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Human adenovirus infection in children with acute respiratory tract disease in Guangzhou, China

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Acute respiratory infections (ARI) are the major worldwide health problem due to associated high morbidity and mortality rates. Adenovirus (Adv) is one of the most common causes of viral ARI, and thus calls for specific diagnosis and better understanding of the epidemiology and clinical characteristics. Our aims were to find out the status of Adv infection in children <14 years with ARI, analyze the epidemiology and clinical characteristics among the Adv-infected children in Guangzhou, China, and to provide some basis for the research of Adv. The throat and pharyngeal swabs were collected among the children with acute respiratory tract infections in outpatient department from September 2006 to August 2008. The samples were analyzed by PCR and the sequences were blasted with the sequences of Adv in GenBank. Clinical data were analyzed along with virological data by using appropriate statistical methods. Adv was detected in 25 out of 512 (4.9%) children. The genome types of 23 samples were determined after analysis of the gene sequence. The most prevalent Adv type was species B type 3. Among the patients, 10 were of Ad3 (43.5%), three were of Ad1 (1.3%), five were of species C Ad2 (21.7%), and five were of species E Ad4 (21.7%). A higher incidence of positive results was found during the summer season, thus showing a pattern of seasonality. There exists Adv infection in children with acute respiratory system diseases in Guangzhou area. No significant differences were found among different age groups and gender groups. Co-infections with other respiratory virus were detected in 64% of the Adv positive samples.

Key words: Adenovirus; virus respiratory infection; incidence.

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Respiratory virus is the most frequent cause of respiratory tract infection for infants and young children in developed countries, but less is known in developing countries (1). It is estimated that adenovirus (Adv) causes 5~15% infection among all respiratory diseases in children. Adenoviruses (Advs) are categorized by species (A–F), further by serotype (Ad1–Ad51), and even further by genome type. These distinctions correlate strongly with antigencity, clinical presentation, and epidemiological character (2) and the same groups are similarly clustered on the basis of genetic homology (3). Respiratory virus infection is caused mainly by species B, C, and E. Advs of species B (Ad3 and Ad7) and species C (Ad1, Ad2, and Ad5) are commonly associated with respiratory tract infections and may persist in children without causing symptoms for years. Species D (Ad8, Ad19, andAd37) usually causes conjunctivitis. Identification of these serotypes is helpful in preventing outbreaks of

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epidemic keratoconjunctivitis. Ad4, the only member of species E, has been reported in both respiratory and ocular infections but is especially related to conjunctivitis outbreaks. Ad40 and Ad41 of species F usually cause severe gastroenteritis. Ad52 has recently been characterized as a new serotype and represents a new species G that is associated with gastroenteritis outbreaks.

High rates of mortality and morbidity attributed to respiratory diseases occur all over the world and the same situation is found in Guangzhou, Southern China. Outbreak of respiratory Adv infection was reported in China, but few data about the molecular epidemiology of Adv in developing countries, such as China, are available and no surveillance system has been established. The aim of this study is to evaluate the Adv incidence and seasonality in respiratory infections from outpatients' children; the clinical features of the Adv infection were also analyzed.

PATIENTS AND METHODS

Patients

From September 2006 to August 2008, consecutive children <14 years who sought care at the Second Affiliated Hospital of Guangzhou Medical College in Guangzhou City with symptoms of an ARI were eligible for participation. Symptoms of ARI were defined as developing of a new illness within the past 3 days with fever, cough, sinus pain, sore throat or congestion. Trained research assistants continuously reviewed triage note to identify potentially eligible patients. Once the patients were identified, a form was used to register the patient's age, clinical signs, and symptoms.

Adenovirus detection using PCR techniques

At the time of enrollment, throat and pharyngeal swabs were collected and placed in 3 mL viral transport media then were immediately transferred to the laboratory and aliquoted into multiple sterile tubes, which will be used for nucleic acid extraction, virus isolation, and storage at −70 °C respectively.

