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Original Article

Molecular characteristics of the structure protein VP1 in Coxsackievirus A10 Isolates from China

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Abstract

Introduction: Coxsackievirus A10 (CVA10) is a non-enveloped, positive-sense single-stranded RNA virus classified within the *Enterovirus* genus in the *Picornaviridae* family. It is among the pathogens that can cause hand, foot and mouth disease. This study aimed to analyze the temporal and spatial distribution of CVA10 in China to understand its epidemiological characteristics of CVA10.

Methodology: We collected the VP1 sequences of CVA10 from January 1, 2004, to December 31, 2019, from the GenBank database and created the global map using MapChart. We selected 56 known CVA10 genotype sequences. Then, MEGA6.06 was used to construct a phylogenetic tree with the collected gene sequences and the known reference sequences for comparative analysis to assess the distribution of CVA10 genotypes in different countries between 2004 and 2019.

Results: CVA10 has been widely detected or reported globally. In China, the prevalent genotype of CVA10 was mainly genotype B before 2008 and genotype C after 2009. In other countries, the prevalence of genotype D was dominant, followed by genotypes C and F, and the prevalence of CVA10 varied from continent to continent.

Conclusions: Monitoring CVA10 genotypes or evolutionary branches should be strengthened, and the study of epidemic genotype characteristics should be enhanced. This will serve as a basis for further research and development of monovalent CVA10 or polyvalent vaccines designed for effective disease prevention.

Key words: Coxsackievirus A10; hand-foot-mouth disease; VP1.

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Introduction

Coxsackievirus A10 (CVA10) is a non-enveloped, positive-sense, single-stranded RNA virus belonging to the *Enterovirus* genus in the *Picornaviridae* family [1]. The capsid of CVA10 is composed of four structure proteins (VP1-4). VP4 is situated on the inner side of the mature virus particle, whereas VP1-VP3 are positioned on the outer side of the mature virus. Therefore, the antigenic determinant of the virus is mainly positioned on VP1~VP3. At present, the genotyping standard in the *Enterovirus* genus is not clear. This is because the complete coding region of VP1 contains many important neutralizing antigenicity sites, and the genotypes between the two sequencing windows usually have the same classification. Thus, VP1 is generally used as the basis for *Enterovirus* subtype typing [2,3]. Moreover, CVA10 is one of the pathogens that can cause hand, foot and mouth disease (HFMD) [4].

HFMD is a common infectious disease that affects early childhood and is caused by enteroviruses. It can be transmitted through the faecal-oral transmission, respiratory tract, and other modes of transmission [5,6]. Preschoolers are most susceptible to this infection [7]. In general, most patients present with fever, macular papules, or small rashes on the skin and mucous membranes of their hands, feet, and mouth. These symptoms usually subside and disappear within a week. However, HFMD can also cause severe diseases such as meningitis, which can cause damage to body functions and even lead to death [6,8,9].

HFMD has become a serious public health issue globally, especially in Asia. In China, *Enterovirus*-A71 (EV-A71) and CVA16 are the most common viruses causing HFMD, but the prevalence of CVA10 has also

increased in recent years [1]. The CVA10 isolates were reported as the main pathogens responsible for herpangina (HA) outbreaks in Aichi Prefecture in 2005 and Kanagawa Prefecture in 2007 in Japan [10]. In 2008, a global epidemic of HFMD emerged, caused by CVA6 and CVA10, with the main symptom being onychomadesis in Finland [11]. Since 2008, HFMD has been prevalent in most areas of China, with reported cases of CVA10 infections occurring throughout the country [12]. An epidemic of HFMD was reported in Guangzhou from 2010 to 2012 [13]. An analysis of the data of patients with HFMD in Shanghai from 2012 to 2013 revealed that CVA10 accounted for 7.5% of cases [14]. CVA10 became the pathogen with the highest prevalence rate of 52.8% in the HFMD outbreak in Wuhan. The epidemiological data suggested that the pathogen spectrum of HFMD in China is changing gradually. The infection rates of EV-A71 and CVA16 showed a declining trend, while CVA6 exhibited an increasing trend. Meanwhile, the infection rate of CVA10 was also increasing with small outbreaks [1,15,16]. EV-A71 and CVA16 usually have similar clinical manifestations, but CVA10 can lead to onychomadesis, HA, and other fatal complications such as neuromyelitis [17].

