Norovirus GII.4 Detection in Environmental Samples from Patient Rooms during Nosocomial Outbreaks

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Running head: NoV GII.4 environmental contamination in patient rooms (48), #Address correspondence to Nancy P. Nenonen, nancy.nenonen@microbio.gu.se

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Abstract

Norovirus (NoV) is an important cause of nosocomial gastroenteric outbreaks. This 5-month study was designed to determine NoV contamination and airborne dispersal in patient rooms during hospital outbreaks. Air vents, overbed tables, washbasins, dust, and virus-traps designed to collect charged particles from air, were swabbed to investigate NoV contamination in patient rooms during outbreaks in seven wards, and an outbreak-free ward. Symptomatic inpatients were also sampled. Nucleic acid extracts of samples were examined for NoV RNA in genogroup (G)I and GII real-time RT-PCR. NoV strains were characterized on RT-PCR, sequencing, and phylogenetic analysis of RNA-dependent RNA-polymerase-N/S-capsid-coding region (1040 nt). Patient strains from two outbreaks in one ward were sequenced across the RNA-dependent-RNA-polymerase-major-capsid-coding region (2.5 kb) including the hyper-variable P2 domain. In outbreak wards NoV GII was detected in 48 of 101 (47%) environmental swabs, and 63 of 108 patients (58%); NoV genotype II.4 was sequenced from 18 environmental samples, dust (n=8), virus-traps (n=4), surfaces (n=6), and 56 patients. In contrast, NoV GII was detected in 2 (GII.4) of 28 (7%) environmental samples, and 2 (GII.6, GII.4) of 17 patients in the outbreak-free ward. Sequence-analyses revealed high similarity (>99.5%, 1040 nt) between NoV GII.4 environmental and patient strains from a given ward, at given time. Strains clustered on 11 sub-branches of the phylogenetic tree with strong correlation to location and time. High nucleotide similarity between NoV GII.4 strains from patients and their hospital-room environment provided molecular evidence of GII.4 dispersal in air and dust, interventional cleaning studies are justified. (250)
Norovirus (NoV) are a common cause of recurring nosocomial outbreaks of acute gastro-enteritis, and can inflict potentially life-threatening infections in the immune-compromised, elderly, or newborn (1-3). Characterized by sudden onset of projectile vomiting and diarrhea, NoV infections are usually of short duration in healthy individuals. These small, non-enveloped, non-cultivable, genomically diverse, single stranded positive sense RNA viruses of approximately 7.5 kb genome, are highly infectious, and remarkably robust in different environmental settings (4-6). On ingestion, NoV pass through the gastric juices, multiply in the small intestine, and are excreted in the feces at high concentrations (7). Therefore NoV are readily transmitted by the fecal-oral route: through hand-to-hand contact, fecal contamination of food and water (8-10) and also, importantly, by aerosol dispersal from vomitus with subsequent heavy contamination of the surrounding environment (11, 12).

The significance of such contamination is strongly indicated in reports where NoV RNA in environmental samples from hospitals (13), cruise ships (14, 15), restaurants (16), and houseboats (17) could be correlated with recurring or ongoing outbreaks. The high viral load in vomitus and feces (7), low infectious dose (18), and short-term immunity (19) that typify NoV gastroenteritis increase the risk of prolonged outbreaks and environmental spread in semi-closed units, where strains of NoV genotype (G) II.4 or GII.3 tend to dominate (20). With no effective antiviral or vaccine therapy to counteract infection and transmission of NoV GII strains, preventative measures are based on strict hygiene control, and isolation of infected patients (21).
Although contamination from aerosols derived from vomitus in NoV outbreaks associated with hotel restaurant diners (16), airplane travellers and crew (22), and nursing staff (23), is strongly suggested, absence of a suitable culture system makes it difficult to demonstrate infectious virus in air or other environmental samples (4). However, advances in molecular analyses permit more detailed examination of such samples for NoV RNA (14). In this study of environmental distribution of NoV during hospital outbreaks, molecular methods were used to investigate NoV contamination, including airborne dispersal of NoV in dust from patient rooms, and molecular epidemiology of the outbreaks.

Materials and Methods

Study Outline. Patient and environmental sampling was carried out in eight wards in four separate buildings of Sahlgrenska University Hospital, Gothenburg, Sweden (Table 1). Infection control nurses provided ward outbreak reports throughout the five-month study. Ward nursing staff were also investigated in studies to be reported elsewhere.

