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Prevalence and genetic characterization of noroviruses in children with acute gastroenteritis in Senegal, 2007–2010

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Abstract

Norovirus is the leading cause of sporadic and epidemic acute gastroenteritis (AGE) in children and adults around the world. We investigated the molecular diversity of noroviruses in a pediatric population in Senegal between 2007 and 2010 before the rotavirus vaccine implementation. Stool samples were collected from 599 children under 5 years of age consulting for AGE in a hospital in Dakar. Specimens were screened for noroviruses using the Allplex™ GI-Virus Assay. Positive samples were genotyped after sequencing of conventional reverse transcription-polymerase chain reaction products. Noroviruses were detected in 79 (13.2%) of the children, with GII.4 (64%) and GII.6 (10%) as the most frequently identified genotypes. Our study describes the distribution of genotypes between 2007 and 2010 and should be a baseline for comparison with more contemporary studies. This could help decision-makers on possible choices of norovirus vaccines in the event of future introduction.

Keywords

diarrhea; genotype diversity; GII.4; GII.6; Norovirus; Senegal

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AUTHOR CONTRIBUTIONS

Kader Ndiaye and Ousmane Kebe conceived the study. Ousmane Kebe, Boris-Enock Zinsou, and Ndack Ndiaye performed research. Ousmane Kebe, Maria-Dolores Fernandez-Garcia, and Amary Fall analyzed the data. Ousmane Kebe, Maria-Dolores Fernandez-Garcia, Amary Fall, Amadou Diop, Jan Vinjé, and Kader Ndiaye interpreted the data. Ousmane Kebe and Kader Ndiaye wrote the manuscript. All authors reviewed and approved the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

1 | INTRODUCTION

Norovirus is one of the leading causes of viral sporadic and outbreak-associated acute gastroenteritis (AGE) in human infections across all age groups and is associated with approximately 18% of all cases of AGE worldwide.[1]

Although humans of all ages are at risk for norovirus infection, children and the elderly are especially vulnerable to severe disease. Norovirus gastroenteritis is defined by a sudden onset of diarrhea and/or vomiting, but may also include nausea, stomach pain, fever, headache, and body aches.[2] In healthy adults, norovirus disease is typically self-limiting, resolving within 1–3 days. More serious outcomes are possible and are typically associated with severe dehydration among children, immunocompromised individuals, and the elderly.[3]

Noroviruses have a 7.5 kb linear, positive-sense, single-stranded RNA genome enclosed in a nonenveloped icosahedral capsid. The norovirus genome is organized into three open reading frames (ORFs). Of these, ORF2 encodes the major capsid protein (VP1), which is also the basis for classifying noroviruses into 10 genogroups (G) of which most infections in humans are caused by GI and GII viruses,[4] which can further be divided into 35 genotypes (9 GI and 26 GII).[4–6] GII.4 viruses caused the majority of infections over the last 20 years.[7]

Several candidate norovirus vaccines are in clinical trials[8] and as no genotype data are available from Senegal, we assessed norovirus activity and circulating genotypes in children under 5 years of age.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

Stool specimens were collected through national routine surveillance activities supported by Word Health Organization (WHO) for public health purposes. WHO and the Ministry of Health approved all technical and ethical aspects. The protocol and oral consent were determined as routine surveillance activity by the steering committee of WHO in compliance with all applicable national regulations governing the protection of human subjects. The methods were carried out in accordance with the principles of the Declaration of Helsinki.

2.2 | Samples collection

Stool samples were collected from children under 5 years of age with AGE attending for consultation at the Albert Royer Children's Hospital Paediatric Institute in Dakar (Senegal) between 2007 and December 2010 and were stored at −20°C until nucleic acid extraction.

2.3 | RNA extraction and viral detection

A 10% (wt/vol) clarified stool suspension was prepared in phosphate-buffered saline solution after removing the solids particles by centrifugation at 3000g for 15 min. Viral RNA was extracted from 140 μl of clarified suspension using a QIAamp viral RNA Minikit (QIAGEN) according to the manufacturer's instructions. After the final wash step, viral RNA was eluted in 60 μl of RNase-free water and immediately used or stored at −80°C. Nucleic acid was tested by Allplex™ GI-Virus Assay (GI9701Y; Seegene), a multiplex

one-step real-time reverse transcription-polymerase chain reaction (RT-PCR) targeting six viruses involved in AGE (rotavirus, norovirus GI, norovirus GII, sapovirus, astrovirus, and adenovirus)[9] using a CFX96 real-time PCR system (Bio-Rad).

