

MOLECULAR CHARACTERIZATION OF VP7, VP4, AND NSP4 GENES OF P[8]G9 ROTAVIRUS STRAINS DETECTED IN SAUDI ARABIA: First characterization of Phylogenetic and Sequencing analysis in the Middle East

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ABSTRACT

Objectives: Serotype G9 rotaviruses have emerged as one of the leading causes of gastroenteritis in children worldwide since the mid-1990s, suggesting that G9 is a globally important serotype.

Methodology: The molecular epidemiology of G9 rotaviruses in two Saudi's cities, Maddina and Gizan, from 2004 to 2005, was investigated in this study.

Results: These G9 strains belonged to P[8] lineage 2 (P-like) VP4, genetic group B (Wa-like) NSP4, and long electropherotype. The Saudi Arabian G9 VP7 gene had a 98% nucleotide sequence identity and grouped with the recently circulated G9 strains belonged to the third lineage.

Conclusion: Molecular characterization of VP7, VP4, NSP4 and PAGE suggest that rotavirus G9 could have been introduced to Saudi as a novel G9P[8] strain. This study is the first to describe Molecular characterization of G9 Strains in the Middle East.

KEY WORDS: Rotavirus, G9, Saudi Arabia.

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INTRODUCTION

Group A rotavirus is the most important cause of severe, dehydrating gastroenteritis in infants and young children worldwide.¹ The two outer capsid proteins are VP7 and VP4, which independently elicit neutralizing antibody responses, form the classification system based on VP7 (G) and VP4 (P) types. Globally, G-serotypes G1, G2, G3 and G4 have predominated in most countries. However, the pattern of G types appears to be changing, as some unusual types namely G5, G8, G9, and G12 have been increasingly reported.¹

The G9 rotavirus was first reported as the causative agent of diarrhea in hospitalized

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children in Philadelphia, USA in 1983-1984. However since 1995, G9 has been recognized as one of the most widespread emerging genotypes in several regions around the world.¹⁻⁹ The G9 rotavirus had never been reported in the region before 2006¹⁰, however since then; G9 has been reported in three countries namely Iraq, Iran and Kuwait.^{2,6,8}

In two recently completed studies of viral gastroenteritis in children in two Saudi cities, Maddina & Gizan and reported elsewhere,¹¹⁻¹³ G9 was identified as an emerging serotype, accounting for 11% of circulating rotavirus strains. These studies were the first to describe serotype G9 in Saudi Arabia. Despite the fact that these studies were the third to describe serotype G9 in the region, only one study described briefly the sequencing characterization circulated in the region and the characterization of VP4 and NSP4 are completely lacking. Given the local importance of this strain together with recent reports of serotype G9 rotavirus globally, it is important to examine this strain in more detail.

METHODOLOGY

Subject enrolment, specimen collection and laboratory investigations were conducted as described previously.¹¹⁻¹³ Briefly, in both studies, faecal specimens were collected from patients who were either admitted to the hospitals or given oral rehydration therapy as outpatients. In Maddina the study was conducted during April 2004 and April 2005 targeting children less than five years old, whereas in Gizan it was carried out from November 2004 to March 2005 including children and adults. Selected rotavirus strains of serotype G9 obtained from patient aged 10 months was chosen for culture adaptation in MA104 cells using a previously described method.¹⁴

For VP7, primers Beg9 and End 9¹⁵ were used for amplification to obtain full-length genome segment 9. For VP4, primers con2 and con3 were used to amplify an 877-bp fragment covering the VP8* fragment of genome segment 4.¹⁶ A 739-bp fragment of the NSP4 gene (genome segment 10) was amplified by using primers described previously.¹⁷

Amplicons obtained for each genome segment were purified by using a QIAquick Spin kit (Qiagen Inc, Valencia, CA). In order to obtain the full sequence in both directions for each genome segment, a series of internal primers were used with slightly modification. Specifically, primer 1205 designed by Ramachandran et al¹⁸, was modified to be as follow: TCGATGTTGTTGATGGTG. The nucleotide sequences for strain MD28 have been submitted to the Gene Bank. The accession numbers of the VP7 and VP4 genes of MD28 are AB297791 and AB297792, respectively.

The nucleotide sequences thus obtained were compared with sequences deposited in the DNA databases using the BLAST program and the genotypes were determined based on the deduced amino acid sequences that showed the highest identity. Phylogenetic trees were constructed based on the neighbour-joining method¹⁹ in the Clustal W software package.²⁰

Statistical analyses were performed by EPInfo version 6. The difference in prevalence between two groups was calculated by the Chi square test. A P value of <0.05 was considered significant.

RESULTS

The detection rate of G9 ranked third commonest in the two cities as 11% of strains in Maddina and 2% in Gizan. Most G9 cases (18/21: 86%) were among children less than 2 years ($P < 0.01$). The average age (SD) of the patients with G9-associated and non-G9 rotavirus diarrhoea was 13.6 (11.8) and 15 (15) months respectively. G9 was also predominant among inpatient cases (20/21: 95)($P < 0.01$).

