Comparative evaluation of a chromogenic agar medium – PCR protocol with a conventional method for isolation of *Vibrio parahaemolyticus* strains from environmental and clinical samples

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Abstract: Screening for pathogenic *Vibrio parahaemolyticus* has become routine in certain areas associated with foodborne outbreaks. This study is an evaluation of the CHROMagar Vibrio (CV) medium – PCR protocol and the conventional method (TCBS (thiosulfate – citrate – bile salts – sucrose) agar plus biochemical and Wagatsuma agar tests) for detection of *V. parahaemolyticus* in shrimp, water, sediment, and stool samples collected for biosurveillance in an endemic area of northwestern Mexico. A total of 131 environmental and clinical samples were evaluated. The CV medium – PCR protocol showed a significantly improved ability ($P < 0.05$) to isolate and detect *V. parahaemolyticus*, identifying isolates of this bacteria missed by the conventional method. Although some other bacteria, distinct from pathogenic *V. parahaemolyticus*, produced violet colonies similar to that of *V. parahaemolyticus* on CV medium, we were able to detect a superior number of samples of *V. parahaemolyticus* with the CV medium – PCR protocol than with the conventional method. The Kanagawa phenomenon is routinely determined on Wagatsuma agar for the diagnosis of *V. parahaemolyticus* (pathogenic) positive for thermostable direct hemolysin (TDH) in developing countries. In our results, Wagatsuma agar showed low sensitivity (65.4% at 24 h and 75.6% at 48 h) and specificity (52.4% at 48 h) for identifying *V. parahaemolyticus* positive for TDH. Overall, our data support the use of the CV medium – PCR protocol in place of the conventional method (TCBS – biochemical tests – Wagatsuma agar) for detection of pathogenic *V. parahaemolyticus*, both in terms of effectiveness and cost efficiency.

Key words: methods, isolation, *Vibrio parahaemolyticus*, biosurveillance, public health.

Résumé : Le dépistage de *Vibrio parahaemolyticus* pathogène est réalisé de routine dans certaines régions associées à l'éclosion de maladies alimentaires. Cette étude consiste à évaluer le protocole de gélose CHROMagar Vibrio (CV) – PCR et la méthode conventionnelle (gélose TCBS (« thiosulfate – citrate – bile salts – sucrose ») plus tests biochimiques et gélose Wagatsuma) pour détecter *V. parahaemolyticus* dans des échantillons de crevettes, d'eau, de sédiments et de selles, récoltés pour la surveillance biologique d'une zone d'endémicité du Nord-Ouest du Mexique. Un total de 131 échantillons environnementaux et cliniques a été évalué. Le protocole de gélose CV–PCR a montré une capacité significativement améliorée ($P < 0,05$) d'isoler et de détecter *V. parahaemolyticus*, en identifiant des isolats de cette bactérie non repérés par la méthode conventionnelle. Même si quelques bactéries, distinctes de *V. parahaemolyticus* pathogène, produisent des colonies violettes similaires à *V. parahaemolyticus* sur gélose CV, nous avons pu détecter un plus grand nombre d'échantillons contenant *V. parahaemolyticus* avec le protocole de gélose CV–PCR qu'avec la méthode conventionnelle. Le phénomène de Kanagawa est habituellement déterminé sur une gélose Wagatsuma afin de diagnostiquer le *V. parahaemolyticus* positif au TDH (« thermostable direct hemolysin » pathogène) dans les pays en voie de développement. Dans nos conditions, la gélose Wagatsuma était moins sensible (65,4 % a 24 h et 75,6 % à 48 h) et moins spécifique (52,4 % à 48 h) dans l'identification de *V. parahaemolyticus* positif au TDH. Globalement, nos données appuient l'utilisation du protocole de gélose CV–PCR plutôt que les méthodes conventionnelles (gélose TCBS – tests biochimiques – gélose Wagatsuma) pour détecter *V. parahaemolyticus* pathogène, tant en terme d'efficacité que de coût/rendement.

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Mots-clés : méthodes, isolement, *Vibrio parahaemolyticus*, surveillance biologique, santé publique.

