JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2003, p. 913–914 Vol. 41, No. 2
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.2.913–914.2003

Molecular Epidemiology of Rotaviruses in Nigeria: Detection of Unusual Strains with G2P[6] and G8P[1] Specificities

We noted with interest the recent report by Adah and colleagues on the detection of G2P[6] rotavirus strains in Nigeria (1). We have also been conducting epidemiological surveillance of rotavirus strains recovered from young children with diarrheal disease in Lagos, Nigeria (2), and identified similar strains during 2001.

Adah and colleagues identified 12 strains that had the unusual profile of VP7 serotype G2 and VP4 genotype P[6]. Most G2 rotavirus strains bear a specific VP4 genotype of P[4] (4), which is well recognized as belonging to the DS-1 genogroup (7). However, Adah did not extend the characterization of these strains to analyze the potential reassortment of various genes. The DS-1 genogroup can be phenotypically measured on at least three structural genes and one nonstructural gene. These include the VP6 subgroup, VP7 serotype, and VP4 genotype, as well as the migration of the NSP5 gene (7). DS-1 genogroup rotaviruses typically share subgroup I, VP7 serotype G2, and VP4 genotype P[4], and the strains carry a short RNA electropherotype. Analysis of these four markers would provide valuable epidemiological data on the potential reassortment of these unusual rotavirus strains.

In our studies, we have continued the surveillance and characterization of rotavirus strains from young children admitted with acute infantile diarrhea requiring hospitalization (2). In the present report, we identified 21 cases of rotavirus among 150 children (14%).

Fecal samples were collected from children less than 5 years of age with acute diarrhea attending the Gbaja Health Center, Massey Street Children's Hospital, and Lagos University Teaching Hospital (all within Lagos State, Nigeria) between January and December 2001. Ten percent suspensions of the samples in phosphate-buffered saline were tested for the presence of group A rotavirus antigen by using a commercial enzyme immunoassay (Rotavirus IDEIA; Dako, Cambridge, United Kingdom).

All 21 strains were examined for the diversity of the RNA electropherotypes by polyacrylamide gel electrophoresis. Standard RNA extraction was performed by phenol-chloroform treatment and ethanol precipitation as described in detail elsewhere (8). Silver staining was used to identify the double-stranded RNA segments as described previously (8).

In addition, the VP6 subgroup specificity of the rotavirus strains was determined by using the VP6 monoclonal antibodies developed by Greenberg et al. (6). These monoclonal antibodies, specific for subgroup I (255/60) and subgroup II rotaviruses (631/9) rotaviruses, have been extensively used in studies worldwide. The methods for their use have been described in detail elsewhere (6).

The VP4 types were identified as described by Gentsch et al. (2, 3). The viral RNA was extracted by treatment with Genetron and purified by RNAid (Bio 101, Inc., La Jolla, Calif.) before analysis by the reverse transcription-PCR (RT-PCR) method with primers *con2* and *con3*. The PCR products were then typed with a cocktail of primers for the different human VP4 genotypes (3).

The VP7 genotypes were examined by the RT-PCR typing method of Gouvea et al. (2, 5). The purified RNA was reverse transcribed, and primers directed to the terminal sequences were used to amplify the entire gene (5). These techniques have been described in detail elsewhere, and similar conditions were used here (2).

In this study, the unusual G2P[6] strains constituted 8 of 21 rotavirus-positive specimens (38%). Analysis of the two other epidemiological markers showed that all eight strains carried a VP6 subgroup I specificity and were characterized by a short RNA electropherotype, although two variations were seen (Fig. 1).

Taken together, these observations indicate that the P[6] VP4 gene may have reassorted onto the DS-1 genogroup background of these strains. However, further analysis is required to identify whether other genes also reassorted during the generation of these unusual rotaviruses. Further-

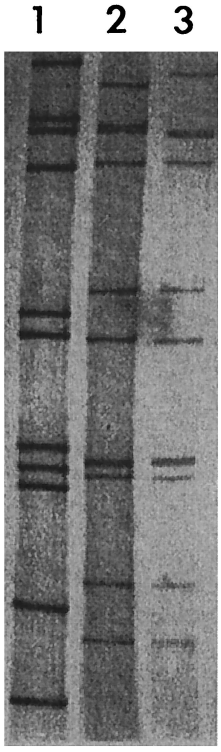

FIG. 1. Polyacrylamide gel electrophoresis of the unusual G2P[6] rotavirus strains recovered in Lagos. The two short strains that carried this unusual genotypic configuration (lanes 2 and 3) are illustrated with a long G8P[1] strain (lane 1) for comparison.

more, this report shows the importance of including all epidemiological markers when characterizing unusual rotavirus strains.

REFERENCES

1. **Adah, M. I., A. Wade, and K. Taniguchi.** 2001. Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. J. Clin. Microbiol. **39:**3969–3975.
2. **Audu, R., S. A. Omilabu, M. C. de Beer, I. Peenze, and A. D. Steele.** 2002. Diversity of human rotavirus VP6, VP7 and VP4 in Lagos State, Nigeria. J. Health Popul. Nutr. **20:**59–64.
3. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B. K. Das, and M. K. Bhan.** 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1365–1373.
4. **Gentsch, J. R., P. A. Woods, M. Ramachandran, B. K. Das, J. P. Leite, A. Alfieri, R. Kumar, M. K. Bhan, and R. I. Glass.** 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. **174:**530–536.
5. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clarke, B. Forrester, and Z. Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
6. **Greenberg, H., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh.** 1983. Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. Infect. Immun. **39:**91–99.
7. **Nakagomi, O., T. Nakagomi, K. Akatani, and N. Ikegami.** 1989. Identification of rotavirus genogroups by RNA-RNA hybridisation. Mol. Cell. Probes **3:**251–261.
8. **Steele, A. D., and J. J. Alexander.** 1987. Molecular epidemiology of rotavirus in black infants in South Africa. J. Clin. Microbiol. **25:**2384–2387.

O. B. Salu
R. Audu
Nigerian Institute for Medical Research
Yaba, Lagos, Nigeria

A. Geyer
A. D. Steele*
MRC Diarrhoeal Pathogens Research Unit
Medunsa 0204
Pretoria, South Africa

A. O. B. Oyefolu
Microbiology Department
University of Lagos
Lagos, Nigeria

*Phone: 27 12 521 5720
Fax: 27 12 521 3535
E-mail: adsteele@medunsa.ac.za