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Elucidation of Echovirus 30's Origin and Transmission during the 2012 Aseptic Meningitis Outbreak in Guangdong, China, through Continuing Environmental Surveillance

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An aseptic meningitis outbreak occurred in Luoding City of Guangdong, China, in 2012, and echovirus type 30 (ECHO30) was identified as the major causative pathogen. Environmental surveillance indicated that ECHO30 was detected in the sewage of a neighboring city, Guangzhou, from 2010 to 2012 and also in Luoding City sewage samples (6/43, 14%) collected after the outbreak. In order to track the potential origin of the outbreak viral strains, we sequenced the VP1 genes of 29 viral strains from clinical patients and environmental samples. Sequence alignments and phylogenetic analyses based on VP1 gene sequences revealed that virus strains isolated from the sewage of Guangzhou and Luoding cities matched well the clinical strains from the outbreak, with high nucleotide sequence similarity (98.5% to 100%) and similar cluster distribution. Five ECHO30 clinical strains were clustered with the Guangdong environmental strains but diverged from strains from other regions, suggesting that this subcluster of viruses most likely originated from the circulating virus in Guangdong rather than having been more recently imported from other regions. These findings underscore the importance of long-term, continuous environmental surveillance and genetic analysis to monitor circulating enteroviruses.

Enteroviruses (EVs; family *Picornaviridae*) are small RNA viruses, some of which are associated with several human diseases (1). Based on the similarity of their VP1 nucleotide sequences, EVs are currently grouped as enterovirus species A to J and rhinovirus species A to C (2).

In most cases, human EV infection is generally asymptomatic or causes only mild symptoms; however, EVs can sometimes cause a broad spectrum of clinical illnesses, including aseptic meningitis, acute flaccid paralysis (AFP), acute encephalitis, and hand, foot, and mouth disease (3–5). As most EV infections are asymptomatic, the circulation of EVs cannot be identified without specialized surveillance. Environmental surveillance of EVs provides a supplemental method to elucidate the trend of virus distribution and variation in the corresponding area over a specific period of time (6, 7). Enteroviruses that are shed from affected individuals are most frequently detected in raw sewage samples during environmental surveillance.

Echovirus type 30 (ECHO30), which belongs to the EV-B species, is recognized as the leading cause of viral aseptic meningitis in both children and adults. In the last few decades, repeated outbreaks and nationwide epidemics of ECHO30 have occurred in Europe (8–11), Asia (12–14), and America (15–17). In China, high frequencies of ECHO30 detection in meningitis cases have been documented in the last few years in different coastal provinces, including Zhejiang (18), Jiangsu (14), and Shandong (19, 20). Recently, we reported that an aseptic meningitis outbreak occurred in Luoding City (Guangdong Province, China) in May of 2012, and ECHO30 was identified as the causative pathogen of this outbreak (21). Guangzhou is the capital city of Guangdong Province, and it is adjacent to Luoding City, where the outbreak of meningitis occurred. In environmental surveillance, we previously described the serotype distribution and circulation patterns of non-polio EVs (NPEVs) in sewage collected from Guangzhou from 2009 through 2012 (7). Four strains of ECHO30 were continuously isolated from 2010 through 2012, indicating the virus circulation in the population. However, the relationship between virus strains isolated from the environmental surveillance and the outbreak in the human population has not been identified.

In this study, we investigated the genetic characteristics of ECHO30 that caused an aseptic meningitis outbreak in Luoding City in 2012 and compared them with the ECHO30 isolates identified in raw sewage from 2010 to 2012 in Guangzhou City as well as with the viral isolates from Luoding City sewage that were collected after the outbreak. Sequence alignments and phylogenetic

