# Importance of Cholera and Other Etiologies of Acute Diarrhea in Post-Earthquake Port-au-Prince, Haiti

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*Abstract.* We estimated the proportion of diarrhea attributable to cholera and other pathogens during the rainy and dry seasons in patients seen in two urban health settings: a cholera treatment center (CTC) and oral rehydration points (ORPs). During April 1, 2011–November 30, 2012, stool samples were collected from 1,206 of 10,845 patients who came to the GHESKIO CTC or to the community ORPs with acute diarrhea, cultured for *Vibrio cholerae,* and tested by multiplex polymerase reaction. *Vibrio cholerae* was isolated from 409 (41.8%, 95% confidence interval [CI] = 38.7–44.9%) of the 979 specimens from the CTC and in 45 (19.8%, 95% CI = 14.8–25.6%) of the 227 specimens from the ORPs. Frequencies varied from 21.4% (95% CI = 16.6–26.7%) during the dry season to 46.8% (95% CI = 42.9–50.7%) in the rainy season. *Shigella*, enterotoxigenic *Escherichia coli*, rotavirus, and *Cryptosporidium* were frequent causes of diarrhea in children less than five years of age.

## INTRODUCTION

The first cases of cholera in Haiti were detected in the Artibonite River Valley region in October 2010,[1,2] nine months after the country had experienced one of the most devastating earthquakes in its history. The cholera epidemic challenged an already fragile health infrastructure and threatened gains that had been made in controlling infantile diarrhea.[3]

Compared with other cholera epidemics in the region, the Haiti epidemic was more than three times worse in its peak incidence year than the one in Peru, which had been considered the most severe in the region (4.80 cases per 1,000 in Haiti versus 1.46 cases per 1,000 in Peru).[4,5] The number of cases reported countrywide in the first year of the epidemic, 476,714, well exceeded the 383,227 cases reported by the World Health Organization (WHO) for South America in 1991, the year the seventh cholera pandemic reached that continent.[6] In 2010, Haiti contributed 57% of all cholera cases and 53% of all cholera deaths reported to WHO,[5] although the true percentage may be much less because Bangladesh does not report to the WHO. As of August 27, 2013, the Ministry of Health (Ministère de la Santé Publique et de la Population [MSPP]) has reported 673,444 cases and 8,248 deaths.[7] The *Vibrio cholerae* strain responsible for the Haiti epidemic was characterized as toxigenic *V. cholerae*, serogroup O1, serotype Ogawa, biotype El Tor, a strain that shares similar genetic characteristics with the South Asian strain.[8–10]

Dwellers in the large urban slums of Port-au-Prince are particularly at risk for diarrheal diseases.[11,12] GHESKIO (Haitian Group for the Study of Kaposi Sarcoma and Opportunistic Infections) is a local institution dedicated to human immunodeficiency virus–acquired immunodeficiency syndrome (HIV-AIDS) care, research and training. In the slums near the GHESKIO campus (Cité de Dieu, Cité l'Eternel, and Cité Plus), poor sanitation conditions, scarce access to clean water, coupled with inadequate hygiene practices, make diarrheal illness an ever-present threat. After the earthquake and before the cholera epidemic, GHESKIO partnered with MSPP and other organizations to tackle the spread of diarrheal illnesses and of other communicable diseases in those communities by establishing clean water stations, providing community health education, and treating those with acute diarrheal illness. In the wake of the cholera epidemic, GHESKIO joined the MSPP country response to this major health emergency, strengthened its community efforts, and led a highly successful pilot vaccine campaign by using Shanchol in a multipronged effort to control the cholera epidemic in these most vulnerable communities.[13–15]

The main objective of this study was to determine the contribution of cholera to cases of acute diarrheal illness in an urban setting during the dry and rainy season and to compare the causes of acute diarrhea among individuals who presented to the GHESKIO–cholera treatment center (CTC) versus those who presented to the community oral rehydration points (ORPs). As a secondary objective, we surveyed other etiologic agents responsible for acute diarrheal illness using the xTAG GPP polymerase chain reaction (PCR).