The VP4 genotype was determined as P[8] for all 21 strains. In addition, 17 (81%) of the 21 G9 strains could be assigned to electropherotypes (all long). A representative sample (MD28) was selected for culture adaptation. This strain was further characterized and confirmed as G9P[8] belong to NSP4 genetic group B (i.e. Wa-like).

The nucleotide (nt) and deduced amino acid (aa) sequences of the gene segment encoding VP7 were determined for MD28. Sequence

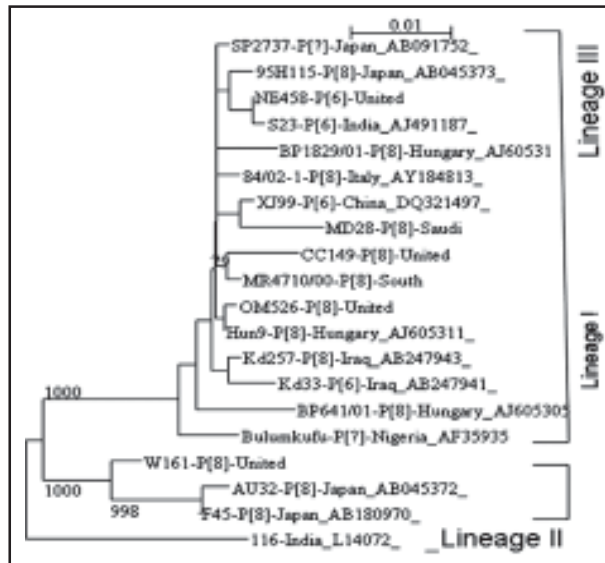


Fig-1: Phylogenetic tree for the G9 VP7 genes constructed by the neighbor-joining method. The horizontal distance connecting two VP7 sequences is proportional to the genetic distance between these two VP7 sequences.

comparisons (Data not shown) indicated that the VP7 sequences of MD28 was most closely related to recently human serotype G9 rotaviruses circulating globally (lineage 3). The strain exhibited closest identity with the VP7 gene of the Chinese serotype G9 strain XJ99 (Accession: DQ321497). On the other hand, MD28 showed low identities at the nucleotide and amino acid levels (less than 90%) compared with the F45 (lineage 1), and the 116E (lineage 2). The phylogenetic analysis (Figure 1) from the VP7 gene the Saudi G9 strain showed that it is closely related with other G9P[8] and G9P[6] strains recently isolated worldwide (lineage 3). Additionally, Saudi G9P[8] grouped with the Chinese serotype G9P[6] strain XJ99 detected six years earlier rather than both Iraqi G9P[8] and G9P[6] circulated in the same period.

Partial gene 4 sequence, encoding VP4, was determined for MD28 showed closest homology to rotaviruses of P[8] VP4 genotype, with greatest identity to a P[8] strain a from the Congo, Hun9 (99% nt and 100% aa identities). The phylogenetic analysis of VP8* Saudi G9P[8] and the other co-circulated Saudi G12P[8] grouped together and with other globally strains in lineage 2 (P-like) strains (Fig-2).

Part of gene 10 (NSP4) of MD28 strain showed

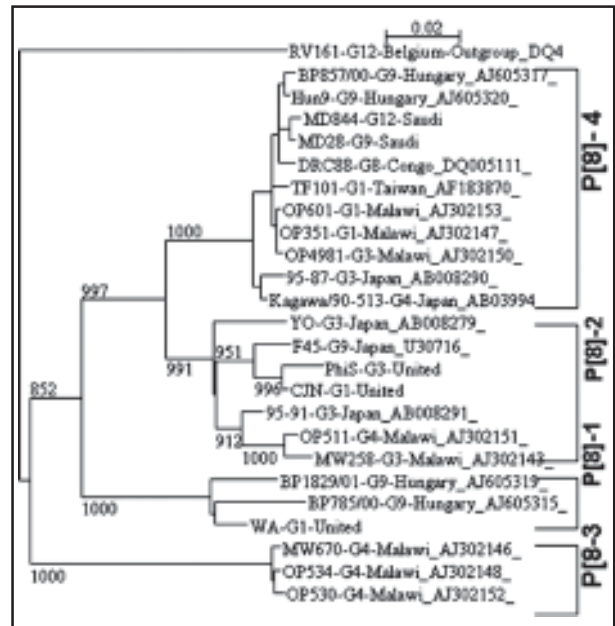


Fig-2: Phylogenetic tree for the P[8] VP4 genes constructed by the neighbor-joining method.

greatest nucleotide and amino acid NSP4 homologies with genetic group B (Wa-like) viruses, with greatest identity to a strain from South Korea (P[8]G9) strain CAU202 (99% nt identity).

