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.

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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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responsible for asymptomatic or mild infections [1]. 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 Cambérène, 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 separa-

tion 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 Gene-JET 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-

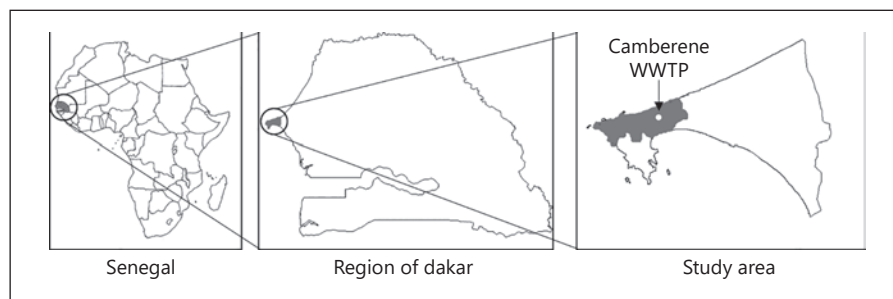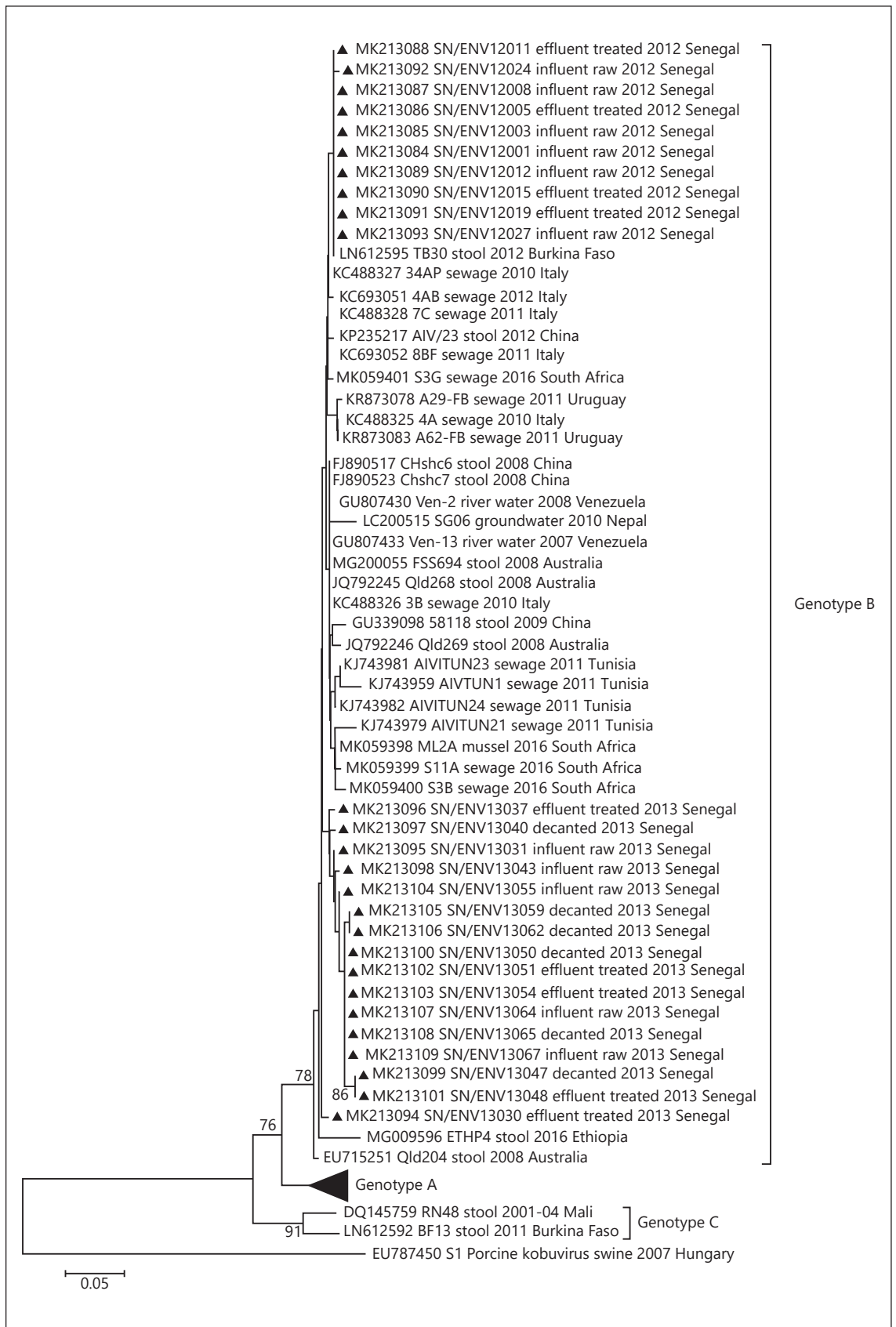**Fig. 1.** Geographical location of the study area.

