Chapter

Norovirus Genotypic Variability in Brazil

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Abstract

Norovirus (NoV) has been recognized as the most common etiological agent of acute gastroenteritis (AGE) in various epidemiological settings worldwide. The virus displays a high genetic diversity that can be classified into genogroups, genotypes, and recombinant strains. Only genogroups I, II, and IV have been found to infect humans. Variants of genogroup II genotype 4 are the most widely circulating strains and have been responsible for all NoV outbreaks globally since the mid-1990s. Several studies from different Brazilian regions have been conducted to detect and genetically characterize NoV from sporadic AGE cases and outbreaks. In this chapter, we have summarized the data that focused on the genetic variabilities of NoVs and thus highlight the value of a surveillance system in assessing not only the true burden of the disease, but also the detection and characterization of emerging novel variants.

Keywords: norovirus, gastroenteritis, genetic diversity, recombinant variants

1. Introduction

Noroviruses (NoVs) are small, non-enveloped viruses with icosahedral symmetry and diameters ranging between 27 and 40 nm [1] belonging to the family Caliciviridae and the genus Norovirus. The virus genome consists of simple, positive-sense RNA strands of approximately 7.4 to 7.7 kb and contains three open reading frames (ORFs) [2, 3]. ORF1 encodes non-structural proteins, such as RNA-dependent RNA polymerase (RdRp), ORF2 encodes the major capsid VP1 protein, and ORF3 encodes a minor structural protein VP2 [4]. The VP1 protein has the N-terminal (N), shell (S), and protruding (P) domains, and the current classification of NoVs into 10 genogroups (Gl to GX) has been based on these gene sequences. These viruses have been further classified into 49 and 60 confirmed genotypes and types based on amino acids of the complete VP1 and partial nucleotide sequences of RNA-dependent RNA polymerase regions, respectively [5]. Among the genogroups, only GI, GII, and GIV contain strains that infect humans. GII genotype 4 (GII.4) is the predominant NoV genotype causing gastroenteritis outbreaks worldwide, with periodic emergence and pandemic spread of novel lineages of the NoV GII.4 variants [6]. Mutations and recombination frequently occur within NoV genomes, and these evolutionary forces contribute to the emergence of new GII.4 variants every two to three years capable of re-infecting individuals already exposed to the virus [4].

Recently, a new variant of the virus (Gll.17) that has never been reported before was detected in several countries in Asia, Europe, and North and South America, including Brazil, during outbreaks of acute gastroenteritis (AGE) [4].

NoVs are strongly associated with acute non-bacterial gastroenteritis; they are highly infectious and can affect all ages. The main routes of transmission of these viruses vary between fecal-oral, contact with infected people, ingestion of contaminated food and/or water, and aerosol produced by vomiting [2, 7]. Infection with these viruses is usually acute and limited: vomiting, diarrhea, and nausea are the main symptoms. However, in immunocompromised and hospitalized patients, there may be prolonged viral excretion and clinical complications due to virus infection [8].

2. Norovirus variants in Brazil

It is well known that NoVs are responsible for outbreaks, sporadic cases, and hospitalizations in Brazil [9]. However, generally in Brazil, the investigations of NoVs in hospital studies have been primarily focused on epidemiological surveys of diarrheal diseases caused by Rotavirus as a cause of severe gastroenteritis [8]. The first molecular detection of NoVs in Brazil occurred in the early 1990s and, since then, the presence of Gl and Gll has been reported in several regions of the country [10]. The tremendous diversity of NoV GII.4 variants with recombinant genotypes has been reported throughout the five geopolitical regions (North, Northeast, Central-West, South, and Southeast) of Brazil [11–18] as depicted in **Figure 1**. It has been suggested that the GII.17 variant detected in 2015 might have been introduced during the soccer World Cup event held in Brazil in 2014 [4].

