27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P040 Epidemiology of viral infections

Category: 1f. Viral molecular epidemiology (other than hepatitis & HIV)

23 April 2017, 13:30 - 14:30
P0880

Molecular epidemiology of norovirus GII.4 strains in Greece

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Background: Noroviruses constitute a major cause of non-bacterial gastroenteritis, affecting both children and adults in healthcare and community settings. Despite that >40 different genotypes have been described to infect humans, genotype GII.4 strains have been causing the overwhelming majority of norovirus-related, sporadic and epidemic cases of gastroenteritis worldwide during the past 2 decades. This is attributed to antigenic variants of GII.4 that emerge in a cyclic fashion, initiating pandemics every 2-3 years. The present study analyzed the molecular epidemiological trends of GII.4 noroviruses in Greek children in correlation with the currently circulating pandemic GII.4 strains.

Material/methods: The study involved 69 norovirus strains, detected in stools of children (17 days - 14 years old) hospitalized in two pediatric hospitals for gastroenteritis between 1/2013 and 6/2015. Genotyping was performed on the basis of ORF2 (VP1 capsid) gene sequences corresponding to part of the conserved shell (S) domain, using the RIVM Norovirus Genotyping tool and the BLAST alignment software. Comparative phylogenetic analysis of nucleic acid and the respective amino acid sequences between the different genotypes, which included both norovirus strains detected in this

study and closely associated strains circulating worldwide, was conducted by MEGA software, version 6.0.

Results: The results revealed the circulation of a diverse variety of norovirus genotypes. GII.4 was the predominant genotype (74%) and most GII.4 strains (44/51) originated from stool samples collected between March and December 2013, indicating a possible ongoing epidemic. Frequency of GII.4 strains diminished in 2014 and 2015, as well as that of clustered cases that indicated possible outbreaks, with the emergence of other genotypes causing sporadic cases of gastroenteritis. The partial ORF2 of most GII.4 strains (46/51) was highly similar (99-100% nucleotide and 97-99% amino acid sequence identity) to the respective sequence of norovirus GII.4 "Sydney 2012" strains circulating worldwide. However, of note, 5 GII.4 strains, 4 of which being detected in August 2013, showed nucleotide and remarkable amino acid sequence divergence from the “2012 variant” strains (97% and 90% respectively).

Conclusions: The molecular epidemiology of norovirus GII.4 strains was tested for the first time in Greece, showing the predominance of the “Sydney 2012" variant that has circulated worldwide either in outbreaks and sporadic cases of gastroenteritis, or in the environment during the last 4 years. These strains were responsible for possible outbreaks of gastroenteritis during the 1st year of the study, as well as for sporadic cases recorded later. The 5 detected divergent strains may represent an emerging GII.4 variant, something that warrants further molecular analysis regarding the more variable P1 and P2 regions of VP1.