To understand the epidemiological characteristics of CVA10, we analyzed the temporal and spatial distribution of CVA10 in China. We also analyzed the annual genotype distribution and amino acid mutation of CVA10 for further study based on the VP1 region, providing a basis for preventing and treating HFMD.

Methodology

Sequence acquisition of VP1 region

All the VP1 gene sequences of CVA10 isolated between January 1, 2004, and December 31, 2019, were retrieved and downloaded from the GenBank database, and the global map was created using MapChart (www.mapchart.net). Further, 2190 isolates of CVA10 were collected globally, and 56 reference sequences were chosen for genotype analyses.

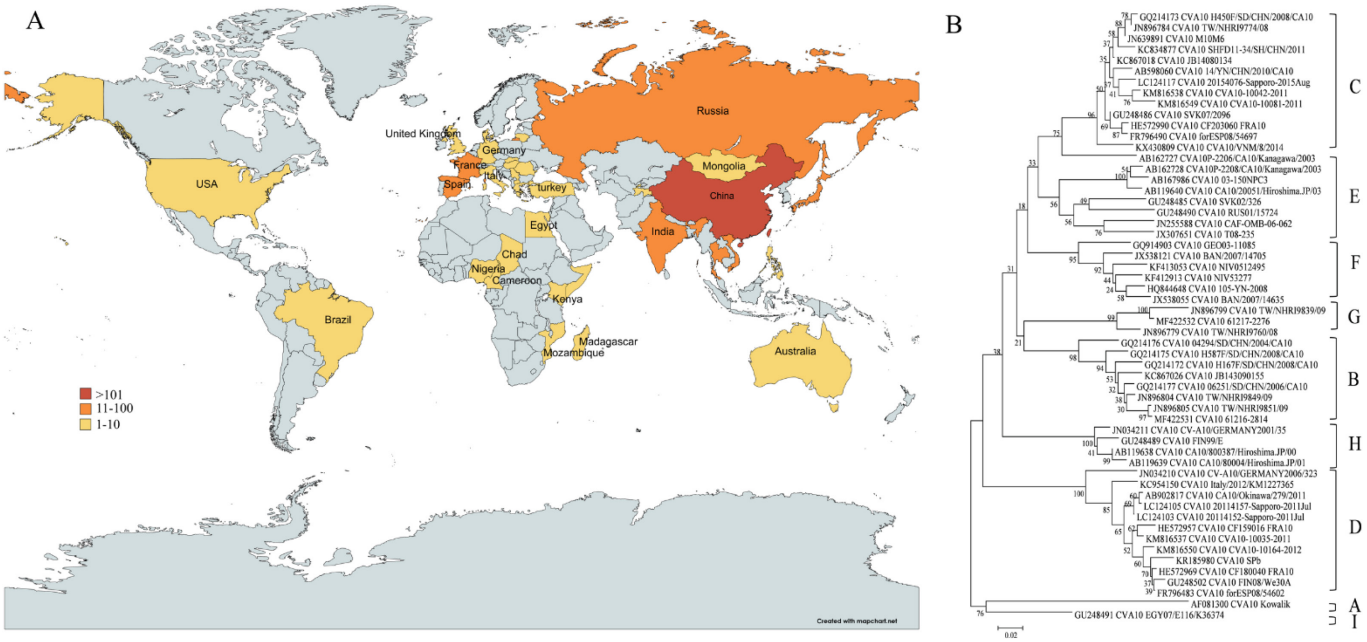
Reference sequences chosen for the VP1 region

Generally, the nucleotide sequence of the VP1 gene is 894 bp, and CVA10 can be classified into seven genotypes: A, B, C, D, E, F, and G, based on a nucleotide difference rate between genotypes greater than 15%. However, some studies believed that CVA10 could be classified into nine genotypes, such as A, B, C, D, E, F, G, H, and I, with an average group distance between 14.5% and 24.6% based on the 239 bp VP1 gene [4]. Relevant reference sequences were selected based on the nine-genotypes classification method (Figure 1B).

Phylogenetic analysis of VP1 region

Because the VP1 sequences of CV-A10 obtained from the GenBank database were not all complete, only

Figure 1. Distribution of CVA10 in the world and phylogenetic analysis of reference sequences.