DISCUSSION

The detection rate of G9 in Saudi Arabia is interesting as it is higher than the worldwide figure of 4.1%¹ but similar to the Kuwaiti (10.2%)⁸ and Iraqi results (11%)² and less than Iranian figures (15%).⁶ However, previous studies performed in Ghana, Kenya and elsewhere, G9 rotaviruses were reported to be the most common circulating strains.^{3,5,7}

The age distribution of patients with G9-associated and non-G9 rotavirus diarrhea appeared to be very similar. In contrast, compared to 1995-1996 studies in which a significant portion occurred in children younger than 6 months,²¹ we did not find sero-type age differences. However, all G9 detected in Saudi Arabia were G9P[8] while the major epidemic strain in 1995s was G9P[6]. We can speculate that previous existing maternal P[8] specific antibodies might provide heterotypic protection in younger children. However, serotype G9 strains have also been detected among older children

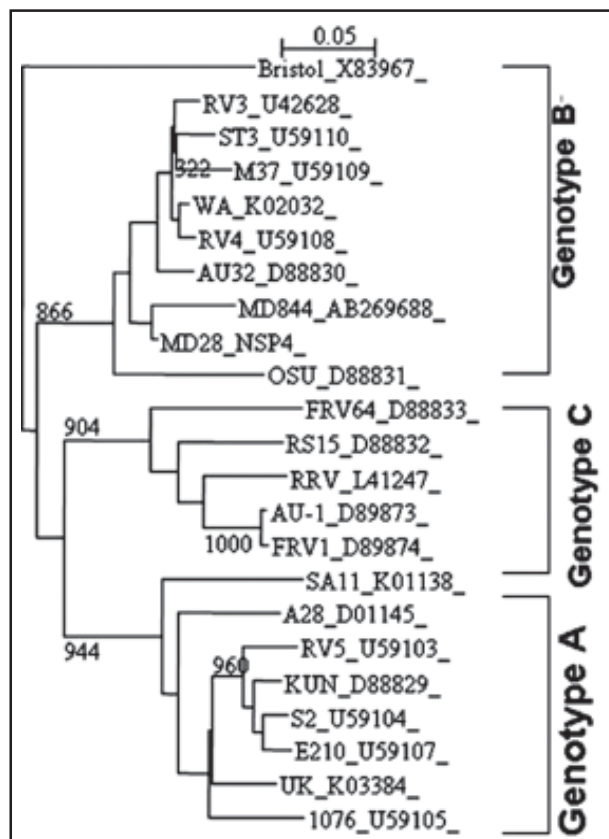


Fig-3: Phylogenetic tree for the NSP4 genes constructed by the neighbor-joining method.

in many countries including the UK.²² Unfortunately, no detailed clinical data were collected, however, it should be noted that 95% of G9 cases were severe to require hospitalization. In some early reports, infections with the novel G9 strains were associated with unusual epidemiological and clinical features. In the UK, Iturriza-Gomara et al²² found that recently emerged G9P[6] strains caused symptomatic infections in children over 5 years of age, where pre-existing immunity was expected to provide sufficient heterotypic protection against common and, perhaps, rare.

The VP7 gene of the selected Saudi strain was virtually identical and up to 98% (nt) similar to recently identified G9 rotaviruses. The VP7 gene appears to be stable as revealed by the high identity (98%) between MD28 and G9 lineage 3 such as a Belgian G9 strain XJ99, recovered 8 years previously. This is in agreement with the observation made by Lin et al.²³ However, analysis of a global collection of G9 strains has

shown high genetic variation between G9 rotaviruses of recent isolation and the reference G9 strains isolated during the 1980s.¹⁸ This indicates that Saudi G9 rotaviruses might have been introduced into Saudi recently as a G9P[8] rather than represent reassortants between G9P[6]. This conclusion is supported by the results of VP4, NSP4 genes and RNA migration pattern.

It is of interest and as noted by Banyai et al²⁴, the phylogenetic tree of VP7 gene from G9 strains shows a correlation of P-type, location and year of detection among strains from single lineage. In this study however, the VP7 sequence of Saudi G9P[8] grouped with the Chinese serotype G9P[6] strain XJ99 detected six years earlier more closely than both Iraqi G9P[8] and G9P[6] circulated in the same period. Unfortunately, G9 strains detected in Iran and Kuwait were not sequenced and the sequencing of VP4 and NSP4 circulating in the region is completely lacking.

The G9P[8] strain is not currently included in second-generation rotavirus vaccines, however, in regard to RotaRix vaccine, it has demonstrated efficacy against G9 strains particularly against serotypes that shared a P type.²⁵ In addition, the Merck (RotaTeq) vaccine is composed of 5 bovine-human reassortants intended to induce serotype-specific protection against the 4 main G- types, (G1, G4,) plus the common P[8] antigens. In Saudi Arabia, these G9 strains had a P[8] antigen, which is included in both vaccines, so severe disease due to this strain should be preventable.

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