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Genetic diversity of rotavirus strains in children with diarrhea in Lagos, Nigeria

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ABSTRACT

Objective: To describe the genetic diversity of rotavirus strains circulating in Lagos, Nigeria.**Methods:** A total of 302 stool samples were obtained from diarrheic children on admission to four hospitals in Lagos and screened for rotavirus antigen by enzyme immunoassay, while rotavirus VP4 and VP7 genotypes were determined by multiplex semi-nested RT-PCR using recognized primers and methods.**Results:** Overall, the globally important genotypes, G1, G2, G3, G4 and G9, associated with P[4], P[6] and P[8] were detected. A rotavirus carrying G12 specificity was also detected at low frequency. The different rotavirus strains found in this study were G[2]P[6], G[2]P[8], G[1]P[8], G[1]P[6], G[3]P[6], G[9]P[6], G[2]P[4], G[4]P[4], G[12]P[8] and some mixed infections.**Conclusions:** The study highlights the wide diversity of rotavirus strains and the potential emergence of unusual rotavirus in this region. It is therefore important to continue the epidemiological studies to monitor rotavirus strains associated with gastroenteritis in hospitals before and after the introduction of rotavirus vaccine.

1. Introduction

Rotavirus is a non-enveloped virus with icosahedral symmetry belonging to the family Reoviridae. The virion consists of three layers of proteins with the viral genome consisting of 11 segments of double-stranded RNA, which encodes six structural proteins, namely, viral proteins (VP) 1–4, VP6 and VP7 and six non-structural proteins, non-structural proteins 1–6[1,2]. The outer capsid is composed of two independent neutralization antigens, namely VP4 which determines P-genotype and VP7 which is denoted as G-serotype. VP7, the glycoprotein or G-protein encoded by gene 7, 8 or 9 depending on the strain, and VP4, the protease-cleaved or P-protein encoded by gene segment 4, determine the serotype specificity and form the basis of the binary classification (G and P types) of rotaviruses[3]. Both G and P proteins induce neutralizing antibodies and may be involved in protective immunity[4]. The most common G serotypes are G1, G2, G3, G4, and G9, in which G1 is

the most prevalent one and G9 emerges fastest worldwide. At least 27 G genotypes and 35 P genotypes have been identified in human rotaviruses whereof genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] are responsible for 80%–90% of the childhood rotavirus disease burden globally[5].

The VP6 protein located in the inner capsid of the virus contains the antigenic determinants which classify them into seven serogroups of A to G. Groups A, B and C rotaviruses have been found both in humans and animals while groups D, E and F have been found only in animals. Group A rotaviruses have clearly been established as causing severe diarrhoeal disease in the young. Group B includes those viruses associated with animal epidemics of severe diarrhea primarily in adults in China. Group C viruses have been found in sporadic cases and outbreaks of diarrhoea in piglets and children[6].

Human group A rotaviruses are the most frequently identified etiologic agent in children hospitalized with acute, severe and dehydrating diarrhea worldwide[7]. It is estimated that 400 000–600 000 children, most of who live in developing countries, die from rotavirus infections each year. Children in sub-Saharan Africa account for over 40% of these deaths[8–10].

The epidemiology of rotaviruses has been reported in some studies in Nigeria[11–14]. However, to update our knowledge of the diversity of rotavirus strains infecting young children in Nigeria, prior to the official introduction of rotavirus vaccines, it is imperative to conduct rotavirus surveillance to determine the emergence of new or unusual

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The study protocol was performed according to the Helsinki declaration and approved by the Lagos State Hospital Management Board and the local ethical committees of the hospitals. Informed written consent was obtained from the parents/guardians of the children recruited in this study.

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genotypes that may have resulted from possible reassortment of co-circulating strains of different genotypes which may have implications on the existing vaccines.

2. Materials and methods

2.1. Subjects and sample collection

A total of 302 samples were collected from children less than 5 years of age presenting with acute diarrhoea, who attended Massey Street Children Hospital, Orile Agege General Hospital, Surulere General Hospital and Ikorodu General Hospital during the period August 2007–July 2008 in Lagos State, Nigeria. A diarrhea case in this study was defined as a child passing loose, watery or bloody stool three or more times in the past 24 hours. Basic demographic data, histories of the illness and clinical information about the children were obtained from their caregivers using questionnaires. The samples were collected and stored frozen at -20 °C in the Department of Microbiology, University of Lagos before transported to Noguchi Memorial Institute of Medical Research, University of Ghana, Legon, Ghana for analyses.