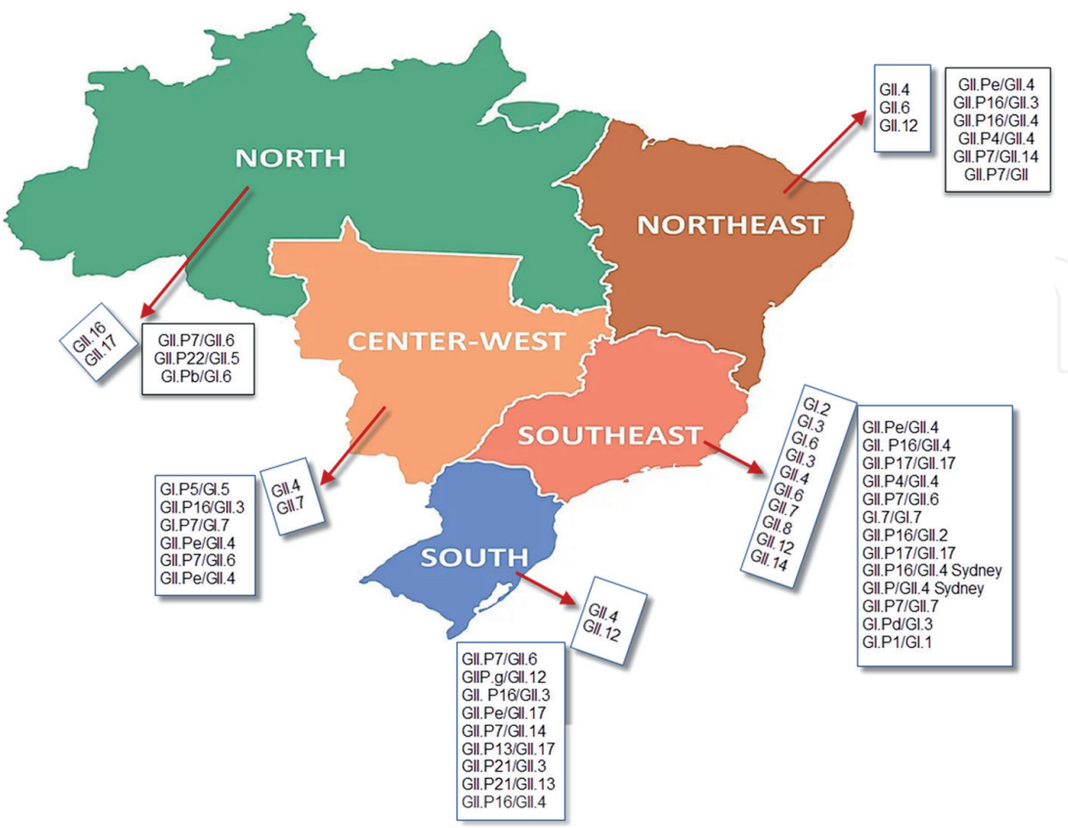

Figure 1.
Distribution of NoVs genotypes and recombinants detected in the five geopolitical regions of Brazil.

2.1 South region

The South region of Brazil is made up of three states: the Rio Grande do Sul, Santa Catarina, and Paraná. The region covers an area of 576,409.6 km2, making it the smallest region in the country. It is considered the third most populated and second most densely populated region in Brazil, as it has a population of 29.4 million people and a population density of 50 individuals per km2. Several studies have been conducted to determine the prevalence of NoV-associated gastroenteritis as well as the genotypic characterization of the virus circulating in the southern region of Brazil [9, 19]. documented the circulation of eight different NoV recombinant strains responsible for AGE outbreaks in the southern region from 2004 to 2011. These strains were identified by the recombination tools as GII.P7/GII.6, GIIP.g/GII.12, GII.P16/GII.3, GII.Pe/GII.17, GII.P7/GII.14, GII.P13/GII.17, GII.P21/GII.3, and GII.P21/GII.13. Among these strains, the GII.P7/GII.6 was the strain with the most frequent recombination, circulating from 2004 to 2010, followed by GII.Pg/GII.12, which was only detected in 2009. In 2016, Débora Maria Pires Gonçalves Barreira and colleagues [11] reported for the first time on the circulation and predominance of the newly emergent GII.P16-GII.4 Sydney strain along the southeastern coast of Brazil. Other studies confirmed the circulation of the pathogenic GIV genotype in both clinical and environmental samples [20, 21].

2.2 Southeast region

This region is made up of four states: Rio de Janeiro, São Paulo, Espírito Santo, and Minas Gerais. It encompasses an area of 924,511.3 km2 and has a population of 86.3 million people, which ranks as the fourth largest region by area and the most populated. It is also the most densely populated region, with a population density of 87 individuals per km2. Various studies have been conducted in this region to highlight the diversity of the circulating NoV genotypes. For example, an earlier report on sporadic infections in Brazilian children in the state of São Paulo revealed a high prevalence of GII and that the majority of the sequenced strains were phylogenetically clustered with GII.4. The same study also reported different potential recombinant strains [13]. In the state of Espírito Santo, an emerging recombinant NoV genotype was detected between January 2015 and July 2016; in the first year, the study reported a predominance of GII.Pe/GII.4 Sydney 2012 and, in the second year, a high prevalence of the GII. P16/GII.4 recombinant strain was observed [11]. The same study also reported the detection of GII.P17/GII.17 in three samples in 2016, which had already been detected in the country in 2015. A recent study by Cantelli et al. [22] aimed to scrutinize the genetic divergence of noroviruses in fecal specimens obtained from children with or without acute diarrheic episodes from a low-income urban area, the Manguinhos community, in the state of Rio de Janeiro. The results of this study showed 10 different genotypes circulating in this community between November 2014 and April 2018: GII.P4/GII.4, GII.P7/GII.6, and GI.7/GI.7 were the most frequent, followed by GII.P16/GII.2, GII.P17/GII.17, GII.P16/GII.4 Sydney, GII.P and GII.4 Sydney, GII.P7/GII.7, GI.Pd/GI.3, and GI.P1/GI.1. In addition, the frequent detection and genetic diversity of NoV observed in children who did not have episodes of acute diarrhea may mean frequent exposure to the virus.

