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Diagnostic performance of rectal swab versus bulk stool specimens for the detection of rotavirus and norovirus: Implications for outbreak investigations☆

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Abstract

Background—In January of 2008, during the peak of the rotavirus season in Guatemala, a gastroenteritis outbreak with high mortality among infants was reported in Guatemala. Despite extensive efforts, the investigation was limited by the lack of bulk stool specimens collected, particularly from the more severely dehydrated or deceased children.

Objectives—We evaluated the diagnostic performance of rectal swab specimens compared with bulk stool for the detection of rotavirus and norovirus.

Study design—Patients with diarrhea (≥3 loose stools in 24 h) were enrolled through an ongoing surveillance system in Guatemala. From January through March 2009, we attempted to enroll 100 patients <5years old captured by the diarrhea surveillance, and collected paired bulk stool and rectal swabs specimens from them. Specimens were tested for norovirus using real-time reverse transcription-polymerase chain reaction and for rotavirus via enzyme immunoassay.

Results—We enrolled 102 patients with paired specimens; 91% of 100 paired specimens tested for rotavirus yielded concordant results positive for rotavirus with a negativity rate of 83%. Among 100 paired specimens tested for norovirus, 86% were concordant norovirus detection and the negativity rate was 85%. The diagnostic performance for rotavirus and norovirus detection did not differ significantly between the two specimen types.

☆*Disclaimer*: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Competing interests

None declared.

Ethical approval

The protocol received approval from the institutional review boards of the Universidad del Valle de Guatemala (Guatemala City, Guatemala) and US Centers for Disease Control and Prevention (Atlanta, GA, Protocol #5150) and was approved by the Guatemalan Ministry of Public Health and Welfare.

Conclusions—Testing of properly collected fecal specimens using rectal swabs may be a viable alternative to bulk stool for detection of rotavirus and norovirus, particularly during outbreaks where collection of bulk stool may be difficult.

Keywords

Norovirus; Rotavirus; Diagnosis; Concordance; Surveillance; Guatemala

1. Background

An estimated four billion cases of diarrhea and over one million diarrhea-related deaths occur worldwide annually [1]. Rotavirus alone causes approximately half a million deaths each year among children aged <5 years, with most deaths occurring in developing countries [2]. Norovirus is a leading cause of diarrheal disease among older children and adults, and the leading cause of diarrheal disease outbreaks worldwide [3]. Because the clinical features of acute gastroenteritis caused by different enteric pathogens are similar, etiological confirmation of the infection requires laboratory testing of fecal specimens. Laboratory confirmation of enteric pathogens is essential for disease surveillance, and early diagnosis of outbreaks could help determine the source of transmission and rule out other etiologies that may be managed differently, thus providing critical guidance for the implementation of effective control measures [4].

Though the detection of enteric bacteria and parasites typically rely on culture and microscopy techniques, the most widely method used for the detection of rotavirus is antigen detection in the stool by enzyme immunoassay (EIA) [5], but for the detection of norovirus this method lacks adequate sensitivity [6]. Thus, detection of norovirus relies primarily on molecular techniques such as real-time reverse transcription polymerase chain reaction (RT-qPCR) [3]. Both EIA and RT-qPCR typically use bulk stool specimens for testing. At least one study has demonstrated better performance from bulk stool compared to rectal swab specimens for the detection of rotavirus [7]. Another study showed that rectal swab specimens were comparable to bulk stool for diagnosis of acute norovirus infection during outbreak settings [8].

In January of 2008, during the peak of the rotavirus season in Guatemala, an acute gastroenteritis outbreak with high mortality resulting in 23 confirmed deaths in children <5 years of age was reported by the Department of Santa Rosa, Guatemala (K. Lindblade, unpublished data). Despite extensive efforts, the investigation and etiological identification was limited by the lack of bulk stool specimens collected, particularly from the more severely dehydrated or deceased children. Whereas rectal swab specimens could be collected from these children for microbiologic testing of bacterial enteric pathogens, testing for norovirus and rotavirus requires bulk stool specimens. The limited bulk stool specimens collected during this outbreak were negative for rotavirus, but there were insufficient bulk stools to test for norovirus. The lack of bulk stool specimens during this outbreak possibly limited the detection of norovirus and rotavirus and, ultimately, precluded confirmation of the etiology of the outbreak.

