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Persistent Norovirus Outbreaks in a Hospital Setting – the Role of Environmental Contamination

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Dear Editor,

A recent review highlighted the healthcare burden of norovirus infections in China, prior to 2017. Norovirus infections occurred all year round, with 22-29% detection rates in children (6-35 months old) and adults (including the elderly), predominantly of norovirus GII.4 (70.4%).[1] Similarly, in England and Wales in 2016 there were 7795 laboratory reported cases of norovirus and acute gastroenteritis, which was estimated to cost the National Health Service up to £86 million annually in bed closures and staff absences,[2,3] making norovirus the second-largest contributor of gastrointestinal disease hospital burden in England.[4]

Norovirus is the world's leading cause of gastroenteritis. Three of its seven genogroups (GI, GII, GIV) cause human infections and GII.4/Sydney/2012 is the current, dominant lineage globally. Transmission is faeco-oral, resulting in nosocomial outbreaks of diarrhoea and vomiting, typically peaking during winter. Multiple norovirus cases and outbreaks have occurred at our hospital throughout the non-winter months over several years, suggesting possible environmental contamination and reinfection of patients.[5] To investigate this, a retrospective analysis of recent norovirus cases and selected environmental sampling on an outbreak ward were performed.

All laboratory-confirmed (on real-time PCR testing) norovirus positive clinical and environmental samples (with sufficient sample volume) from Jan2017-Jun2018 were included, and sent to the national reference laboratory (Enteric Virus Unit, PHE) for genotyping by sequencing methods used for national surveillance.[6] Partial norovirus capsid protein VP1 (ORF2) sequences from these samples were aligned and edited with similar GenBank sequences. Maximum likelihood phylogenetic trees were constructed to reveal any correlation between the patient and environmental samples.

A total of 216 samples (mostly from general medical wards) were laboratory-confirmed norovirus positives during 2017-2018, of which 133 (41/66 from 2017; 92/146 from 2018) were sequenced. Of these, 6/216 (2.78%) were GII.7 and 127/216 (58.80%) were GII.4 viruses. The remainder of the samples (83/216, 38.42%) could not be sequenced, either due to insufficient sample

remaining, the sample having already been discarded, or a sequencing failure at the reference laboratory. Sequences consisted of partial VP1 sequences of approximately 300 bp in length.

In addition, environmental swabs (see **Fig 1**) were taken for norovirus detection and sequencing from one of the worst affected wards (Ward X, a care of the elderly ward). This included pre- and post-clean samples (**Figs 1a, 1b**) taken from one shared 6-bedded bay, one shared patient bathroom and two single patient isolation rooms. Routine cleaning for norovirus was performed manually on all surfaces, using clean, single-use, cloths dipped in a commercial troclosene sodium (sodium dichloroisocyanurate, NaDCC)-based cleansing agent ('Chlor-Clean', Guest Medical Ltd., Kent, England) at a concentration of 1000 parts per million. Both patient and environmental samples were taken using a commercial swab made of polyurethane foam collected into proprietary virus transport medium (Sigma Virocult, MWE Ltd., Corsham, England).

Of the total number of environmental swabs taken (100: 47 pre- and 53 post-clean), 28/100 were positive for norovirus RNA and contained sufficient virus for sequencing, of which 21/28 (75%) were pre-clean (all taken on 7/3/2018), and 7/28 (25%) were post-clean (all taken on 22/3/2018). None of the Ward X patients that were on this ward during this study period were actually admitted with vomiting and diarrhoea. Therefore these norovirus infections were acquired from the ward, either sporadically or during a ward outbreak, after their admission for other reasons.

The top of the phylogenetic tree (**Fig S1**, purple font) shows that separate GII.7 virus outbreaks occurred on the adult haematology (Ward H, Sep2017) and paediatric respiratory (Ward P2, Feb/Mar2018) wards, not thought to be epidemiologically linked. The UHL sequences are distinct (SH support 0.75) from the closest similar sequence from GenBank (black). No environmental samples were taken from these wards. However, the majority of the UHL GII.4 patient (blue) and Ward X environmental viruses detected during Dec2017-May2018 are most closely related to a 2016 GII.4 virus from Thailand, detected in human stool. Only 15 pre-clean (red) and 5 post-clean (green) environmental, and 86 patient samples were of sufficient sequence quality for inclusion in this final phylogenetic tree. Note that all virus detection was performed by PCR testing, which does not give any indication of virus viability.