## METHODS

**Study population and specimen selection.** The CTCs, cholera treatment units (CTUs), and community ORPs, which were coordinated by MSPP, were set up all over Haiti to care for patients with acute diarrhea. Patients with moderate-to-severe diarrhea were admitted to CTCs and CTUs for care; those with mild diarrhea symptoms were seen at their community ORPs.

Medical care to patients with acute diarrhea was provided according to the guidelines set forth by MSPP. The GHESKIO cholera network included 2 CTCs, one at each of the GHESKIO main centers, GHESKIO-INLR and GHESKIO-IMIS, which were located, respectively at the southern and northern tip of the capital, 8 CTUs, and 10 ORPs.

This study evaluated only specimens collected during April 1, 2011–November 30, 2012 at one CTC, the GHESKIO-INLR CTC and the ORPs located in the surrounding area. Every tenth consecutive patient seen at either the CTC or the ORP had a stool specimen collected for cholera culture. Every other consecutive specimen collected for culture was also tested by using the xTAG GPP PCR. Stools were collected during the dry and rainy seasons from the GHESKIO-CTC and the ORPs

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over the course of the study and were sent to the GHESKIO Laboratory for *V. cholerae* culture and for xTAG GPP PCR testing (Luminex Corp., Toronto, Ontario, Canada). Demographics and basic clinical information were recorded in the laboratory electronic registry.

**Conventional stool culture and biochemical test procedures.** One stool sample was collected at the bedside during hospitalization and transported to the laboratory in coolers for culture. Stool specimens were inoculated in alkaline peptone water and plated on thiosulfate citrate bile sucrose agar for identification of *Vibrio cholerae*. Suspicious colonies were selected for further identification on MacConkey or blood agar.[16] After culture for *V. cholerae*, stool specimens that would normally be discarded were aliquoted into sterile vials, archived, and frozen at –80°C.

**Nucleic acid extraction.** Every other specimen sent to the laboratory for cholera culture was selected for the Luminex test: 635 tests for patients from the CTC and 131 tests for those from the ORPs. Total nucleic acid was isolated from 10% stool suspensions by mechanical disruption using Precellys Soil Mix Beads (Bertin Corp., Rockville, MD), followed by purification using the EasyMAG (BioMerieux, Craponne, France) automated extraction system, and elution in 25 µL of buffer. MS2 bacteriophage was added to each specimen before nucleic acid extraction as recommended by the manufacturer.

**Nucleic acid amplification and Luminex xTAG GPP PCR.** Multiplexed reverse transcription PCR was performed with Luminex xTAG GPP reagents designed for the simultaneous amplification of sequences corresponding to targets listed in Table 1. The MS2 phage sequences were simultaneously amplified in the same reaction mixtures with pathogen sequences to confirm success of nucleic acid extraction and absence of endogenous inhibitors of amplification. Target-specific PCR products were detected by using Luminex xMAP technology with fluorescent microspheres and the MAGPIX microfluidics system. Data were analyzed by using xPONENT 3.1 and TDAS LSM software packages. All work was approved by the GHESKIO and the Weill Cornell Medical College institutional review boards.

**Data collection and analysis.** Data collection was done using a Microsoft Excel spreadsheet (Microsoft, Redmond, WA). The STATA 12.1 (StataCorp, College Station, TX) software package was used for data analysis. The primary

TABLE 1
Luminex xTAG GPP polymerase chain reaction target pathogens*

| Bacteria | Viruses | Parasites |
|---|---|---|
| *Salmonella* spp. | Adenovirus 40/41 | *Giardia lamblia* |
| *Shigella* spp. | Rotavirus A | *Cryptosporidium* |
| *Campylobacter* spp. | Norovirus GI/GII | *Entamoeba histolytica* |
| *Yersinia enterolytica* | | |
| Enterotoxigenic *Escherichia coli* (ETEC) LT/ST | | |
| *E. coli* O157:H7 | | |
| Shiga-like toxin–producing *E. coli* (STEC) stx1/stx2 | | |
| *Clostridium difficile* toxin A/B | | |
| *Vibrio cholera* | | |