2.2. Virus detection

Ten percent suspension of all the diarrhea stool samples in phosphate buffered saline were tested for the presence of group A rotavirus antigen using commercially available enzyme immunoassay kit (Rotavirus IDEIATM, Dako, UK). The test was carried out according to the manufacturer's instructions.

2.3. VP7 and VP4 typing by RT-PCR

A total of 58 samples which were rotavirus antigen positive by EIA were further analyzed by PCR genotyping. Briefly, rotavirus double stranded RNA was extracted from these samples and purified using the RNaid kit (Bio 101 system, Qiogene Carlsbad, USA). The extracts were used as templates for RT-PCR, *i.e.* the first and second round amplifications using random primers. For G-typing, a full-length 1 (1062 bp) gene segment 9, encoding the VP7 glycoprotein of human group A rotaviruses, was amplified with the forward primer Beg9 (5'GGCTTTAAAAGAGAGAATTTCCGTCTGG3') and the reverse primer End9 (5'GGTCACATCATACAATTCTAATCTAAG3'). This was followed by semi-nested PCR using G-serotype specific primers: aAT8 (5'GTCACACCATTTGTAAATTCG3'; nt 178–198), aBT1 (5'CAAGTACTCAAATCAATGATGG3'; nt 314–335), aCT2 (5'CAATGATATTAACACATTTCTGTG3'; nt 411–435), aDT4 (5'CGTTTCTGGTGAGGAGTTG3'; nt 480–498), aET3 (5'CGTTTGAAGAAGTTGCAACAG3'; nt 689–709), aFT9 (5'CTAGATGTAACACTACAAC3'; nt 757–776) and a G12 specific pair of primers [5'TCGTCATGCTGCCATTTA3'; nt 173–190 (forward) and 5'GTCCAGTCGGGATCAGTT3'; nt 327–344 (reverse)], to identify G types [15–17]. In a similar manner, PCR for P typing was carried out in two steps, *i.e.* first and second amplifications, as described by Armah *et al.* [18]. Briefly, a DNA fragment of 876 bp was amplified from gene segment 4, encoding VP4 protein using primers Con2 (5'ATTCGGACCATTATAACC3') in the forward direction and

Con3 (5'TGGCTTCGCCATTTATAGACA3') in the reverse. The VP4 genotypes of rotavirus strains were then determined for samples with successfully amplified full length gene segment 4 (876 bp fragment) using rotavirus specific primers 1T-1 (5'ACTTGGATAACGTGC3') KU(P8), 2T-1 (5'CTATTGTTAGAGGTTAGAGTC3') RV5(4), 3T-1 (5'TGTTGATTAGTTGGATTCAA3') I 076 (P6), 4T-1 (5'TGAGACATGCAATTGGAC3', K8(P9), 5T-1 (5'ATCATAGTTAGTAGTCGC3') 69M (P10) and consensus primer Con2 [19]. All PCR products were electrophoresed at 100 V for 60 min in 2% agarose gels containing 2 µL ethidium bromide, viewed under UV illuminator and documented using the AlphaDigitDoc™ RT imaging system (Alpha Innotech Corporation, USA). The characteristic of G and P genotypes amplicons was determined against the 100 bp molecular size DNA marker (Promega).

3. Results

Three hundred and two stool specimens were analyzed for rotavirus, in which 78 (25.8%) tested positive for rotavirus antigen by EIA. Among these positive samples, 58 were typed using RT-PCR.

3.1. Distribution of G type

Ninety percent of the rotavirus isolates were assigned VP7 G-type specificity while 10% showed amplification failure. Six different rotavirus VP7 serotypes including the G9 and G12 were detected. In the study, genotypes G1 and G2 occurred predominantly with an equal proportion of 31.0% for each, followed by G3 (8.6%), G4 (5.2%), G9 (5.2%) and G12 (1.7%). Mixed infection (G1 + G2) and (G1 + G3) were also detected in 3 (5.2%) and 1 (1.7%) of the samples, respectively, while 6 (10.4%) were nontypeable (Table 1).

3.2. Distribution of P type

Out of the 58 amplified samples, 56 (96.6%) could be assigned to VP4 genotype. The VP4 genotypes detected during this study included three of the recognized human rotavirus VP4 alleles, P[4], P[6] and P[8] as well as strains with dual P types P[4 + 6], P[4 + 8], P[6 + 8] and mixed infection of the three VP4 alleles P [4 + 6 + 8]. The most predominant P type was P[6] in 25 (43.1%) isolates, followed by P[8] in 9 (15.5%) and P[4] in 5 (8.6%). Seventeen (29.4%) were mixed P genotypes and 2 (3.5%) could not be assigned VP4 genotype (Table 1).