A) Distribution of CVA10 in the world; B) Reference sequences for genotype analyses and phylogenetic analysis.

the VP1 sequences containing the core (239 bp) were retained to construct the phylogenetic tree to better study the evolution of CV-A10 in China and the global epidemic trend. Sequence alignment was performed using MAFFT with the default settings, and phylogenetic trees were generated using the Neighbor-Joining method with 1000 bootstrap resamplings of the alignment data sets and visualized using the program MEGA6.06 [18].

Results

Global distribution of CV-A10

CVA10 has exhibited a global presence, with a high prevalence in China, followed by Russia, India, France, and Spain. Sporadic outbreaks have been recorded in other countries, such as the United States, Australia, and the United Kingdom (Figure 1A). The phylogenetic tree based on the VP1 region of 56 reference sequences is depicted in Figure 1B to prove the appropriate selection of reference sequences.

Global distribution of CV-A10 genotypes

A phylogenetic tree was constructed based on the VP1 nucleic acid sequences of 423 isolates from other countries and 56 reference sequences of CVA10. The analysis results indicated that in other countries, the prevalence of genotype D was dominant, followed by genotypes C and F. Additionally, the prevalence of CVA10 varied from continent to continent. In other Asian countries except China, the prevalence of CVA10 was dominated by genotype F, with genotype C as the second most common, followed by genotypes D, E, H, and I (genotype F: 88 isolates, genotype C: 74 isolates, genotype D: 38 isolates, genotype E: 19 isolates, genotype H: 9 isolates, and genotype I: 1 isolate). Genotype F was prevalent in Bangladesh, Georgia, India, and other countries, whereas genotype D was prevalent in Japan and Cyprus, and genotype E was more prevalent in Mongolia and Tajikistan. In Vietnam, Thailand, and the Philippines, genotype C was dominant, similar to China. Besides Asia and Europe, the other continents presented fewer CVA10 isolates. In Africa, genotypes C, E, and F were more prevalent, while genotypes D, G, and I were less frequent. These results were illustrated in Figure 2.

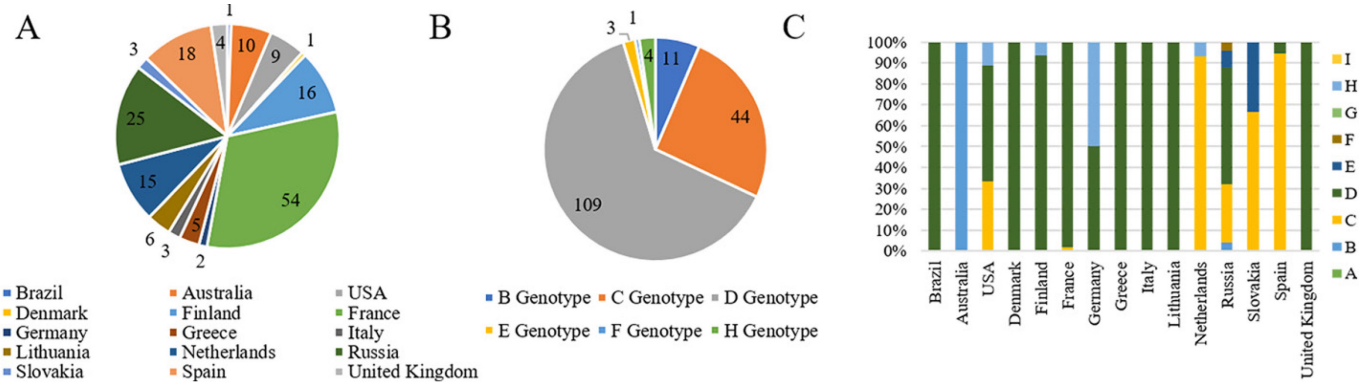
In Europe, the most prevalent genotype of CVA10 was D, followed by genotype C, with the occasional presence genotypes E, H, B, and F (genotype D: 103 isolates, genotype C: 41 isolates, genotype E: 3 isolates, genotype H: 3 isolates, genotype B: 1 isolate, and genotype F: 1 isolate). In Denmark, Finland, France, Greece, Italy, Lithuania, the United Kingdom, and Russia genotype D was predominant. However, in the Netherlands, Slovakia, and Spain genotype C was the most prevalent. Besides Asia and Europe, fewer virus isolates were isolated from the other continents. In South America, the isolated genotype of CVA10 was D. North America was dominated by genotype C, followed by genotypes D and G isolated occasionally. In Australia, genotype B was the dominant isolate. The Kowalik isolate from the United States in 1950 was the only representation of genotype A. These results are shown in Figure 3.

