Prevalence and Genetic Diversity of *Aichi Virus 1* from Urban Wastewater in Senegal

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Abstract

*Aichi virus 1 (AiV-1)* has been proposed as a causative agent of human gastroenteritis. In this study, raw, decanted, and treated wastewater samples from a wastewater treatment plant in an urban area of Dakar, Senegal, were collected. AiV-1 was detected in raw (70%, 14/20), decanted (68.4%, 13/19), and treated (59.3%, 16/27) samples, revealing a noticeable resistance of AiV-1 to chlorine-based treatment. Phylogenetic analysis revealed that all sequences clustered within genotype B. Our study presents the first report on the detection of AiV-1 in the environment of Dakar and constitutes indirect evidence of virus circulation in the population.

Keywords

*Aichi virus 1* · Senegal · Sewage · Wastewater treatment plant · Molecular epidemiology

Introduction

*Aichi virus 1* (AiV-1) is a human enteric virus of the genus *Kobuvirus* within the *Picornaviridae* family. It is a small, non-enveloped, positive-sense, single-stranded RNA virus of approximately 8.3 kb in length [1]. AiV-1 is classified into 3 genotypes (A, B, and C) using the conserved nucleotide sequences of the 3C and 3D (3CD) junction region of the genome [2, 3]. It has been proposed as a causative agent of human gastroenteritis potentially transmitted primarily by the fecal-oral route [1]. AiV-1 shedding in human feces may contaminate aquatic environments directly or after discharge of raw or treated sewage [4–7]. AiV-1 is known to be resistant to different treatment procedures, and once in the environment, it can persist for a very long time [4, 8]. The presence of AiV-1 in aquatic environments has been reported in previous studies that detected AiV-1 in river water [5, 7, 9], in sewage [6, 9–14], in sewage sludge [15], in groundwater [9, 16], and shellfish [10, 13, 17–19]. AiV-1 detection in humans was initially reported as the likely cause of an oyster-associated gastroenteritis outbreak in Aichi, Japan, in 1989 [20]. Since then, the virus has been found at low incidence in patients with gastroenteritis in different regions of the world, including Asian [2, 21–24], European [3, 25–29], South American [25], and African [30, 31] countries. The seroprevalence of AiV-1 reaches 80–95% by the age of 30–40 years [1, 32, 33]. The incongruity between low detection rates in gastroenteritis cases and high seroprevalence suggests that the virus might be...
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Wastewater surveillance is a useful tool to understand the prevalence and circulation of enteric viruses within a human population as wastewater contains viruses shed from all individuals within a service area regardless of symptoms [34]. In this regard, examining environmental samples is especially useful for viruses such as AiV-1 that are underdiagnosed and potentially hazardous to health [35]. In Africa, AiV-1 has been detected in sewage samples in Tunisia and South Africa [10, 12, 13], in gastroenteritis cases in Tunisia and Burkina Faso [30, 31], and from fecal samples of healthy children in Ethiopia [36]. To date, there are no documented reports on the molecular epidemiology of AiV-1 in Senegal. For this aim, we investigated the occurrence and genetic diversity of AiV-1 in raw and treated sewage samples from a WWTP located in a major urban area of Dakar.

Materials and Methods

Samples of this study were collected at the WWTP of Cambréne, Dakar, Senegal (Fig. 1). The WWTP process for wastewater consists of (1) screening fat, oil grease, and sand removal (raw sewage); (2) decantation (decanted sewage); and (3) chlorination (treated sewage) (level of chlorine dose 10 g/L, chlorine residual 0.5 mg/L). A total of 66 wastewater samples (20 raw, 19 decanted, and 27 treated) were collected once per week from November 2012 to March 2013 in the morning between 9.00 a.m. and 11.00 a.m. using the grab method as described in the WHO Guidelines for Environmental Surveillance of Poliovirus [37]. The concentration of wastewater samples was performed using the two-phase separation method using polyethylene glycol 6000 and dextran 40 as previously described [37, 38]. RNA was extracted from 140 μL of the concentrated virus solution using a commercial QIAamp Viral RNA Kit (Qiagen Inc., Germany) according to the manufacturer’s instructions. The presence of AiV-1 RNA was assessed using the primer set Ai6261 and Ai6779 specific for AiV-1, targeting a 519-base pair fragment at the 3CD junction [2]. RT and PCR were performed in a 1-step procedure, using the QIAGEN® One-Step RT-PCR Kit. Amplified PCR fragments were purified using the GeneJET Gel Extraction (Thermo Fisher Scientific) according to the manufacturer’s instructions. Genotyping was performed by direct sequencing of amplified products by the Sanger method using Ai6261 and Ai6779 primers. Nucleotide sequences were deposited in the GenBank database (MK213084 to MK213109). All sequences were aligned by using ClustalW multiple alignment program within the BioEdit Sequence Alignment Editor package, version 7.0.9.0. Phylogenetic studies and genetic distances were calculated by using the Molecular Evolutionary Genetics Analysis 6 (MEGA6) software.