*GI = group I; GII = group II; LT = heat-labile toxin; ST = heat-stable toxin.

measures in our study were the frequencies of stool specimens in the sampled populations in the CTC and the ORPs that were positive for *V. cholerae*. In addition, we calculated estimated frequencies of specimens in the sampled populations that were positive for each of the pathogens in the xTAG GPP PCR (Table 1). Categorical variables (sex, age group, *V. cholerae* culture results, xTAG GPP PCR results, and HIV status) were summarized as proportions and frequencies and confidence intervals (CIs) were calculated at the 95% level. Differences between groups were assessed by using the chi-square test. Odd ratios (ORs) and *P* values were two-tailed; *P* values ≤ 0.05 were deemed statistically significant.

Stepwise binary logistic regression was used to assess differences in infection type in the cohort less than five years of age. Age < 5 years versus age ≥ 5 years was the dependent variable, and each major pathogen in the xTAG GPP PCR was represented as a separate variable. The same approach was used to evaluate infection type and HIV status.

## RESULTS

**Characteristics of the study population.** During April 1, 2011–November 30, 2012, GHESKIO cared for 10,845 patients with acute diarrhea, 7,372 at its CTC and 3,473 at its ORPs. Demographic characteristics of the population admitted to the GHESKIO-CTC and ORPs and those of the patients whose specimens were tested in bacteriology and by the xTAG GPP PCR are summarized in Table 2. The demographics of the patients sampled for *V. cholerae* and for the xTAG GPP PCR were similar with respect to sex and age < 5 years to those of the entire patient population. Children less than five years of age made up 19.5% of the entire patient population; 14.5% were from the CTC and 30.1% were from the ORPs. More than 70% of patients who came to the CTC were more than 15 years of age. Significant differences were observed for female sex among the ORP groups (*P* = 0.0018), in the 5–15 years and >15 years age groups among the CTC groups (*P* = 0.0007 and *P* < 0.0005, respectively), and in the totals for children less than five years of age and HIV status (*P* = 0.0017). The HIV voluntary counseling and testing in the CTC reported that 8.7% (95% CI = 7.6–9.9%) of the population was positive for HIV.

***Vibrio cholerae* culture results.** Four hundred nine of the 979 stool specimens (41.8%, 95% CI = 38.7–44.9%) from the GHESKIO-CTC grew *V. cholerae* in culture, whereas 45 of the 227 specimens (19.8%, 95% CI = 14.8–25.6%) from the ORPs were positive for *V. cholerae* in culture. When culture and xTAG GPP PCR results were taken as valid test results for cholera, the percentages in those sampled with both assays increased to 48.4% (95% CI = 45.2–51.6%) in the CTC and 21.6% (95% CI = 16.4–27.5%) in the ORPs.

Proportions of cultures positive for *V. cholerae* over the course of the study period are shown in Figure 1. Peaks in the proportions of *V. cholerae*-positive cultures correspond to the rainy months in the Port-au-Prince metropolitan area. The proportion of stool specimens positive for *V. cholerae* in culture ranged from 21.4% (59 of 276, 95% CI = 16.6–26.7%) during the months with low average rainfall (< 5 mm; January, February, July, November, and December), to 32.1% (88 of 274, 95% CI = 26.6–38.0%) during the months with moderate average rainfall (5–20 mm; March, June, and August), and to 46.8% (307 of 656, 95% CI = 42.9–50.7%) during the months