2. Objectives

We conducted a study during the following rotavirus season to assess the diagnostic performance of rectal swab specimens preserved in phosphate buffered saline (PBS) solution versus bulk stool specimens for the detection of rotavirus and norovirus among children enrolled with diarrhea through ongoing facility-based surveillance in Guatemala.

3. Study design

3.1. Diarrhea surveillance

The US Centers for Disease Control and Prevention's (CDC) International Emerging Infections Program (IEIP), in collaboration with the Guatemalan Ministry of Public Health and Welfare and the Universidad del Valle de Guatemala (UVG), initiated active facility-based surveillance for diarrheal, respiratory, febrile, and acute infectious neurological diseases in Santa Rosa, Guatemala, in 2007. The Department of Santa Rosa has a population of 308.522 persons and is located 80 km southeast of Guatemala City. The main objectives of the laboratory-based surveillance system are to determine the etiology-specific burden of the diseases under surveillance. The surveillance system operates within the public healthcare structures, and captures patients of all ages presenting to the only government hospital in the Department of Santa Rosa and the ambulatory clinics in the municipality of Nueva Santa Rosa.

Trained surveillance nurses identified patients admitted with signs or symptoms suggestive of diarrhea by reviewing ward registers for diarrhea-related admission diagnoses or by determining the chief complaints of patients waiting to be admitted from the emergency department or seen at ambulatory clinics. A case of diarrhea was defined as ≥3 loose stools in a 24-h period during the last seven days prior to the current visit in a person of any age admitted to the hospital or presenting to the health center or posts under surveillance. Clinical and epidemiologic data were collected using standardized questionnaires, and, in the case of hospitalized patients, chart extractions were also conducted. All specimens were tested for enteric viruses, bacteria, and parasites.

3.2. Rectal swab performance study

From January through March 2009, we attempted to collect paired rectal swab and a bulk stool specimen from each patient <5 years of age meeting the case definition for diarrhea. This time period was selected as it corresponds to the rotavirus season based on laboratory-based surveillance data [9]. Both specimen types were collected simultaneously and within 24 h of admission to the hospital or during the ambulatory clinic visit. Rectal swabs were collected directly from the patient by trained nurses, using Fisher-brand polyester tipped applicators (Thermo Fisher Scientific Inc, NH, USA). Nurses were instructed to moisten the rectal swab in sterile transport medium, insert swab gently into the rectal sphincter approximate 2–3 cm, rotate to rectal swab 360°, and gently remove the swab. After checking for presence of visible feces in the rectal swab, the swab was immediately inserted in a Falcon polypropylene conical-bottom tube with a dome seal screw cap (Becton, Dickinson, and Company, CA, USA) containing 5 ml of phosphate buffered saline (PBS) solution. Bulk stool specimens were collected in a plastic cup with a cap. All specimens were kept at 4 °C

after collection, and transported for processing and testing within 24 h of collection. All specimens were tested using a commercial qualitative EIA for the detection of rotavirus (Group A) (IDEIA Rotavirus test kits, Dako Ltd., Ely, United Kingdom) following manufacturer's instructions, and for norovirus genogroups I and II using a standard monoplex RT-qPCR [10]. To compare the viral load between bulk stool and rectal swab specimens, the cycle threshold (Ct) values of each positive norovirus RT-qPCR result were compared. Details for the extraction of nucleic acids, Ct-value cut-offs for positive and negative specimens, and RT-qPCR detection limits are described elsewhere [11]. The laboratory testing procedures did not differ by specimen type. Since the study was nested within an ongoing surveillance system fir diarrhea. All laboratory testing was conducted within a week of specimen collection.