2.4 | Conventional RT-PCR

All real-time PCR positive samples (with $C_t < 40$) were characterized by two separate conventional RT-PCR assays. The partial capsid region was amplified using previously described genogroup-specific (GSK) oligonucleotide primers targeting the 5′-end of ORF2 (capsid)[10] and oligonucleotide primers targeting a partial (327 nt) region of polymerase gene of GI and GII noroviruses.[11] Amplification conditions included reverse transcription at 50°C for 30 min followed by inactivation of the RT-enzyme at 95°C for 15 min. PCR was conducted for 40 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 1 min for the GSK primers or 40 cycles at 94°C for 1 min, 37°C for 1 min, 72°C for 1 min for the JV primers. The amplification products were analyzed by 1.5% agarose gel electrophoresis and visualized by UV illumination after ethidium bromide staining.

2.5 | Sequencing and genotyping of norovirus

PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific) and sent to a genomics company service (GENEWIZ) for sequencing by the Sanger method. All sequences were aligned using ClustalW within the BioEdit Sequence Alignment Editor package (version 7.2.5). The norovirus genotypes were determined by submitting sequences to the online CDC norovirus typing tool (https://norovirus.ng.philab.cdc.gov/bctyping.html) and sequences were also analyzed using online NCBI databases https://blast.ncbi.nlm.nih.gov/Blast.cgifor confirmation. Phylogenetic trees were generated by using the Molecular Evolutionary Genetics Analysis (MEGA X version 10.1) software package. The reliability of the tree topologies was estimated by bootstrap analysis with 1000 replicates. The norovirus sequences determined in this study have been deposited submitted to GenBank (accession numbers: MT322850–MT322856 for norovirus GI and MW279338–MW279412 for norovirus GII).

3 | RESULTS

A total of 599 stool samples (2007 [$n = 109$], 2008 [$n = 275$], 2009 [$n = 124$], and 2010 [$n = 91$]) were available for testing (Table 1). Of these, 79 (13.2%) samples tested positive for norovirus by real-time RT-PCR assay including 12 (15.2%) for GI and 67 (84.8%) for GII. The prevalence ranged from 13.8% in 2007, 15.6% in 2008, 9.7% in 2009 and 9.9% in 2010 (Table 1). Using the polymerase oligonucleotide primers, 25 samples (31.6%) produced a band of the appropriate size on a gel of which 23 samples were successfully sequenced. Sixteen (69.6%) samples could be typed as GII.P4, three (13.0%) as GII.P30, and one (4.4%) as GII.P13. One sample collected in 2007 tested positive for GII.P4 and GII.P30. Three strains could not be P-typed. Using the GSK oligonucleotide primers, 7 (58%) GI samples and 52 (77.6%) of the 67 GII samples were successfully genotyped (Table 1). For 20 GII strains, sequences for both the polymerase region and the capsid region could be obtained. These included GII.4 [P4] ($n = 15$, 73.7%), GII.2 [P30] ($n = 4$, 21.0%) and GII.6 [P6], 5.3% ($n = 1$). Of the 15 GII.4 [P4] strains, 6 samples were typed

as GII.4Yerseke_2006a [P4], 5 as GII.4 New Orleans [P4], 2 as GII.4Sydney [P4], and 1 as GII.4Osaka 2007[P4] and 1 as GII.4 [Apeldoorn P4].

Overall, 33 (57.9%) of the 57 sequenced strains could be typed as GII.4 followed by GI.3 (n = 5, 8.8%), GII.6 (n = 5, 8.8%), GII.17 (n = 4, 7.0%), GII.2 (n = 2, 3.5%), and GII.13 (n = 2, 3.5%) (Figures 1) and 1 each of GI.5, GI.9, GII.7, GII.8, GII.9, GII.16, and GII.21. For one specimen (strain 531) we found a coinfection GII.4 and GII.6 (Supporting Information File). GII.4 Yerseke2006a viruses were the predominant genotype during the entire study period (Figure 2). Of the 33 GII sequences, 16 could not be typed using the genotyping tool likely because the sequences were not complete. Using BLAST, 12 samples had the closest match with GII.4 Yerseke2006a, 1 with GII.4 Osaka2007, 1 with GII.4 Apeldoorn, and 2 with GII.4 New Orleans with nucleotide identities ranging between 96% and 100%. Based on the partial polymerase sequences, GII.P4 viruses were detected throughout the study period with prevalence ranging from 57% to 100% (Figure 3).

