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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 <5 years 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.
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.

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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

☆ *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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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 Fisherbrand 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