One subset of UHL GII.4 patient viruses detected during Dec2017-Feb2018 are most closely related to a 2016 GII.4 virus from Bangladesh (detected in sewage) or a 2015 GII.4 virus from Australia, detected in human stool. A second subset of UHL GII.4 patient viruses detected during Jul2017 and Feb2018 appear to be more closely related to 2015/2016 GII.P16 GII.4 viruses from the USA, detected in human stool.

A closer examination of **Fig S1** (i.e. within the red boxes) shows that very similar viruses were found across several hospital wards during Mar2017-May2018, suggesting the long-term persistence of these viruses during this 15-month period, in either the environment and/or hospitalised patient population. Such long-term viral persistence may potentially infect future patients being admitted to these wards and this hospital.[7] Deeper analysis of genetic similarity was limited by the use of an existing partial sequencing approach that was designed for another purpose (national surveillance); partial sequencing of both polymerase and capsid genes, or whole genome sequencing, could provide greater resolution in investigating potential transmission chains in the future.[8-10]

Therefore, the main findings from this investigation are i): both GII.4 and GII.7 noroviruses have been detected in UHL patients during Jan2017-Jun2018; ii) some of the patients from the 2017 and 2018 outbreaks were infected with very similar viruses, suggesting persistence of these same viruses in the environment or patient population for over a year; iii) the ward environmental contamination with norovirus may persist after deep cleaning.

As a result of the last finding, since mid-2018, we have reviewed and enhanced our ward cleaning methods at our hospital. One component of this included updating our hospital 'norovirus toolkit' which includes team action cards that identify the type of clean required. These now also include items that might have been missed previously, e.g. replacing patient anti-slip socks and the cleaning of any hospital-related patient footwear (e.g. Repose Boots) when they are moved into a clean bed-space.

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Figure 1a. Ward map showing layout and contents of patient rooms with selected sites for environmental sampling – before cleaning.

