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Research Paper

Prevalence of Human Noroviruses in Commercial Food Establishment Bathrooms

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ABSTRACT

Although transmission of human norovirus in food establishments is commonly attributed to consumption of contaminated food, transmission via contaminated environmental surfaces, such as those in bathrooms, may also play a role. Our aim was to determine the prevalence of human norovirus on bathroom surfaces in commercial food establishments in New Jersey, Ohio, and South Carolina under nonoutbreak conditions and to determine characteristics associated with the presence of human norovirus. Food establishments (751) were randomly selected from nine counties in each state. Four surfaces (underside of toilet seat, flush handle of toilet, inner door handle of stall or outer door, and sink faucet handle) were swabbed in male and female bathrooms using premoistened macrofoam swabs. A checklist was used to collect information about the characteristics, materials, and mechanisms of objects in bathrooms. In total, 61 (1.5%) of 4,163 swabs tested were presumptively positive for human norovirus, 9 of which were confirmed by sequencing. Some factors associated with the presence of human norovirus included being from South Carolina (odd ratio [OR], 2.4; 95% confidence interval [CI], 1.2 to 4.9; $P < 0.05$) or New Jersey (OR, 1.7; 95% CI, 0.9 to 3.3; $0.05 < P < 0.10$), being a chain establishment (OR, 1.9; 95% CI, 1.1 to 3.3; $P < 0.05$), being a unisex bathroom (versus male: OR, 2.0; 95% CI, 0.9 to 4.1; $0.05 < P < 0.10$; versus female: OR, 2.6; 95% CI, 1.2 to 5.7; $P < 0.05$), having a touchless outer door handle (OR, 3.3; 95% CI, 0.79 to 13.63; $0.05 < P < 0.10$), and having an automatic flush toilet (OR, 2.5, 95% CI, 1.1 to 5.3; $0.05 < P < 0.10$). Our findings confirm that the presence of human norovirus on bathroom surfaces in commercial food establishments under nonoutbreak conditions is a rare event. Therefore, routine environmental monitoring for human norovirus contamination during nonoutbreak periods is not an efficient method of monitoring norovirus infection risk.

Key words: Bathrooms; Environment; Fomites; Norovirus; Restaurants; Retail food

Human noroviruses are the leading cause of acute gastroenteritis and foodborne disease in the United States, sickening between 19 and 21 million people every year *(24, 49)*. Although human norovirus is primarily spread from person to person (69% of infection cases) or via food (23%), an increasing body of epidemiological evidence suggests that environmental surfaces also play an important role in norovirus transmission *(9, 15, 19, 24, 34, 64)*.

The most common setting for norovirus outbreaks is long-term care facilities (60%), and the second most common setting is food establishments (22%), such as restaurants, catering, and banquet facilities. The route of transmission of norovirus in food establishments is different from that in long-term care facilities; exposure is commonly attributed to the consumption of contaminated food (48%) rather than person-to-person *(24)*. Food often becomes contaminated through contact by an infected food worker who handles ready-to-eat foods with bare hands. Another underrecognized route of transmission may be environmental surfaces that become contaminated via contact with contaminated hands or with vomitus or feces either directly or through settling of aerosolized particles *(15, 19, 64)*. Contaminated environmental surfaces in shared spaces, such as bathrooms, are especially likely to be a source of norovirus.

Bathrooms in most commercial food establishments are considered shared spaces because they may be used by both customers and employees. Shared bathroom surfaces could become contaminated with norovirus particles after use by an infected individual. These contaminated surfaces could then serve as a source to spread norovirus to others in the facility, leading to an outbreak. The presence of noroviruses on shared bathroom surfaces in food establishments under both outbreak and nonoutbreak conditions have been reported *(10, 11, 61)*. In a systematic literature review, human noroviruses were found on bathroom surfaces under outbreak ($n = 11$) and nonoutbreak ($n = 5$) conditions *(36)*. Swab samples from high-touch surfaces such as toilet seats, toilet flush handles, sink faucet handles, and bathroom door

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handles were most likely to be positive for norovirus. Some researchers further examined the relationship between select factors and the presence of human noroviruses. Boxman et al. *(10)* reported that population density had a borderline significant effect on the presence of human norovirus. Verhoef et al. *(61)* found that small commercial food establishments were more likely than large establishments to have human norovirus on surfaces, and Boxman et al. *(10)* reported that the number of employees did not have a significant effect on norovirus presence. In other studies, improper cleaning and disinfecting was linked to the prevalence of human norovirus on environmental surfaces *(15, 19)*.

