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BRIEF REPORT

Poor Sensitivity of Stool Culture Compared to Polymerase Chain Reaction in Surveillance for *Vibrio cholerae* in Haiti, 2018–2019

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We report on the sensitivity and specificity of stool culture compared to polymerase chain reaction for detecting *Vibrio cholerae* in Haiti during the waning period of the initial outbreak in 2018–2019. We found that stool culture (with a sensitivity of 33.3% and specificity of 97.4%) may not be sufficiently robust in this context.

Keywords. cholera; culture; Haiti; PCR; *Vibrio cholerae*.

Stool culture is often challenging to perform in the regions where cholera outbreaks occur, and this was the situation in rural central Haiti, where a major cholera epidemic occurred between 2010 and 2019. By 2018, cases of cholera continued to be reported in Haiti, though at a substantially lower rate than prior years (25.5 cases per 100 000 in 2018) [1], and the last reported cholera case by the Haitian Ministry of Health was sampled on 4 February 2019 in the Artibonite department [2], until a new outbreak was detected in September 2022. We implemented a surveillance protocol for acute watery diarrhea between 2018 and 2019 in the Centre department that used both stool culture and polymerase chain reaction (PCR) for *Vibrio cholerae*. This brief report describes the sensitivity and specificity of stool culture relative to PCR for *V cholerae* detection in patients with acute watery diarrhea in central Haiti.

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MATERIALS AND METHODS

Beginning in September 2018, we enrolled, with informed consent, subjects who were admitted to a medical facility and who met clinical definition of cholera (3 or more episodes of acute watery diarrhea in 24 hours without blood) in an epidemiologic study in Mirebalais, Haiti, as part of an institutional review board–approved protocol by Mass General Brigham in the United States (protocol number 2018P000350) and Zanmi Lasante in Haiti (protocol number 113). Fresh stool samples were collected in clean, nonchlorinated disposable containers and transported in a cool box to the medical facility's laboratory for processing and storage. The samples for cultures were immediately placed into Cary-Blair media and refrigerated at 4°C–8°C until transport in cold storage to a local enteric diseases laboratory run collaboratively by the Haitian Ministry of Health and a nongovernmental organization within a week of collection [3, 4]. A standard stool culture on thiosulfate citrate bile salt sucrose (TCBS) agar (BD Difco) was performed by a well-trained and experienced laboratory technician who had worked on similar studies throughout the cholera epidemic [5, 6]. Inoculated TCBS plates were incubated overnight at 35°C–37°C. Colonies suggestive of *V cholerae* (yellow with opaque centers and translucent peripheries) were subcultured on heart infusion agar (BD Difco), with further screening of isolates by Gram staining and oxidase or string testing. Serogrouping was confirmed by a standard slide agglutination method using polyvalent O1 antisera, followed by monovalent Ogawa and Inaba antisera for serotyping [7].

In addition to the standard stool culture performed in the local laboratory, all enrolled cases had undiluted stool spotted on filter paper, dried, and stored at ambient temperature in individual bags with desiccant. Samples were transported within 14 days of collection to our laboratory in Boston, Massachusetts, for testing by PCR. If shipping was not possible within 14 days, the spotted cards were frozen at −20°C until transport [8]. Evaluation for the presence of *V cholerae* was performed in Boston using a multiplexed PCR assay, based on amplification of the toxin-encoding *ctxAB* gene and the O antigen–encoding *rfb* gene. The multiplex reaction included primer sets specific for both the O1- and O139-encoding *rfb* genes (though no toxigenic O139 infection has been detected in Haiti) [9]. This protocol has been validated and used previously in other laboratories and, in a study using Bayesian latent class modeling, was found to have a sensitivity and specificity similar to microbiological culture [10]. Each PCR run included positive and negative controls. Laboratory-cultured strains of O1, O139, and nontoxigenic *V cholerae* were used as positive controls. Sample elution buffer was used as the negative

Table 1. Clinical Characteristics of Study Patients Included in the Analysis

Characteristic	No. of Patients (%)
Age, y (n = 60)	
<5	12 (20.0)
5–15	6 (10.0)
>15	42 (70.0)
Sex (n = 60)	
Male	28 (46.7)
Female	32 (53.3)
Dehydration (n = 43)	
Severe	10 (23.3)
Moderate	22 (51.2)
None or mild	11 (25.6)
Antibiotics received at the medical facility after sample collection (n = 43)	
Yes	34 (79.1)
No	9 (20.9)

Table 2. Detection of *Vibrio cholerae* in Stool Samples by Culture and Polymerase Chain Reaction Methods

Results by Culture	Results by PCR, No. of Samples		
	Positive	Negative	Total
Positive	7	1	8
Negative	14	38	52
Total	21	39	60

Abbreviation: PCR, polymerase chain reaction.

control. Additionally, each sample extract was tested with a 16S ribosomal DNA PCR to confirm the presence of bacteria and absence of PCR inhibitors. The controls allowed for confirmation of each of the multiplexed reactions.