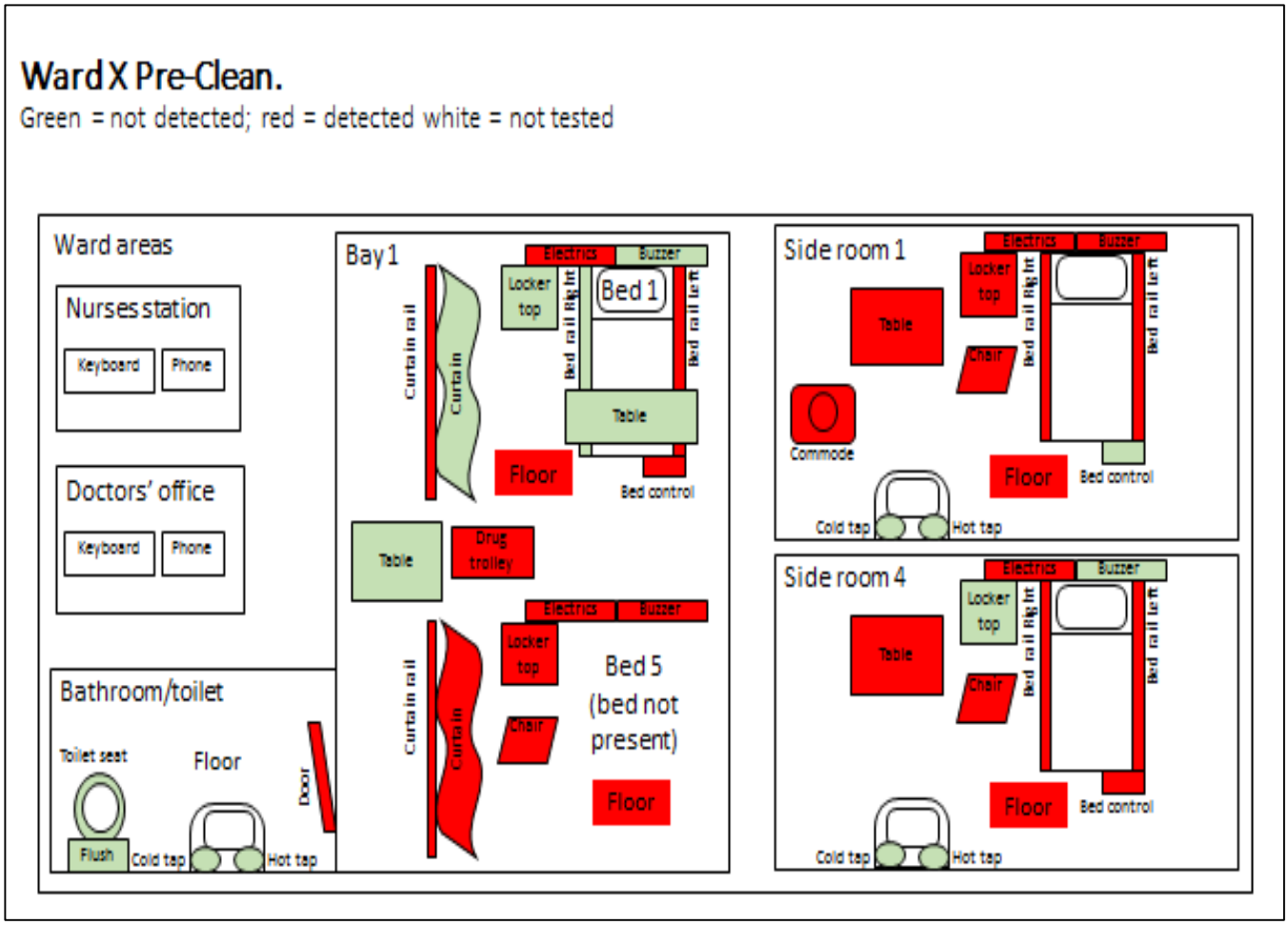


Figure 1b. Ward map showing layout and contents of same patient rooms with selected sites for environmental sampling – after cleaning.