The aim of the present study was to determine the presence of human noroviruses on bathroom surfaces in commercial food establishments under nonoutbreak conditions in three U.S. states representing three geographic regions: New Jersey, Ohio, and South Carolina. Our objectives were to determine (i) the presence of human noroviruses on four types of surfaces commonly found in bathrooms and (ii) the characteristics associated with the presence of human noroviruses. To our knowledge, this is the first study to monitor human noroviruses on environmental bathroom surfaces in food establishments in multiple states using power calculations to determine sample size. Our results will help researchers fine-tune current risk models and highlight the importance of proper cleaning and disinfecting procedures for commercial food establishment bathrooms and the need for training food workers on how to properly clean and disinfect bathroom surfaces.

MATERIALS AND METHODS

Statistical power calculation of sample size. The sample size was calculated using the method presented by Naing et al. *(41)*. Expected norovirus prevalence estimates of 1, 2, and 4% were used when calculating the sample size with a 95% confidence interval (CI) and a precision of 0.05, 0.01, and 0.02, respectively. Estimates of 1 and 4% were selected based on data reported by Boxman et al. *(10)*. The authors used 1% as their expected norovirus prevalence but observed a 4% prevalence in commercial and institutional food establishments under nonoutbreak conditions *(10)*. We used 2% as a middle value between 1 and 4%, resulting in a calculated necessary sample size of 750.

Sample distribution. The 750 sites included in this study were commercial food establishments distributed proportionately across three states in the United States—New Jersey, Ohio, and South Carolina—according to the number of food establishments per state. Commercial establishments were chosen because bathrooms in these types of facilities are generally spaces open to the public, and no special permission was required to gain access. Proportionality was determined using the number of food establishments in each state as reported by the National Restaurant Association in fall 2012 (http://www.restaurant.org). Of the 750 food service establishments, 38% (285) were in New Jersey, 46% (345) were in Ohio, and 16% (120) were in South Carolina.

Sample site selection. Nine counties in each state were selected to make visiting food establishments more efficient. All counties in each state were classified by population density into categories of high, medium, and low population density, and three counties were randomly selected from each category using SAS 9.3 software (SAS Institute, Cary, NC). Because the size and populations of the three selected states differed greatly, the definition of counties with high, medium, and low population densities was allowed to differ by state. Percentiles (33 and 66) were used as an objective measure to break up population density without looking for natural breaks in the data. For New Jersey, low-density counties had an average of 0 to 535 residents per square mile, medium-density counties had 536 to 1,772, and high-density counties had 1,773 to 13,883. For Ohio, low-density counties had an average of 0 to 93 residents per square mile, medium-density counties had 94 to 166, and high-density counties had 167 to 2,779. For South Carolina, low-density counties had 0 to 56 residents per square mile, medium-density counties had 57 to 147, and high-density counties had 148 to 588 *(55)*.

A list of all food establishments in each county was obtained from the appropriate regulatory agency or agencies in each state. All lists were reviewed, and any facilities that were not commercial food establishments (e.g., schools, long-term care facilities, and country clubs) were removed. Sampling sites were chosen randomly from the final lists using SAS 9.3 and distributed proportionally based on the number of food establishments in each of the three population density categories per state. In New Jersey, approximately 50% of establishments were located in high-density counties and 25% each were in medium- and low-density counties. In Ohio and South Carolina, about 75% of food establishments were located in high-density counties, 15% were in medium-density counties, and 10% were in low-density counties. We kept our sampling sites proportional to our source populations to ensure that our samples were as representative as possible. We also oversampled by 30% for each category in the event that we were unable to take samples from a selected site (e.g., the establishment was closed or did not have a public bathroom).

After sample sites were selected, they were randomly divided into two groups. One group contained swab samples collected from both types of bathrooms, i.e., those designated as male and those designated as female. Swab samples in the other group were collected from only one type of bathroom, i.e., bathrooms designated as male or bathrooms designated as female. This approach was necessary based on limited available resources. Bathroom designations for some sites were changed to unisex when such bathroom types were encountered during sampling.

Establishments also were categorized as chain or nonchain. A chain establishment was defined as any food establishment under a single brand name with central headquarters that was 1 of at least 10 units in two or more distinct geographical locations.

Environmental surface swabbing. Swab samples were collected from the selected food establishments during two winter seasons, February to March 2013 and December 2013 to March 2014. Macrofoam swabs (Puritan Medical Products, Guilford, ME) premoistened with a solution of phosphate-buffered saline and Tween 80 (0.02%) at pH 6.5 were used to collect samples from bathroom surfaces as described previously *(46)*.