Results and Discussion

Out of 66 wastewater samples, 43 (65.1%) were found to be positive for AiV-1 RNA. AiV-1 was detected in 14/20 (70%) raw, 13/19 (68.4%) decanted, and 16/27 (59.3%) treated wastewater samples, demonstrating high and constantly abundant AiV-1 detection rates in influent and effluent wastewater. The significant percentage (59.3%) of AiV-1 prevalence in effluent wastewater highlights the difficulty in eliminating AiV-1 by the current treatment methods of chlorination. This result is in agreement with the established environmental stability of AiV-1.
1 and its known low-pH resistance [8]. The study supports others in providing further evidence of the high persistence of AiV-1 during similar wastewater treatments [5, 12, 35, 39, 40]. The observation of a high detection rate in the dry cold season (season of this study) is in accordance with results reported in previous studies in Africa [12, 31]. This can be explained by the influence of temperature on the persistence of enteric viruses in aquatic environments [12]. Indeed, viral inactivation of enteric viruses is faster when the water temperature is very high. In particular, for AiV-1, inactivation has been reported to occur at 56°C for 20 min by heat treatment [8].

A total of 28 out of 43 AiV-1-positive samples were used for genotyping. Majority (26/28, 92.8%) were successfully genotyped. Sequence and phylogenetic analysis of the 3CD nucleotide sequences of the 26 strains indicated that all strains belonged to genotype B (Fig. 2). This is in agreement with previous studies in the African region (Ethiopia, South Africa, Burkina Faso, and Tunisia) reporting the predominance of genotype B in clinical and wastewater samples [12, 13, 30, 36]. Nevertheless, genotype A has also been reported in Africa in clinical and wastewater samples [3, 10, 30]. Interestingly, the only 3 AiV-1 strains from genotype C published in GenBank are from Africa (Mali and Burkina Faso) [3, 30]. However, genotype C has never been isolated from wastewater. A notable observation was segregation of study sequences in 2 clusters that occurred sequentially in time: strains from 2012 grouped in 1 cluster different from those of 2013 (Fig. 2). However, the low mean p-distance between both clades (0.008 SE ± 0.003) and the low bootstrap values (63% for the 2012 cluster and 33% for the 2013 cluster, data not shown) suggest low genetic divergence among them. Despite the low degree of variability, this evident temporal shift of AiV-1 strains in a restricted geographical region agrees with what has been observed in other studies [10, 41]. The 10 AiV-1 strains of the 2012 cluster showed 99% nucleotide sequence identity (mean p-distance of 0.002 SE ± 0.001) to an AiV-1 strain (LN612595) detected in a stool specimen from a child with gastroenteritis in Burkina Faso in 2012, which clustered in the same monophyletic group (Fig. 2). This high similarity among the AiV-1 strain detected in a human clinical case and the AiV-1 strains detected in sewage suggests a direct link between contaminated water and human infection with AiV-1. Senegalese strains were found to be genetically related (90–99% of nucleotide identity) to AiV-1 strains detected in different geographical areas such as China [22], Italy [11], Venezuela [7], or Tunisia [12], evidencing the circulation of closely related strains worldwide.

**Conclusion**

This study describes for the first time the occurrence and genetic diversity of this virus in the environment of Senegal. Results reported here revealed a high frequency of AiV-1 in effluent wastewater, suggesting that chlorine-based sewage treatment is only partially effective for the removal of viruses. In the WWTP of Cambérène, the effluent wastewater is partly drained to the ocean and partly used for irrigating agricultural areas that provide vegetables in several markets of Dakar [42]. Discharge of the virus into the marine environment or the use of contaminated effluent wastewater for irrigation may represent a risk on human health and ecology of aquatic environments. Further studies are warranted in order to assess the potential role of AiV-1 discharge in the natural environment as a causative agent of gastroenteritis in Senegal.

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**Statement of Ethics**

This article does not contain any studies with human participants or animals.

**Conflict of Interest Statement**

The authors declare that there are no conflicts of interest.

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**Author Contributions**

Kader Ndiaye and Ousmane Kebe conceived the study. Amary Fall, Hamet Dia, and Maxime Bidalot performed research. Ousmane Kebe and Maria-Dolores Fernandez-Garcia analyzed the data. Ousmane Kebe, Maria-Dolores Fernandez-Garcia, Amary Fall, Katia Ambert-Balay, and Kader Ndiaye interpreted the data. Ousmane Kebe and Maria-Dolores Fernandez-Garcia wrote the manuscript. All authors reviewed and approved the manuscript.
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