TABLE 2
Demographic characteristics of the CTC and ORP patients evaluated for cholera, Port-au-Prince, Haiti*

| Characteristic | Entire population | | | Tested by bacteriologic analysis | | | Tested by xTAG GPP polymerase chain reaction | | |
|---|---|---|---|---|---|---|---|---|---|
| | CTC (n = 7,372) | ORP (n = 3,473) | Total (n = 10,845) | CTC (n = 979) | ORP (n = 227) | Total (n = 1,206) | CTC (n = 635) | ORP (n = 131) | Total (n = 766) |
| Female sex | 3,514 (47.7) | 2,227 (64.1)† | 5,741 (52.9) | 498 (50.9) | 127 (56.0)† | 625 (51.8) | 315 (49.6) | 69 (52.7)† | 384 (50.1) |
| Age group, years | | | | | | | | | |
| < 5 | 1,066 (14.5) | 1,047 (30.1) | 2,113 (19.5)† | 118 (12.1) | 72 (31.7) | 190 (15.8)† | 79 (12.4) | 44 (33.6) | 123 (16.1)† |
| 5–15 | 1,079 (14.6)† | 410 (11.8) | 1,489 (13.7) | 111 (11.3)† | 34 (15.0) | 145 (12.0) | 67 (10.6)† | 20 (15.3) | 87 (11.4) |
| > 15 | 5,227 (70.9)† | 2,016 (58.0) | 7,243 (66.8)† | 750 (76.6)† | 121 (53.3) | 871 (72.2)† | 489 (77.0)† | 67 (51.1) | 556 (72.6)† |
| HIV status | | | | | | | | | |
| Tested | 2,400 (32.6) | 109 (3.1) | 2,509 (23.1) | 786 (80.3) | 10 (4.4) | 796 (66.0) | 573 (90.9) | 4 (3.1) | 577 (75.3) |
| Positive | 218 (9.1) | 0 (0) | 218 (8.7)† | 92 (11.7) | 0 (0) | 92 (11.6)† | 75 (13.1) | 0 (0) | 75 (13.0)† |

*Values are no. (%). CTC = cholera treatment center; ORP = oral rehydration point; HIV = human immunodeficiency virus.
†Significant difference by chi-square test ($P < 0.05$).

with high average rainfall (> 20 mm; April, May, September, and October) ($P < 0.0005$). Overall, the proportion of specimens positive for *V. cholerae* by culture was higher during the rainy seasons than during the dry seasons (395, 87.0% versus 59, 13.0%; $P < 0.0005$).

**Detection of causes of acute diarrhea by the xTAG GPP PCR.** The xTAG GPP PCR detected bacterial pathogens in 351 (45.8%, 95% CI = 42.3–49.4%) specimens, viruses in 80 (10.4%, 95% CI = 8.4–12.8%) specimens, and protozoan parasites in 98 (12.8%, 95% CI = 10.5–15.4%) specimens. As shown in Figure 2A, the most frequently detected bacterial pathogens in patients from the GHESKIO-CTC patients were *V. cholerae* (231, 35.9%, 95% CI = 32.2–39.6%), *Shigella* spp. (83, 13.1%, 95% CI = 10.4–15.7%), and enterotoxigenic *E. coli* (ETEC) (50, 7.9%, 95% CI = 5.8–10.0%), whereas in the ORPs, the most frequently detected bacterial pathogens were ETEC (27, 20.6%, 95% CI = 13.6–27.6%), *V. cholerae* (23, 17.6%, 95% CI = 11.0–24.2%), and *Shigella* spp. (21, 16.0%, 95% CI = 9.7–22.4%). Among the viruses detected in the CTC specimens were norovirus (26, 4.1%, 95% CI = 2.5–5.6%), rotavirus (25, 3.9%, 95% CI = 2.4–5.4%), and adenovirus (16, 2.5%, 95% CI = 1.3–3.7%), whereas in the ORP specimens, the viruses detected were norovirus (17, 13.0%, 95% CI = 7.1–18.8%), rotavirus (1, 0.8%, 95% CI = 0.7–2.2), and adenovirus (2, 1.5%, 95% CI = 0.6–3.7%). Twenty (76.9%, 95% CI = 56.4–91.0%) of the 26 cases of rotavirus were detected in specimens collected during the dry months. As for protozoan parasites, the xTAG GPP PCR detected 55 (8.7%, 95% CI = 6.5–10.9) cases of *Giardia* and 21 (3.3%,

95% CI = 1.9–4.7%) cases of *Cryptosporidium* in the CTC; in the ORP, the assay detected 21 (16.0%, 95% CI = 9.7–22.4%) cases of *Giardia* and 3 (2.3%, 95% CI = 0.3–4.9%) cases of *Cryptosporidium*. No cases of infection with *Entamoeba histolytica* were detected.