Fig. 2. Phylogenetic analysis of AiV-1 strains recovered from a WWTP in an urban area in Dakar and a representative global set of AiV-1 strains from the GenBank database, based on a 301-base pair fragment of the genomic 3CD junction region. The tree was inferred with a neighbor-joining method. Distances were computed using the Kimura 2-parameter model in the package MEGA6. The robustness of the nodes was tested by 1,000 bootstrap replica-

tions. Bootstrap support values >75% are shown in nodes. Black triangles indicate the 26 strains from this study. Scale bar represents nucleotide substitutions per site. A value of 0.05 substitutions per site is equivalent to 5% changes. Abbreviations of virus names indicate GenBank accession number, strain name, source, year of collection, and country of origin. AiV-1, *Aichi virus 1*.

(For figure see next page.)

▲ MK213088 SN/ENV12011 effluent treated 2012 Senegal
▲ MK213092 SN/ENV12024 influent raw 2012 Senegal
▲ MK213087 SN/ENV12008 influent raw 2012 Senegal
▲ MK213086 SN/ENV12005 effluent treated 2012 Senegal
▲ MK213085 SN/ENV12003 influent raw 2012 Senegal
▲ MK213084 SN/ENV12001 influent raw 2012 Senegal
▲ MK213089 SN/ENV12012 influent raw 2012 Senegal
▲ MK213090 SN/ENV12015 effluent treated 2012 Senegal
▲ MK213091 SN/ENV12019 effluent treated 2012 Senegal
▲ MK213093 SN/ENV12027 influent raw 2012 Senegal
LN612595 TB30 stool 2012 Burkina Faso
KC488327 34AP sewage 2010 Italy
KC693051 4AB sewage 2012 Italy
KC488328 7C sewage 2011 Italy
KP235217 AIV/23 stool 2012 China
KC693052 8BF sewage 2011 Italy
MK059401 S3G sewage 2016 South Africa
KR873078 A29-FB sewage 2011 Uruguay
KC488325 4A sewage 2010 Italy
KR873083 A62-FB sewage 2011 Uruguay
FJ890517 CHshc6 stool 2008 China
FJ890523 Chshc7 stool 2008 China
GU807430 Ven-2 river water 2008 Venezuela
LC200515 SG06 groundwater 2010 Nepal
GU807433 Ven-13 river water 2007 Venezuela
MG200055 FSS694 stool 2008 Australia
JQ792245 Qld268 stool 2008 Australia
KC488326 3B sewage 2010 Italy
GU339098 58118 stool 2009 China
JQ792246 Qld269 stool 2008 Australia
KJ743981 AIVITUN23 sewage 2011 Tunisia
KJ743959 AIVTUN1 sewage 2011 Tunisia
KJ743982 AIVITUN24 sewage 2011 Tunisia
KJ743979 AIVITUN21 sewage 2011 Tunisia
MK059398 ML2A mussel 2016 South Africa
MK059399 S11A sewage 2016 South Africa
MK059400 S3B sewage 2016 South Africa
▲ MK213096 SN/ENV13037 effluent treated 2013 Senegal
▲ MK213097 SN/ENV13040 decanted 2013 Senegal
▲ MK213095 SN/ENV13031 influent raw 2013 Senegal
▲ MK213098 SN/ENV13043 influent raw 2013 Senegal
▲ MK213104 SN/ENV13055 influent raw 2013 Senegal
▲ MK213105 SN/ENV13059 decanted 2013 Senegal
▲ MK213106 SN/ENV13062 decanted 2013 Senegal
▲ MK213100 SN/ENV13050 decanted 2013 Senegal
▲ MK213102 SN/ENV13051 effluent treated 2013 Senegal
▲ MK213103 SN/ENV13054 effluent treated 2013 Senegal
▲ MK213107 SN/ENV13064 influent raw 2013 Senegal
▲ MK213108 SN/ENV13065 decanted 2013 Senegal
▲ MK213109 SN/ENV13067 influent raw 2013 Senegal
▲ MK213099 SN/ENV13047 decanted 2013 Senegal
86 ▲ MK213101 SN/ENV13048 effluent treated 2013 Senegal
▲ MK213094 SN/ENV13030 effluent treated 2013 Senegal
MG009596 ETHP4 stool 2016 Ethiopia
EU715251 Qld204 stool 2008 Australia

Genotype B

78
76

Genotype A

DQ145759 RN48 stool 2001-04 Mali
91 LN612592 BF13 stool 2011 Burkina Faso
Genotype C

EU787450 S1 Porcine kobuvirus swine 2007 Hungary

0.05

2

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.

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.

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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