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Introduction

Vibrio parahaemolyticus is reported as an agent of foodborne illness around the globe (Nair et al. 2007). Human infection with this pathogen is associated most frequently with the consumption of seafood, primarily raw or improperly cooked shellfish (Blake et al. 1980; Wong et al. 2000). Consumption of sufficiently high numbers of organisms of virulent *V. parahaemolyticus* strains can cause gastroenteritis (with *tdh* and (or) *trh* toxins), septicemia, and even death (Nair et al. 2007). Infections caused by *V. parahaemolyticus* have increased globally in the last 5 years (Cabanillas-Beltrán et al. 2006; Chowdhury et al. 2000). To establish effective control measures to reduce the risk of *V. parahaemolyticus* infection and to ensure the safety of foods, efficient analytical methods (protocols) for the detection of *V. parahaemolyticus* in clinical samples, foods, and the environment must be available. The use of TCBS (thiosulfate – citrate – bile salts – sucrose) agar, a differential and selective plating medium for *Vibrio* spp., and of a biochemical test for bacterial identification is the conventional method most widely used today in public health laboratories in both developing and some developed countries. Additionally, the Kanagawa phenomenon is routinely determined on Wagatsuma agar for the diagnosis of *V. parahaemolyticus* positive for thermostable direct hemolysin (TDH) (pathogenic). However, the conventional method is complicated because the procedure for isolating the bacterium and confirming its pathogenicity is very labor-intensive and time consuming, thus final results may not be available for 5–8 days (Fig. 1).

Chromogenic agars have recently been developed to facilitate recognition of *Vibrio* spp. directly on primary media (Hara-Kudo et al. 2001). On the other hand, a PCR technique has been applied for the identification of different pathogens like *V. parahaemolyticus* (Bej et al. 1999; Kim et al. 1999; Matsumoto et al. 2000; Wong and Lin 2001; Hara-Kudo et al. 2003). In this study, we aimed to find a fast and reliable strategy (protocol) for the detection and confirmation of the presence of *V. parahaemolyticus* in clinical, shrimp, seawater, and sediment samples, and apply it to biosurveillance. We studied the effectiveness of a method involving CHROMagar Vibrio (CV) medium and PCR (*tdh* toxigenic gene).

Materials and methods

Bacterial cultures

Twenty-four strains of non-*Vibrio* bacteria (including *Listeria monocytogenes*; *Aeromonas sobria* and *Aeromonas caviae*; *Staphylococcus aureus* ATCC 29213; *Streptococcus pyogenes*; *Shigella flexneri*; *Streptococcus pneumoniae*; *Pseudomonas* spp.; *Escherichia coli* ATCC 25922; uropathogenic *E. coli*; diarrheagenic *E. coli* pathotypes, which include enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli*, diffusely adherent *E. coli*, and enteroaggregative *E. coli*) and 61 strains of *Vibrio* species (*V. parahaemolyticus*, *Vibrio fluvialis*, and *Vibrio metschnikovii*) were used in the study. All cultures were provided by the Sinaloa State Public Health Laboratory, Culiacán Sinaloa, México.

Sample collection

Stool samples (n = 57) were taken from persons (The selection of donor of human samples were performed as recommended by ethics committee of The Sinaloa State Public Health Laboratory.) with gastroenteritis who had eaten seafood; environmental samples (from shrimp, seawater, and sediments; n = 74) were taken from routine biosurveillance by the Sinaloa State Public Health Laboratory and the Ministry of Health between September and October 2006. All samples were processed as described in the *Bacteriological Analytical Manual* of the Food and Drug Administration (Kaysner and DePaola 1998). The shrimp (n = 25) and sediment (n = 20) samples were homogenized in a Stomacher-400 circulator, and each homogenate was placed in alkaline peptone water. Seawater (n = 29) samples were added to alkaline peptone water and incubated at 37 and 42 °C for 6–24 h. The non-bloody stool samples were collected in Cary–Blair transport medium and transported at room temperature to the laboratory within 2 h. These specimens were also enriched in alkaline peptone water (pH 8.6) for 6 h at 37 °C. The enrichment broths were streaked onto thiosulfate – citrate – bile salts – sucrose (TCBS) agar plates and (or) CV medium (CHROMagar, Paris, France) and incubated at 37 °C for 18–24 h. Figure 1 summarizes the protocol.