3.3. G and P type combination

Twenty distinct strains were identified with the predominant combinations being strains with genotypes G[2]P[6], G[1]P[8] and G[1]P[6] and each having same proportions (14%). These were followed by G[4]P[4], G[3]P[6], G[9]P[6] and G[2]P[4], occurring in the proportions of 6%, 4%, 4% and 4%, respectively. It is important to note that all the three samples that were identified as G4 were also typed as P4 genotype. The following unusual genotypes: G[4]P[4] (6%), G[12]P[8] (2%), G[2]P[8] (2%) and so many mixed infections were also observed (Table 1).

Table 1

Circulating rotavirus (G and P) genotypic strains in children in Lagos.

Genotype	G1	G2	G3	G4	G9	G12	G1 + G2	G1 + G3	G (nt)	Total [n (%)]
P[4]	0	2	0	3	0	0	0	0	0	5 (8.6)
P[6]	7	7	2	0	2	0	2	1	4	25 (43.1)
P[8]	7	1	0	0	0	1	0	0	0	9 (15.5)
P[4 + 6]	0	3	1	0	0	0	0	0	0	4 (6.9)
P[4 + 8]	1	1	0	0	1	0	1	0	0	4 (6.9)
P[6 + 8]	2	4	0	0	0	0	0	0	1	7 (12.1)
P[4 + 6 + 8]	1	0	0	0	0	0	0	0	1	2 (3.5)
P[nt]	0	0	2	0	0	0	0	0	0	2 (3.5)
Total [n (%)]	18 (31.0)	18 (31.0)	5 (8.6)	3 (5.2)	3 (5.2)	1 (1.7)	3 (5.2)	1 (1.7)	6 (10.4)	58 (100.0)

nt: Nontypeable.

4. Discussion

Rotavirus gastroenteritis continues to be the single most important cause of dehydration in young children. This research study showed the importance of human rotavirus in infantile diarrhea in Lagos. This is the first hospital-based study of long duration in Lagos to report rotavirus serotype G4 as well as the unusual strain G12 specificities, even though Oluwatoyin Japhet *et al.*[14] had previously reported rotavirus G12 from diarrheic stool samples of children in Ile-Ife, one of the neighboring cities around Lagos. Rotavirus G12 has recently been identified as an emerging genotype[20]. But generally from the African continent, G12 in combination with P[6] or P[8] had been reported from Nigeria, South Africa, Malawi and Gabon[14,21-23].

Earlier studies have implicated genotype G1 as the predominant strain[24-26]. However, in this study, not only G1 was identified as the predominant serotype but also G2 occurring in the same proportion as G1. This is in conformity to the findings of Iyoha and Abiodun[27] in Benin City which reported the prevalence of serotype G1. Likewise, Aminu *et al.*[28] identified the same G1 as the most common serotype. However, Lekana-Douki *et al.*[23] reported that G6 serotype was the predominant strain from a study in Gabon. This implies that there are diversities of prevalent strains in different parts of Africa. In the past, rotavirus G4 had never been detected until in early 20th century when the first G4 serotype was isolated in Nigeria. This strain was detected in a study carried out in Ilorin, Kwara State where a single sample was identified as G4[11]. In the current study, 3 (5%) of the rotavirus isolates were typed as G4 as against (1.7%) reported earlier in Nigeria[11].

The G9 strain, usually in combination with P[8] or P[6], has gained epidemiological relevance in the past years[29]. In this study, G9 serotype was found to be in association with P6. However, in the previous study, in Ile Ife, Nigeria, serotype G9 was found in association with P[8][14]. The G9 serotype has become ubiquitous and is now the fourth most common strain worldwide, accounting for 4.1% of the infection[5].

Among the five most common VP4 genotypes, P[6] is known to be associated with asymptomatic infection in newborn nurseries[30]. However, in this study P[6] was the most common strain found circulating among symptomatic children in Lagos. This has also been reported in South Africa[31].