Four swab samples were collected from each bathroom: (i) the underside of the toilet seat where it connects to the toilet bowl, (ii) the flush handle of the toilet, (iii) the inner door handle of the stall door or, when there was no stall door, the inner door handle of the outer door, and (iv) the hot water knob of the sink faucet. For irregular surfaces (i.e., door handle, flush handle of toilet, and sink faucet handle), the entire surface was swabbed. For flat surfaces such as the toilet seat, an area ca. 10 by 10 cm was swabbed. Swabs were kept in a cooler at 4°C during overnight transport to Clemson University and stored at −80°C until analysis.

TABLE 1. *Oligonucleotide primers and probes used in this study (13)*

Name	Virus target	DNA sequence (5′–3′)
Cog1F	GI	CGYTGGATGCGITTYCATGA
Cog1R	GI	CTTAGACGCCATCATCATTYAC
Ring 1E	GI	FAM-TGG ACA GGR GAY CGC-MGBNFQ[a]
Cog2F	GII	CARGARBCNATGTTYAGRTGGATGAG
Cog2R	GII	TCGACGCCATCTTCATTCACA
Ring 2	GII	Cy5-TGGGAGGGCGATCGCAATCT-BHQ
MS2F	MS2	TGGCACTACCCCTCTCCGTATTCACG
MS2R	MS2	GTACGGGCGACCCCACGATGAC
MS2P	MS2	HEX-CACATCGATAGATCAAGGTGCCTACAAGC-BHQ2

[a] MGBNFQ, minor groove binder and nonfluorescent quencher.

A checklist was used to collect information about the characteristics, materials, and mechanisms of objects in the bathroom, including the outer door handle, stall door handle, toilet flush handle, toilet seat, sink faucet, hand washing signage, soap type, hand drying devices, and cleaning schedule. Photographs of bathrooms were taken as a reference for any data missing from the checklist. All bathroom checklists were verified against their corresponding photographs when available.

Viral RNA extraction, concentration, and purification. Swabs were thawed at room temperature approximately 20 to 30 min prior to RNA extraction. Viral RNA was extracted directly from macrofoam swabs, with bacteriophage MS2 (ATCC 15597-B1) as an internal process control *(13)*. UNEX lysis buffer (Microbiologics, St. Cloud, MN) was combined with an MS2 working solution prepared from ATCC 15597-B1 using *Escherichia coli* (Migula) ATCC 15597 as the host at a ratio of 600:1 (v/v), and 3 mL of this buffer mixture was added to each swab. After mixing by vortexing, excess liquid was removed by pressing the swab against the tube wall, and the swabs were removed from their tubes and discarded. After 10 min at room temperature, 2 mL of absolute ethanol was added to each tube. All liquid (ca. 4.5 mL) was transferred to a HiBind RNA Midi column (Omega Bio-tek, Norcross, GA). The Midi columns were centrifuged at 5,000 × *g* for 5 min, washed twice with 70% ethanol, and spun dry, and 250 μL of prewarmed (70°C) TE buffer (10 mM Tris pH 8.0 and 1 mM EDTA pH 8.0) was used to elute RNA bound to the Midi column. Extracted nucleic acid was concentrated to 25 μL with a Zymospin IC RNA Clean and Concentrator kit (Zymo Research, Irvine, CA) with slight modifications to the manufacturer's instructions, including use of TE buffer instead of water for the final elution.

Human norovirus TaqMan real-time RT-PCR. A previously reported multiplex reverse transcription TaqMan real-time PCR (RT-PCR) assay for the detection of genogroup I (GI) and genogroup II (GII) human norovirus *(13)* was carried out on a CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA) using the AgPath kit (Applied Biosystems, Carlsbad, CA). The assay included oligonucleotide primers and probes for the detection of GI, GII, and the internal extraction control MS2 (Table 1). Cycling conditions were reverse transcription for 10 min at 45°C, denaturation for 10 min at 95°C, and then 45 cycles of 15 s at 95°C and 1 min at 60°C. Samples with a threshold cycle (C_T) value of ≥30 for MS2 (expected value 28) were retested at 1:10 and 1:100 dilutions. A sample was presumed positive for norovirus when the amplification curve had typical S-shape and the C_T value was ≤40.