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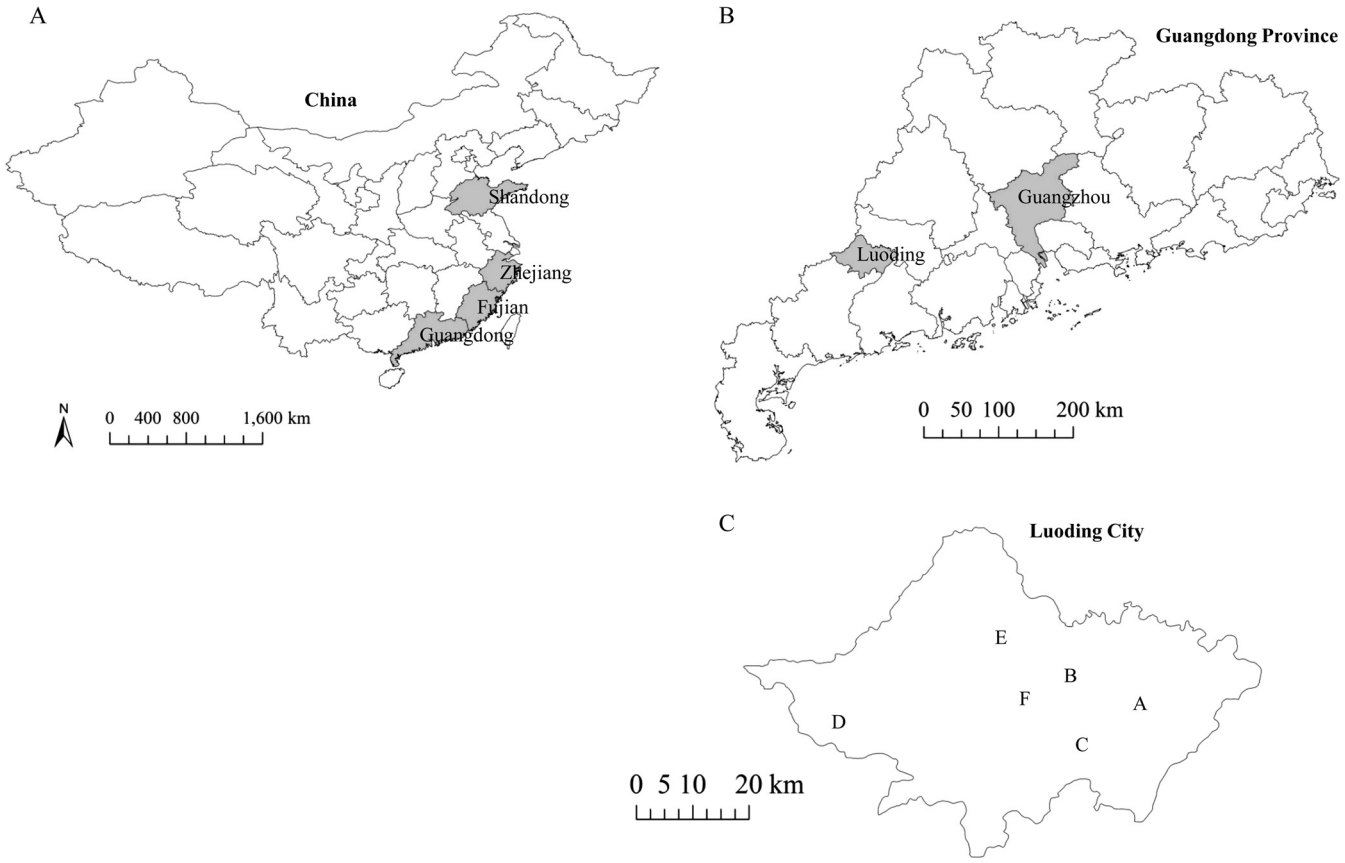

FIG 1 Geographic information of ECHO30 sampling. (A) Distribution of ECHO30 outbreaks in China. Four coastal cities with reported ECHO30 outbreaks are indicated in gray. (B) Location of Luoding and Guangzhou City. (C) Clinical samples were collected from six (A to F) different towns in Luoding City. Detailed information for each ECHO30 strain and identifications of the towns are given in Table 1. Maps were created using ArcGis (ArcMap 9.3) with data from the National Administration of Surveying, Mapping and Geoinformation of China.

analyses of the entire VP1 gene sequences were performed to present a genetic overview of ECHO30 isolates and shed light on the transmission and evolution of ECHO30 in Guangdong Province, China.

MATERIALS AND METHODS

Ethics statement. The study was approved by the institutional ethics committee of Center for Disease Control and Prevention of Guangdong Province (Guangdong CDC). This work did not include direct contact with patients or volunteers, and research focused on previously collected samples. Thus, there was no need for ethical approval or informed consent. Patient records were coded and deidentified prior to analysis. No identifying details are included in this article.

Clinical viral isolates collected from the patients of aseptic meningitis. Luoding City is located in the central west region of Guangdong Province and is 200 km from the provincial capital city, Guangzhou (Fig. 1). From May to June 2012, an outbreak of aseptic meningitis in Luoding was reported by the Guangdong Provincial Center for Disease Control and Prevention (21, 22). A total of 121 cerebrospinal fluid (CSF) specimens were collected from patients who presented with aseptic meningitis and analyzed to detect EV based on WHO guidelines (23). Briefly, nucleic acid was first extracted from the collected samples with a QIAamp Viral RNA minikit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. One-step reverse transcription-PCR (RT-PCR) was performed by using a Qiagen OneStep RT-PCR kit with pan-enterovirus primers (PE-F, 5′-TCCGGCCCCTGAATGCGGCTAATCC-3′; PE-R, 5′-ACACGGACACCCAAAGTAGTCGGTCC-3′). Amplification products were analyzed by polyacrylamide gel electrophoresis (PAGE), and positive products were detected at the expected size (114 bp). The EV-positive CSF samples were selected and inoculated on rhabdomyosarcoma (RD) and HEp-2 cell lines for virus isolation.

Environmental viral isolates collected from sewage. Raw sewage samples were collected monthly from January 2009 to December 2012 from the primary sedimentation tanks at the Liede wastewater treatment plant (WWTP) in Guangzhou City (7). Four samples (1 liter each) were obtained from the inlets of the primary sedimentation tanks on a routine basis each month. The samples were immediately transported to the laboratory, and sample treatment was started within 2 h after arrival. Forty-three sewage samples were also collected from WWTPs 1 and 2 of Luoding City 1 month after the outbreak began. Raw sewage from WWTP 1 of Luoding City is sourced from household sewage and industrial wastewater, and WWTP 2 collects household sewage and hospital wastewater. Viruses in the sewage samples were concentrated and isolated as previously described (7). The 1-liter sewage sample was first concentrated through improved negative-charge filter membrane absorption, and virus was eluted in 10 ml of a 3% beef extract solution (pH 9.6) after sonication and centrifugation (24). Thereafter, 200 μl of each concentrated eluent was used for inoculating the standard monolayer of cells for virus isolation.