Total nucleic acid was extracted from respiratory specimens using the QIAamp minElute virus spin kit (Qiagen, Hombrechtikon, Germany). All the pre-PCR processing was undertaken in a separate location for PCR and post-PCR analysis. Conventional PCR assay was performed according to Echavarria et al. (4), with primers for Adv targeting the hexon gene as follows: Hex1885-5′GCCGCAG TGGTCTTACATGCACATC, Hex1913-5′CAGCA CGCCGCGGATGTC AAAGT.

Detection of other respiratory pathogens

Besides detecting Adv, each sample was also analyzed for respiratory syncytial virus (RSV), influenza virus A and B, parainfluenza virus types 1–4, human metapneumovirus, human coronavirus OC43 and 229E, and picornavirus(inclusive of rhinovirus and enterovirus) by RT-PCR (5).

Phylogenic tree analysis

The PCR products were purified using QIAquick Gel Extraction kit (Qiagen, Hombrechtikon, Germany) and subsequently sequenced directly on both strands using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA) with ABI prism 3130 analyzer(Applied Biosystems). Phylogenic trees were constructed using MEGA version 4.0.

Data analysis

SPSS version 12.0 (SPSS, Chicago, IL, USA) was used for statistical analysis by applying Chi-squared and Fisher's exact test, p-values <0.05 was considered statistically significant.

RESULTS

Respiratory virus detection

The study involved 512 clinical samples collected from 2006 to 2008 to analyze the incidence and disease in the region. By screening with PCR, Adv was detected in 25 of 512 children samples. Frequencies of the different virus are presented in Table 1. RSV was the most prevalent, which was detected in 126 samples (24.6%) and showed the highest co-infection with Picornavirus in 20of 126 samples (15.9%). Adv infection appeared as the fifth highest frequency, of which 25 of 512 (4.9%) were detected and showed co-infection rate of 8(32%), 2(8%), 1(4%), 2(8%), 1(4%), 1(4%) and 1(4%) out of 25 with RSV, influenza A, influenza B, human metapneumovirus, picornavirus, human parinfluenza, and human coronavirus, respectively. Adv co-infections with other respiratory viruses were detected in 18 (72%) of positive samples.

Table 1. Total results of simple infection and co-infections in children with ARI

	RSV	hMPV	HPIV	Adv	FluA	FluB	CoV	Picorna	Total
RSV	**66**	5	5	8	14	7	1	20	186
hMPV		**24**	1	2	1	1	0	10	54
HPIV			**21**	1	0	0	0	2	26
Adv				**20**	2	1	1	1	30
FluA					**46**	4	0	5	64
FluB						**20**	0	5	30
Coro							**5**	0	5
Picorna								**79**	79

Number of samples containing each set of viruses (row × column). In boldface, simple infections.
Adv, adenovirus; ARI, acute respiratory infections; Flu A,influenza A virus; FluB, influenza B virus; CoV, human coronavirus (229E and OC43); hMPV, human metapneumovirus; HPIV, human parinfluenza; Picorna, Picornavirus; RSV, respiratory syncytial virus.

Genotyping by PCR and sequencing

From the 25 Advs which were screened positive by PCR, 23 samples genome types were determined after analysis of the gene sequence. Ten were of species B serotype Ad3, eight were of species C serotypes (Ad1 and Ad2), and five were of species E serotype Ad4.

Phylogenetic analyses of Adv strains

Amplified PCR products of the Adv hexon gene were visualized on 2.0% gels. The PCR products from each positive specimen were sequenced and were identical with Adv. The 23 Adv strains detected in this study were classified into four serotypes, three strains belonged to Ad1, five strains belonged to Ad2, ten belonged to Ad3, and five belonged to Ad4 (Fig. 1).

Epidemiological data associated with Adv infection

Based on the sampling date, we were able to obtain a graph of the positive rate to the Adv in each month of the year (Fig. 2), showing the seasonality of the Adv from September 2006 to August 2008 (Table 2). Fourteen (20.9%) patients were infected with Adv in summer, which was significantly higher than those of the other seasons, including spring (6.5%), autumn (1.2%), and winter (1.8%).