Nested PCR and genotyping of norovirus. All samples positive for norovirus with the RT-PCR assay ($C_T \leq 40$) were tested by nested PCR targeting the 5′-region of the capsid gene (region C) *(32),* and negative samples were further tested by RT-PCR targeting a small region of the polymerase gene (region A) *(60)*. PCR products of appropriate size (region C: 330 bp for GI and 344 bp for GII; region A: 327 bp) were visualized after separation on a 2% agarose gel (Seakem-ME, Lonza, Allendale, NJ) containing Gel Red (Biotium, Fremont, CA) and gel purification by ExoSAP-IT (Affymetrix, USB, Cleveland, OH) or by using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Sanger sequencing was conducted (Eurofins MWG Operon, Louisville, KY), and norovirus genotypes were assigned after phylogenetic analysis using the unweighted pair group method with arithmetic means and reference sequences in CaliciNet *(13, 59)* for capsid genotyping.

Data analysis. Descriptive statistics, including odds ratios (ORs), were computed to compare norovirus prevalence by establishment and bathroom characteristics, such as whether an establishment was chain or nonchain and the gender type of the bathroom. A logistic regression model was used to examine the effects of state, population density, and the interaction between state (*s*) and population density (*p*) on norovirus prevalence in food establishments:

$$y_{ij} = \left[1 + e^{-(s_i + p_j + sp_{ij})}\right]^{-1}$$

where y_{ij} is the probability that norovirus is present at a food establishment in state *i* and population density *j*.

ORs were also used to compare the odds of norovirus prevalence in food establishments across states without adjusting for population density. This approach was used because of the lack of swab samples positive for norovirus for particular state–population density combinations (e.g., no swabs were positive for norovirus in medium-density counties in Ohio or in low-density counties in South Carolina). A chi-square test was used to examine the odds of norovirus between counties with different population densities. When expected value was less than 5 or the observed number was 0 in at least one cell of the resulting 2 × 2 contingency table, the *P* value for Fisher's exact test was computed and a small sample correction was applied before calculating confidence intervals (i.e., 0.5 was added to each cell of the contingency table). A significance level of 0.05 was used for all tests of significance.

TABLE 2. *Number of samples collected by state and number of samples positive for human norovirus as determined by real-time RT-PCR*

State	Sites visited	Bathrooms sampled	Surfaces sampled	No. of presumptive-positive samples[a]			% positive[b]
				GI	GII	Total	
New Jersey	286	377	1,505	14	13	27	1.8
Ohio	345	496	1,977	11	7	18	0.9
South Carolina	120	171	681	4	12	16	2.3
Total	751	1,044	4,163	29	32	61	1.5

[a] Number of swab samples that were positive after analysis. GI, genogroup I noroviruses; GII, genogroup II noroviruses.
[b] Number of positive swabs divided by the total number of swabs collected.

RESULTS

Swab sample results. Although our goal was to visit 750 commercial food establishments, we actually visited 751 establishments, in which 1,044 bathrooms and 4,163 surfaces were swabbed (Table 2). Of the 4,163 swabs collected, 61 (1.5%) were presumed positive for human norovirus (29 GI and 32 GII). Overall, 54 (7.2%) of the 751 food establishments had at least one swab that was positive for norovirus (Table 3). In most establishments with a positive result, only one of the four swabs was positive. However, one South Carolina establishment and three New Jersey establishments had positive swabs from multiple surfaces. Only 9 of the 61 real-time presumed positive swabs were confirmed by sequencing.

Significant risk factors across the three states combined. The ORs for all states combined revealed that samples positive for norovirus were approximately 2.4 times more likely to be found in South Carolina establishments than in Ohio establishments (95% CI, 1.15 to 4.87; $P < 0.05$) and approximately 1.7 times more likely to be found in New Jersey establishments than in Ohio establishments (95% CI, 0.92 to 3.25; $0.05 < P < 0.10$) (Table 4). Based

TABLE 3. *Results of swab sample analysis based upon state, establishment, and bathroom characteristics*

