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Evidence of False Positivity for *Vibrio* Species Tested by Gastrointestinal Multiplex PCR Panels, Minnesota, 2016–2018

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Background. Syndromic gastrointestinal multiplex polymerase chain reaction (PCR) panels (GMPPs) are used by an increasing number of clinical laboratories to identify enteric pathogens. *Vibrio* species are included on GMPPs, but because of the low prevalence of vibriosis, performance characteristics for these panels have been difficult to measure.

Methods. All *Vibrio* spp. cases identified by GMPPs in Minnesota during 2016–2018 (n = 100) were assessed to identify differences between culture-confirmed cases and those that were PCR-positive only.

Results. Overall, 47% of cases had *Vibrio* species recovered by culture. Two GMPPs were used in Minnesota, Verigene EPT and FilmArray GIP, and the recovery rate of *Vibrio* spp. was significantly different between these platforms (Verigene EPT 63%, compared with FilmArray GIP 28%). No distinct seasonality was identified among GMPP-positive, culture-negative cases, whereas culture-confirmed case incidence peaked during July and August. Among cases with no other pathogen detected by the GMPP, confirmed cases reported a lower rate of bloody diarrhea (odds ratio [OR], 0.7; $P = .004$) and were less likely to have a symptom duration >14 days (OR, 0.3; $P = .04$). Confirmed cases were also more likely to include reports of consuming food items typically associated with *Vibrio* spp. infection or to have another likely source of infection (eg, international travel or contact with an untreated body of fresh or salt water or marine life; OR, 9.6; $P = .001$).

Conclusions. The combined findings indicate that cases identified by GMPP that did not have culture confirmation were less likely to include symptoms or exposures consistent with vibriosis. These findings emphasize the need for improvements to testing platform specificity and the importance of combining clinical and exposure information when diagnosing an infection. This study underscores the importance of maintaining the ability to culture *Vibrio* species to aid in accurate diagnoses.

Keywords. CIDT; gastrointestinal illness; multiplex PCR panel; public health; vibriosis.

Gastrointestinal illness caused by *Vibrio* spp. is commonly associated with the consumption of raw or undercooked seafood. Vibriosis is uncommon in the United States (0.4 cases/100 000 population in 2015), but is likely vastly underdiagnosed because routine stool culture methods for gastroenteritis are suboptimal to detect *Vibrio* spp. [1]. For every laboratory-confirmed *V. parahaemolyticus* case, it is estimated that there are 142 additional cases [2]. However, *Vibrio* spp. can now be detected by several gastrointestinal multiplex PCR panels (GMPPs), a type of culture-independent diagnostic test (CIDT) that gives clinical laboratories the ability to routinely test for *Vibrio* spp. directly from stool specimens.

GMPPs have been reported to be more sensitive than culture for detection of certain enteric bacterial pathogens [3–6]. As use of GMPPs increases by clinical laboratories, the number of positive laboratory tests for *Vibrio* spp. reported to public health agencies is also increasing [7]. This increase might be attributable to increased sensitivity of GMPPs, increased testing by health care providers, false-positive tests, or some combination of these factors. From clinical and public health perspectives, the assessment of whether a positive GMPP result for *Vibrio* spp. likely represents a true positive or a false positive is important. In Minnesota, an increase in *Vibrio* spp. detections by GMPPs was identified through reportable disease surveillance during 2016–2018. This study used clinical and epidemiologic data gathered through routine surveillance to assess the performance characteristics of GMPPs for *Vibrio* spp.

METHODS

The study period was January 1, 2016, to December 31, 2018. Vibriosis is a reportable disease in Minnesota; state law mandates that clinical laboratories submit an isolate or clinical specimen from a CIDT-positive test to the Minnesota Department of Health (MDH). Clinical laboratories sent CIDT-positive stool specimens in transport medium to MDH according to the

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Association of Public Health Laboratories (APHL) CIDT interim guidelines [8].