Distribution of CV-A10 genotype in different years in China

A total of 1767 isolates of CVA10 were collected from the GenBank database of mainland China from January 1, 2004, to December 31, 2019. Subsequently, 1501 viruses in China with complete VP1 sequences were screened and retained for further study.

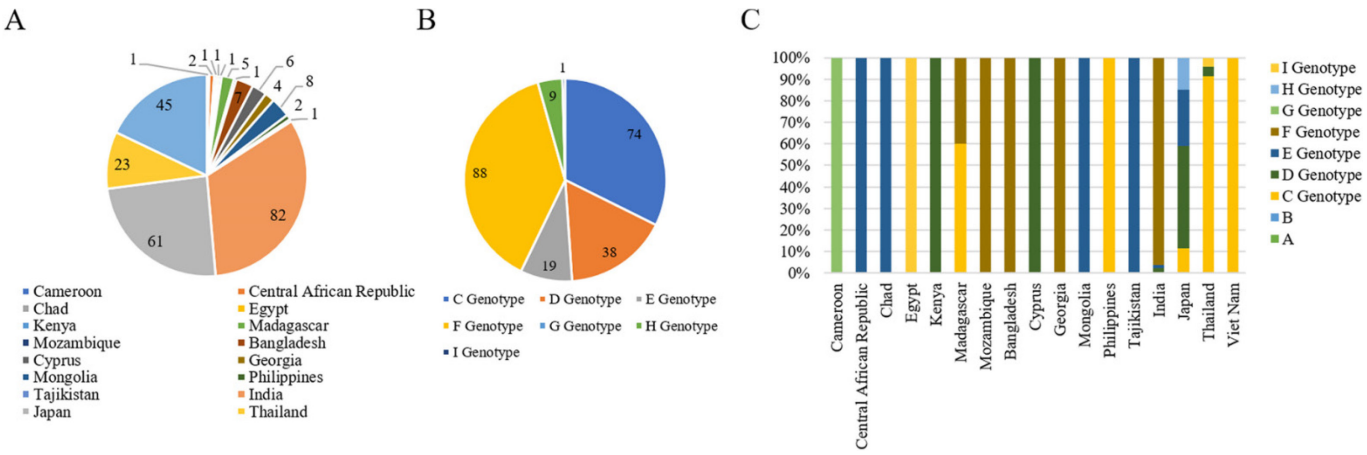
The temporal and geographical distribution of CVA10 in China showed that before 2008, CVA10 was sporadic in a few regions and provinces, with a small

Figure 2. Distribution of CVA10 genotypes in Africa and Asia (except China).



A) The number of CVA10 isolates in Africa and Asia; B and C) The number of CVA10 genotypes in Africa and Asia.

Figure 3. Distribution of CVA10 genotypes in South America, North America, Oceania and Europe.



A) The number of CVA10 isolates in South America, North America, Oceania and Europe. B and C) The number of CVA10 genotypes in South America, North America, Oceania, and Europe.