Virus isolation. Enterovirus-positive specimens from the outbreak and the concentrated sewage samples were selected for virus isolation. Human rhabdomyosarcoma (RD) and human laryngeal epidermoid carcinoma (HEp-2) cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were used for virus isolation with

TABLE 1 Details of ECHO30 strains isolated and sequenced in this study

GenBank accession no.	Isolate name	Sample type	Collection date (mo-day-yr)	Location[a]	Cluster no. in phylogenetic tree
KM034782	C3	CSF	5-10-2012	Luoding A	4
KM034783	C8	CSF	5-11-2012	Luoding A	1
KM034787	C15	CSF	5-11-2012	Luoding A	3
KM034788	C16	CSF	5-9-2012	Luoding A	4
KM034789	C17	CSF	5-17-2012	Luoding A	3
KM034796	C25	CSF	5-11-2012	Luoding A	4
KM034798	C29	CSF	5-20-2012	Luoding A	2
KM034784	C11	CSF	5-13-2012	Luoding B	1
KM034785	C13	CSF	5-17-2012	Luoding B	1
KM034786	C14	CSF	5-13-2012	Luoding B	4
KM034792	C21	CSF	5-6-2012	Luoding B	4
KM034793	C22	CSF	5-14-2012	Luoding B	1
KM034794	C23	CSF	5-15-2012	Luoding C	4
KM034795	C24	CSF	5-11-2012	Luoding C	1
KM034790	C19	CSF	5-10-2012	Luoding D	2
KM034799	C33	CSF	5-20-2012	Luoding D	2
KM034781	C1	CSF	5-17-2012	Luoding E	3
KM034791	C20	CSF	5-14-2012	Luoding E	2
KM034797	C26	CSF	5-10-2012	Luoding F	2
KC897073	EM161	CSF	6-9-2012	Luoding F	4
KM034800	2113	Sewage	5-30-2012	WWTP 1, Luoding	3
KM034801	2313	Sewage	5-30-2012	WWTP 2, Luoding	1
KM034802	2314	Sewage	5-30-2012	WWTP 2, Luoding	1
KM034803	2412	Sewage	5-30-2012	WWTP 2, Luoding	4
KM034804	5012	Sewage	8-18-2010	Liede WWTP, Guangzhou	
KM034805	4111	Sewage	10-19-2011	Liede WWTP, Guangzhou	1
KM034806	3221	Sewage	7-11-2012	Liede WWTP, Guangzhou	1

[a] Towns in Luoding are indicated as follows: A, Sulong; B, Luochen; C, Luoping; D, Longwan; E, Fuchen; F, Shengjiang.

standard procedures (25). Cell culture supernatant was collected as a positive isolate when cytopathic effect (CPE) was observed.

Molecular typing. For molecular typing, nucleic acid was first extracted from the positive isolates with a QIAamp Viral RNA minikit, and RT-PCR was performed according to the method developed by Nix et al. (26). Briefly, cDNA was synthesized by using a PrimeScript II 1st Strand cDNA synthesis kit (TaKaRa, Dalian, China) with 1 μM each cDNA primer (primers AN32, 5′-GTYTGCCA-3′; AN33, 5′-GAYTGCCA-3′; AN34, 5′-CCRTCRTA-3′; and AN35, 5′-RCTYTGCCA-3′) in a 20-μl system. Following reverse transcription, 2.5 μl of cDNA was then used in the first PCR (PCR1; final volume, 25 μl) by using a *Taq* PCR master mix kit (Qiagen) with 0.5 μM (each) primers 224 and 222 targeting a highly conserved motif in the VP3 and VP1 genes, respectively (224, 5′-GCIAT GYTIGGIACICAYRT-3′; 222, 5′-CICCIGGIGGIAYRWACAT-3′). After the amplification, 2.5 μl of the PCR1 product was used as a template in the second-round PCR with 0.5 μM (each) primers AN88 and AN89 targeting the partial VP1 gene (AN88, 5′-CCAGCACTGACAGCAGYNGARA YNGG-3′; AN89, 5′-TACTGGACCACCTGGNGGNAYRWACAT-3′). The final PCR products were analyzed on 1.2% agarose gels, and the positive products (~350 to 400 nucleotides [nt]) were purified using a QIAquick PCR purification kit (Qiagen) and sent for sequencing by using primer AN88 or AN89. The sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) server at the National Center for Biotechnology Information (NCBI), and the serotype of each isolate was determined according to a previously described molecular typing method (27). In general, a pending EV was classified as the same serotype as the prototype strain if it had >75% nucleotide identity and >85% amino acid sequence identity in the coding region of the VP1 gene; the pending EVs were classified into different serotypes if they had <70% nucleotide identity and <85% amino acid sequence identity.