3.3. Human subjects

Caregivers of children who met the case definition were requested to provide written, informed consent for the participation of their children. All data were stored and managed in a manner that protected all personal identifying information. The protocol received approval from the institutional review boards of the UVG (Guatemala City, Guatemala) and CDC (Atlanta, GA).

3.4. Data collection and analysis

Data were collected primarily using hand-held personal digital assistants (PDAs), and were managed and stored using SQL Server (Microsoft Corporation, Seattle, WA). We analyzed data using Statistical Analysis System version 9.1 (SAS Institute, Cary, NC). Frequencies were generated for categorical data, and means, medians, ranges, and ranges for continuous variables. We compared concordance of laboratory results from the two specimen types using McNemar test statistics (X^2) with their respective p-values. Mean Ct values were compared by Student's independent t-test.

4. Results

We enrolled 102 patients <5 years of age with diarrhea, of which 98 had paired bulk stool and rectal swab specimens tested for both norovirus and rotavirus, two had paired specimens tested only for rotavirus, and two had paired specimens tested only for norovirus. Median age was one year (range: 0–4 years). Among the 100 patients with paired specimens tested for rotavirus, 38 (38%) patients were enrolled from ambulatory clinics and 62 (62%) from the hospital. Fifty-six (56%) were positive for rotavirus; 51 from rectal swab and 52 from bulk stool specimens. Thirty-six (58%) hospitalized patients and 20 (53%) ambulatory patients tested positive for rotavirus by either specimen type. The median age of children positive for rotavirus was one year (range: 0–4 years); 62 (62%) were male. Mean optical density values were not significantly different by specimen type. Overall, 91 (91%) of paired specimens yielded concordant results for rotavirus and there was no significant difference in diagnostic performance between bulk stool and rectal swab specimens ($p = 0.8$). Concordance in detection of rotavirus from rectal swab and bulk stool specimens did not vary significantly by age groups, among hospitalized or ambulatory patients, or with respect to clinical presentation (Table 1).

Among the 100 patients with paired specimens tested for norovirus, 37 (37%) patients were enrolled from ambulatory clinics and 63 (63%) from the hospital. Twenty-two (22%) tested positive for norovirus by either specimen type; 16 from rectal swab and 14 from bulk stool specimens. Eleven (17%) hospitalized patients and five (14%) patients from ambulatory clinics tested positive for norovirus. The median age of children positive for norovirus was one year (range: 0–4 years); 60 (60%) were male. Mean Ct values were not significantly different by specimen type. Norovirus detection was concordant using rectal swab and bulk stool specimens in 86 (86%) patients; six patients were positive by bulk stool only and eight patients were positive by rectal swab only. Eight (36%) specimens were positive for norovirus from both bulk stool and rectal swab specimens. Thus, overall diagnostic performance for norovirus detection did not differ significantly between the two specimen types ($p = 0.6$). Likewise, diagnostic performance of bulk stool and rectal swab specimens for norovirus did not vary significantly by age group, among hospitalized or ambulatory patients, or with regard to clinical characteristics (Table 2). Among the patients positive for norovirus by either specimen type, 8 (36%) had concordant results. In contrast, among patients positive for rotavirus by either specimen type, 47 (84%) had concordant results.

Among the 22 specimens positive for norovirus, 8 (36%) were genogroup I and 14 (64%) were genogroup II positive. The mean Ct value of norovirus positive rectal swab specimens did not differ significantly among patients positive by bulk stool versus those with negative bulk stool specimens (24.2 and 27.6, respectively; $p = 0.4$). Similarly, the mean Ct value of bulk stool specimens positive for norovirus were similar among patients with positive rectal swabs and those with negative rectal swabs (28.5 and 30.1, respectively, $p = 0.6$).