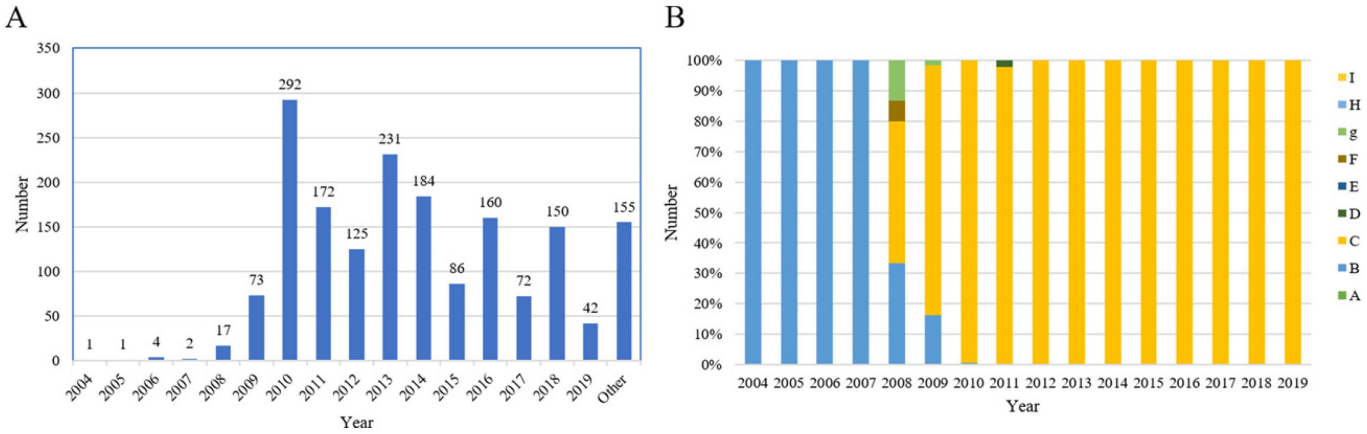
number of cases. The number of cases started increasing in 2009. After 2014, it showed an increasing and then decreasing yearly trend (Figure 4A). Subsequently, a phylogenetic tree was constructed based on the VP1 nucleic acid sequences of 1501 isolates from China and 56 reference sequences of CVA10. The analysis results revealed that genotype C was dominant, followed by genotype B, and genotypes D, F, and G were occasionally present among these CVA10 isolates. The genotype distribution of 1350 CVA10 isolates in different years did not show precisely the same pattern. Six isolates isolated from China were all genotypes B before 2008. Genotypes C appeared, accompanied by genotypes F and G in 2008, and one isolate belonging to genotype G was isolated in 2009. In 2011, genotype B disappeared, and the prevailing body began to change to genotype C, a new genotype D appeared in that year. Genotype C became the most common prevalent isolate of CVA10 between 2012 and 2019. These findings were presented in Figure 4B.

Discussion

The prevalence of HFMD in China is mainly caused by EV-A71 and CVA16. However, CVA6 and CVA10 outbreaks have reportedly increased in recent years [6]. Some studies have found that both CVA10 and EV-A71 can cause serious complications, accounting for a higher proportion of severe cases of HFMD. This suggests that CVA10 may be a new pathogen causing severe HFMD and needs urgent attention for its prevention and treatment [1].

Based on the perspective of epidemiology, the prevention of infectious diseases is generally based on three aspects: the source of infection, the route of transmission, and the susceptible population. This

Figure 4. The number of CVA10 isolates and genotypes in China every year.



A) The number of CVA10 isolates in China. B) The number of CVA10 genotypes in China.

approach also applies to the prevention of CVA10. At present, vaccines are mainly used for prevention purposes. In 2015, the inactivated *Enterovirus* vaccine was approved for marketing, but this vaccine had no preventive effects against other enteroviruses causing HFMD except EV-A71 [19]. Therefore, it is imperative to accelerate the research on other *Enterovirus* vaccines, and the study of multivalent vaccines focusing on EV-A71, CVA16, CVA6, and CVA10 is of great significance in preventing HFMD [20,21].

This study showed that genotype B has been a dominant factor of CVA10 infection in China since 2004. In 2008, genotype C began to appear and gradually increased. Genotype C became the dominant genotype in China by 2009, whereas other genotypes only appeared occasionally. Different from mainland China, the prevalent genotype in Taiwan was mainly genotype C, with a certain proportion of genotypes B, D, and G. The analysis revealed that in other countries, the prevalence of genotypes D was dominant, followed by genotypes C and F, and the prevalence of CVA10 varied from continent to continent. This implies that continuous monitoring of CVA10 is essential for the timely detection of changes in virus type or subtype. Such monitoring helps control the prevalence of CVA10 and carry out the corresponding preventive measures. The epidemic CVA10 isolate in China is evolving and is significantly different from that in other countries. Therefore, the designs of vaccine specific to varying subtypes in different countries are more effective in preventing diseases.

In conclusion, HFMD is a serious threat to the physical and mental health of people, especially children, among which CVA10 has gradually become a viral infection factor of HFMD in China. This study analyzed the genotypes and amino acid mutations based on the VP1 region of CVA10 to further understand its main epidemic characteristics and provide a reference basis for treating HFMD and preparing vaccines in China. It is also suggested that monitoring of CVA10 genotypes or evolutionary branches should be strengthened, and the study of epidemic genotype characteristics should be increased. This will provide the basis for the research and development of monovalent or polyvalent CVA10 vaccines suitable for China.

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