Results of the 766 specimens tested for *V. cholerae* by culture and the xTAG GPP PCR are summarized in Table 3. Compared with culture, the sensitivity and specificity of the xTAG GPP PCR for detection of *V. cholerae* in our study were 74.6% (95% CI = 68.6–79.9%) and 86.8% (95% CI = 83.6–89.6%), respectively.

**Pathogens detected by the xTAG GPP PCR in children less than five years of age.** Frequencies of the pathogens detected by the xTAG GPP PCR in the CTC and the ORP specimens according to age group are shown in Figure 2B. The most frequently detected pathogens in the specimens from children less than five years of age in the CTC were *Shigella* spp. (17, 21.5%, 95% CI = 13.1–32.2%), ETEC (16, 20.3%, 95% CI = 12.0–30.8%), rotavirus (5, 19.0%, 95% CI = 11.0–29.4%), *Cryptosporidium* (11, 13.9%, 95% CI = 7.2–23.5%), and *V. cholerae* (8, 10.1%, 95% CI = 4.5–19.0%). The pathogens most frequently detected in the specimens from children less than five years of age in the ORP were ETEC (12, 27.3%, 95% CI = 15.0–42.8%), norovirus (11, 25%, 95% CI = 13.2–40.3%), *Giardia* (11, 25%, 95% CI = 13.2–40.3%), *Shigella* spp. (8, 18.2%, 95% CI = 8.2–32.7%), and *Campylobacter* spp. (8, 18.2%, 95% CI = 8.2–32.7%). Children less than five years of age with diarrhea were less likely to have cholera (OR = 0.17, 95% CI = 0.10–0.27, $P < 0.0005$) but more likely to have viral pathogens detected (OR = 6.0, 95% CI = 3.7–9.8, $P < 0.0005$). Rotavirus was more frequently detected in children less than five years of age than in the older age groups (OR = 8.0, 95% CI = 3.6–17.8, $P < 0.0005$). Overall, non-cholera causes of diarrhea detected by the xTAG GPP PCR were more frequently found in children less than five years of age than in the older age groups (OR = 4.9, 95% CI = 3.5–7.0, $P < 0.0005$).

**HIV status and pathogens detected by the xTAG GPP PCR.** Ninety-two (11.6%, 95% CI = 9.4–14.0%) of the 796 patients for whom HIV results were available were HIV positive. Fifty-two (56.5%) patients were newly given a diagnosis of infection with HIV. The HIV-positive patients were more likely to have diarrhea caused by invasive bacteria (OR = 2.4, 95% CI = 1.4–4.1, $P = 0.001$). The HIV status was not correlated with the detection of protozoan parasites.

**Co-infection with more than one pathogen.** The xTAG GPP PCR detected two or more enteric pathogens in 170 (22.2%, 95% CI = 19.3–25.3%) of the 766 specimens tested.
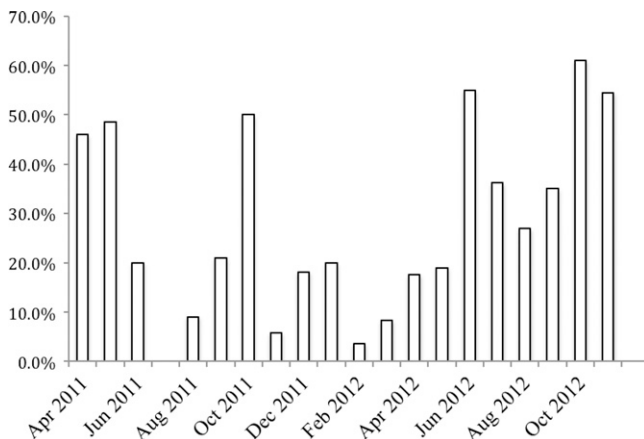


FIGURE 1. Proportion of specimens positive for *Vibrio cholerae* in cultures per month during the study period, Port-au-Prince, Haiti.