In this study of NoV hospital infections and dispersal of NoV in patient rooms, an outbreak ward was defined as a ward where two or more cases of suspect or verified NoV gastroenteritis occurred in patients, and where spread of infection occurred within the ward (24). Patient rooms were predominantly two to four bedded rooms with communal patient toilets in ward corridors; these toilets were accessible to visitors. Each ward also had two to four single rooms with individual toilets.

Sampling of symptomatic inpatients and their hospital room environment began in January 2012, the onset of seasonal nosocomial NoV outbreaks, continuing through May
Seven outbreak wards, and one outbreak-free ward with two isolated cases of acute NoV gastroenteritis occurring seven weeks apart, were examined. An administration office room in another hospital building (E) with no wards was sampled for presence of airborne virus particles, as control (Table 1). Environmental swabs and patient samples were processed separately, at different time points, in two different laboratories dedicated to environmental, or patient sample preparation. Strict precautions were taken to prevent cross-contamination of samples and PCR products including use of UV work stations, dedicated pipettes, and water controls (25).

**Patient samples.** Feces and/or vomitus were obtained from inpatients (n=125) on routine diagnostic indications of acute gastroenteritis. A total of 125 patient samples were examined; 108 from outbreak wards, and 17 from outbreak-free ward (Table 1). Suspensions (10%) were prepared in phosphate-buffered saline (PBS, pH 7·4) (26), and 250 μl was mixed with 2 ml of NucliSENS® lysis buffer (Biomérieux, Marcy-l’Étoile, France) before total nucleic acid (TNA) extraction (110 μl) on the NucliSENS® EasyMAG extractor (Biomérieux).

**Environmental samples.** One investigator used standardized procedures to collect samples (total n=135, Table 1) from the eight wards, and the administration office room. Sterile, cotton-tipped swabs, pre-wetted with PBS, were drawn back and forth, once, across 25 cm surface; circular sweeps were used to swab washbasin drains, and air vents. Swabs were transported to the laboratory in 2 ml NucliSENS® lysis buffer, and vortexed vigorously prior to TNA extraction (110 μl) on the NucliSENS® EasyMAG extractor.
Swabs (n=91) from over-bed tables, washbasins, and air vents were collected from patient rooms in eight wards (Table 1). Dust was sampled from rooms in two wards in May; outbreak ward A1, and outbreak-free ward D (Table 1). Dust swabs (n=10), one per room, were collected from locker-tops in nine patient rooms and the nurses’ office in ward A1. Dust (n=10) was also sampled from locker-tops in 10 patient rooms in ward D.

Virus-trap sampling (VTs) was based on an ionizer prototype manufactured by Airpoint Ab, Stockholm, Sweden. The device was modified by installing a disposable plastic cup with conductive surface 47 mm in diameter as collector plate (GP plastic industry, Gislaved, Sweden). At 12 volts the ionizer generates negative ions, surface molecules of virus particles in the air become negatively charged, and are thus attracted to the positively charged collector plate. The ionizer was placed on a shelf above the bed 2–3 meters from the patient’s head, and exposed for 3 h at room temperature. The sample cup was washed with PBS (1 ml), and rubbed gently with a sterile swab. PBS aliquots (250 μl) were transferred to 2 ml NucliSENS® lysis buffer prior to TNA extraction (110 μl). VTs (n=12) were collected from six patient rooms, two samples per room, in four outbreak wards A1, B1, B2, B3 (Table S1). Three patient rooms in the outbreak-free ward, D, were also examined by VTs (n=6). Control VTs (n=6) was carried out in an administration office in building E (with no wards) on three consecutive days, two samples per day, during the study period.

Real-time reverse transcription-RT-PCR. Validated real-time reverse transcription RT-PCR (rRT-PCR) assays were used to detect NoV GI and NoV GII, Rotavirus (RoV), Astrovirus (HuAstV) (6, 27), Sapovirus (HuSaV) (28), and Adenovirus (HuAdV) (29) in duplicate TNA extracts (10 μl) of environmental and patient samples, as described on July 7, 2017 by guest http://jcm.asm.org/ Downloaded from
previously (6, 27). rRT-PCR controls included virus positive feces, and a plasmid construct designed to control viral amplification. Cycle threshold values (Ct) ≤ 39·5 were recorded as positive.