RESULTS

Between September 2018 and March 2019, 60 cases of acute watery diarrhea meeting clinical case definition of cholera [11] were enrolled in the study. The clinical characteristics of these cases are shown in Table 1. There were 28 (46.7%) male and 32 (53.3%) female patients, ranging in age from 7 months to 80 years, with a median age of 31 years. Of 43 subjects with complete clinical information, 22 (51.2%) presented with moderate dehydration and 10 (23.3%) with severe dehydration. Stool culture and PCR results are presented in Table 2. Stool culture was 33.3% (7/21) sensitive and 97.4% (38/39) specific for detecting the presence of *V cholerae,* when PCR was considered the gold standard.

DISCUSSION

When compared with PCR, stool culture in this cohort of 60 samples from patients with acute watery diarrhea in central Haiti in a period of waning cholera incidence during the first Haitian cholera outbreak performed worse than would be expected in the diagnosis of *V cholerae* in stool. This finding is important because, as cholera cases wane over the course of a big outbreak, rigorous laboratory-based surveillance must correspondingly increase to identify ongoing transmission, detect outbreaks early, and allow public health officials to be on alert for new surges. While many studies have shown culture to be overall concordant with rapid diagnostic tests (RDTs) and PCR [12–15], others have found it to be less sensitive under certain conditions [9, 16, 17], albeit not to the extent found in our study. Culture may have nonetheless remained the practical gold standard because other diagnostic tools such as RDTs can have low to moderate specificity [15, 16, 18] and PCR requires more expensive equipment and skilled staff [9, 16].

Upon extensive review of the laboratory practice, no technical or process-related reasons were identified to explain the discrepancy in sensitivity between culture and PCR. Because *V cholerae* was detected by PCR in our subjects' stool and they had dehydrating diarrhea meeting clinical case definition for cholera, we considered them to have cholera, despite a negative stool culture, although historically stool culture is considered a gold standard diagnostic tool. Subjects' stool specimens in our study were collected before the administration of facility-prescribed antibiotics, but we could not exclude the use of antibiotics prior to presentation for care. Off-prescription antibiotic medications are widely available in both the informal sector and over the counter in Haiti [19] and may have been a factor contributing to poor stool culture sensitivity. If this factor played a role in reducing the sensitivity of stool culture during surveillance for cholera, it would have important implications for national surveillance by this method. Other possible explanations for the low culture sensitivity are the presence of lytic phage (ICP2), which was previously identified in a cholera patient sample in Haiti [20] and has been shown to interfere with diagnostic results [16, 21], or storage procedures including refrigeration or lack of routine enrichment in alkaline peptone water, which can enhance the isolation of *V cholerae* when few organisms are present. Some, but not all, study samples were enriched.

Thousands of stool cultures were undertaken in Haiti between 2019 and 2022 by the Haitian National Laboratory and no *V cholerae* was detected after 4 February 2019 [2]. Similarly, we did not detect *V cholerae* either by culture or PCR after January 2019, despite continued surveillance by our group through 2020. Our study is limited by being at a single site, with a limited sample size, given that the epidemic was already waning at the time of enrollment. Its finding should therefore be interpreted with caution. Although there is no standardized method for the preservation of stool samples on filter paper, the method used in our study is based on the well-

established techniques used for preserving nucleic acids from dried blood spots [8], where sample desiccation and cold storage are the main factors in preserving quality.

CONCLUSIONS

Remarkable public health efforts in Haiti during the first cholera outbreak resulted in an extended period from 2019 to 2022 without reported cholera cases and a declaration that Haiti was cholera-free [22, 23]. While stool culture is an important component of surveillance for diarrheal disease, our study raises the possibility that the use of more convenient, low-maintenance sample collection and preservation tools (eg, filter paper) and the addition of more sensitive testing methods (eg, PCR) that are stable to the rigors of a rural environment and transportation delays that are typical in areas where cholera is a serious threat may contribute to a better alert system for the emergence of *V cholerae*.

Notes

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Potential conflicts of interest. All authors report no potential conflicts.