Amplification and sequencing of the VP1 gene of ECHO30. After molecular typing, 27 ECHO30 strains were selected, and viral RNA was extracted and reverse transcribed with random primers. The entire VP1 gene, VP1-F (5′-ACAAGYATYGTGACGCCACCAGA-3′; positions 2331 to 2354, relative to ECHO30 strain Bastianni), and VP1-R (5′-AAGTAY ACACCTGTGGWRCACTGGCA-3′; positions 3501 to 3526, relative to the ECHO30 Bastianni strain) were used for PCR amplification. The PCR products were gel purified and then sequenced twice in both directions using the same forward and reverse primers as those used in the PCR.

Sequence analysis. Full-length VP1 gene sequences (876 nt) were aligned with Clustal W (BioEdit) software. Genetic distances between and within clusters were calculated using the Kimura two-parameter substitution model in the software MEGA (version 6.06). Phylogenetic trees were constructed with MEGA using the maximum-likelihood (ML) method based on entire VP1 gene sequences (28). To assess the robustness of individual nodes on phylogenetic trees, 1,000 bootstrap replicates were performed. The nucleotide sequences of ECHO30 strains were downloaded from the GenBank database (accessed 15 April 2014), and 34 strains from other countries were selected to represent known lineages.

Nucleotide sequence accession numbers. Sequences obtained in this study have been deposited in the GenBank database under the accession numbers listed in Table 1.

RESULTS

Outbreak description. An aseptic meningitis outbreak in 246 patients occurred in Luoding City from 1 May to 30 June 2012. Seventy-five of the 121 collected CSF samples were EV positive, as identified by real-time PCR. All EV-positive CSF samples were inoculated in RD and HEp-2 cell lines for virus isolation. A cytopathic effect (CPE) was observed in 40 specimens (53.3%); among them, 32 were identified as ECHO30, and 8 were identified as ECHO6. Twenty ECHO30 isolates were randomly chosen to obtain entire VP1 gene sequences. These 20 ECHO30 strains were