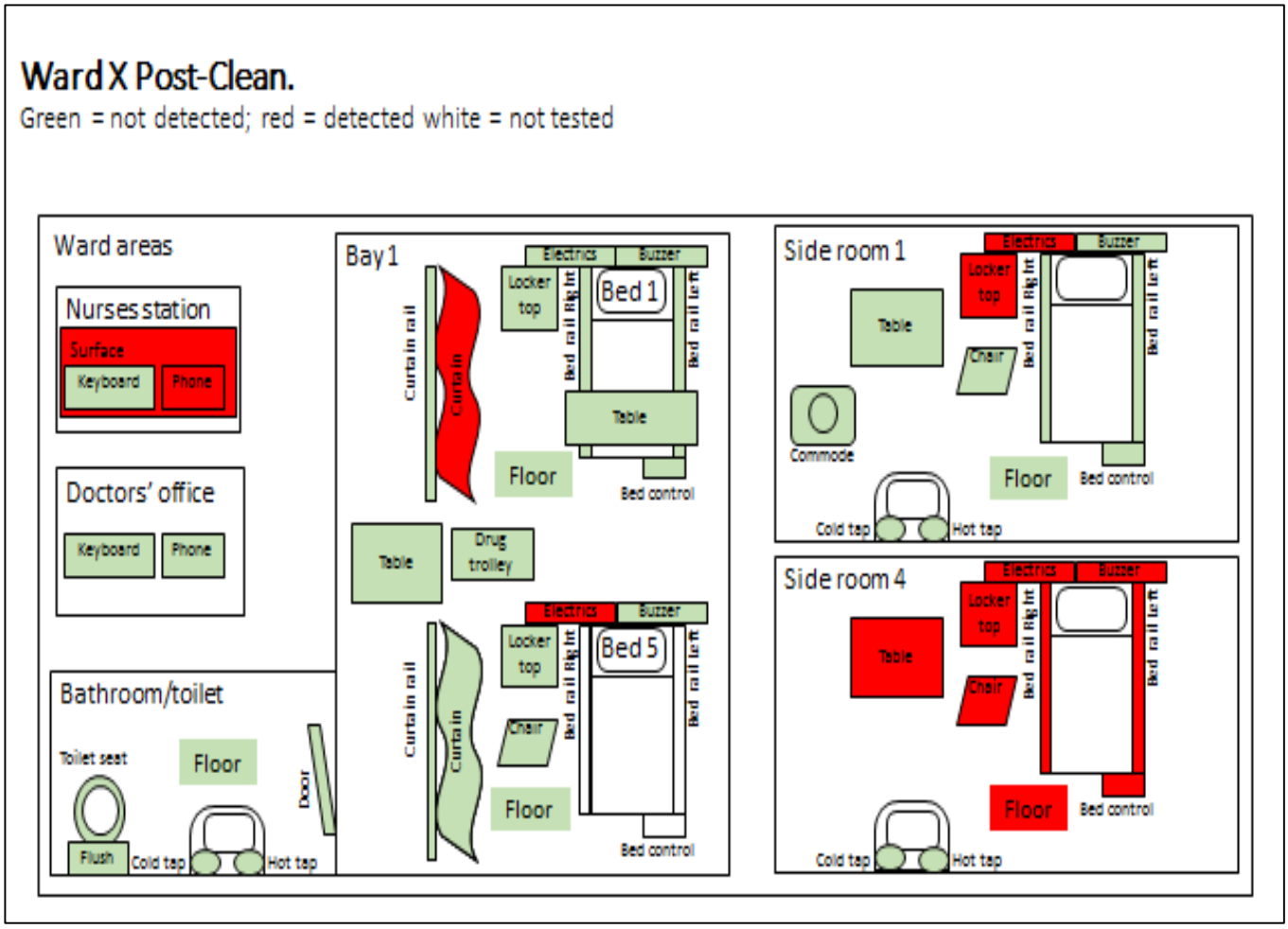