Biochemical identification

Biochemical tests for the identification of *Vibrio* species were carried out following standard procedures (Kaysner and DePaola 1998). Briefly, the strains exhibiting the following characteristics were identified as *V. parahaemolyticus*: gram-negative rods; oxidase positive; arginine dihydrolase negative; ornithine and lysine decarboxylase positive; growth at 8% NaCl but not at 0%; negative for sucrose, lactose, ONPG (*o*-nitrophenyl-β-D-galactopyranoside), urease, and Voges–Proskauer; positive for arabinose, D-mannitol, D-mannose, indole, gelatinase, and citrate.

Kanagawa hemolysin testing

Wagatsuma agar was prepared according to the Miyamoto method (Miyamoto et al. 1969). Briefly, the constituents of Wagatsuma's medium are as follows: yeast extract, 0.3%; bactopeptone (Difco), 1%; NaCl, 7%; K_2HPO_4, 0.5%; agar, 1.5%; with distilled water added to a final volume of 1 L. After dissolving by heating (heat sterilization should be avoided), mannitol is added to a concentration of 1%, 0.1% crystal violet alcohol solution to 0.1%, and human defibrinated blood (or saline suspension of red blood cells) to 5%. Each plate was inoculated with *V. parahaemolyticus* strains; positive reactions were recorded as a zone of β-hemolysis

Fig. 1. Schematic representation of the protocols of the methods used in this study to isolate *Vibrio parahaemolyticus* from different sources. APW, alkaline peptone water; PBS, phosphate-buffered saline; TCBS, thiosulfate – citrate – bile salts – sucrose agar; TSA, tryptic soy agar; TDH, thermostable direct hemolysin.

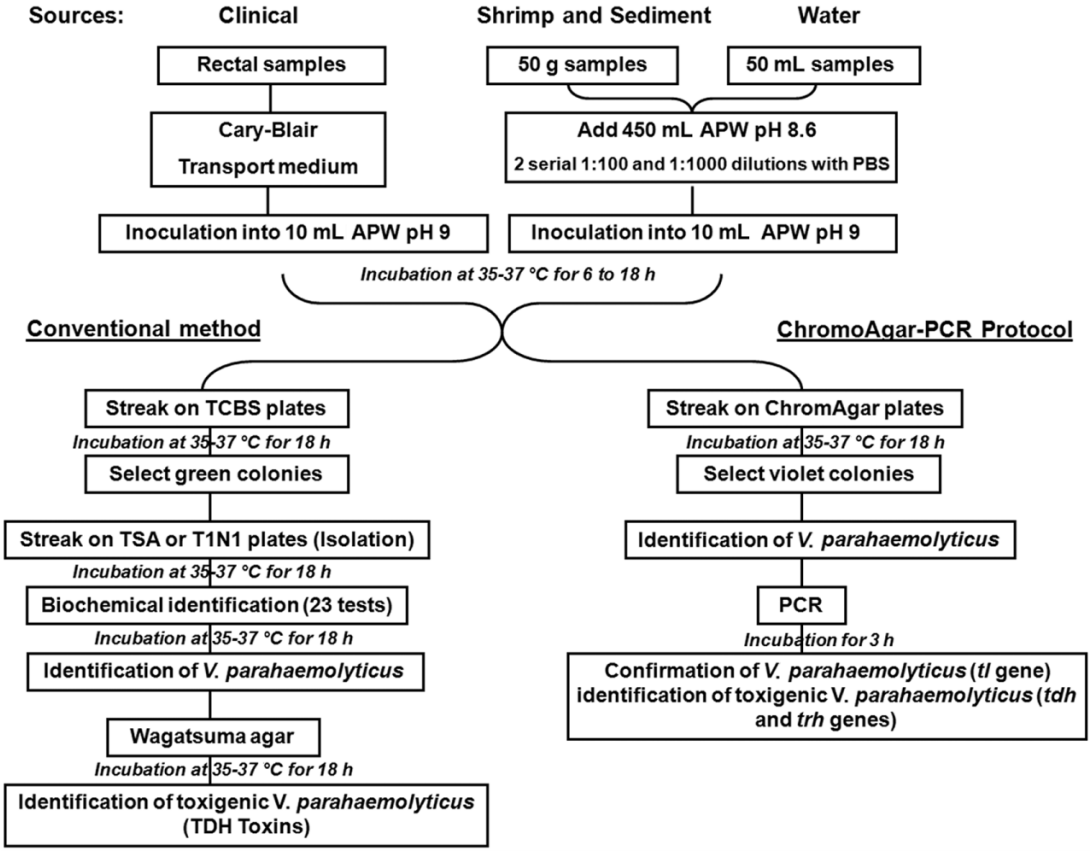


Table 1. Specific primers used in *Vibrio parahaemolyticus* gene amplification, melting temperature, and size of fragments.