The clinical features of Adv infections are summarized in Table 3. When physical symptoms were compared with adv infection no significant association was found, both simple infection and co-infection often consisted of headache and angina, followed by catarrh, fever, and cough, but no chest radiograph change was detected. Table 3 also presents the

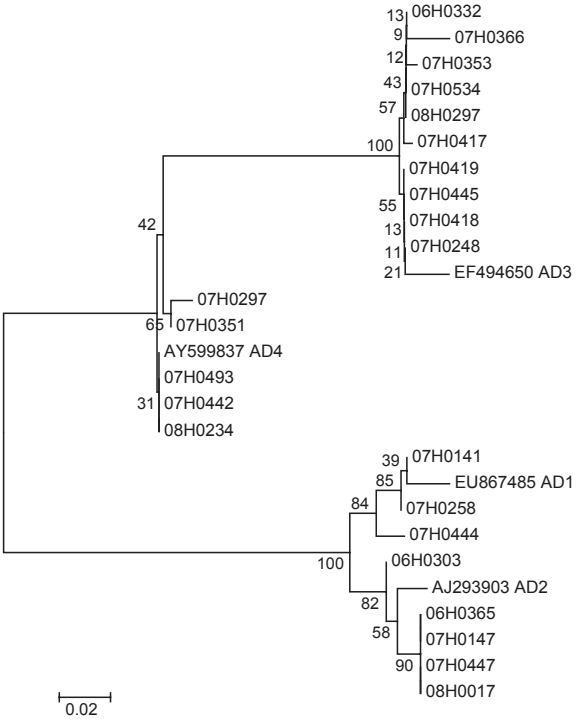

Fig. 1. Phylogenetic analysis of the hexon genes of human adenovirus in China children, which was reconstructed using neighbor-joining method. Bootstrap proportions (1000 replicates) are plotted at the branches of phylogram to show support values. Sequences are available from Genbank under accession No.FJ404725-FJ404747. The accession numbers of reference sequence and their origins are: EU867485, AJ293903, EF494650, AY599837.

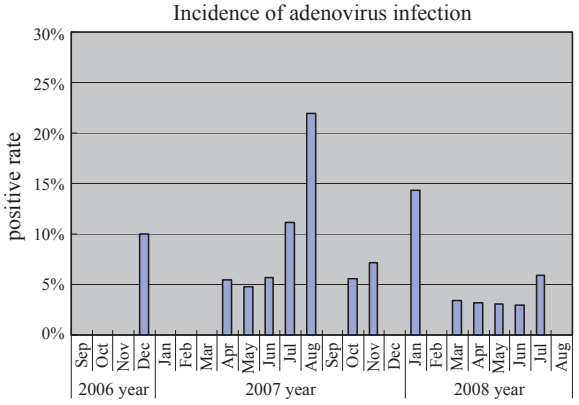

Fig. 2. Incidence of adenovirus infection from children with acute respiratory infection.

age and the gender distribution along the period of the study; age and gender were not relevant in outpatient children with adv infection.

Table 3. Demographic and clinical data of the children with acute respiratory infection

Variable	No. of detected	No. of positive	Positive rate
Age (years)			
0˜3	255	10	3.92%
4˜6	150	5	2.98%
7˜14	107	10	9.35%
p-value			0.053
Gender			
Male	297	17	5.72%
Female	215	8	3.42%
p-value			0.299
Clinical symptom			
Fever	512	25	5.19%
Cough	317	14	4.42%
Catarrh	311	19	6.11%
Headache, angina	84	7	8.33%
Chest radiograph change	53	0	0%
p-value			0.456[1]

[1]Applied by fisher's exact test.

DISCUSSION

This study describes the molecular epidemiology of Adv associated with respiratory infection in Guangzhou and provides a basis for future survey studies on the changes of this virus.

In summary, our results have shown a rate of Adv detection by PCR of 4.88% in throat and pharyngeal swabs from outpatient children with mild respiratory symptoms, most of which were caused by species B Ad3. This prevalence is significantly lower than that reported recently from Germany (12.9%), Brazil (6%), but is higher than that from India (1.5%) (6–8). However, among these studies, only in the German study RT-PCR was used. The last two employed immunofluoresence and viral culture, respectively. The differences among the prevalence rates can be due to a variety of factors, including age of the patients, geographic location, and severity of disease, type of sample, diagnostic method, and time of the year.