Category	New Jersey		Ohio		South Carolina		Total	
	No. positive[a]/total	% positive	No. positive/total	% positive	No. positive/total	% positive	No. positive/total	% positive
Ownership[b]								
Chain	6/70	8.6	14/181	7.7	10/58	17.2	30/309	9.7
Nonchain	16/216	7.4	4/161	2.5	4/62	6.5	24/439	5.5
Total	22/286	7.7	18/342[c]	5.3	14/120	11.7	54/748[c]	7.2
Service type[b]								
Table service	10/141	7.1	5/128	3.9	3/43	7.0	18/312	5.8
Counter service	5/114	4.4	7/124	5.6	6/45	13.3	18/283	6.4
Self-service	4/25	16.0	5/73	6.8	3/25	12.0	12/123	9.8
Take-out	2/4	50.0	0/5	0.0	0/0	0.0	2/9	22.2
Multiple service	1/2	50.0	1/12	8.3	2/7	28.6	4/21	19.0
Total	22/286	7.7	18/342[c]	5.2	14/120	11.7	54/748[c]	7.2
Bathroom type[d]								
Male	12/165	7.3	11/229	4.8	3/76	3.9	26/470	5.5
Female	8/146	5.5	5/240	2.1	11/83	13.3	24/469	5.1
Unisex	7/65	10.8	2/27	7.4	2/12	16.7	11/104	10.6
Total	27/376[e]	7.2	18/496	3.6	16/171	9.4	61/1,043[e]	5.8
Surfaces[f]								
Toilet seat	14/377	3.7	10/495	2.0	6/171	3.5	30/1,043	2.9
Toilet flush handle	6/376	1.6	5/493	1.0	2/171	1.2	13/1,040	1.3
Inner door handle	5/377	1.3	1/496	0.2	5/169	3.0	11/1,042	1.1
Sink faucet handle	2/375	0.5	2/493	0.4	3/170	1.8	7/1,038	0.7
Total	27/1,505	1.8	18/1,977	0.9	16/681	2.3	61/4,163	1.5

[a] Presumptive-positive results.
[b] Characteristics at the establishment level (at least one positive swab in the entire establishment).
[c] Ownership and service type could not be determined for three establishments.
[d] Characteristics at the bathroom level (in each establishment, samples could be collected from one or two bathrooms).
[e] Gender designation was not recorded for one bathroom.
[f] Characteristics at the swab level (four surfaces swabbed in each bathroom).

TABLE 4. *Significant and borderline significant factors for the presence of human norovirus in food establishments across New Jersey, Ohio, and South Carolina*

Risk factor	Category comparison pair	No. with norovirus[a]	No. without norovirus	Odds ratio (95% CI)	*P* value
State	South Carolina vs	14	157	2.37 (1.15, 4.87)	0.0162[b]
	Ohio	18	478		
	New Jersey vs	23	345	1.73 (0.92, 3.25)	0.0873[c]
	Ohio	18	478		
Chain	Chain vs	30	279	1.86 (1.06, 3.25)	0.0273[b]
	Nonchain	24	415		
Service type	Multiple vs	4	17	4.09[d] (1.31, 12.77)	0.0405[b]
	Table	18	294		
	Multiple vs	4	17	3.69[d] (1.18, 11.52)	0.0542[c]
	Counter	18	265		

[a] Presumptive-positive results.
[b] Significant at $P < 0.05$.
[c] Borderline significant at $0.05 < P < 0.10$.
[d] When one observed frequency was 0 or at least one expected frequency was <5, a small sample correction was applied (0.5 added to each observed frequency), and the *P* value for Fisher's exact test is reported.

on the logistic regression analysis, none of the factors (state, population, or state by population interaction) were significant at predicting human norovirus prevalence in food establishments.

Of the 751 establishments visited, 309 were chain and 439 were nonchain (ownership could not be determined for 3 establishments) (Table 3). Positive swabs were approximately 1.9 times as likely to be found in chain establishments than in nonchain establishments (95% CI, 1.06 to 3.25; $P < 0.05$) when data from all states were combined (Table 4). Most establishments visited were classified as table service (312), followed by counter service (283), and then self-service (123) with very few take-out (9) or multiple service (21) establishments (service type could not be determined for 3 establishments) (Table 3). Positive swabs were more likely to be found in multiple service establishments than in establishments classified as table service (OR, 4.1; 95% CI, 1.31 to 12.77; $P < 0.05$) or counter service (OR, 3.7; 95% CI, 1.18 to 11.52; $0.05 < P < 0.10$) (Table 4).

Of the 1,043 bathrooms for which gender type was recorded, 470 were male, 469 were female, and 104 were unisex (Table 3). Positive swabs were approximately 1.9 times more likely to be found in unisex bathrooms than in bathrooms for males (95% CI, 0.89 to 4.10; $0.05 < P < 0.10$) and approximately 2.6 times as likely to be found in unisex bathrooms as in bathrooms for females (95% CI, 1.16 to 5.73; $P < 0.05$) for all states combined (Table 5). Almost half (30) of positive swabs were found on the underside of the toilet seat (Table 3). About 20% were found on the toilet flush handle (13) and the inner handle of the stall or outer door (11). Only 11% (7) of positive swabs were found on the sink faucet handle. The likelihood of a norovirus-positive sample from the underside of the toilet seat was significantly different from the likelihood of a positive sample from any other surface: toilet seat versus toilet flush handle (OR, 2.4; 95% CI, 1.2 to 4.6), toilet seat versus inner door handle (OR, 2.8; 95% CI, 1.4 to 5.6), and toilet seat versus sink faucet handle (OR, 4.4; 95% CI, 1.9 to 10.1) ($P < 0.05$).