**NoV classic RT-PCR and nucleotide sequencing.** NoV rRT-PCR positive samples were amplified in semi-nested RT-PCR of the NoV RNA-dependent RNA-polymerase-N/S-capsid-coding region (RdRp-N/S-capsid-coding region) (1040 nt) as described previously for NoV GII strains (9). Amplicons were purified, and cycle-sequenced in unilateral master mixes with NoV GII primers and Big Dye Kits (v3.1 or v1.3, Applied Biosystems, Foster City, CA) (9).

NoV GII.4 patient strains from two outbreaks in ward A1 (January, May) were amplified and sequenced across the RdRp-N/S-major-capsid-coding region (2·5 kb) as described for long-fragment analysis of NoV GII strains (9). This region of the NoV GII genome included the partial RdRp of ORF (open reading frame) 1, entire ORF2 encoding major capsid-protein, and partial minor capsid protein-coding region in ORF3 (2·5 kb).

**Comparative sequence and phylogenetic analyses.** NoV sequences were analyzed and aligned with GenBank reference strains by using Sequencher 4.9 (Gen Codes Corp., Ann Arbor, MI) and Basic Local Alignment Search Tool (BLAST®, NCBI). Phylogenetic analysis included neighbor-joining methods using MacVector 7.2 software (Accelrys, Inc., San Diego, CA).

**Nucleotide sequences.** The sequences described in the study have GenBank accession numbers KF768469-KF768544.

**Results**
Outbreaks of acute gastroenteritis occurred in seven of eight wards monitored for nosocomial NoV infections and environmental contamination during the 5-month study (Table 1). Samples from inpatients and their hospital room environment were examined in rRT-PCR assays designed for laboratory diagnosis of enteric virus infections (6, 27). NoV GII detection according to building, ward, patient, room, day of outbreak sampling, Ct values, and sequence-based genotyping is detailed in Table S1.

Patients. NoV GII infections were detected in 63 of 108 (58%) patients sampled in outbreak wards (Ct range 13.0–37.8, median 20.0, IQR 5.3). Sequence-based genotyping revealed NoV GII.4 infections in 56 of the 63 NoV GII positive patients; seven strains could not be sequenced because of poor amplification, or inadequate sample material (Table S1). One case of RoV gastroenteritis was detected in ward B3.

In outbreak-free ward D, NoV infection was detected in 2 of 17 patients (12%), (Table 1). The first patient (P27, GII Ct=34.7) developed NoV gastroenteritis within 24 hours of admission in mid-March (Table S1). NoV strain P27 genotyped on sequencing as GII.6. Short time-delay between admission and onset of diarrheal symptoms indicated that this patient might have been admitted early in acute stage of infection. No prior NoV infections were recorded in ward D during the study period, and no secondary spread was noted between March and May. The second case P61 occurred early in May, 49 days after the first. Strain P61 (Ct=20.9) sequenced as GII.4. No further cases of NoV gastroenteritis were reported from ward D.

The epicurve showed onset of NoV GII infections in outbreak wards A1, A2, B1, B2, B3, C1, and C2, during the five month study, including the single cases of NoV GII
infections (GII.6 and GII.4) that occurred in outbreak-free ward D (Figure 1). Evidence of NoV GII outbreaks over time was noted particularly for wards A1, A2 and B1. A1 outbreaks occurred on weeks 1-2 (January), and again on weeks 18-19 (May); A2 in late February and May; and B1 outbreaks were reported on four occasions, in January, February, March, and April.

Environment. NoV GII RNA was detected in 48 of 101 (47·5%) environmental swabs from seven outbreak wards (Ct range 25·4–39·5, median=33·7, IQR=4·4), and in 2 of 28 (7·1%) samples (Ct=28·0, 35·8) from the outbreak-free ward.

NoV GII RNA (Ct range 26·7–39·5, median 34·3, IQR 5·1) was detected in 29 of 79 (37%) environmental swabs from overbed tables, washbasins, and air vents in the seven outbreak wards (A1, A2, B1, B2, B3, C1, C2; Tables1, S1). Sequence-based genotyping (1040 nt) revealed NoV GII.4 strains in 6 of 10 rRT-PCR positive swabs from wards B1 and C1. NoV was not detected in surface samples (n=12) from three rooms in the outbreak-free ward.