**A**



**B**
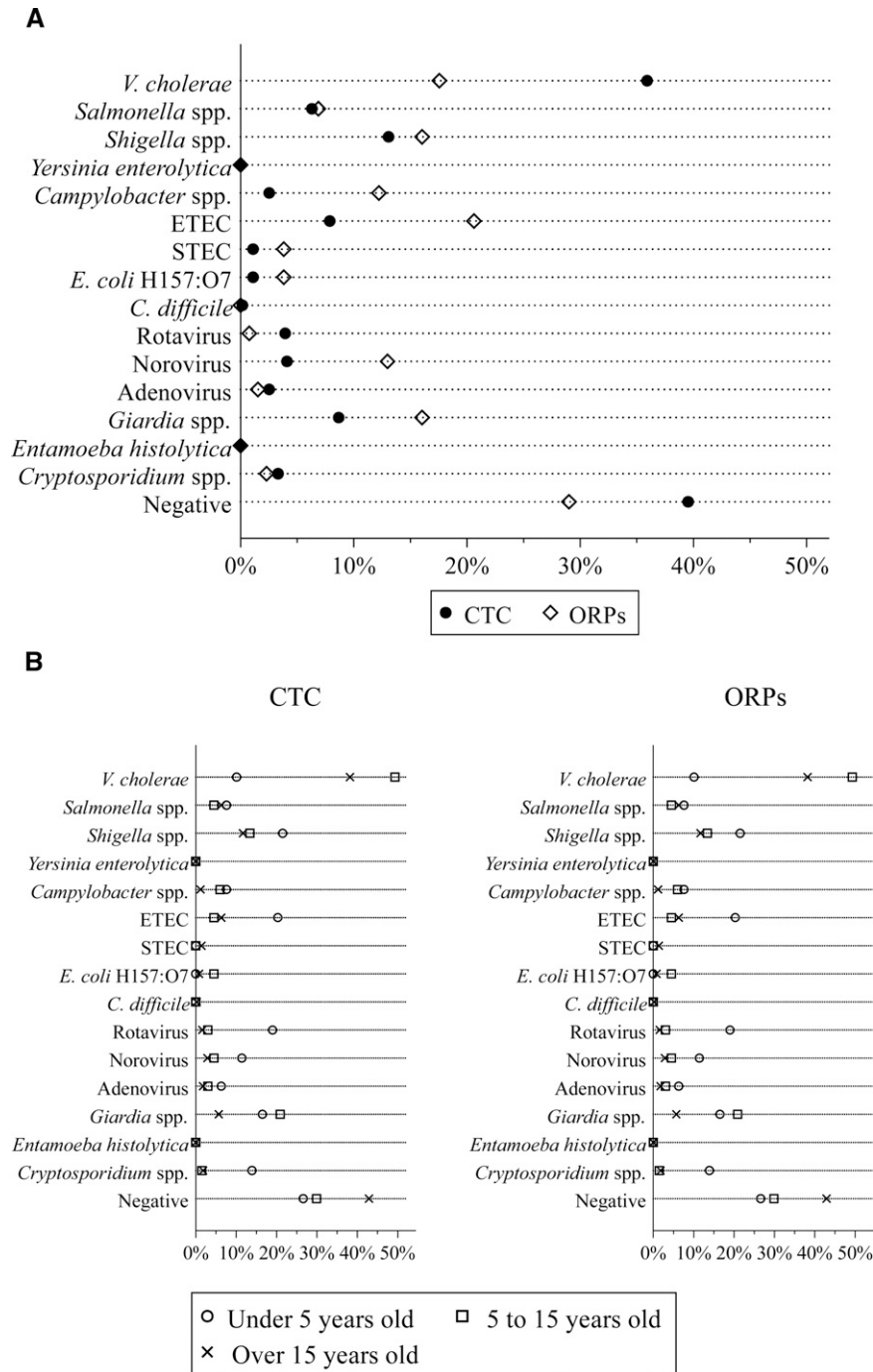
CTC                    ORPs



FIGURE 2.   **A**, Frequencies of pathogens detected by the xTAG GPP polymerase chain reaction (PCR) from patients seen in a cholera treatment center (CTC) versus those seen at oral rehydration points (ORPs), Port-au Prince, Haiti. **B**, Frequencies of pathogens detected by the xTAG GPP PCR in different age groups in the CTC and in the ORPs. ETEC = enterotoxigenic *Escherichia coli*; STEC = Shiga toxin–producing *E. coli*.

TABLE 3

*Vibrio cholerae* test results of 766 specimens tested by culture and the xTAG GPP polymerase chain reaction, Port-au-Prince, Haiti*

| Variable | *V. cholerae* culture result | | | Sensitivity (%), (95% CI) | Specificity (%), (95% CI) |
|---|---|---|---|---|---|
| xTAG GPP PCR result | Positive | Negative | Total | | |
| Positive | 182 | 69 | 251 | 74.6, (68.6–79.9) | 86.8, (83.6–89.6) |
| Negative | 62 | 453 | 515 | | |
| Total | 244 | 522 | 766 | | |

*PCR = polymerase chain reaction; CI = confidence interval.

*Vibrio cholerae* and at least one another pathogen were detected in 79 (10.3%, 95% CI = 8.3–12.7%) of the 766 specimens tested. In these 79 specimens from patients with cholera, the most frequently detected pathogens were *Shigella* spp. (29, 17.1%, 95% CI = 11.7–23.5%), *Giardia* (28, 16.5%, 95% CI = 11.2–22.9%), ETEC (17, 10.0%, 95% CI = 5.9–15.5%), and *Salmonella* spp. (9, 5.3%, 95% CI = 2.4–9.8%). Specimens from the ORPs were more likely to have two or more pathogens detected by the xTAG GPP PCR than those from the CTC (38, 29.0% versus 132, 20.8%; *P* = 0.039). Factors associated with the detection of two or more pathogens were age less than five years (OR = 2.6, 95% CI = 1.5–4.4, *P* = 0.001) and HIV seropositivity (OR = 2.0, 95% CI = 1.2–3.6, *P* = 0.012).