Positive swabs were more likely to be found in bathrooms that had outer door handles that must be touched (e.g., knob or handle) than in bathrooms with outer door handles that could be touchless (e.g., flat plate) (OR, 3.3; 95% CI, 0.79 to 13.63; $0.05 < P < 0.10$) (Table 5). Bathrooms with automatic flush toilets were more likely to have positive swabs than were bathrooms with manual flush toilets (OR, 2.5; 95% CI, 1.14 to 5.33; $0.05 < P < 0.10$). Positive swabs also were more likely to be found in bathrooms with trash cans attached to the paper towel dispenser than in bathrooms with trash cans not attached to the paper towel dispenser (OR, 4.8; 95% CI, 2.28 to 10.02; $P < 0.05$).

Nucleotide sequencing of presumptive-positive samples. A total of 61 samples were presumed positive for human norovirus (29 GI and 32 GII) by RT-PCR ($C_T < 40$) (Table 2). Quality sequences were obtained for nine of these samples. Eight samples were identified to genotype using the nested region C assay (GI.3, $n = 3$; GII.3, $n = 1$; GII.7, $n = 1$; GII.13, $n = 1$; GII.14, $n = 1$), and one sample was typed as GII.Pe using the region A polymerase sequence. One nested PCR sample was positive for both GI.6 and GII.14. The majority of the samples positive by the real-time RT-PCR assay that could not be confirmed by sequencing had C_T values >35 for GI and >37 for GII viruses.

DISCUSSION

Our results support previous findings that human noroviruses are rarely present on bathroom surfaces in commercial food establishments under nonoutbreak conditions. Norovirus was present on 1.5% of bathroom surfaces sampled in this study, which is consistent with the 1.7 and 1.9% prevalence reported in The Netherlands in 2011 and 2015, respectively *(10, 11)*. In a recent systematic review of seven articles published from 1980 to 2014 *(36),* only three included reports of norovirus-positive samples from bathroom surfaces in commercial and institutional settings under nonoutbreak conditions *(10, 48, 61)*. One reason for the low

TABLE 5. *Significant and borderline significant factors for the presence of human noroviruses in bathrooms of food service establishments across New Jersey, Ohio, and South Carolina*

Risk factor	Category comparison pair	No. with norovirus[a]	No. without norovirus	Odds ratio (95% CI)	*P* value
Bathroom type	Unisex vs	10	91	1.91 (0.89, 4.10)	0.0916[b]
	Male	26	452		
	Unisex vs	10	91	2.58 (1.16, 5.73)	0.0163[c]
	Female	19	446		
Outer door handle type	Handle vs	15	182	4.37 (0.98, 19.47)	0.0359[c]
	Flat plate	2	106		
	Touch vs	52	842	3.27 (0.79, 13.63)	0.0848[b]
	Touchless	2	106		
Stall door handle latch type	Slide vs	19	227	3.12 (0.91, 10.78)	0.0584[b]
	Turn	3	112		
Toilet flush mechanism	Automatic vs	8	65	2.46[d] (1.14, 5.33)	0.0515[b]
	Manual	47	901		
Soap type	Foam vs	23	317	1.66 (0.94, 2.93)	0.0780[b]
	Liquid	28	640		
	Bar vs	2	1	37.45[d] (4.78, 294.12)	0.0056[c]
	Liquid	28	640		
	Bar vs	2	1	22.52[d] (2.85, 178.57)	0.0147[c]
	Foam	23	317		
Trash can type[e]	Attached vs	10	44	4.78[d] (2.28, 10.02)	0.0004[c]
	Not attached	42	861		

[a] Presumptive-positive results.
[b] Borderline significant at $0.05 < P < 0.10$.
[c] Significant at $P < 0.05$.
[d] When one observed frequency was 0 or at least one expected frequency was <5, a small sample correction was applied (0.5 added to each observed frequency), and the *P* value for Fisher's exact test is reported.
[e] Trash can either attached or not attached to the paper towel dispenser.

prevalence in our study could be that human norovirus was present in such low numbers that they were below the limit of detection of our multiplex real-time RT-PCR *(13)* assay. The detection limit is approximately 20 copies per PCR for either GI or GII viruses (J.V., personal communication), and lower levels of contamination may not have been detected. A second reason could be that individuals experiencing gastroenteritis symptoms (e.g., vomiting and diarrhea) are less likely to leave the house, and the highest levels of virus are shed during the symptomatic phase of infection *(2)*. The analytical methods, such as the recovery method and sensitivity of real-time PCR, also could affect results.