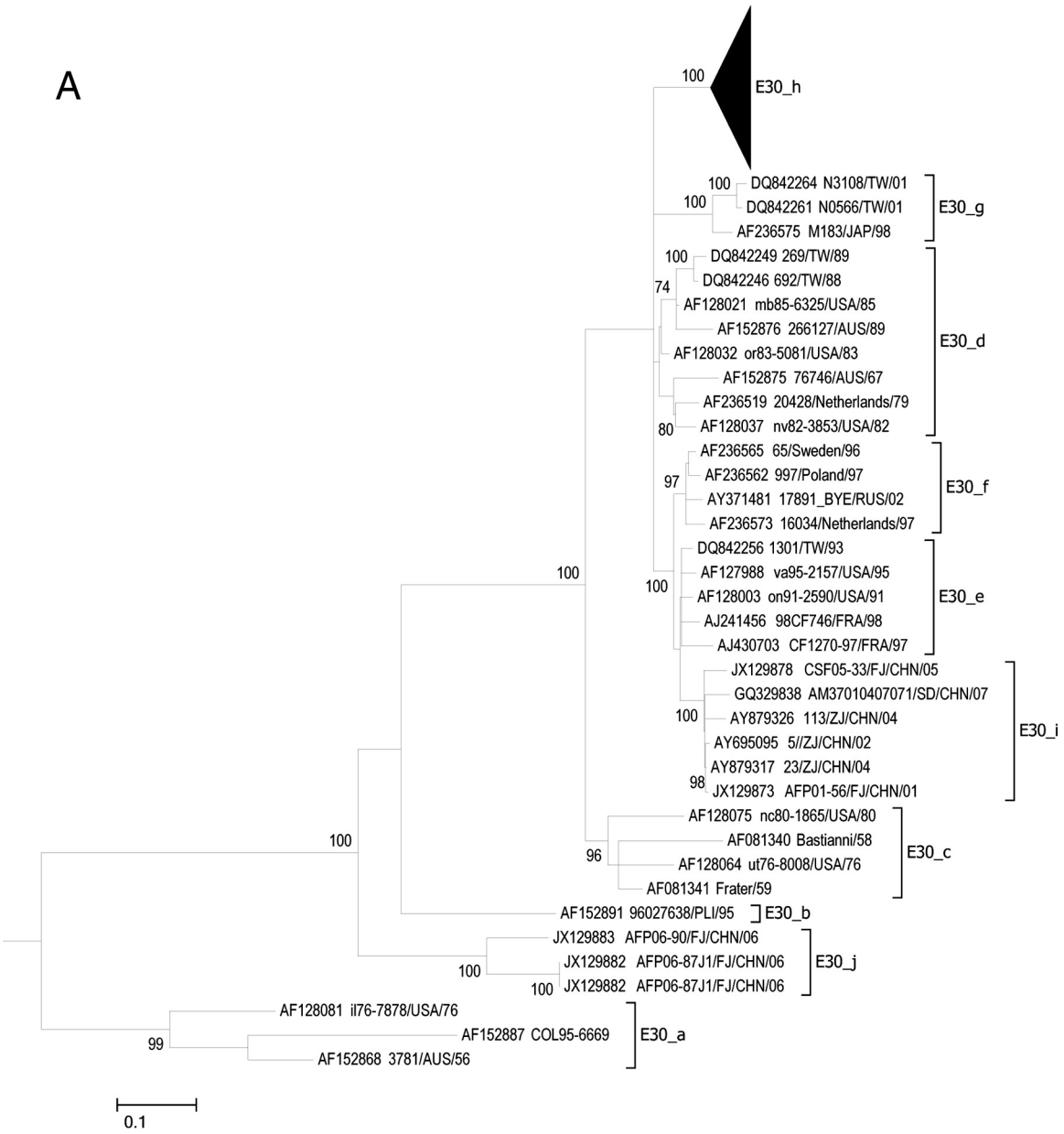

FIG 2 (A) Phylogenetic relationships of ECHO30 isolates based on the entire nucleotide sequence of the VP1 gene. (B) Phylogenetic relationships of ECHO30 lineage h based on entire nucleotide sequence of the VP1 gene. In panel B, the ECHO30 strains isolated from clinical samples in this study are marked with filled circles. The strains isolated from Luoding and Guangzhou sewage samples are marked with filled and open triangles, respectively. Bars in both panels indicate nucleotide distances as substitutions per site. Only bootstrap values of over 70% are shown.

collected from six different towns in Luoding from 9 to 15 May 2012 (Fig. 1 and Table 1).

Environmental surveillance. EV environmental surveillance in Guangzhou started in the middle of 2008 (7). During environmental surveillance from January 2009 to December 2012, a total of 947 EV-positive isolates were collected. Molecular typing was successfully conducted on 916 isolates, and the serotypes for the other 31 EV-positive isolates needed to be determined due to the failure of nested PCR amplifications. In total, 17 NPEV serotypes were identified based on the molecular typing method of a 340-bp fragment sequence in the VP1 gene (7). According to the environmental surveillance, ECHO30 was first detected in Guangzhou in August 2010. Then, one and two ECHO30 strains were successfully isolated from sewage samples in 2011 and 2012, respectively (Table 1). All of these ECHO30 strains were isolated from HEp-2 cells. Among them, one virus strain isolated in 2012 was identified as a mixture of ECHO30 and ECHO6, so it was not included for further sequencing in this study.

On 30 May 2012, nearly 1 month after the outbreak began in Luoding, 17 and 26 sewage samples were collected from WWTP 1 and WWTP 2 of Luoding City, respectively. Sewage samples were concentrated and inoculated for virus isolation. Six sewage samples (6/43, 14%) were detected as ECHO30 positive (Table 1). Five ECHO30 strains were successfully isolated