A case was defined as a Minnesota resident with a positive test for *Vibrio* spp. by GMPP at a clinical laboratory where the specimen had reflex culture performed at the clinical laboratory or at MDH-PHL and for whom a specimen or isolate was received at MDH. A confirmed case was defined as one in which *Vibrio* spp. was recovered from the GMPP-positive specimen by culture at either the clinical laboratory or MDH. A probable case was defined as one in which *Vibrio* spp. was not recovered by culture from the GMPP-positive specimen. Cases were considered to have a co-detection if any other pathogen (reportable or nonreportable) was detected on the GMPP at the clinical laboratory.

Interviews were attempted for all cases to ascertain clinical symptoms and risk factors for vibriosis during the week before illness onset using the Cholera and Other *Vibrio* Illness Surveillance report form. Risk factors for vibriosis were defined as travel outside the United States, consumption of seafood, and contact with an untreated body of fresh or salt water or marine life [9]. Illness duration was defined as time from onset to cessation of symptoms.

At MDH, *Vibrio*-positive stool specimens in transport medium were plated to thiosulfate-citrate-bile salts-sucrose (TCBS) agar and used to inoculate alkaline peptone water. After enrichment in alkaline peptone water at 37°C, samples were plated to TCBS agar at 6 hours and 18 hours. TCBS plates were incubated aerobically for 48 hours at 37°C and examined at 24 and 48 hours. Up to 3 suspected *Vibrio* spp. colonies per TCBS plate were screened using the matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF) Biotyper system (Bruker Daltonics, Inc., Billerica, MA, USA) using RUO, version 6903 (2016–2017), and RUO, version 7311 (2018), and confirmed by biochemical tests [10]. *Vibrio* spp. isolates received from clinical laboratories were confirmed by MALDI-TOF and biochemical testing at MDH.

Data were analyzed using SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA). Cases with co-detections on GMPPs were excluded from analyses of illness symptoms and duration. The 2-sided Wilcoxon rank-sum test was used to compare median durations. Categorical variables were analyzed using logistic regression models or the Fisher exact test.

RESULTS

In total, 100 GMPP-positive *Vibrio* spp. cases were identified during 2016–2018 by 21 clinical laboratories, including 47 culture-confirmed at MDH-PHL (35 *V. parahaemolyticus;* 9 *V. cholerae* non-O1, non-O139; 2 *V. cholerae* O1; and 1 *V. parahaemolyticus* and *V. fluvialis* dual infection) and 53 culture-negative at MDH-PHL. Two clinical laboratories submitted 1 isolate each that were from specimens previously positive by GMPP, and these were included in the culture-confirmed case count. *Vibrio* spp. recovered by culture totaled 7 of 15 cases in 2016, 11 of 24 cases in 2017, and 29 of 61 cases in 2018. Ninety of the 100 cases were available for interview.

Two GMPPs were used by clinical laboratories during the study period, Verigene Enteric Panel Test (EPT; Luminex Corporation, Austin, Texas; n = 54 cases) and FilmArray Gastrointestinal Panel (GIP; BioFire Diagnostics, Salt Lake City, UT, USA; n = 46 cases). The EPT uses gene targets *rfbL*, *trkH*, and *tnaA* to identify *Vibrio* spp., while the GIP does not list the gene targets used to identify *Vibrio* spp. in the package insert. The mean and median time from specimen collection to receipt at MDH was 2 days for both platforms (Verigene EPT: range, 1–5 days; and FilmArray GIP: range, 0–7 days). The culture recovery rate for specimens positive by Verigene EPT was 63%, compared with 28% for FilmArray GIP (P = .006).

Seasonality

The distribution of confirmed cases by month varied (median, 1.5 cases in each month; range, 0–17). Confirmed cases were more likely to be identified during summer, compared with other seasons after adjusting for testing platform (adjusted odds ratio [aOR], 3.1; 95% CI, 1.3–7.3; P = .01), and less likely to be identified during spring (aOR, 0.1; 95% CI, 0.03–0.5; P = .004). The distribution of probable cases did not differ by month (median, 5 cases in each month; range, 1–7).