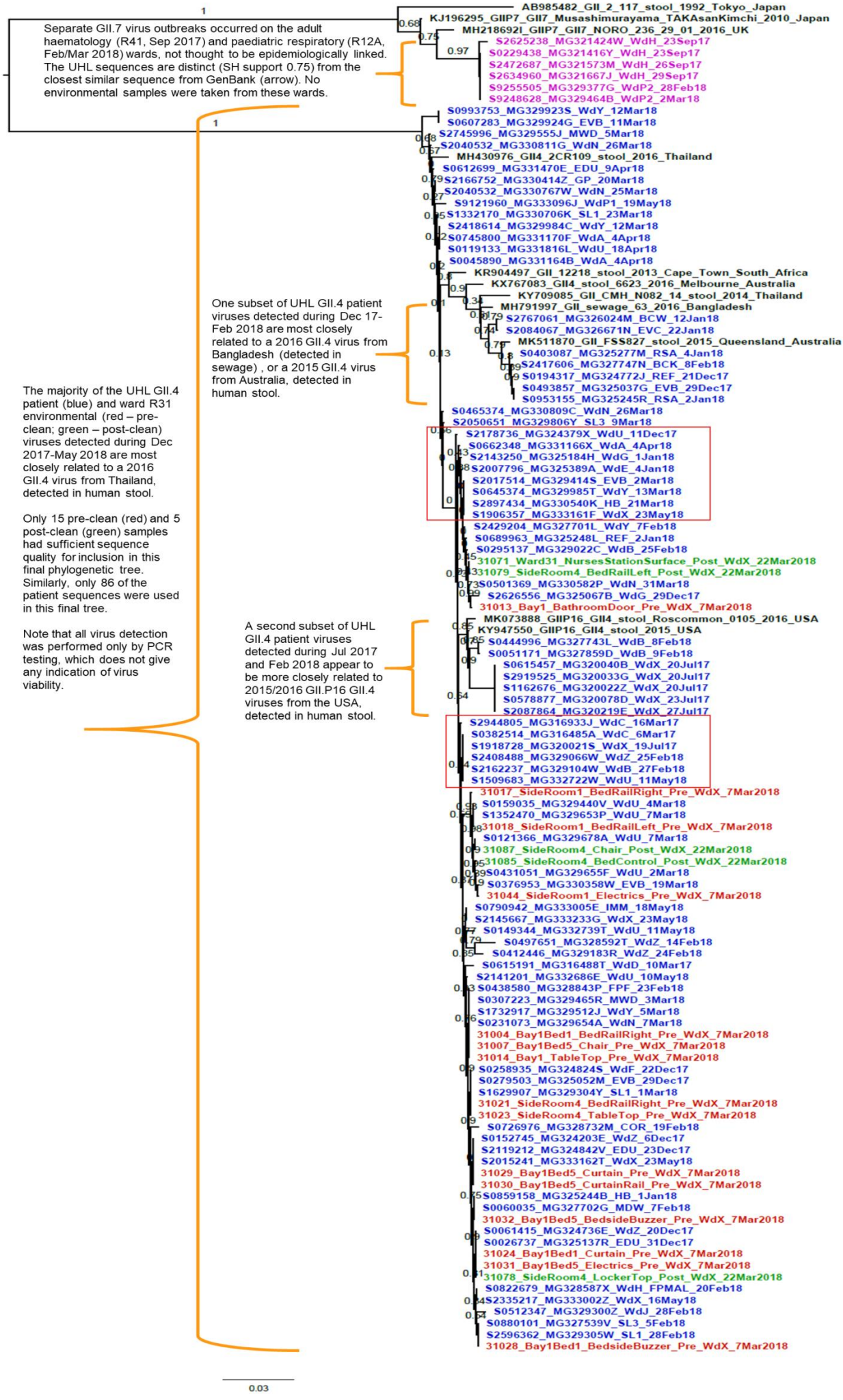