Rotavirus classification is based on the differences in the VP7 (G) and VP4 (P) capsid proteins, with G serotypes 1–4 and P

genotypes P[8] and P[4] predominated worldwide[5,32]. A review of G and P types from over 2700 specimens found that the P[8] genotype was almost always with either G1, G3 or G4 and that P[4] was almost always associated with G2[5]. In this study, 90% of the isolates were genotyped, of which 64% were from faecal specimens with single strains of these four common combinations. The P[4] genotype which is usually associated with rotavirus carrying VP7 serotype G2 specificity was found in association with G4 in this study. Uncommon strains were detected, amongst were G[4]P[4], G[2]P[8] and strain G[12]P[8] which are being detected for the first time in Lagos, Nigeria. A significant proportion (36%) of the specimens had multiple G and/or P types as it has also been reported elsewhere [33,34]. This is consistent in infections with more than one rotavirus strains. One potential consequence of the presence of multiple G and/or P types in specimens is a high chance for the reassortment during natural infections or it could be as a result of mixed infections. This study adds to the growing knowledge on the diversity of rotavirus strains in circulation and also provides data to buttress the need for introduction of rotavirus vaccines in National Immunization Programme in Nigeria.

In conclusion, the report of an unconventional serotype G12 and some nontypeable G strains in this study stresses the need for continual surveillance to monitor the emergence of unusual rotavirus strains which may not be protected by the existing vaccines, although a monovalent vaccine (Rotatrix) has been reported to show a high efficacy against severe rotavirus gastroenteritis among African infants[35].

Conflict of interest statement

I declare that I have no conflict of interest.

References

- Estes MK, Kang G, Zeng CQ, Crawford SE, Ciarlet M. Pathogenesis of rotavirus gastroenteritis. *Novartis Found Symp* 2001; **238**: 82-96.
- Paesi S, Da Rosa CE, Marocco Dornelles PR, Da Luz F, Schenkel CA, Zampieri D. Evaluation of a latex agglutination kit for detecting rotavirus in piglets. *Acta Sci Vet* 2012; **40**(1): 1014.
- Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med* 2012; **13**(68): 85-97.
- Pesavento JB, Crawford SE, Estes MK, Prasad BV. Rotavirus proteins: structure and assembly. *Curr Trop Microbiol Immunol* 2006; **309**: 189-