Symptom Profiles

Ninety cases provided clinical information on interview. Of those, 30 had a co-detected pathogen and 60 did not. Probable cases were more likely to include reports of bloody diarrhea than were confirmed cases (Table 1). For cases who had recovered by the time of interview, no significant difference was reported in median illness duration between confirmed and probable cases. However, durations for probable cases (3–180 days) had a much greater range than that observed for confirmed cases (5–22 days); confirmed cases were more likely to report illness durations ≤14 days than probable cases (Table 1). Assessing differences in symptom profiles by testing platform was limited by the low number of confirmed cases tested on the FilmArray GIP platform who were able to be interviewed and did not have another pathogen detected (n = 3) (Table 2).

Risk Factors

Confirmed cases were significantly more likely to include reports of eating any seafood, raw seafood specifically, and oysters (Table 1). Forty-one (95%) of 43 confirmed cases reported exposure to ≥1 risk factor for *Vibrio* spp. infection during the week before illness onset, compared with 32 (68%) of 47 probable cases (OR, 9.6; P = .001). For cases tested on Verigene EPT, 30 (100%) confirmed cases reported exposure to ≥1 risk factor for *Vibrio* spp. infection during the week

Table 1. Symptoms, Seasonality, and Risk Factors of Gastrointestinal Multiplex PCR Panel–Positive *Vibrio* spp. Cases (n = 100)

		No. Interview Responses	Confirmed, No. (%)	Probable, No. (%)	OR (95% CI)[a]	*P* Value
Illness characteristics[b]	Diarrhea	60	31 (100)	27 (93)	Undefined	.23
	Vomiting	60	10 (32)	12 (41)	1.5 (0.5–4.3)	.59
	Abdominal cramps	57	23 (77)	22 (81)	1.3 (0.4–4.9)	.75
	Fever	57	10 (33)	5 (19)	2.2 (0.6–7.5)	.24
	Bloody diarrhea	59	1 (3)	9 (32)	0.7 (0.01–0.6)	.004
	Illness duration ≤7 d	57	12 (41)	8 (29)	1.8 (0.6–5.3)	.41
	Illness duration ≤14 d	53	22 (81)	14 (54)	3.8 (1.1–13)	.04
	Illness duration, median (range),[c] d	47	8 (5–22)	12 (3–180)	-	.17
Seasonality[d]	Specimen collection season[e]					
	Winter	10	2 (20)	8 (80)	0.3 (0.06–1.7)	.19
	Spring	18	3 (20)	15 (80)	0.1 (0.03–0.5)	.004
	Summer	46	29 (63)	17 (37)	3.1 (1.3–7.3)	.01
	Fall	26	13 (50)	13 (50)	1.4 (0.5–3.8)	.47
Risk factors and exposures[f]	International travel	89	8 (19)	7 (15)	1.3 (0.4–3.9)	.78
	Any seafood consumption[g]	87	38 (90)	28 (62)	5.8 (1.7–19.0)	.003
	Any raw seafood consumption[g]	82	35 (83)	6 (15)	28.3 (8.6–92.9)	<.001
	Oyster consumption[a]	86	30 (71)	8 (18)	11.3 (4.1–31.1)	<.001
	Untreated water or marine life contact[a]	83	15 (36)	9 (22)	2.0 (0.8–5.2)	.23
	At least 1 exposure to a known risk factor for vibriosis[a]	90	41 (95)	32 (68)	9.6 (2.0–45.1)	.001

Abbreviations: OR, odds ratio; PCR, polymerase chain reaction.

[a]Mantel-Haenszel odds ratio and Fisher exact test 2-sided *P* value.

[b]Excludes *Vibrio* spp. cases with any other pathogen detected on the gastrointestinal multiplex PCR panel.

[c]Wilcoxen 2-sample test *P* value.

[d]Specimen collection season was compared with all other seasons and adjusted for testing platform in a logistic model.

[e]Winter = January 1–March 31; spring = April 1–June 30; summer = July 1–September 30; and fall = October 1–December 31.

[f]The 90 cases who were available for interview were included in risk factor analysis. Known risk factors included consumption of any seafood, contact with an untreated body of water, contact with marine life, and international travel.