4 | DISCUSSION

The aim of our study was to determine and characterize the prevalence of norovirus in hospitalized children with AGE and characterize the circulating strains in Dakar between 2007 and 2010. The presence of noroviruses in Senegal was previously described with limited data regarding the prevalence and genetic diversity.[12] Over the study period of 4 years, we found an overall norovirus prevalence of 13% compared to other studies in the African region.[13] The relative incidence of norovirus has increased due to the successful implementation of rotavirus vaccines in many countries.[13,14] This suggests that more recently collected samples should be tested in Senegal where a rotavirus vaccine was introduced in November 2014.

The predominant norovirus genogroup detected in our study was GII. These findings are similar to what has been reported globally.[13] In addition, we found that GII.4 viruses were the most predominant genotype in this study which with 66% is close to the average prevalence found in other African countries (26). GII.4 Yerseke2006a viruses started to decline after 2009 and were replaced by GII.4 New Orleans. In Burkina Faso, Ouedraogo et al.[13] found a higher prevalence (71.4%) for GII.4 viruses. In contrast, GII.6 viruses were predominant (37.5%) in our study in 2009 and were genetically very close to strains that had been detected in Senegal in 1976[12] and Burkina Faso in 2011.

Among GI viruses, GI.3 has been most frequently detected in developing countries.[15] In sub-Saharan Africa, children under 17 years of age were mostly infected with GI.7 viruses (33%) followed by GI.3 and GI.5 with 21% and 17%, respectively.[16]

Our study has several limitations. With the lack of clinical data, we were not able to identify possible risk factors for infection and since samples from nondiarrheal control children were not collected, we were not able to assess the true association between detection of norovirus and gastroenteritis symptoms in children as in several studies the prevalence of norovirus in nondiarrheal controls have been reported to be in the same range as in children with diarrhea.[17] We used two different RT-PCR assays for genotyping of strains including one

targeting a partial region of the polymerase gene and one targeting a partial region of the capsid gene. In addition to the poor performance of the polymerase assay, the polymerase and capsid region should ideally be amplified in a single robust GSK RT-PCR assay.[18]

5 | CONCLUSION

This is the first report on the prevalence of norovirus and genetic strain diversity in Senegal showing that norovirus is a major cause of AGE in Senegalese children. The distribution of genotypes confirms studies globally and for future norovirus vaccine introduction, it will be important to test norovirus samples from more recent years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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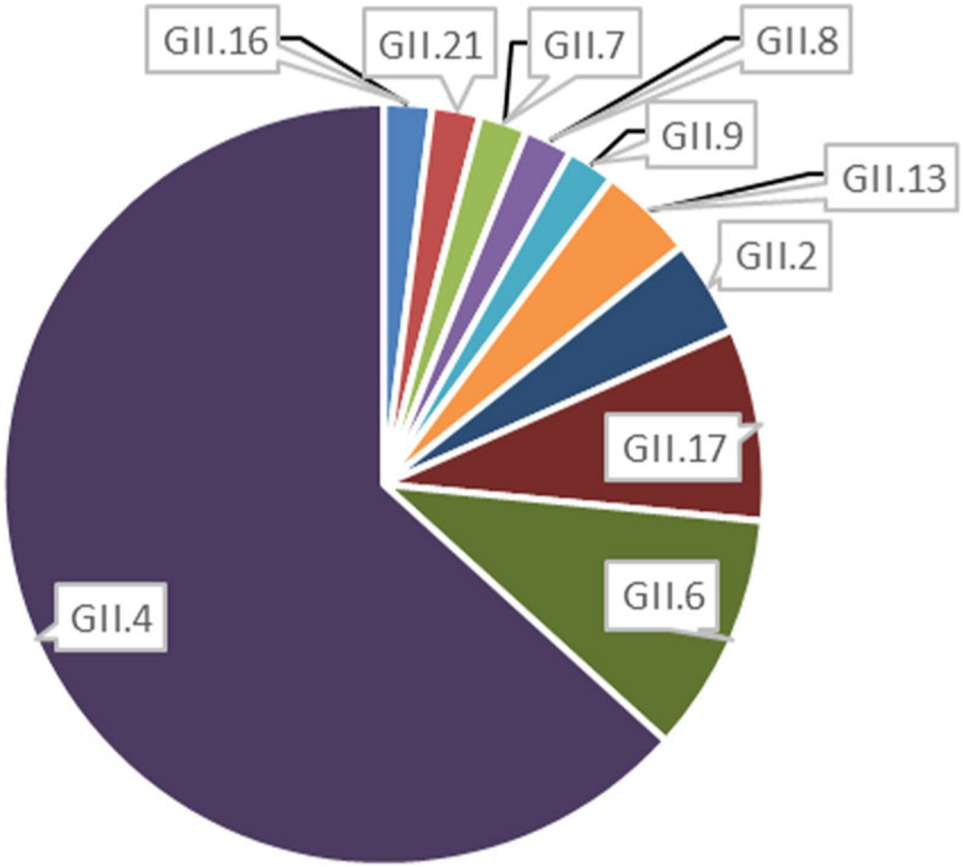