5. Discussion

Our results showed a good overall diagnostic performance for the detection of rotavirus from rectal swab specimens compared to results obtained by testing bulk stool specimens. In contrast, a study among US children found that a bulk stool specimen was better than a rectal swab specimen for rotavirus detection by EIA testing, with a rate of detection from bulk stools (49%) significantly higher than that from rectal swabs (27%) [7]. This difference could be attributed to several factors. First, our study was conducted using highly trained surveillance nurses dedicated to the specific task of enrolling patients with diarrhea and collecting appropriate samples and carefully maintaining a cold chain, and thus it is possible that the rectal swabs in our study contained more fecal material with viable viruses. Second, in the US study, both a rectal swab and a bulk stool specimens were not obtained from the same patient, and while patients with swabs and bulk stool were matched for age and season, it is possible that the specimens were not directly comparable [7]. Another study conducted among hospitalized patients in Guinea-Bissau that evaluated paired specimen from the same patient found that, similarly to our study, the use of rectal swab appeared to have a similar detection rate for rotavirus infection [12]. Finally, it is possible that the level of viral shedding during acute rotavirus illness was greater among children in Guatemala compared with the United States and thus enough viral antigen was present in the swabs to test positive by EIA, although we do not have any specific data to test this hypothesis.

We also found that the overall diagnostic performance of rectal swab specimens for the molecular detection of norovirus was similar to that of bulk stool specimens. However, the two specimen types frequently yielded discordant results which could not be explained by differences in Ct values between specimen types. Thus, both specimen types appear to have limitations with regard to detection of norovirus, and from an epidemiological perspective neither could appropriately be classified as a gold standard for comparative purposes. Similarly, for discordant results among specimens tested for rotavirus could not be explain by analysis of optical density values.

The findings in this study are subject to several limitations. First, although attempts were made to collect the two types of specimens simultaneously, we did not collect detailed information on the times of specimen collection. Future studies should attempt to collect these data, as it would be important to determine, specifically for the discordant patients for whom rectal swabs tested positive whereas bulk stools tested negative, whether the rectal swabs could have been collected significantly earlier in the clinical presentation versus bulk stools. Second, our sample size and study period were based primarily on the detection of rotavirus and the number of specimens positive for norovirus was relatively smaller. The lower detection rates for norovirus resulted in insufficient power to conclusively asses the relative performance for norovirus detection from each specimen type. Finally, specimen collection using rectal swabs was conducted by trained staff specifically hired for this task, and this may not reflect the reality, particularly during field outbreak investigation. Also, many clinical laboratories use rectal swabs that are commonly placed in a bacterial transport medium to collect samples for bacteriological cultures, and the ability of these media transport media to preserve enteric viruses in rectal swab specimens was not assessed by this study and should also be evaluated.

Despite these limitations, our findings highlight the potential benefits of using rectal swabs for detection of rotavirus and norovirus, particularly during outbreak situations when multiple specimens with a presumably common etiology are available. Use of rectal swab specimens allows increased ease in collecting, short-term storing, and transporting of clinical specimens. This is particularly important in outbreak settings where the logistics for collecting specimens can be extremely challenging, especially when attempting to obtain specimens from severely ill, dehydrated, or recently deceased patients. Even for surveillance purposes, ease of specimen collection using rectal swabs could result in a higher rate of specimen collection, particularly in countries where surveillance is conducted by clinical staff that is already overwhelmed with other responsibilities. Unlike bulk stool specimens which can be stored at 4 °C for many years without affecting viral titers, storing specimens in diluted form as with swabs could affect viral stability, and this should be considered when retrospective testing of specimens for nororvirus or other viruses is expected. Although we did not compare the feasibility of successfully sequencing and geno-typing rectal swab with bulk stool specimens, the mean Ct values derived from PCR analyses of norovirus positive rectal swab and bulk stool specimens did not differ significantly suggesting that collection of rectal swabs would not limit the capacity to conduct these additional laboratory tests when needed to further characterize the norovirus etiology.