2.3 Center-west region

The Brazilian Central-West region includes the following states: Mato Grosso, Mato Grosso do Sul, Goiás, and the Federal District (which includes Brasília, the

capital of Brazil). Few studies on the variability of NoVs from human clinical samples have been performed in this region. In the State of Goiás, feces were collected from children with or without symptoms of AGE in the periods 2009–2011 and 2014–2015 to characterize the ORF1 - RdRp and ORF2 - VP1 regions of the NoV genome. This study identified GII.P7-GII.6 (the most frequent recombination), GII. Pe/GII.4 (the second most frequent recombination in this study and characterized as Sydney 2012 variant), and GII. P16-GII.3 [23]. The data from the same study indicate that the distribution of NoV genotypes circulating in this region varies over time and that some recombinant strains had different recombinant breakpoints in samples obtained at different periods.

2.4 North region

The Brazilian North region includes the following states: Tocantins, Pará, Amapá, Amazonas, Roraima, Acre, and Rondônia. This is the largest region in Brazil, corresponding to 45.27% of the national territory. It is the least inhabited region of the country, with only 3.8 inhabitants per km^2. Recombinant strains of NoVs have been previously detected in a few clinical cases or in outbreaks that occurred in this region. However, in the Amazon states, between 2011 and 2014, the circulation of a recombinant strain GII.P7 / GII.6 was documented, which may indicate that this strain is already established in the population [2]. A recent study on samples collected during 2015 and 2016 from children under 5 years of age in Pará and Amazonas states demonstrated an increase in the circulation of the emerging GII.17_2014 strain in the Amazon region, and their phylogenetic approach suggests a single introduction of this genotype to the Amazon region [7]. Another strain that has also been detected in Amazonas is Gll.4, including variants GII.4 New Orleans_2009, and Sydney_2012 [2]. The same genotype detected in the Amazon region (GII.17_2014) was also detected in children with gastroenteritis in Belém, the capital of the state of Pará (neighboring the state of Amazonas) in 2016 [24]. However, a few years prior in this same region, more precisely during the period 2012–2015, stools and blood samples were collected from children hospitalized with acute gastroenteritis, and the viral load in the serum was very low when compared to the stools. In this study, a recombinant strain considered unusual was detected (GII.P13 / GII.17). However, while the main strain detected was GII.4 Sydney 2012, strains GII.P7 / GII.6, GII.P22 / GII.5, and GI.Pb /GI.6 were also reported [8]. An older study, by Siqueira et al. [25], also conducted in Belém, which lasted for almost 30 years (1982–2011), had followed both children in the community and outpatients and patients in hospitals to identify NoV genotypes related to cases of acute gastroenteritis. The Gll.4 (or GII.P4) genotype was the unique variant detected in all collected samples during the period 1998 to 2011. However, between 1982 and 1986, the GII genotypes were highlighted. P6 and GII.P7 were prevalent during the period 1990–1992, and GII.P3, GII.6, and GII.7 were reported during the period 1992–1994, with GII.P3 noted as the most prevalent variant. The GII.P21 genotype also had a wide circulation and was detected more frequently from the end of September 1998 until 2011, and was widely detected between the years 2001–2002 and 2008–2011.

2.5 Northeast region

The Brazilian Northeast region includes the following states: Bahia, Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará, Piauí, and Maranhão. In several cities in the state of Pernambuco, between 2014 and 2017, stool samples were collected from individuals with acute gastroenteritis, with children up to

3 years old being the most affected by the virus. After sequencing the ORF1–ORF2 regions of 20 strains circulating in the state, four different GII genotypes were found: GII.Pe-GII.4, GII.P16-GII.3, GII.P16-GII.4, and GII.P4-GII.4, of which GII. Pe-GII.4 was the most prevalent in the region In the semi-arid region of North-eastern Brazil, which has the lowest income and the largest income disparity in the country, a study was conducted between 2009 and 2012 to determine the distribution of NoV genotypes in children ranging in age from 2 to 36 months with diarrhea and living in the cities in the states of Paraíba, Ceará, Pernambuco, or Piauí. In the study subjects, 45.2% of the individuals were positive for NoV, with genogroups GII and Gl being detected in 94.6% and 5.3% of positive samples, respectively. Based on the polymerase region, the most frequent genotypes were GII.P7 and GII.P16, while based on the capsid region, the main genotypes were GII.3, GII.14, and GII.4 New Orleans_2009. However, when both regions were analyzed, the authors observed a high frequency of recombinant strains classified as GII.P16-GII.3, GII.P7-GII.14, and GII.P7-GII, of which GII.P16-GII.3 had the highest prevalence [26].

3. Conclusions

Overall, this chapter has shown the circulation of multiple NoV strains in Brazil, which may lead to the occurrence of novel recombinant strains. Therefore, efforts to improve the national surveillance system are warranted to facilitate early detection of novel emergent variants, preparedness for upcoming epidemics, and the development and production of vaccines.

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Conflict of interest

The authors declare that there is no conflict of interest.

Notes/thanks/other declarations

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