doi:10.1086/522188
14. Hakim FA, Tleyjeh IM (2008) Severe adenovirus pneumonia in immunocompetent adults: a case report and review of the literature. Eur J Clin Microbiol Infect Dis 27(2):153–158. doi:10.1007/s10096-007-0416-z
15. Herbert FA, Wilkinson D, Burchak E, Morgante O (1977) Adenovirus type 3 pneumonia causing lung damage in childhood. Can Med Assoc J 116(3):274–276
16. Hierholzer JC (1989) Adenoviruses. In: Schmidt NJ, Emmons RW (eds) Diagnostic procedures for viral, rickettsial and chlamydial infections, 6th edn. American Public Health Association, Washington DC, pp 219–264, Chapter 10
17. Hong JY, Lee HJ, Piedra PA, Choi EH, Park KH, Koh YY, Kim WS (2001) Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features, and prognosis. Clin Infect Dis 32(10):1423–1429
18. James AG, Lang WR, Liang AY, Mackay RJ, Morris MC, Newman JN, Osborne DR, White PR (1979) Adenovirus type 21 bronchopneumonia in infants and young children. J Pediatr 95(4):530–533
19. Kim SH, Huh JH, Bae SY, Kim JS, Yoon SY, Lim CS, Cho Y, Kim YK, Lee KN, Lee CK (2006) Epidemiology of respiratory viral infection in 2004–2006. Korean J Lab Med 26(5):351–357
20. Lee J, Choi EH, Lee HJ (2010) Clinical severity of respiratory adenoviral infection by serotypes in Korean children over 17 consecutive years (1991–2007). J Clin Virol 49(2):115–120. doi:10.1016/j.jcv.2010.07.007
21. Lee J, Choi EH, Lee HJ (2010) Comprehensive serotyping and epidemiology of human adenovirus isolated from the respiratory tract of Korean children over 17 consecutive years (1991–2007). J Med Virol 82(4):624–631. doi:10.1002/jmv.21701
22. Lo JY, Tsang TH, Leung YH, Yeung EY, Wu T, Lim WW (2005) Respiratory infections during SARS outbreak, Hong Kong, 2003. Emerg Infect Dis 11(11):1738–1741
23. Moro MR, Bonville CA, Suryadevara M, Cummings E, Faddoul D, Kobayaa H, Branigan PJ, Domachowske JB (2009) Clinical features, adenovirus types, and local production of inflammatory mediators in adenovirus infections. Pediatr Infect Dis J 28(5):376–380. doi:10.1097/INF.0b013e31819075a6

24. Murtagh P, Giubergia V, Viale D, Bauer G, Pena HG (2009) Lower respiratory infections by adenovirus in children. Clinical features and risk factors for bronchiolitis obliterans and mortality. Pediatr Pulmonol 44(5):450–456. doi:10.1002/ppul.20984
25. Murtagh P, Kajon A (1997) Chronic pulmonary sequelae of adenovirus infection. Pediatr Pulmonol Suppl 16:150–151
26. Odievre MH, Danekova N, Picard C, Mesples B, BenCheikha Z, Avran D, De Blic J, Leruez-Ville M, Parez N (2011) Pneumonia due to adenovirus type 7: a case report in a healthy infant. Arch Pediatr 18(7):772–777. doi:10.1016/j.arcped.2011.04.005
27. Ou ZY, Zeng QY, Wang FH, Xia HM, Lu JP, Xia JQ, Gong ST, Deng L, Zhang JT, Zhou R (2008) Retrospective study of adenovirus in autopsied pulmonary tissue of pediatric fatal pneumonia in South China. BMC Infect Dis 8:122. doi:10.1186/1471-2334-8-122
28. Shann F (1992) WHO definition of tachypnea in children. Lancet 339(8786):176–177
29. Simila S, Linna O, Lanning P, Heikkinen E, Ala-Houhala M (1981) Chronic lung damage caused by adenovirus type 7: a ten-year follow-up study. Chest 80(2):127–131
30. Sly PD, Soto-Quiros ME, Landau LI, Hudson I, Newton-John H (1984) Factors predisposing to abnormal pulmonary function after adenovirus type 7 pneumonia. Arch Dis Child 59(10):935–939
31. Spigelblatt L, Rosenfeld R (1983) Hyperlucent lung—long-term complication of adenovirus type-7 pneumonia. Can Med Assoc J 128(1):47–49
32. Sung RY, Chan PK, Tsen T, Li AM, Lam WY, Yeung AC, Nelson EA (2009) Identification of viral and atypical bacterial pathogens in children hospitalized with acute respiratory infections in Hong Kong by multiplex PCR assays. J Med Virol 81(1):153–159. doi:10.1002/jmv.21364
33. Sung RY, Cheng AF, Chan RC, Tam JS, Oppenheimer SJ (1993) Epidemiology and etiology of pneumonia in children in Hong Kong. Clin Infect Dis 17(5):894–896
34. Videla C, Carballal G, Misirlian A, Aguilar M (1998) Acute lower respiratory infections due to respiratory syncytial virus and adenovirus among hospitalized children from Argentina. Clin Diagn Virol 10(1):17–23
35. Yoon JS, Kim HH, Lee Y, Lee JS (2007) Cytokine induction by respiratory syncytial virus and adenovirus in bronchial epithelial cells. Pediatr Pulmonol 42(3):277–282. doi:10.1002/Ppul.20574
36. Yuksel H, Yilmaz O, Akcali S, Sogut A, Ciftdogan DY, Urk V, Ertan P, Sanlidag T (2008) Common viral etiologies of community acquired lower respiratory tract infections in young children and their relationship with long term complications. Mikrobiyol Bul 42(3):429–435