In conclusion, testing of specimens from rectal swabs could be a viable alternative to bulk stool for detection of rotavirus, and this could be particularly useful during outbreaks and other settings where collection of bulk stool may be difficult. Although the high rate of discordance among patients positive for norovirus by either specimen type suggests sub-optimal sensitivity of both, this may be overcome in an outbreak setting by collection of either specimen type from multiple patients, thus allowing confirmation of the etiology of the outbreak. Additional studies should be conducted in other settings with sample size calculations based on the prevalence of norovirus in order to conclusively describe the performance of rectal swabs for norovirus detection before changing current specimen collection recommendations [13].

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Table 1

Concordance between rectal swab and bulk stool specimens for detection of rotavirus, Santa Rosa, Guatemala, 2009.

	Concordant Specimens			Discordant Specimens			*P*-value[a]
	Swab+/bulk+	**Swab−/bulk−**	**Total concordance (95% CI)**	**Swab+/bulk−**	**Swab−/Bulk+**	**Total discordance (95% CI)**	
Characteristic							
Overall (*n* = 100)	47	44	91% (85–97%)	4	5	9% (3–15%)	0.8
Age group							
<1 year (*n* = 40)	19	18	93% (84–100%)	0	3	8% (0–16%)	0.1
1 to <2 years (*n* = 39)	19	14	85% (73–96%)	4	2	15% (4–27%)	0.5
2 to <5 years (*n* = 21)	9	12	100% (NA)	0	0	0%	1.0
Healthcare setting							
Ambulatory (*n* = 38)	16	18	89% (80–99%)	1	3	11% (1–20%)	0.4
Hospital (*n* = 62)	31	26	92% (85–99%)	3	2	8% (1–15%)	0.7
Clinical characteristic							
Onset <1.5 days (*n* = 50)	24	22	92% (84–100%)	2	2	8% (0–16%)	1.0
Current diarrhea (*n* = 89)	42	39	91% (85–97%)	4	4	9% (3–15%)	1.0
Vomiting (*n* = 75)	38	30	91% (84–97%)	3	4	9% (3–16%)	0.7
<1 year old + vomiting (*n* = 30)	14	13	90% (79–100%)	0	3	10% (0–21%)	0.1

CI = confidence interval; NA = not applicable.

[a]Based on McNemar test for difference between paired proportions (specimen type).

Table 2

Concordance between rectal swab and bulk stool specimens for detection of norovirus, Santa Rosa, Guatemala, 2009.

	Concordant specimens			Discordant specimens			*P*–value[a]
	Swab+/bulk+	**Swab−/bulk−**	**Total concordance (95% CI)**	**Swab+/bulk−**	**Swab−/Bulk+**	**Total discordance (95% CI)**	
Characteristic							
Overall (*n* = 100)	8	78	86% (79–93%)	8	6	14% (7–21%)	0.6
Age group							
<1 year (*n* = 41)	5	28	80% (68–93%)	5	3	20% (7–32%)	0.5
1 to <2 years (*n* = 39)	3	31	87% (77–98%)	3	2	13% (2–23%)	0.7
2 to <5 years (*n* = 20)	0	19	95% (85–100%)	0	1	5% (0–15%)	0.5
Healthcare setting							
Ambulatory (*n* = 37)	1	31	86% (75–98%)	4	1	14% (3–25%)	0.2
Hospital (*n* = 63)	7	47	86% (77–94%)	4	5	14% (6–23%)	0.7
Clinical characteristic							
Onset <1.5 days (*n* = 50)	3	38	82% (71–93%)	5	4	18% (7–29%)	0.8
Current diarrhea (*n* = 89)	7	68	84% (77–92%)	8	6	16% (8–23%)	0.6
Vomiting (*n* = 76)	8	59	88% (81–95%)	4	5	12% (5–19%)	0.8
<1 year old with vomiting (*n* = 31)	5	22	87% (75–99%)	2	2	13% (1–25%)	1.0

CI = confidence interval; NA = not applicable.

[a]Based on McNemar test for difference between paired proportions (specimen types).