FIGURE 1.
Distribution of norovirus GII genotypes detected in this study

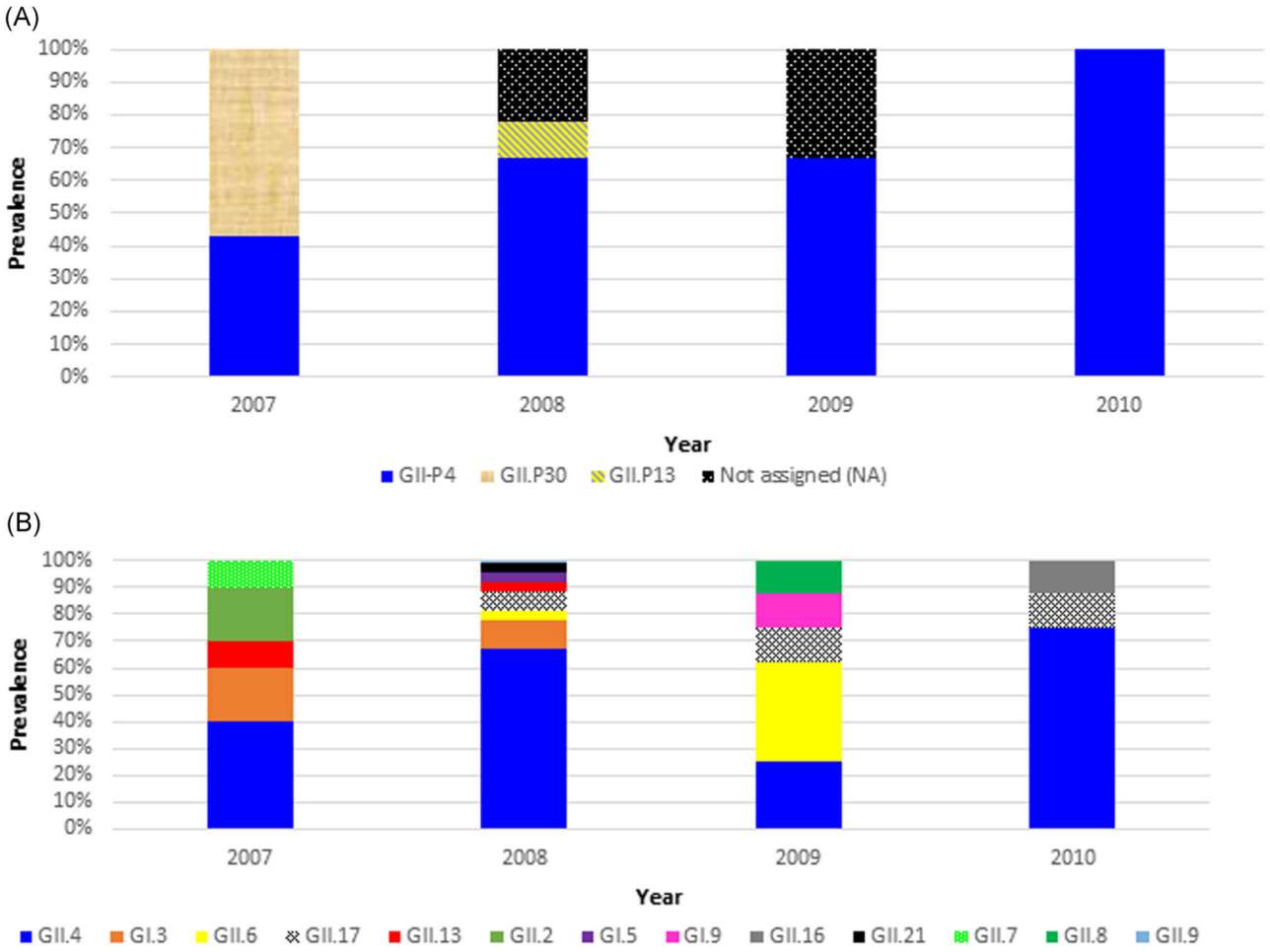

FIGURE 2.
Trends of norovirus GII.4 variants in this study

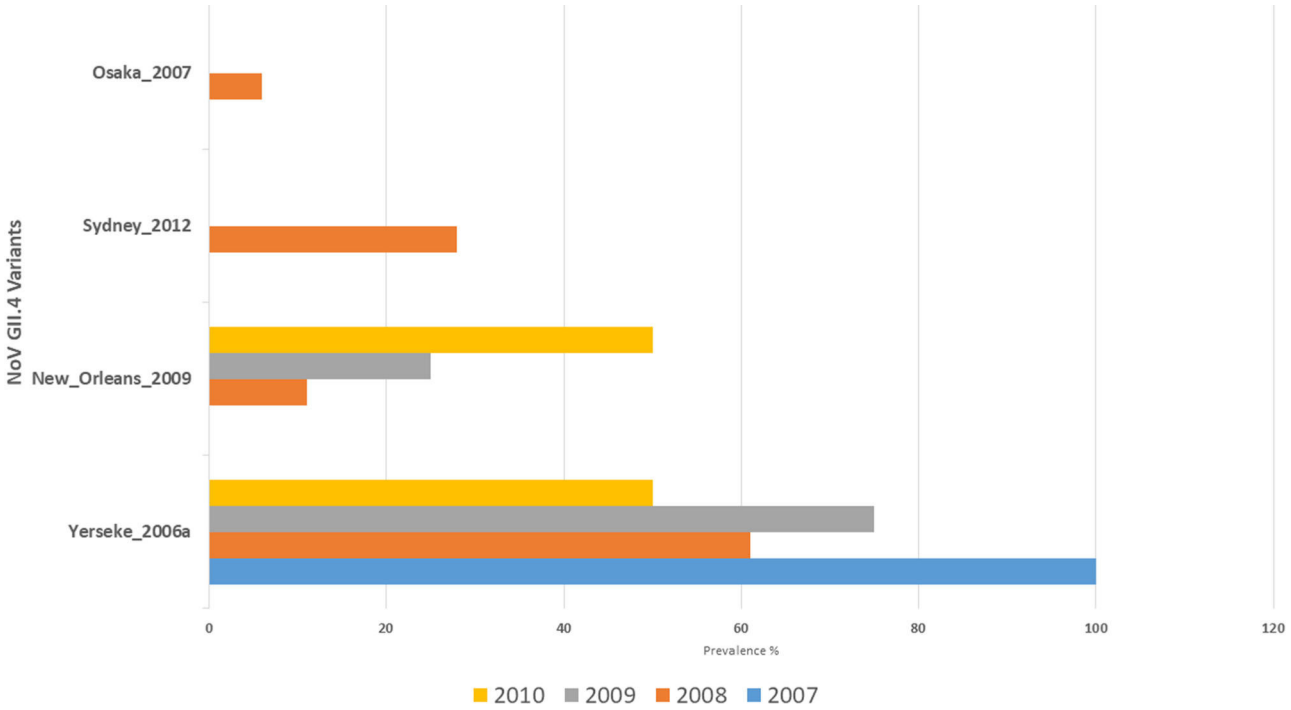

FIGURE 3.

Norovirus genotype distribution in hospitalized children with acute gastroenteritis between 2007 and 2010. (A) partial polymerase region and (B) Partial capsid region

TABLE 1

Norovirus results obtained by real-time and conventional RT-PCR for 599 stool samples from children with acute gastroenteritis in Senegal, 2007–2010

				Conventional RT-PCR								
	Real time RT-PCR			GI and GII polymerase gene			GI capsid gene			GII capsid gene		
Years	No. tested	Pos	%	No. tested	Pos	%	No. tested	Pos	%	No. tested	Pos	%
2007	109	15	13.8	15	7	46.7	4	2	50.0	11	9	81.8
2008	275	43	15.6	43	9	20.9	6	4	66.7	37	25	67.6
2009	124	12	9.7	12	3	25.0	2	1	50.0	10	9	90.0
2010	91	9	9.9	9	6	66.7	0	0	0	9	9	100
Total	599	79	13.0	79	25	33.3	12	7	58.3	67	52	77.6