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The Application of Nanopore Sequencing Technology to Characterize Rotavirus Genotypes from Young Children with Diarrhea in the United Arab Emirates

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Background

- In the United Arab Emirates, rotavirus A (RVA) remains a major cause of gastroenteritis in young children despite the universal use of rotavirus vaccines (*Alsuwaidi AR et al. BMC Infect Dis. 2021*). Monitoring the genetic diversity of circulating strains is essential to better understand the disease burden.
- Although simplex/multiplex-PCR is a popular method for RVA genotyping, some clinically significant strains may not be accurately identified by this technique.
- Nanopore sequencing is a third-generation single-molecule sequencing technology that can generate long-read data in real-time and at competitive cost, which has been successfully applied in the real-time surveillance of viruses and bacteria.

Objectives

- To investigate the use of nanopore sequencing and simplex-PCR methods to identify RVA genotypes

Methods

- Thirty-three RVA isolates, obtained from stool specimens collected from children < 5 years old who presented with diarrhea (December 2017 - April 2019); (*Alsuwaidi AR et al. BMC Infect Dis. 2021*), were selected for genotyping by PCR and nanopore sequencing; *Figure 1*.
- Thirteen isolates were genotyped by simplex TaqMan-based qRT-PCR based on VP7 (G) and VP4 (P) genes and confirmed by nanopore sequencing.
- Fifteen isolates were genotyped by the nanopore method alone.
- Five isolates were excluded due to poor RNA quality.
- Phylogenetic analysis was performed using Molecular Evolutionary Genetic Analysis (MEGA11) software. Phylogenetic trees were generated for both VP7 and VP4 genes.

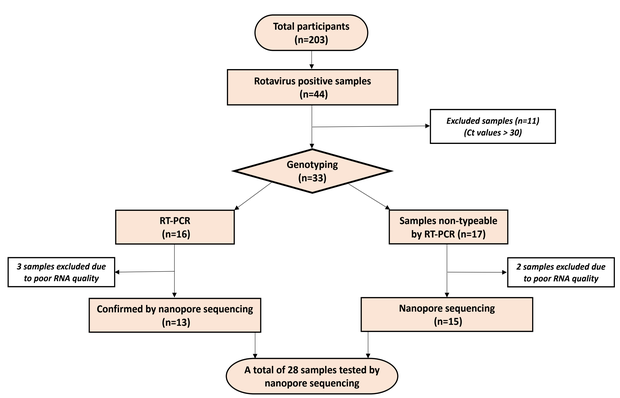

Figure 1: Flowchart representing the selection of samples for RT-PCR and sequencing

Results

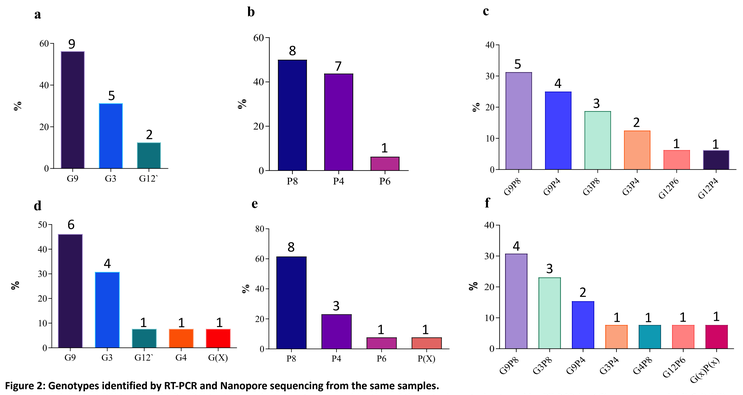

Figure 2: Genotypes identified by RT-PCR and Nanopore sequencing from the same samples.
Upper panel: (a) G (VP7) typing, (b) P (VP4) typing, and (c) genotypes combination of the rotavirus strains typed by RT-PCR (n=16). *Lower panel*: (d) G (VP7) typing, (e) P (VP7) typing, and (f) genotypes combination by nanopore sequencing (n=13, 3 samples were excluded because of poor RNA quality). The graph presents % of rotavirus strains of G and P genotypes detected in the children. (X: non-typeable)