[g]"Any seafood consumption" includes consumption of raw seafood and oysters. "Any raw seafood consumption" includes consumption of oysters if consumed raw. The categories are not exclusive.

before illness onset, compared with 12 (71%) of 17 probable cases (Table 2). For cases tested on FilmArray GIP, consumption of any raw seafood and raw oysters was significantly associated with culture confirmation of *Vibrio* spp. infection, but exposure to at least 1 known risk factor for vibriosis was not significantly associated with culture confirmation (OR, 2.8; $P = .29$) (Table 2).

DISCUSSION

Minnesota observed a 4-fold increase in *Vibrio* spp. cases diagnosed by GMPPs during 2016–2018, but fewer than half were recovered by culture. Some of this increase might be attributable to a true increase in the number of *Vibrio* spp. infections [11] and clinicians' ability to more easily test specifically for *Vibrio* spp. There were several clinical and epidemiologic differences between culture-confirmed and culture-negative (probable) cases. Culture-confirmed cases more often reported an illness duration ≤14 days and less often reported bloody diarrhea, which are both consistent with the clinical syndrome description of vibriosis [12]. Additionally, confirmed cases were more likely to include reports of well-documented risk factors for vibriosis, including raw seafood consumption, foreign travel, and contact with potentially contaminated water, than were culture-negative cases. Furthermore, there was a distinct summer seasonality for confirmed cases, which fits the known epidemiology of vibriosis [11, 13]; conversely, the number of probable cases did not change by season.

The differences between culture-positive and culture-negative cases indicate that a substantial proportion of the culture-negative cases represent false-positive tests for *Vibrio* spp. by GMPPs. In this study, we observed higher culture recovery rates from specimens tested by Verigene EPT (63%) compared with the FilmArray GIP (28%). The reasons for this difference were not determined; more research is needed to assess performance of these GMPP platforms in clinical practice.

The utility of comparing clinical and epidemiological characteristics of culture-positive vs culture-negative cases to evaluate the positive predictive value of CIDT tests has been demonstrated for other pathogen–CIDT combinations [14, 15]. This study is among the first to apply this method with GMPPs. Similar research has also shown potential problems with false-positive results from GMPPs for other pathogens, specifically *Salmonella enterica* [4, 16].

Table 2. Symptoms, Seasonality, and Risk Factors of Gastrointestinal Multiplex PCR Panel–Positive *Vibrio* spp. Cases by Testing Platform

		Verigene EPT (n = 47)					FilmArray GIP (n = 43)				
		No. Interview Responses	Confirmed, No. (%)	Probable, No. (%)	OR (95% CI)[a]	*P* Value	No. Interview Responses	Confirmed, No. (%)	Probable, No. (%)	OR (95% CI)[a]	*P* Value
Illness characteristics[b]	Diarrhea	39	28 (100)	10 (91)	Undefined	.28	21	3 (100)	17 (94)	Undefined	1.00
	Vomiting	39	8 (29)	5 (45)	0.48 (0.1–2.0)	.45	21	2 (67)	7 (39)	3.1 (0.2–41.5)	.55
	Abdominal cramps	37	20 (74)	8 (80)	0.7 (0.1–4.2)	1.00	19	3 (100)	2 (13)	Undefined	.01
	Fever	38	7 (26)	3 (27)	0.9 (0.2–4.4)	1.00	20	3 (100)	14 (82)	Undefined	1.00
	Bloody diarrhea	38	1 (4)	3 (30)	0.09 (0.01–1.0)	.05	21	0 (0)	6 (33)	Undefined	.53
	Illness duration ≤7 d	39	12 (43)	3 (27)	2.0 (0.4–9.2)	.48	18	0 (0)	5 (29)	Undefined	1.00
	Illness duration ≤14 d	38	22 (81)	6 (55)	3.7 (0.8–17.0)	.12	15	-	8 (53)	Undefined	-
	Illness duration, median (range),[c] d	35	8 (5–22)	12 (6–53)	-	.18	12	-	12 (3–180)	Undefined	-
Seasonality[d]	Specimen collection season[e]										
	Winter	54	0 (0)	3 (15)	Undefined	.05	46	2 [15]	5 (15)	1.0 (0.2–6.0)	1.00
	Spring	54	2 (6)	8 (40)	0.09 (0.02–0.5)	.003	46	1 [8]	7 (21)	0.3 (0.3–2.8)	.41
	Summer	54	23 (68)	6 (30)	4.9 (1.5–16.1)	.008	46	6 (46)	11 (33)	1.7 (0.5–6.3)	.5
	Fall	54	9 (26)	3 (15)	2.0 (0.5–8.7)	.50	46	4 (31)	10 (30)	1.0 (0.3–4.1)	1.00
Risk factors and exposures[f]	International travel	46	5 (17)	2 (13)	1.4 (0.2–8.2)	1.00	43	3 [23]	5 (17)	1.5 (0.3–7.5)	.68
	Any seafood consumption[g]	46	28 (93)	11 (69)	6.4 (1.1–37.8)	.04	41	10 (83)	17 (59)	3.5 (0.7–19.1)	.17
	Any raw seafood consumption[g]	45	26 (87)	2 (13)	42.3 (6.8–261.6)	<.001	37	9 (75)	4 (16)	15.8 (2.9–85.2)	.001
	Oyster consumption	46	24 (80)	3 (19)	17.3 (3.7–81.0)	<.001	40	6 (50)	5 (17)	4.6 (1.0–20.4)	.06
	Untreated water or marine life contact	46	9 (30)	4 (25)	1.3 (0.3–5.1)	1.00	37	6 (50)	5 (20)	1.0 (0.9–17.9)	.12
	At least 1 exposure to a known risk factor for vibriosis[a]	47	30 (100)	12 (71)	Undefined	.004	43	11 (85)	20 (67)	2.8 (0.5–14.9)	.29

