

Genetic analysis of Ghanaian G1P[8] and G9P[8] rotavirus A strains reveals the impact of P[8] VP4 gene polymorphism on P-genotyping

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Background

The World Health Organisation rotavirus surveillance networks have documented and shown eclectic geographic and temporal diversity in circulating G- and P- genotypes identified in children <5 years of age. To effectively monitor vaccine performance and effectiveness, robust molecular and phylogenetic techniques are essential to detect novel strain variants that might emerge due to vaccine pressure. This study inferred the phylogenetic history of the VP7 and VP4 genes of previously non-typeable strains and provided insight into the diversity of P[8] VP4 sequences which impacted the outcome of our routine VP4 genotyping method.

Methods

Near-full-length VP7 gene and the VP8* fragment of the VP4 gene were obtained by Sanger sequencing and genotypes were determined using RotaC v2.0.

Results

Forty-eight of the 57 (84.2%) had the P[8] specificity, of which 43 (89.6%) were characterized as P[8]a subtype and 5 (10.4%) as the rare OP354-like subtype. The VP7 gene of 27/39 samples were successfully sequenced and their G-genotypes confirmed as G1 (18/27) and G9 (9/27). Phylogenetic analysis of the P[8]a sequences placed them in sub-cluster IIIc within lineage III together with contemporary G1P[8], G3P[8], G8P[8], and G9P[8] strains detected from across the world from 2006 – 2016. The G1 VP7 sequences of the study strains formed a monophyletic cluster with African G1P[8] strains detected previously in Ghana and Mali during Rotateq vaccine trial and from neighbouring Togo. The G9 VP7 sequences of the study strains formed a monophyletic cluster with contemporary African G9 sequences from neighbouring Burkina Faso within the major sub-cluster of lineage-III. Mutations identified in the primer binding region of the VP8* sequence of the Ghanaian P[8]a strains may be responsible for genotyping failure since an alternative primer designed taking into consideration the mutations successfully genotyped the previously non-typeable P[8] strains.

Conclusion

In summary, the G1, G9, and P[8]a sequences were highly similar to contemporary African strains at the lineage level. The study also resolved the methodological challenges of the standard genotyping techniques and highlighted the need for regular evaluation of the multiplex PCR-typing method especially in the post-vaccination era.