- 219.
- [5] Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* 2005; **15**(1): 29-56.
- [6] O’Ryan M. The ever-changing landscape of rotavirus serotypes. *Pediatr Infect Dis J* 2009; **28**(3 Suppl): S60-2.
- [7] Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. *Lancet* 2013; **381**(9875): 1405-16.
- [8] Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et al. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 2009; **200**(Suppl 1): S9-15.
- [9] Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ* 2008; **86**(9): 710-7.
- [10] Chandola TR, Taneja S, Goyal N, Rathore SS, Appaiahgari MB, Mishra A, et al. Descriptive epidemiology of rotavirus infection in a community in North India. *Epidemiol Infect* 2013; **141**(10): 2094-100.
- [11] Audu R, Omilabu SA, de Beer M, Peenze I, Steele AD. Diversity of human rotavirus VP6, VP7, and VP4 in Lagos State, Nigeria. *J Health Popul Nutr* 2002; **20**(1): 59-64.
- [12] Adah MI, Wade A, Taniguchi K. Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. *J Clin Microbiol* 2001; **39**(11): 3969-75.
- [13] Aminu M, Esona MD, Geyer A, Steele AD. Epidemiology of rotavirus and astrovirus infections in children in North-western Nigeria. *Ann Afr Med* 2008; **7**(4): 168-74.
- [14] Oluwatoyin Japhet M, Adeyemi Adesina O, Famurewa O, Svensson L, Nordgren J. Molecular epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: high prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype. *J Med Virol* 2012; **84**(9): 1489-96.
- [15] Benati FJ, Maranhão AG, Lima RS, da Silva RC, Santos N. Multiple-gene characterization of rotavirus strains: evidence of genetic linkage among the VP7-, VP4-, VP6-, and NSP4-encoding genes. *J Med Virol* 2010; **82**(10): 1797-802.
- [16] Banerjee I, Ramani S, Primrose B, Iturriza-Gomara M, Gray JJ, Brown DW, et al. Modification of rotavirus multiplex RT-PCR for the detection of G12 strain based on characterization of emerging G12 rotavirus strains from South Indian. *J Med Virol* 2007; **79**(9): 1413-21.
- [17] Griffin DD, Nakagomi T, Hoshino Y, Nakagomi O, Kirkwood CD, Parashar UD, et al. Characterization of nontypeable rotavirus strains from the United States: identification of a new rotavirus reassortant (P2A[6],G12) and rare P3[9] strains related to bovine rotaviruses. *Virology* 2002; **294**(2): 256-69.
- [18] Armah GE, Pager CT, Asmah RH, Anto FR, Oduro AR, Binka F, et al. Prevalence of unusual human rotavirus strains in Ghanaian children. *J Med Virol* 2001; **63**(1): 67-71.
- [19] Sudarmo SM, Shigemura K, Athiyah AF, Osawa K, Wardana OP, Darma A, et al. Genotyping and clinical factors in pediatric diarrhea caused by rotaviruses: one-year surveillance in Surabaya, Indonesia. *Gut Pathog* 2015; **7**:3.
- [20] Trimis G, Koutsoumbari I, Kottaridi C, Palaiologou N, Assimakopoulou E, Spathis A, et al. Hospital-based surveillance of rotavirus gastroenteritis in the era of limited vaccine uptake through the private sector. *Vaccine* 2011; **29**(43): 7292-5.
- [21] Cunliffe NA, Ngwira BM, Dove W, Nakagomi O, Nakagomi T, Perez A, et al. Serotype G12 rotaviruses, Lilongwe, Malawi. *Emerg Infect Dis* 2009; **15**(1): 87-90.
- [22] Page NA, de Beer MC, Seheri LM, Dewar JB, Steele AD. The detection and molecular characterization of human G12 genotypes in South Africa. *J Med Virol* 2009; **81**(1): 106-13.
- [23] Lekana-Douki SE, Kombila-Koumavor C, Nkoghe D, Drosten C, Drexler JF, Leroy EM. Molecular epidemiology of enteric viruses and genotyping of rotavirus A, adenovirus and astrovirus among children under 5 years old in Gabon. *Int J Infect Dis* 2015; **34**: 90-5.
- [24] Pitzer VE, Patel MM, Lopman BA, Viboud C, Parashar UD, Grenfell BT. Modeling rotavirus strain dynamics in developed countries to understand the potential impact of vaccination on genotype distributions. *Proc Natl Acad Sci U S A* 2011; **108**(48): 19353-8.
- [25] Moyo SJ, Blomberg B, Hanevik K, Kommedal O, Vainio K, Maselle SY, et al. Genetic diversity of circulating rotavirus strains in Tanzania prior to the introduction of vaccination. *PLoS One* 2014; **9**(5): e97562
- [26] Esona MD, Armah GE, Steele AD. Rotavirus VP4 and VP7 genotypes circulating in Cameroon: identification of unusual types. *J Infect Dis* 2010; **202**(Suppl 1): S205-11.
- [27] Iyoha O, Abiodun PO. Human rotavirus genotypes causing acute watery diarrhea among under-five children in Benin City, Nigeria. *Niger J Clin Pract* 2015; **18**(1): 48-51.
- [28] Aminu M, Page NA, Ahmad AA, Umoh JU, Dewar J, Steele AD. Diversity of rotavirus VP7 and VP4 genotypes in Northwestern Nigeria. *J Infect Dis* 2010; **202**(Suppl 1): S198-204.
- [29] Payne DC, Staat MA, Edwards KM, Szilagyi PG, Gentsch JR, Stockman LJ, et al. Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. *Pediatrics* 2008; **122**(6): 1235-43.
- [30] Linhares AC, Mascarenhas JD, Gusmão RH, Gabbay YB, Fialho AM, Leite JP. Neonatal rotavirus infection in Belém, Northern Brazil: nosocomial transmission of a P[6] G2 strain. *J Med Virol* 2002; **67**(3): 418-26.
- [31] Pager C, Steele D, Gwamanda P, Driessen M. A neonatal death associated with rotavirus infection-detection of rotavirus dsRNA in the cerebrospinal fluid. *S Afr Med J* 2000; **90**(4): 364-5.
- [32] Bányai K, László B, Duque J, Steele AD, Nelson EA, Gentsch JR, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. *Vaccine* 2012; **30**(Suppl 1): A122-30.
- [33] Odiit A, Mulindwa A, Nalumansi E, Mphahlele MJ, Seheri LM, Mwenda JM, et al. Rotavirus prevalence and genotypes among children younger than 5 years with acute diarrhea at Mulago National Referral Hospital, Kampala, Uganda. *Pediatr Infect Dis J* 2014; **33**(Suppl 1): S41-4.
- [34] Hokororo A, Kidenya BR, Seni J, Mapaseka S, Mphahlele J, Mshana SE. Predominance of rotavirus G1[P8] genotype among under-five children with gastroenteritis in Mwanza, Tanzania. *J Trop Pediatr* 2014; **60**(5): 393-6.
- [35] Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010; **362**(4): 289-98.