All dust samples (n=10), collected on outbreak day 131 from 10 rooms (one sample per room) in ward A1, tested positive in NoV GII rRT-PCR (Ct range 25·4–35·8, median 30·6, IQR 5·5). Nine of these samples were from the patient rooms; the tenth was from the nurses’ office. Results were verified by repeat extraction and assay of the dust. Sequencing revealed NoV GII.4 RNA (1040 nt) in dust from 8 of the rooms, mixed with HuAstV contamination (Ct=33·7, 35·9) in 2 rooms, with HuSaV (Ct=35·2, 39·1) in another 2 rooms, and HuAdV (Ct=38·0, 38·0, 38·9) in 3 rooms (Table S1).
NoV GII (Ct=28·0, 35·8) was detected in dust from locker-tops in 2 of 10 rooms in the outbreak-free ward. These dust samples were collected on day 59 after onset of symptoms in the first case of NoV gastroenteritis to occur in the ward (P27, NoV GII.6), and 10 days after the single case of GII.4 infection (P61). GII strains detected in dust from these 2 rooms sequenced as GII.4 across short 100 nt regions; NoV GI (Ct=31·8) was also detected in dust from one of these rooms (Table S1).

VTs (n=12) from six patient rooms (two samples per room) in outbreak wards A1, B1, B2, and B3 were examined for NoV RNA (Table S1). Nine of ten VTs from five of these rooms were positive in NoV GII rRT-PCR assays (Ct range 29·5–39·0, median 32·1, IQR 6·6). Sequencing revealed NoV GII.4 RNA (1045 nt) in VTs from four of these rooms. The patients in these five rooms had symptomatic, laboratory-confirmed NoV infections on the day of VT sampling. VTs (n=2) from the sixth room were NoV rRT-PCR negative. The patients in this room, R12 in ward B3, were symptom-free on the day of VT sampling (Table S1).

VTs (n=6) from three patient rooms in outbreak-free ward, D, tested negative for NoV when sampled after point cleaning with 1% Virkon (Rely+On™ Virkon®, DuPont, UK), following patient discharge (24). One of these rooms had been occupied by P27, a patient with NoV GII.6 infection (Table S1). VT controls (n=6) from the administration office in building E, with no wards were NoV rRT-PCR negative.

Comparative sequence and phylogenetic analyses. Sequence analyses of RdRp-N/S capsid-coding region (1040 nt) showed predominance of NoV GII.4 strains in environmental and patient samples from the seven outbreak wards. NoV GII.4 sequences
detected in environmental swabs and patient samples collected from a given ward during an ongoing outbreak were identical, or had high similarity (>99.5%, 1040 nt), (Figure 2). This level of similarity also held on analyses of the RdRp-N/S-major-capsid-coding region (2.5 kb), and of the 484 nt hyper-variable P2 subdomain (99.8-100%) in patient strains from each of the ward A1 outbreaks (January, May).

All strains but one (NoV GII.6, P27, ward D) sequenced as NoV GII.4, most similar to the pandemic recombinant strain New Orleans 2009. Nucleotide similarity of GII.4 sequences detected in this study ranged between 97.9–98.9% (1040 nt) when compared to New Orleans 2009, GenBank GU445325, and 97.0–98.8% compared to NoV Berowra 2012, JX459902. The variants clustered in a similar pattern when trees were constructed based on either the RdRp region or the major capsid-coding region of the sequences (data not shown).

Eleven NoV GII.4 variants were detected in wards as shown on the phylogenetic tree (Figure 2). Ten variants were from outbreak wards, and one from the outbreak-free ward; the latter variant (P61) was from an isolated case of GII.4 acute gastroenteritis occurring late in the season. These eleven variants showed 0.7–3.7% nt variation across the RdRp-N/S-capsid-coding region (1040 nt). Nt diversity (1.7–1.9%) over the longer 2.5 kb sequences was comparable to the diversity across the 1040 nt region (1.2–1.4%) for the two variants found in patients from ward A1 outbreaks (January, May). As expected, a slightly higher nt diversity (2.2–2.6%) was noted across the hyper-variable P2 subdomain of these variants.
When each of the eleven variants was compared to its nearest-neighboring variant cluster, one to three putative amino acid (aa) substitutions were detected, except for variant P13 with five silent point mutations across the RdRp-N/S major capsid region (1040 nt). The two patient variants found in A1 outbreaks (January and May) showed two aa changes at residues 1521 and 1642 in the partial RdRp region. Analyses of the longer A1 sequences spanning the RdRp-N/S-major-capsid and partial minor capsid-coding region (nt 4313-6820, GenBank GU445325) revealed seven additional aa changes, one in the shell (aa 174), five in the P2 subdomain (aa 282, 291, 294, 297, 372), and one in the partial minor capsid-coding region at position 23.