Abbreviations: OR, odds ratio; PCR, polymerase chain reaction.

[a]Mantel-Haenszel odds ratio and Fisher exact test 2-sided *P* value.

[b]Excludes *Vibrio* spp. cases with any other pathogen detected on the gastrointestinal multiplex PCR panel.

[c]Wilcoxen 2-sample test *P* value.

[d]Specimen collection season was compared with all other seasons and adjusted for testing platform in a logistic model.

[e]Winter = January 1–March 31; spring = April 1–June 30; summer = July 1–September 30; and fall = October 1–December 31.

[f]The 90 cases who were available for interview were included in risk factor analysis. Known risk factors included consumption of any seafood, contact with an untreated body of water, contact with marine life, and international travel.

[g]"Any seafood consumption" includes consumption of raw seafood and oysters. "Any raw seafood consumption" includes consumption of oysters if consumed raw. The categories are not exclusive.

This was not a formal evaluation of the GMPPs where additional measures of the laboratories and laboratorians conducting the test would be reviewed, the transit and storage of specimens would be uniform between the platforms, and additional testing methods could be employed to validate negative culture results. Rather, we used surveillance data to assess test accuracy. The clinical and epidemiological data support the possibility of performance issues with the predictive value of the test.

Potential reasons for false positives for *Vibrio* spp. include specimen contamination with nucleic acid and cross-reactivity with other organisms [17]. Of concern is the detection of low levels of *Vibrio* DNA in agar components of Cary Blair media, a commonly used transport medium for stool samples (BioFire Technical Notes: FLM1-PRT-0239-02).

Nonreproducible CIDT-positive results have been reported previously for *Vibrio* spp. [18, 19]. False-positive diagnostic test results adversely affect clinical diagnosis and public health investigations. Patients might be unnecessarily treated with antibiotics and experience a delay in diagnosis of the true cause of illness. False positives also present a burden to the public health system, because patients as cases with unconfirmed infections still require additional testing at the public health laboratory and epidemiological assessment of exposures.

There is currently no mechanism for postmarket surveillance of diagnostic test efficacy after initial approval from the US Food and Drug Administration. This study suggests a potential need for routine assessments of diagnostic tests to ensure they are meeting accuracy targets. In addition, these findings further demonstrate the need to consider clinical and exposure information along with CIDT results before making a diagnosis of vibriosis. Finally, these data support the recommendation for laboratories to perform reflex culture to confirm CIDT-positive results [8]. This would also provide the added benefit of providing isolates for characterization to conduct public health interventions.

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