Overall 97 (18.9%) of the children had more than one potential pathogen detected in their nasopharyngeal swabs. This is perhaps not surprising as the respiratory viral seasons are often coincident (9), thus increasing the detect range of potential pathogens will reveal more co-infections. In 60 (47.6%) children the copathogens were RSV with one or more other pathogens. In 16 (64%) of the cases of Adv infection it was found together with another potential pathogen. The German study showed that in 5% of the cases when an agent was detected there was more than one potential pathogen and in most cases this was Adv with

Table 2. Seasonal distribution of adenovirus infection from children with actute respiratory infection

No. (%) of positive samples			
Season	September 2006–August 2007	September 2007–August 2008	Total
Spring (March–May)	3 (3.23%)	3 (3.19%)	6 (3.2%)[1]
Summer (June–August)	11 (12.94%)	3 (3.75%)	14 (8.0%)[1]
Autumn (September–November)	0 (0%)	2 (3.7%)	2 (1.02%)[1]
Winter (December–February)	1 (4.17%)	2 (4.65%)	3 (4.5%)[1]

[1]A significant difference ($\chi^2 = 12.272$, $p < 0.05$) between summer and the other seasons.

another pathogen (6). Such co-infection has previously been linked with more severe acute respiratory infections (ARI) in some (10–12) but not all studies (13, 14).

Adenoviral infections cause a wide range of diseases depending on the serotype. In this study, Ad3 was identified as the major causative agent responsible for the respiratory Adv infection in the children. Ad3 of Adv species B is frequently associated with respiratory tract infection in pediatric patients and may cause severe infection in newborns and infant (15). In Korea, successive outbreaks of severe acute respiratory illnesses caused by Ad3 in children during 1990s have been reported (16, 17).The presence of Ad3a was also detected in hospitalized children with acute respiratory infection in Brazil, where Ad7 of the same species B was the predominant serotype for decades (18). In southern Taiwan, Ad3 used to be the predominant serotype for respiratory Adv infection in 1981–1989 (68%), 1990–1998 (44%), 2000 (36%), and 2002 (46%) (19, 20). In Chile serotypes seven, two, and one are the most frequently detected in infants hospitalized for ALRI (21).

Adenovirus infections can occur endemically or as outbreaks. Adv-B group B1 (Ad3, Ad7, and, less frequently, Ad21) and Ad4 have been the causative agents in epidemic outbreaks of respiratory disease in Europe, America, Oceania, and Asia (22–25). Adv infections are highly contagious and common in dense and close populations, such as military training venues and day-care centers. The population of Asia is large and often dense, especially in China and Japan. Consequently, epidemics of ARD caused by Advs occur at high frequency. In July 2004, more than 200 children from an infant school were infected with Ad3 in Guangzhou, southern China (26).

This study revealed the incidence and pattern of seasonal prevalence of Adv for outpatients in Guangzhou. From the two-year studies, it is noticed that the summer season has a higher infection than other seasons. But in Brazil and in other tropical countries, Adv infection was observed year round without a clear seasonality, and it was the same to northern Taiwan that no seasonal variation was noted for Adv. But in Hong Kong and Mexico, Adv infections are usually more frequent in winter and spring. The infections were distributed among the different age groups and no differences were found in the respiratory symptoms between the children positive for Adv positive and with ARI without Adv (27, 28). And there were no differences in age and gender distribution of Adv infection children in our study. Other studies found that children between 1 and 3 years of age were the most common age group affected by Adv. It is important to know the occurrence of the virus and the seasonal pattern, then patients can be managed with specific antiviral drugs and even the inappropriate use of antibiotics could be reduced to reduce the extensive burden of disease. The most important limitation of this study was that it was conducted in a relatively short period and only in outpatient children.

Better therapies and prevention strategies are needed to decrease the burden of ARI particularly those that are caused by Adv. Thus additional molecular epidemiological studies over longer periods are warranted to better determine the role of the different Adv serotypes in the epidemiology and the severity of disease and their inter-relationship with other respiratory pathogens. This could inform better therapeutic approaches and vaccine development.

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