The low prevalence of norovirus found in this study also could be attributed to the frequency and effectiveness of bathroom cleaning. The U.S. Food and Drug Administration Food Code, which has been adopted (at least in part) by all 50 states and the District of Columbia *(57),* does not currently explicitly proscribe the frequency of bathroom cleanings but rather addresses the need to keep all physical facilities clean and properly maintained. However, food establishment guests see bathroom cleanliness as an indicator of kitchen cleanliness and thus the safety of the food *(8)*. Many consumers have reported that they will not return to a food establishment that has bathrooms that appear dirty *(1, 4, 33)*. Thus, many food establishments probably clean and disinfect their bathrooms frequently to ensure customer satisfaction. This frequency may account for the low prevalence of norovirus on bathroom surfaces and for the fact that norovirus-positive swabs were commonly found on only one of the bathroom surfaces sampled instead of multiple surfaces in the same bathroom.

Our results showed a difference in the rate of norovirus detection in establishments across the three states included in our study, which may be attributable to population density. New Jersey has the highest population density of the three states (1,218.1 people per square mile) *(43),* and South Carolina has a relatively low population density (153.9 people per square mile) *(54)*. Ohio falls in the middle, with a population density of 282.3 people per square mile *(53)*. Most norovirus-positive swab samples came from New Jersey establishments and the fewest came from South Carolina establishments, suggesting that population density may have played a role in these results. However, because we set parameters for high, medium, and low population density by state rather than overall, we were unable to determine whether population density was a significant factor. Jarquin et al. *(26)* found that population density did not increase the risk of enteric infections transmitted via environmental surfaces, but Boxman et al. *(10)* found more norovirus-positive samples in regions with higher population densities ($0.05 < P < 0.10$). More studies are needed to clarify the contradictory findings related to population density as a risk factor for transmission of human noroviruses.

Other factors that could affect the distribution of norovirus-positive samples across states are temperature and humidity. Norovirus on hard surfaces survives longer at lower temperatures (4 to 9°C) than at higher temperatures (25 to 40°C) *(17, 31, 35, 37, 39)*. However, findings on the

effect of humidity are conflicting. Colas de la Noue et al. *(17)* and Kim et al. *(31)* found that murine norovirus, a surrogate for human norovirus, survived longer at low relative humidity (10 to 30%) than at high relative humidity (70 to 100%). Conversely, Lamhoujeb et al. *(35)* found that human norovirus survived significantly longer at high relative humidity (86%) than at low relative humidity (30%). Although we did not gather data on specific weather during our sampling time, in general winters in Ohio and New Jersey tend to be colder and have more snow than winters in South Carolina, so more norovirus-positive samples might be expected from Ohio and New Jersey than from South Carolina *(42, 44, 45, 50, 56)*. However, this assumption does not take into consideration indoor heating. More research is needed to determine the effect of temperature and humidity as a risk factor for human norovirus on surfaces under field conditions.

In our study, norovirus-positive samples were more likely to be found in chain food establishments than in nonchain food establishments. This finding differs from that of previous studies in which chain and nonchain food establishments were compared. In two studies, nonchain restaurants were cited for critical food safety violations more often than were chain restaurants *(25, 40),* and Jin and Leslie *(28)* found that hygiene at nonchain restaurants was poorer than that at chain restaurants. These findings most likely stem from the fact that chain establishments typically have their own food safety standards developed by the parent organization and potentially greater financial resources, allowing them to provide more food safety training and sanitary equipment *(20, 47)*. In these studies, only visual indicators of cleanliness and hygiene were examined, whereas in our study we obtained microbiological results. Even when surfaces look clean, pathogens might still be present *(18, 51),* although visible moisture and food debris may be correlated with detectible bacteria *(12)*. One factor that might explain the higher prevalence of norovirus in chain establishments is the number of customers. Chain establishments may have more patrons each day than nonchain establishments, which could result in more exposure of bathroom surfaces to human norovirus. However, we did not gather data on number of customers from the establishments we visited, so we were unable to test this hypothesis.

Risk factors for the presence of human norovirus were determined based on characteristics and equipment in establishment bathrooms. Norovirus-positive samples were more likely to be found in unisex bathrooms than in single-sex bathrooms, possibly because twice as many patrons use unisex bathrooms than use single-sex bathrooms. Additionally, unisex bathrooms tend to be single occupancy instead of multiple occupancy. Thus, more people may use a single unisex toilet than a toilet in a multiple occupancy bathroom. However, no significant difference was found between multiple occupancy bathrooms and single occupancy bathrooms (data not shown), suggesting the need for further research to understand the difference in findings between unisex and single-sex bathrooms.