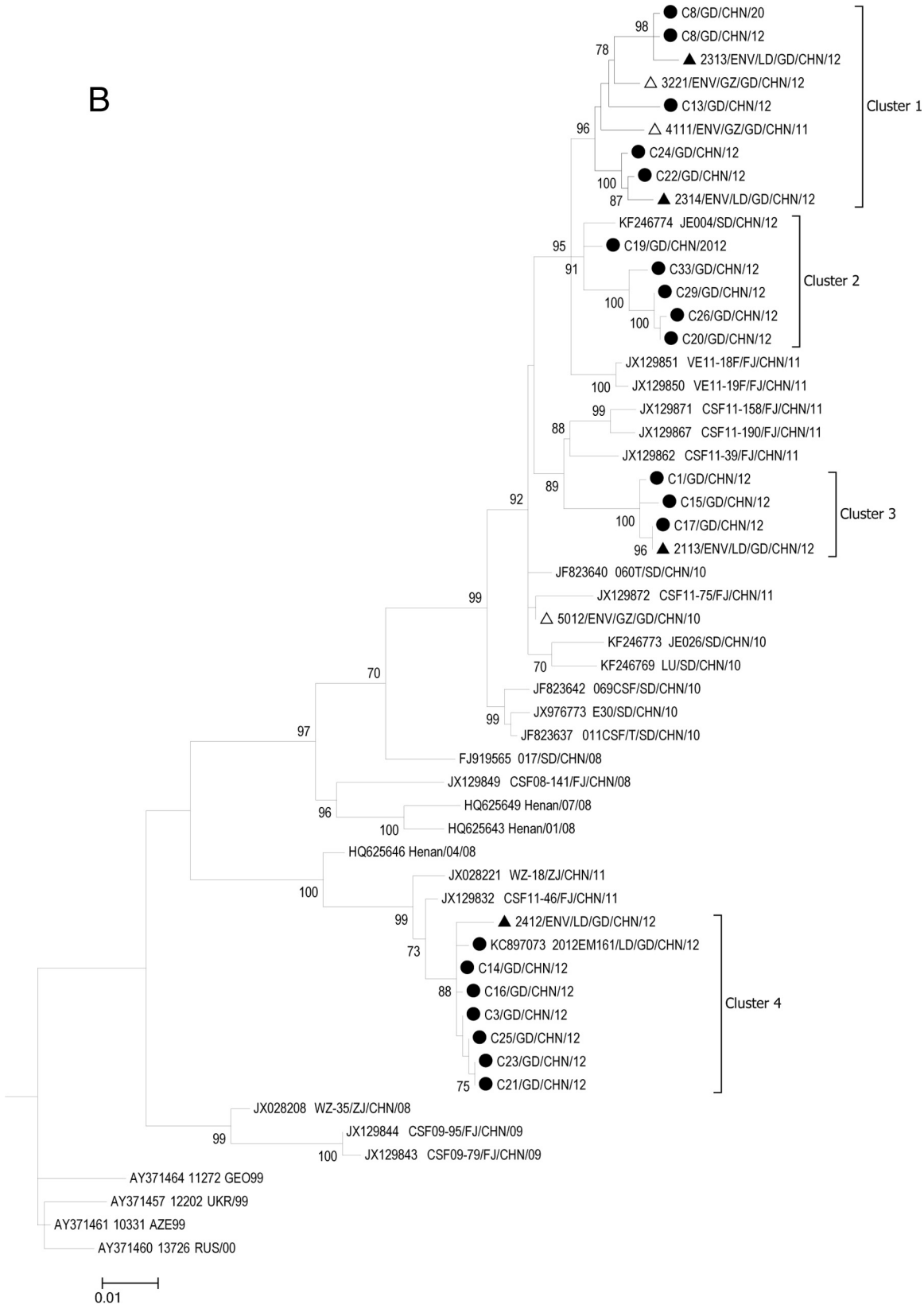

FIG 2 continued

TABLE 2 Nucleotide distances between and within clusters of ECHO30 isolates in Guangdong

Cluster no.	No. of base substitutions/site[a]			
	Cluster 1	Cluster 2	Cluster 3	Cluster 4
2	0.027			
3	0.036	0.036		
4	0.101	0.102	0.1	
Distance within cluster	0.015	0.012	0.004	0.004

[a] The numbers of base substitutions per site from all sequence pairs between and within clusters are shown. Analyses were conducted by using a Kimura two-parameter model.

TABLE 3 Comparison of nucleotide and amino acid sequence identities of ECHO30 strains

Environmental strain	Clinical strain	Nucleotide identity (%)	Amino acid identity (%)
5012/ENV/GZ/GD/CHN/10	060T/SD/CHN/10	99.4	100
4111/ENV/GZ/GD/CHN/11	C24/GD/CHN/2012	98.5	100
3221/ENV/GZ/GD/CHN/12	C8/GD/CHN/2012	98.7	100
2113/ENV/LD/GD/CHN/12	C17/GD/CHN/2012	100	100
2313/ENV/LD/GD/CHN/12	C11/GD/CHN/2012	99.4	100
2314/ENV/LD/GD/CHN/12	C22/GD/CHN/2012	99.4	99.3
2412/ENV/LD/GD/CHN/12	C3/GD/CHN/2012	99.4	99.6

from HEp-2 cells, and one viral strain was isolated from RD cells.

Sequence analysis of clinical and environmental ECHO30 isolates in Guangdong. To investigate the genetic relationship between clinical and environmental ECHO30 isolates, 20 ECHO30 clinical strains from the patients and 7 ECHO30 environmental strains (3 from Guangzhou and 4 from Luoding WWTPs) were isolated for VP1 gene sequencing (Table 1). The phylogenetic trees based on entire VP1 gene sequences are shown in Fig. 2. ECHO30 strains could be divided into 10 lineages based on previously established phylogenetic classification criteria (Fig. 2A, E-30_a to E-30_h) (29, 30). Most of the ECHO30 isolates in China clustered into lineage h, except for several strains that were isolated from Zhejiang, Fujian, and Shandong before 2008, which clustered into lineages i and j.

Twenty-seven viral isolates from the aseptic meningitis outbreak and sewage samples of Guangdong that fell into lineage h could be further divided into four subclusters (clusters 1 to 4) in the phylogenetic tree (Fig. 2B). The bootstrap support value for each cluster was more than 75%. In cluster 1, four clinical strains from three towns in Luoding City clustered together with the environmental strains from both Luoding and Guangzhou sewage samples. In addition, none of the ECHO30 strains from other regions fell into this cluster even when all ECHO30 sequences in the GenBank database were included (Fig. 2B; see also Fig. S1 in the supplemental material). Cluster 2 contained five clinical strains from four towns in Luoding. In clusters 3 and 4, all of the ECHO30 strains that were isolated from the patients clustered together with the environmental strains from sewage samples from Luoding. In contrast to cluster 1, clinical strains in clusters 2, 3, and 4 were clustered with other ECHO30 strains that were recently collected in other Chinese provinces, such as Zhejiang (2011), Shandong (2010 to 2012), and Fujian (2011).

The genetic intra- and intercluster distances were calculated with a Kimura two-parameter substitution model. Consistent with the phylogenetic analysis, the intracluster genetic diversity was greater than that of the intercluster diversity, and the sequences in cluster 4 were more divergent from the sequences in other clusters (genetic distance of $\geq$0.1) (Table 2). The amino acid alignment showed that the variability in clusters 1, 2, and 3 was produced almost entirely by synonymous changes (see Fig. S2 in the supplemental material). The amino acid differences between clusters 1 to 3 and cluster 4 were identified, suggesting that the outbreak in 2012 was caused by genetically different viruses.