References

1. Pan American Health Organization/World Health Organization. Epidemiological update: cholera. **2018**. Available at: https://www.paho.org/en/documents/epidemiological-update-cholera-11-october-2018. Accessed 21 November 2022.
2. Rebaudet S, Dély P, Boncy J, et al. Toward cholera elimination, Haiti. Emerg Infect Dis **2021**; 27:2932–6.
3. Orozco JD, Greenberg LA, Desai IK, et al. Building laboratory capacity to strengthen health systems: the Partners In Health experience. Clin Lab Med **2018**; 38:101–17.
4. Bopp CA, Ries AA, Wells JG. Laboratory methods for the diagnosis of epidemic dysentery and cholera. WHO/CDS/CSR/EDC/99.8. Atlanta, GA: Centers for Disease Control and Prevention; **1999**.
5. Matias WR, Julceus FE, Abelard C, et al. Laboratory evaluation of immunochromatographic rapid diagnostic tests for cholera in Haiti. PLoS One **2017**; 12: e0186710.
6. Franke MF, Jerome JG, Matias WR, et al. Comparison of two control groups for estimation of oral cholera vaccine effectiveness using a case-control study design. Vaccine **2017**; 35:5819–27.
7. World Health Organization (WHO). A manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. WHO/CDS/CSR/RMD/2003.6. Geneva, Switzerland: WHO; **2003**.
8. World Health Organization (WHO). WHO manual for HIV drug resistance testing using dried blood spots. Geneva, Switzerland: WHO; **2020**.
9. Hoshino K, Yamasaki S, Mukhopadhyay AK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. FEMS Immunol Med Microbiol **1998**; 20:201–7.
10. Sayeed MA, Islam K, Hossain M, et al. Development of a new dipstick (Cholkit) for rapid detection of *Vibrio cholerae* O1 in acute watery diarrheal stools. PLoS Negl Trop Dis **2018**; 12:e0006286.
11. Global Task Force on Cholera Control. Cholera outbreak response: field manual. **2018**. Available at: https://www.gtfcc.org/wp-content/uploads/2020/05/gtfcc-cholera-outbreak-response-field-manual.pdf. Accessed 21 November 2022.
12. Ontweka LN, Deng LO, Rauzier J, et al. Cholera rapid test with enrichment step has diagnostic performance equivalent to culture. PLoS One **2016**; 11:e0168257.
13. Mwaba J, Ferreras E, Chizema-Kawesa E, et al. Evaluation of the SD Bioline cholera rapid diagnostic test during the 2016 cholera outbreak in Lusaka, Zambia. Trop Med Int Health **2018**; 23:834–40.
14. Debes AK, Murt KN, Waswa E, et al. Laboratory and field evaluation of the crystal VC-O1 cholera rapid diagnostic test. Am J Trop Med Hyg **2021**; 104:2017–23.
15. Chowdhury G, Senapati T, Das B, et al. Laboratory evaluation of the rapid diagnostic tests for the detection of *Vibrio cholerae* O1 using diarrheal samples. PLoS Negl Trop Dis **2021**; 15:e0009521.
16. Alam M, Hasan NA, Sultana M, et al. Diagnostic limitations to accurate diagnosis of cholera. J Clin Microbiol **2010**; 48:3918–22.
17. Sinha A, SenGupta S, Guin S, et al. Culture-independent real-time PCR reveals extensive polymicrobial infections in hospitalized diarrhoea cases in Kolkata, India. Clin Microbiol Infect **2013**; 19:173–80.
18. Harris JR, Cavallaro EC, de Nóbrega AA, et al. Field evaluation of crystal VC rapid dipstick test for cholera during a cholera outbreak in Guinea-Bissau. Trop Med Int Health **2009**; 14:1117–21.
19. Moise K, Bernard JJ, Henrys JH. Evaluation of antibiotic self-medication among outpatients of the state university hospital of Port-Au-Prince, Haiti: a cross-sectional study. Pan Afr Med J **2017**; 28:4.
20. Seed KD, Yen M, Shapiro BJ, et al. Evolutionary consequences of intra-patient phage predation on microbial populations. Elife **2014**; 3:e03497.
21. Nelson EJ, Grembi JA, Chao DL, et al. Gold standard cholera diagnostics are tarnished by lytic bacteriophage and antibiotics. J Clin Microbiol **2020**; 58: e00412-20.
22. Haitian Ministry of Communications. 4 février 2019–4 février 2022—trois (3) ans sans choléra en Haïti. 2022 [in French]. **2014**. Available at: https://www.communication.gouv.ht/2022/02/4-fevrier-2019-4-fevrier-2022-trois-3-ans-sans-cholera-en-haiti/. Accessed 21 November 2022.
23. Cousins S. Eliminating cholera in Haiti. Lancet **2022**; 399:1928–9.