Most norovirus-positive samples came from the underside of the toilet seat, followed by the toilet flush handle, inner door handle, and the sink faucet. Leone et al. *(36)* found similar results in their literature review of the presence of human norovirus on bathroom surfaces. Norovirus-positive samples were found on toilet seats in five studies *(9, 15, 19, 34, 64)* and on sink faucet handles *(15, 22, 23),* toilet flush handles *(48),* and bathroom door handles *(22, 34, 48)* in fewer studies. These results suggest that areas further away from the toilet are less likely to harbor norovirus contamination; toilet surfaces (especially the underside of the seat) would be closest to vomiting and diarrheal events during which high numbers of norovirus particles could be shed *(3)*. Flushing a toilet can reaerosolize virus particles, allowing them to be deposited onto bathroom surfaces, with the most droplets likely settling onto surfaces near the toilet *(5)*. Surfaces not contaminated by aerosolized droplets (e.g., sink faucets or door handles) could become contaminated by contact with norovirus on users' hands *(6, 52)*.

Our results also indicated that norovirus-positive samples were somewhat more likely to be found in bathrooms with automatic (touchless) flush toilets than in bathrooms with manual (touch) flush toilets ($P = 0.0515$). However, the opposite was true for the door handle mechanism; norovirus-positive samples were more likely in bathrooms with door handles that must be touched than in bathrooms with touchless door handles ($P = 0.0848$). Berry et al. *(7)* found that individuals perceived lower pathogen risk when using bathrooms with an automatic flush toilet than in bathrooms with manual flush toilets, which in turn decreased the likelihood that individuals would wash their hands. Lack of hand washing after using an automatic flush toilet could account for the higher presence of norovirus in those bathrooms due to the spread of pathogens to other bathroom surfaces via contact with contaminated hands. Contaminated hands may also explain the higher presence of norovirus in bathrooms with door handles that must be touched. Results of numerous studies have revealed that norovirus can be readily transferred from contaminated hands to hard surfaces *(27, 29, 52),* and Barker et al. *(6)* found that norovirus can be transferred to up to seven surfaces touched in sequence.

Most norovirus infection outbreaks in the United States are caused by GII noroviruses, specifically GII.4 viruses *(13, 58)* of which 16% of outbreaks have a foodborne etiology based on epidemiologic information. In our study, the prevalence of GI (43%) and GII (57%) noroviruses was very similar, and no GII.4 viruses were detected. In contrast, Boxman et al. *(10)* reported 2 GI-positive and 33 GII-positive surfaces in food establishments, and GII.4 was the most frequently detected genotype. Recent environmental surveillance studies revealed that human noroviruses in the environment are sometimes more genetically diverse than are outbreak strains, suggesting that the genotype distribution of noroviruses associated with sporadic or asymptomatic infections is higher. In sewage samples, the proportion of GI versus GII noroviruses is often similar *(30, 62),* which could also indicate different survival characteristics for GI and GII viruses.

Because we collected samples from only three geographical regions in the eastern United States, our findings may not be generalizable to the entire country. We visited

each establishment only once, so our findings represent only a snapshot in time of the prevalence of human noroviruses in each commercial food establishment during the winter months. Norovirus prevalence likely varies based upon season, patronage volume, effectiveness of sanitation procedures, and the chance that an infected individual would visit a given establishment. Because we chose to set population density parameters by state, we were unable to assess whether population density was a significant factor for norovirus presence.

Human noroviruses are found only rarely on surfaces in bathrooms in food establishments under nonoutbreak conditions. The factors of being a chain establishment, having a unisex bathroom, having automatic flush toilets, and having door handles that must be touched all increased the likelihood of norovirus contamination in food establishment bathrooms. Future research should consider how the layout of bathrooms can affect the presence and spread of microorganisms, e.g., testing the differences between single occupancy and multiple occupancy bathrooms and between high-touch and low-touch bathrooms. Our data suggest that routine environmental monitoring for norovirus during nonoutbreak periods is not a practical way to determine cleanliness. One alternative technique that is commonly used in food processing and health care settings is to assay for ATP bioluminescence *(14, 16, 21, 38, 63)*. Although viruses do not contain ATP, bacteria and fecal matter do. Thus, ATP may be a good indicator of surface cleanliness because assays for this molecule are fast and may be more affordable than other methods.

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