Compared to New Orleans 2009 strain (GenBank reference GU445325), ward A1 strains from January and May outbreaks showed 6 and 3 aa changes respectively. Substitutions found in the A1 January variant were at residues I1521V and A1642S in RdRp; N282S, R297H and D372N in the P2 subdomain of the major capsid-coding region; and N23S in minor capsid. A1 May strains showed changes in the major capsid-coding region at residues S174P, T291I, and P294S. Substitutions at aa sites 294, 297 and 372 were located over the putative blocking epitope A (30).

All ten GII.4 variants from outbreak wards showed high correlation between location and time of outbreak. Building A: ward A1 in January 2012; ward A2 February; wards A1, A2, May. Building B: B1, January; B1, February; B1, March; B1, April; B2, April; B3, April. House C: wards C1, C2, February and March (Figure 2). However, the outbreak strains detected in wards A1 and A2 during May belonged to the same variant, possibly due to the shared dining- and shower-facilities (Figure 2, Table 1). GII.4 strains
Nosocomial outbreaks of NoV GII.4 present a recurring problem of far-reaching consequence for patients and medical staff. Concerned that explosive spread of virus in semi-closed communities could not be accounted for by fecal-oral spread alone, Caul suggested airborne transmission of virus from aerosolized vomit as a potential source of NoV infection, although classical respiratory spread of infections was not documented (31). Molecular methods are now used to monitor NoV contamination on touch surfaces (14, 32) but the question of airborne contamination remains to be elucidated. We undertook this study to further our understanding of NoV contamination and airborne dispersal of NoV in patient rooms during nosocomial outbreaks.

Aware that restrictive measures do not always contain the spread of nosocomial infections, we investigated environmental contamination in patient rooms by surface swabbing, including dust from patient locker-tops, and a VT ionizing device designed to capture airborne virus particles. Substantial NoV GII contamination was detected in rRT-PCR studies of dust (Ct median=30.6), and VT (Ct median=32.1) in outbreak wards as measured by the low Ct values registered in environmental samples: in patient samples Ct values of 30 cycles were estimated to approximate a viral load of $10^6$ genome copies per gram feces (9, 27). Environmental contamination was markedly higher in outbreak wards (47%, total n=101) than in the outbreak-free ward (7%, total n=28). The achievement of identifying long, contiguous sequences from dust, VT, and surface samples argues for
detection of intact, potentially infectious virus, and against the detection of fragmented viral RNA. In the absence of cell-culture, molecular analysis of genomic NoV RNA is the method currently available to investigate environmental contamination (4). So volunteer studies, or more refined molecular tools, such as the rRT-PCR RNAse protection assay used to assess virus capsid integrity (33), may be required to determine viral infectivity, and validate the relevance of our findings in environmental samples.

Genomic diversity and prevalence of NoV GII.4 add to the complexity of nosocomial infections (34), as noted here where eleven NoV GII.4 variants (differing by 0.7–3.7%, 1040 nt) were detected in eight wards in four separate buildings during the five-month study (Figure 1). The phylogenetic tree showed that these eleven variants could be correlated with time of onset of a given outbreak in a given ward, as in wards A1 and B1 where a different GII.4 variant was implicated in each of the outbreaks over time (Figure 2). NoV GII.4 variants from patients clustered on eleven sub-branches of the phylogenetic tree. And environmental strains placed on five of these branches (Figure 2). Comparative sequence analyses revealed identical or high similarity (>99.5%, 1040 nt) between the NoV GII.4 strain detected at a given time-point, in dust, VTs, over-bed tables, washbasins, or air vents, from a given ward room, and the GII.4 strain detected in the symptomatic patient/s occupying the room, at that time. These molecular epidemiological studies strengthen indices that dust in patient rooms could be a repository for NoV GII.4 virus during outbreaks, particularly as transmission of infection by aerosolized vomitus has been indicated previously in hospital (23), and hotel dining-room outbreaks (16). Therefore we suggest that airborne dispersal of NoV, as detected in dust particles and in VT samples from patient rooms, may be a source of contamination...
in nosocomial outbreaks.

NoV sequence variability over the hyper-variable P2 subdomain (454 nt) has been suggested as a marker for tracing outbreak strains in nosocomial settings (35, 36). However, in this study, sequencing of a longer region (1040 nt) that revealed variant-typical sites was as informative as the P2 subdomain in tracing NoV GII.4 patient and environmental strains (36).

Various theories are presented for the prevalence of NoV GII.4 strains where different, emerging subtypes replace one another over time (19, 37) through the suggested greater epidemiological fitness of GII.4 over other circulating genotypes, such as the GII.6 strain observed here in one patient from the outbreak-free ward.