The close relationship between environmental viral isolates and clinical isolates was demonstrated in VP1 nucleotide sequence alignments. Environmental isolates that were collected from the neighboring city (Guangzhou) before the outbreak and from Luoding City after the outbreak share high sequence similarity with the corresponding clinical isolates (nucleotide identity of 98.5% to 100%), except for the strain 5012 collected in Guangzhou City in 2010, which was more closely related to the clinical strain (060T/SD/CHN/10) obtained from Shandong province in the same year (Table 3). The phylogenetic analysis of the VP1 gene suggested that the ECHO30 strains in cluster 1 were separated from the other viral strains. The subsequent nucleotide alignment of the VP1 gene showed that the two nucleotide changes at nt 3236 (T to C) and nt 3254 (C to T) (relative to ECHO30 strain Bastianni) were exclusively observed in virus strains in cluster 1 but not in strains from clusters 2 to 4 (Fig. 3).

DISCUSSION

Environmental surveillance has been proven to be valuable for monitoring circulating EVs in specific communities (6, 7, 31). A surveillance study conducted in Wisconsin from 1994 to 2002 showed that the seasonal and serotype distributions of EVs in sewage were related to those in the affected population. In Wisconsin, the annual peaks of both sewage EV titers and clinical cases occurred in late summer or early fall, and in some years, early spring sewage EVs revealed some of the EVs that would clinically predominate during the following summer. Moreover, most of the EV serotypes that were identified from clinical specimens were also found in sewage samples, and the most commonly detected EV serotypes in sewage were similar to the most commonly detected EV serotypes in clinical samples (31). Compared to Wisconsin, Guangzhou and Luoding cities have high population densities (1,715 people/sq km and 490 people/sq km) but relatively low levels of cleanliness. Therefore, a higher positive rate of EVs and a greater number of serotypes of EVs are observed in sewage samples of Guangzhou City than in Wisconsin (7, 31). Due to a lack of long-term clinical surveillance in Guangdong, we cannot currently compare the prevalence of EVs in sewage samples to that in clinical cases. However, a close phylogenetic relationship between ECHO30 strains isolated from sewage samples and those from outbreaks was demonstrated in this study. The data provided here suggest that sewage surveillance also has a value in monitoring circulating EVs in the communities of cities in developing countries like China.

ECHO30 is one of the most frequently isolated EV serotypes that cause aseptic meningitis. Numerous aseptic meningitis outbreaks that are caused by different lineages of ECHO30 have been reported during the last decade in many countries (15, 21, 32). In

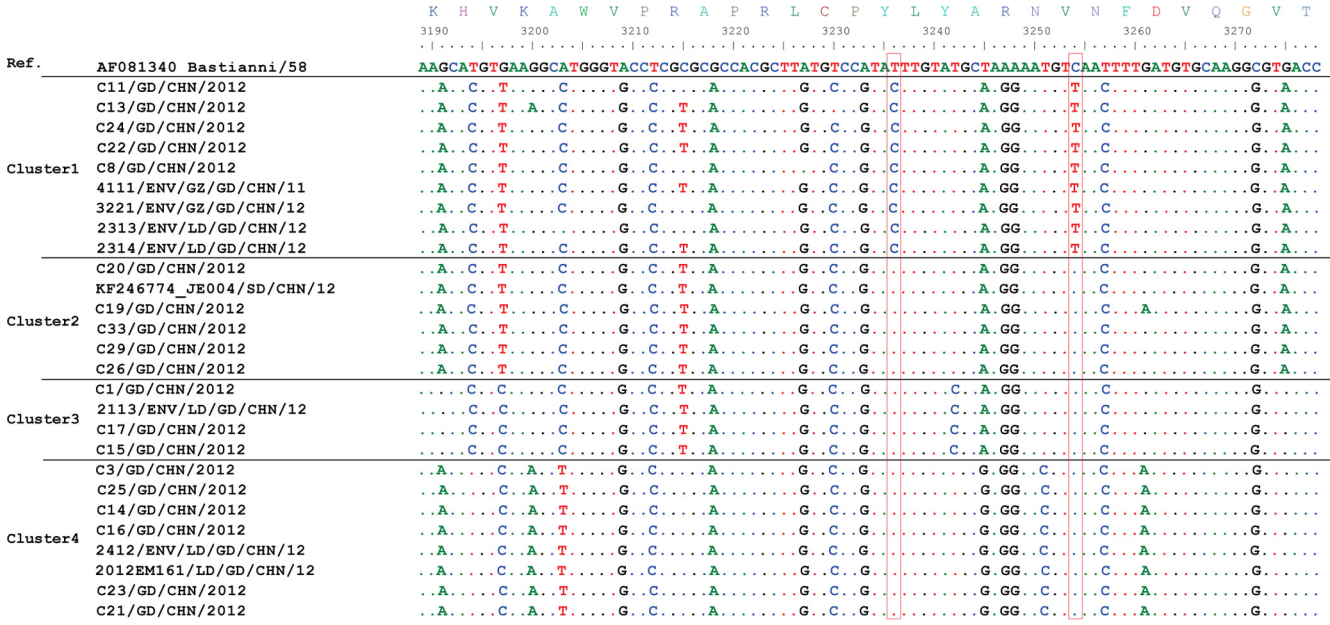

FIG 3 Alignment of VP1 nucleotide sequences (nucleotide position 3189 to 3278, relative to ECHO30 reference strain Bastianni) of clusters 1 to 4. The significant nucleotide differences between cluster 1 and clusters 2 to 4 were identified at positions 3236 and 3254 (boxed).