## DISCUSSION

In our study, we found that 41.8% of the patients sampled who came to the CTC and 19.8% of those seen in the community ORPs had acute diarrhea caused by cholera. This finding was expected because patients with cholera have more severe diarrhea and are more likely to be admitted to a CTC. Our results indicate that cholera still remains a major cause of diarrheal illness in the community, even during the dry season.

A significant proportion of patients had multiple enteric pathogens detected by the xTAG GPP PCR: 20.8% of those in the CTC and 29.0% of those in the ORPs. In addition, we found that children less than five years of age, although less likely to have cholera, were significantly more likely to have diarrhea caused by multiple enteric pathogens.[17–19] These results support the necessity to reinforce water and sanitation interventions to decrease waterborne causes of diarrhea in Haiti. As expected, viruses were more frequently detected in children less than five years of age. Rotavirus diarrhea appeared to follow a seasonal pattern; 20 of 26 cases (76.9%) were found during the dry season, 57.7% of the cases were in children less than five years of age, and 96.2% of the cases were seen in the CTC, indicating that the severity of diarrhea was sufficiently worrisome to warrant a visit to the CTC. The recent introduction of the rotavirus vaccine in Haiti holds much promise in reducing the burden of diarrhea in children less than five years of age.[20,21]

Similarly, most of the cases of infection with *Cryptosporidium* spp. were seen in children less than five years of age in the CTC. A recent study on the etiology of diarrheal disease in infants and young children in developing countries found that *Cryptosporidium,* irrespective of HIV status, was among the top five pathogens responsible for moderate to severe diarrhea in this population.[22]