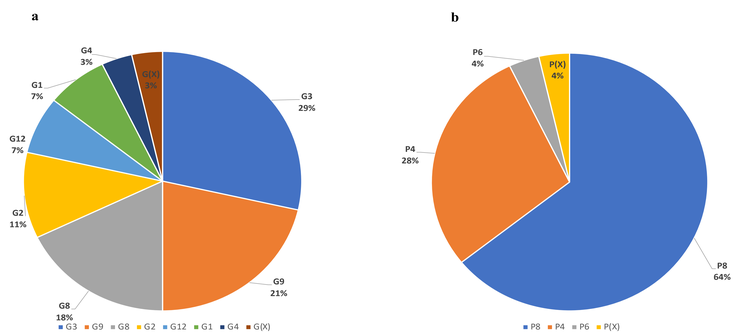

Figure 3: Frequency of VP7 (a) and VP4 (b) genotypes in the samples as identified by nanopore sequencing (n=28).

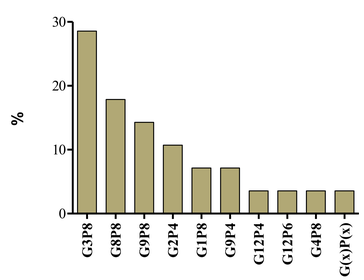

Figure 4: Prevalence of G/P genotype combinations in the samples that were sequenced by nanopore (n=28).

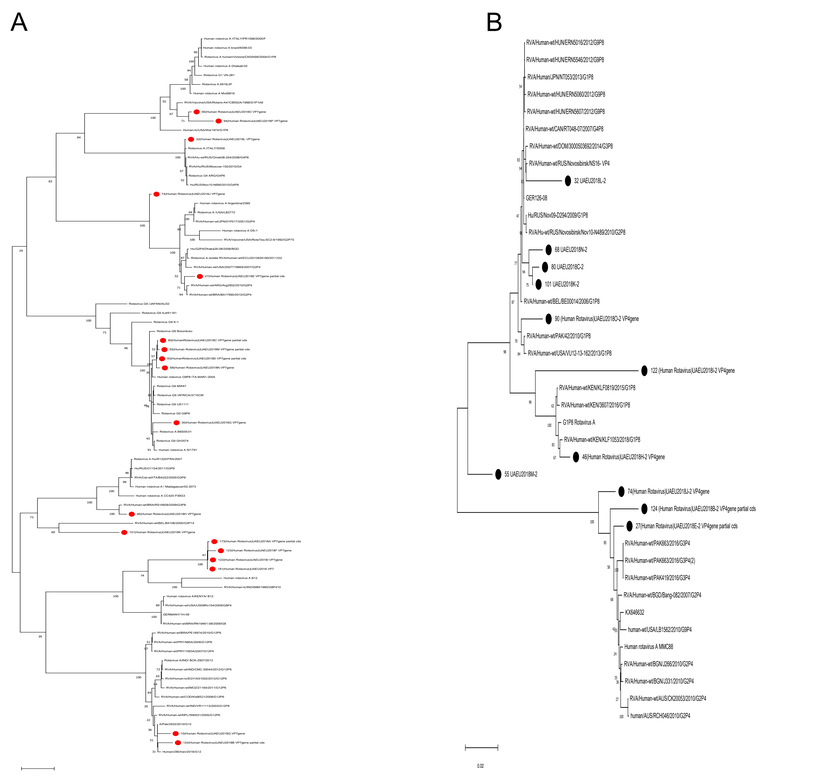

Figure 5:
(A) Phylogenetic tree of rotavirus A strains based on partial VP7 nucleotide sequences. Bootstrap confidence limits are shown at each node; values less than 50 are not shown. Sequences from this study are marked by 🔴
(B) Phylogenetic tree of rotavirus A strains based on partial VP4 nucleotide sequences. Bootstrap confidence limits are shown at each node; values less than 50 are not shown. Sequences from this study are marked by ⚫

Conclusions

- Nanopore sequencing detected various RVA strains that were failed to be identified using PCR method alone including the recently emerging strain, G8P[8].
- Using this method in surveillance studies may advance our understanding of the origin, genetic recombination, and burden of disease.