Figure S1. Leicester norovirus GII.7 Leeds/1990/UK patient viruses (purple font); GII.4 Sydney/2012/AUS viruses (patient viruses – blue font; environmental viruses: red font: pre-clean, green font: post-clean). Sequences in black font are the closest related sequences available from GenBank. Red boxes show the clusters containing both 2017 and 2018 viruses, suggesting possible persistence in the environment, and/or within the hospitalised patient population (ward names have been coded). This tree was reduced from a total of ~6800 similar VP1 sequences from GenBank, and further simplified from larger trees of about 150 GII.7 and 850 GII.4 sequences, respectively, including both partial and full genome VP1 sequences. Poor quality and/or overly short patient or environmental sequences (<200 bp) were manually removed from the final sequence alignment and phylogenetic tree construction (see annotations) using BioEdit v7.0.5: http://www.mbio.ncsu.edu/BioEdit/bioedit.html. Final sequence lengths after alignment and editing 289 bp. The tree was constructed under a GTR model of evolution using maximum likelihood within FastTree v2.1: http://www.microbesonline.org/fasttree/. Branch support: Shimodaira-Hasegawa-like support values.


Separate GII.7 virus outbreaks occurred on the adult haematology (R41, Sep 2017) and paediatric respiratory (R12A, Feb/Mar 2018) wards, not thought to be epidemiologically linked. The UHL sequences are distinct (SH support 0.75) from the closest similar sequence from GenBank (arrow). No environmental samples were taken from these wards.
One subset of UHL GII.4 patient viruses detected during Dec 17-Feb 2018 are most closely related to a 2016 GII.4 virus from Bangladesh (detected in sewage) , or a 2015 GII.4 virus from Australia, detected in human stool.
The majority of the UHL GII.4 patient (blue) and ward R31 environmental (red – pre-clean; green – post-clean) viruses detected during Dec 2017-May 2018 are most closely related to a 2016 GII.4 virus from Thailand, detected in human stool.
Only 15 pre-clean (red) and 5 post-clean (green) samples had sufficient sequence quality for inclusion in this final phylogenetic tree. Similarly, only 86 of the patient sequences were used in this final tree.
Note that all virus detection was performed only by PCR testing, which does not give any indication of virus viability.
A second subset of UHL GII.4 patient viruses detected during Jul 2017 and Feb 2018 appear to be more closely related to 2015/2016 GII.P16 GII.4 viruses from the USA, detected in human stool.
AB985482_GII_2_117_stool_1992_Tokyo_Japan
KJ196295_GIIP7_GII7_Musashimurayama_TAKAsanKimchi_2010_Japan
MH218692I_GIIP7_GII7_NORO_236_29_01_2016_UK
S2625238_MG321424W_WdH_23Sep17
S0229438_MG321416Y_WdH_23Sep17
S2472687_MG321573M_WdH_26Sep17
S2634960_MG321667J_WdH_29Sep17
S9255505_MG329377G_WdP2_28Feb18
S9248628_MG329464B_WdP2_2Mar18
S0993753_MG329923S_WdY_12Mar18
S0607283_MG329924G_EVB_11Mar18
S2745996_MG329555J_MWD_5Mar18
S2040532_MG330811G_WdN_26Mar18
MH430976_GII4_2CR109_stool_2016_Thailand
S0612699_MG331470E_EDU_9Apr18
S2166752_MG330414Z_GP_20Mar18
S2040532_MG330767W_WdN_25Mar18
S9121960_MG333096J_WdP1_19May18
S1332170_MG330706K_SL1_23Mar18
S2418614_MG329984C_WdY_12Mar18
S0745800_MG331170F_WdA_4Apr18
S0119133_MG331816L_WdU_18Apr18
S0045890_MG331164B_WdA_4Apr18
KR904497_GII_12218_stool_2013_Cape_Town_South_Africa
KX767083_GII4_stool_6623_2016_Melbourne_Australia
KY709085_GII_CMH_N082_14_stool_2014_Thailand
MH791997_GII_sewage_63_2016_Bangladesh
S2767061_MG326024M_BCW_12Jan18
S2084067_MG326671N_EVC_22Jan18
MK511870_GII_FSS827_stool_2015_Queensland_Australia
S0403087_MG325277M_RSA_4Jan18
S2417606_MG327747N_BCK_8Feb18
S0194317_MG324772J_REF_21Dec17
S0493857_MG325037G_EVB_29Dec17
S0953155_MG325245R_RSA_2Jan18
S0465374_MG330809C_WdN_26Mar18
S2050651_MG329806Y_SL3_9Mar18
S2178736_MG324379X_WdU_11Dec17
S0662348_MG331166X_WdA_4Apr18
S2143250_MG325184H_WdG_1Jan18
S2007796_MG325389A_WdE_4Jan18
S2017514_MG329414S_EVB_2Mar18
S0645374_MG329985T_WdY_13Mar18
S2897434_MG330540K_HB_21Mar18
S1906357_MG333161F_WdX_23May18
S2429204_MG327701L_WdY_7Feb18
S0689963_MG325248L_REF_2Jan18
S0295137_MG329022C_WdB_25Feb18
31071_Ward31_NursesStationSurface_Post_WdX_22Mar2018
31079_SideRoom4_BedRailLeft_Post_WdX_22Mar2018
S0501369_MG330582P_WdN_31Mar18
S2626556_MG325067B_WdG_29Dec17
31013_Bay1_BathroomDoor_Pre_WdX_7Mar2018
MK073888_GIIP16_GII4_stool_Roscommon_0105_2016_USA
KY947550_GIIP16_GII4_stool_2015_USA
S0444996_MG327743L_WdB_8Feb18
S0051171_MG327859D_WdB_9Feb18
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S2919525_MG320033G_WdX_20Jul17
S1162676_MG320022Z_WdX_20Jul17
S0578877_MG320078D_WdX_23Jul17
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