The finding that the frequency of diarrhea caused by cholera was less among children less than five years of age than that seen in older age group is noteworthy. This finding could be caused by less cholera in young children or a higher frequency of other causes of diarrhea in young children. Additional studies would be required to distinguish between the two possibilities. There are, however, several studies conducted in endemic areas in Asia and Africa that show a high incidence of cholera among children during outbreaks.[23–25] A recent study from Bangladesh showed that the incidence of cholera increased with age for children less than five years of age.[26] This study concluded that increasing age, lower socioeconomic status, and lack of breastfeeding are key correlates of increased risk for cholera hospitalization among children less than five years of age in rural and urban Bangladesh. The children in our study could have been protected from cholera through breastfeeding. A number of studies have shown that breastfed children are less likely to have diarrheal illness and less likely to have cholera during an epidemic.[27–29] An alternative explanation is that children less than five years of age with diarrhea were less likely to have cholera because this was a newly introduced epidemic to which the whole population is immunologically naive.[30]

Of note, 39.5% of the specimens from the CTC and 29.0% of those from the ORP had no pathogens detected by the xTAG GPP PCR, although 14.3% of the CTC cases and 18.4% of the ORP cases were culture positive for *V. cholerae*. The cases that were negative by the xTAG GPP PCR and *V. cholerae* culture could have been caused by pathogens not included in the xTAG GPP PCR panel and/or to emerging or yet unrecognized causes of diarrhea in Haiti. We plan to further investigate these cases for the presence of other pathogens not included in the xTAG GPP PCR panel. Additional studies will focus on the pathogens detected by the xTAG GPP PCR by using classical methods, singleplex PCR, and sequencing.

The seroprevalence of HIV in the CTC patients in our study was relatively high (9.1–13.0%). More than half (56.5%) of the HIV-positive persons were given new diagnoses of infection with HIV at the CTC and had sought care at the CTC rather than at established HIV clinics because of diarrhea. These findings underscore the importance of integrating HIV pre-test counseling services at cholera treatment centers to manage more effectively cases of diarrhea in these patients and refer them for appropriate care after stabilization. The HIV-infected patients were more likely to have invasive bacterial organisms detected by the xTAG GPP PCR. A study conducted in the United States on bacterial causes of diarrhea among HIV-infected persons showed that after *C. difficile*, which accounted for 53.6%, invasive bacteria such as *Shigella* spp., *Campylobacter* spp., and *Salmonella* spp. organisms together accounted for 35.2% of all reported bacterial agents.[31]

There are limitations to our study. First, we did not perform confirmatory testing for all the pathogens in the xTAG GPP PCR. Culture was performed only for *V. cholerae*. Confirmatory testing would have enabled better characterization of some of the pathogens detected by the xTAG GPP PCR. Second, one must also bear in mind that inherent to all molecular testing is the possibility that because of its high sensitivity, the PCR might amplify DNA fragments from organisms that are no longer present, reflecting a previous infection. Molecular testing can also reflect carriage or persistence of an organism that does not cause any pathologic changes in its bearer. Although we ensured that we selected patients with acute diarrhea, we cannot completely exclude the possibility that some patients were carriers of the pathogens detected.

The low sensitivity of the xTAG GPP PCR observed in our study could have been caused by inappropriate handling or storage of the specimens, which were sometimes collected under difficult conditions. In addition, some of the *V. cholerae* culture results could have been negative because an estimated 30% of patients take antibiotics (e.g., tetracycline) before seeking care.

In conclusion, the results of our study show that cholera continues to play a major role as a cause of acute diarrheal illness 6–25 months after the epidemic started in Haiti.

Epidemiologic studies conducted every year in the same CTC and ORPs can provide useful information on the dynamic of the cholera epidemic. The impact of the recently introduced cholera vaccine could also be evaluated. Furthermore, the PCR identified the spectrum of pathogens encountered in patients with acute diarrhea in an urban setting in Haiti. Co-infection with multiple enteric pathogens is common and should be considered for appropriate management of patients with acute diarrhea. Adequate preventive and control measures are essential to alleviate the impact of cholera and other causes of diarrhea on the Haitian population.

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