Recombination and mutation feature strongly in the evolving pandemic NoV GII.4 lineage (38, 39), and GII.4 strains from this study showed high similarity (≥97.0%) to recombinant strains New Orleans 2009, and Berowra 2012. NoV GII.4 variation was evident on analyses of the eleven ward-related variants reported here. When each of the eleven variants was compared to its nearest neighboring cluster, one to three aa substitutions were detected, except for the B1 February variant (P13) with the least distance difference (5 nt) from its neighboring cluster, B3 April (Figure 2). Point mutations found in the longer sequences from A1 January and May variants were scattered across the entire sequence studied (2.5 kb), resulting in aa substitutions in the RdRp, shell, P2 subdomain and partial minor capsid protein. Amino acid substitutions in the hyper-variable P2 subdomain of A1 variants were located close to and across the putative epitope A, a blockade epitope recognized as interacting with potential neutralizing antibodies, and one of several P domain epitopes with potential antigenic and
histo-blood group antigen binding functions reported as changing between epidemic GII.4 strains (37, 40-42).

Our molecular findings raise important questions on effectiveness of current hospital-ward cleaning procedures where contract cleaning has replaced the dedicated ward cleaner (43). Comparative study of environmental contamination with gastro-enteric viruses in pediatric wards, during outbreak-free periods, indicated that environmental surveillance improved staff awareness of the problems, and changes in cleaning protocols reduced environmental contamination, although breakthrough incidents still occurred (32). Major cleaning including “all surfaces above head” is often performed just once a year. Changing emphasis in cleaning policies may contribute to the problem of recurring nosocomial infections, a problem not unique to hospitals. In a three-year study of cruise-ship outbreaks the importance of thoroughness of disinfection cleaning (TDC) in reduction of outbreaks is emphasized, low TDC scores being predictive of subsequent NoV outbreaks (44). In the present study, point cleaning (24) of patient rooms in the outbreak-free ward following discharge of the single case of GII.6 infection, did appear to reduce the level of environmental dispersal of NoV as measured by the negative findings on VT and surface sampling (Table S1). However, avoidance of the rapid spread of NoV GII.4 strains that characterized NoV transmission in other wards may require a more intense cleaning response. We suggest that detection in dust, VT and other environmental samples from patient rooms of NoV GII.4 strains with high sequence similarity to strains found in symptomatic patients occupying the room, indicates the need for interventional studies of hospital-ward cleaning.
In this study of seasonal outbreaks of nosocomial infections, dust and virus-trap sampling provided molecular evidence supporting airborne dispersal of patient-related NoV GII.4 in hospital rooms during outbreaks, a novel approach for investigating the non-cultivable human NoV. Although the question of virus infectivity and potential spread to patients from sources such as dust remains to be determined, we suggest that interventional studies of cleaning policies are justified.

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References


Legends

Figure 1: Epicurve showing distribution of NoV GII cases (n=65) in eight wards A1, A2, B1, B2, B3, C1, C2, and D during seasonal nosocomial outbreaks, weeks 1 to 20, 2012. NoV GII.4 was detected in 57 of 58 NoV GII cases where strains were sequenced, the exception being one case of GII.6 infection detected in outbreak-free ward D.

Figure 2: Neighbor-joining tree based on nucleotide analysis of the RNA-dependent RNA-polymerase N/S capsid-coding region of the NoV genome (1040 nt). P=patient strain sequence; A1, A2, B1, B2, B3, C1, C2, D=wards; R=patient room; Rx=patient room not defined; VT= virus trap; ptab=patient over-bed table; whb=washbasin; airv=air vent. Hospital buildings are represented by four colors red, blue, purple, and orange; and wards are indicated by the symbols ■, ○. Environmental samples are indicated in green type. Relevant Genbank reference strains are included. The bar indicates genetic distance per nucleotide per site per year.
Fig. 1 Nenonen et al
Fig. 2 Nenonen et al
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<sup>a</sup> Total number of samples examined in real-time RT-PCR assays designed for detection of human Norovirus GI and GII, Adenovirus, Astrovirus, Rotavirus, and Sapovirus.

<sup>b</sup> Number of samples with NoV GII positive real-time RT-PCR result.
c Interconnected wards with shared dining-room and shower facilities.

d Denotes dust samples.

e Includes one patient with acute gastroenteritis testing positive for Rotavirus in real-time RT-PCR.

f Outbreak-free ward with no nosocomial infections.