Guangdong Province, China, ECHO30 was first isolated from a sewage sample in 2010 through environmental surveillance; subsequently, this virus was continually but not frequently detected in sewage of Guangzhou City (7). Taking into account that similar methods have been used for sewage concentration and virus isolation in cell cultures (as described in the Materials and Methods section) over the years, it is likely that continuous ECHO30 identification is a reflection of the circulation of the virus in this region.

In this study, strains from Guangzhou sewage samples were sequenced and compared with strains from the aseptic meningitis epidemic in adjacent Luoding. Meanwhile, the sewage samples from two major WWTPs in Luoding were also collected, and a high prevalence of ECHO30 (14%) was identified. Previous studies on the molecular epidemiology of ECHO30 and phylogenetic classification were primarily based on the VP1 gene (29, 30, 33). Therefore, phylogenetic analyses of the clinical strains and the environmental strains were also performed by using VP1 gene sequences according to the classification scheme that was established by Bailly et al. (29).

The ECHO30 h lineage represents the primary outbreak virus strain in China, including strains from meningitis outbreaks in Jiangsu Province in 2003 (14), Shandong Province in 2008, and Fujian Province in 2011 (30). Similarly, the ECHO30 strains identified in Guangdong also fell into lineage h but were segregated into four different subclusters (Fig. 2B, clusters 1 to 4). Nine strains collected from Guangdong (five clinical strains and four environmental strains) belonged to cluster 1 and were separated from the ECHO30 strains from other regions (Fig. 2B; see also Fig. S1 in the supplemental material). The sequence alignment also illustrated the coexistence of two site changes on the VP1 gene (T3236C and C3254T) that were exclusively observed in viral strains of cluster 1 (Fig. 3) and not identified in other ECHO30 strains in the GenBank database (data not shown). In addition, the environmental strain 4111 isolated from sewage from Guangzhou in 2011 is the closest strain to the strain from the outbreak in Luoding in 2012 (nucleotide sequence identity of 98.1% to 98.5%). These observations might suggest that ECHO30 strains in cluster 1 are more likely Guangdong local strains and that the cluster 1 viral strain from the meningitis outbreak in 2012 may have been transmitted from the earlier circulating viral strains (4111-like viruses) in Guangdong Province.

The clinical strains were isolated from patients from six different towns in Luoding City without a specific geographic distribution, according to VP1 gene sequences. More interestingly, the various clusters of ECHO30 strains were isolated from the outbreak in a single town (Table 1 and Fig. 1). For example, strains C8, C29, C15, and C3 from town A fell into four different clusters. These observations confirmed the VP1 sequence diversity of ECHO30 strains that were isolated in this outbreak. In addition, the environmental strains isolated from WWTPs in Luoding after the outbreak share high nucleotide sequence similarity with the clinical strains from the epidemic (nucleotide identity of 99.4% to 100%). These analyses indicate that the viral strains that were isolated from both WWTPs of Luoding City after the outbreak represent the predominant ECHO30 clinical strains in this outbreak.

In conclusion, we analyzed the VP1 gene sequences of ECHO30 strains from the aseptic meningitis outbreak in 2012 in Guangdong, China, and the ECHO30 strains isolated from raw sewage before and after the outbreak. One subcluster of ECHO30 clinical strains were closely related with the Guangdong environmental strain isolated before the outbreak but diverged from strains from other regions, suggesting that this subcluster of viruses was likely to have originated from the circulating virus in Guangdong rather than having been recently imported from other regions. In addition, the high nucleotide sequence identities (98.5% to 100%) shared by the clinical strains from the epidemic and the environmental strains from sewage reinforce the value of

EV environmental surveillance. In fact, active surveillance on clinical specimens is more efficient than surveillance on sewage for detecting and tracking outbreaks. Some EVs, especially EV-As, might have been missed in environmental surveillance because their growth in cells is lower than that of EV-Bs (7). However, a systematic EV disease surveillance system is absent in China, and clinical specimens are hard to obtain. Moreover, many EV infections are asymptomatic or subclinical, and EV serotypes not detected clinically can be identified from local sewage. Hence, currently sewage surveillance is a practical way to inform us of an epidemic background of the circulating EVs in the community and to provide a warning of possible enteroviral disease outbreaks in China. Since high epidemic activity of ECHO30 has been recently reported in China, ECHO30 has the potential to rapidly spread in the future, and high-quality, continuous environmental surveillance of EVs is warranted.

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We declare that we have no competing interests.

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