Gene	Primer sequence (5′–3′)	Amplicon size (bp)	T_m (° C)	Reference
tl	F: AAA GCG GAT TAT GCA GAA GCA CTG	450	58.63	Bej et al. 1999
	R: GCT ACT TTC TAG CAT TTT CTC TGC		51.11	
R72H	F: TGCGAATTCGATAGGGTGTTAACC	387 or 320	71.30	Robert-Pillot et al. 2002; Lee et al. 1995
	R: CGAATCCTTGAACATACGCAGC		69.30	
tdh	F: GTA AAG GTC TCT GAC TTT TGG AC	269	48.58	Bej et al. 1999
	R: TGG AAT AGA ACC TTC ATC TTC ACC		53.27	
trh	F: TTG GCT TCG ATA TTT TCA GTA TCT	500	51.37	Bej et al. 1999
	R: CAT AAC AAA CAT ATG CCC ATT TCC G		58.32	

surrounding the spot of growth on the human blood plate. The interpretation times for the test were 24, 48, 72, and 84 h, and the results were compared with the presence of the gene *tdh* (PCR) in the analyzed strains.

PCR amplification

PCR amplification was performed in a 25 μL volume consisting of 1× GoTaq Green Master Mix (Promega) primer mixture, or individually from genes *tl* 0.02 μmol/L, *R72H* 0.02 μmol/L (Lee et al. 1995; Robert-Pillot et al. 2002), *tdh* 0.02 μmol/L, and *trh* 0.02 μmol/L (Table 1), and 0.5 μg of purified genomic DNA template, with the remaining volume consisting of sterilized water. PCR was routinely carried out in a Thermal Cycler C1000 (BIORAD) under the following cycling conditions: an initial period of DNA denaturation and enzyme activation at 94 °C for 3 min; followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C; and a final extension of 5 min at 72 °C (Bej et al. 1999). Negative control reactions were performed simultaneously with each test run by replacing the template DNA with sterilized water in the PCR mixture. Ten microlitre aliquots of each amplification product were separated by electrophoresis in a 1% agarose gel. Ethidium bromide staining (0.5 mg/mL) allowed for the visualization of DNA fragments with a digital imaging system (Kodak, Model E1 logia 100 imaging system). It was possible to identify virulence by comparison with a 50-bp DNA ladder (Promega DNA step ladder).

The statistical analysis used to determine differences be-

Table 2. Comparison of the CHROMagar – PCR protocol and the conventional method for isolation of *Vibrio parahaemolyticus* from samples from different sources.

	Shrimp (n = 25)		Seawater (n = 29)		Sediment (n = 20)		Clinical (n = 57)		Total (n = 131)	
Protocol	+	–	+	–	+	–	+	–	+	–
CHROMagar										
Violet colony	25 (100)	0	27 (93)	2 (7)	19 (95)	1 (5)	5 (9)	52 (91)	76 (58)	55 (42)
PCR (*tl* gene, *R72H* gene, VP)	23 (92)*	2 (8)	24 (83)	5 (17)	18 (90)	2 (10)	5 (9)	52 (91)	70 (53)*	61 (47)
TCBS										
Green colony	20 (80)	5 (20)	21 (72)	8 (28)	18 (90)	2 (10)	1 (2)	56 (98)	60 (45)	71 (55)
Biochemical tests (VP)	16 (64)*	9 (36)	19 (66)	10 (34)	16 (80)	4 (20)	1 (2)	56 (98)	52 (40)*	79 (60)

Note: Data are the numbers of samples showing growth (+) or no growth (–) according to colony color or to identification of *V. parahaemolyticus* of samples tested, with percentage in parentheses. An asterisk (*) indicates a significant difference ($P < 0.05$) between the results of CHROMagar Vibrio medium and TCBS (thiosulfate – citrate – bile salts – sucrose) agar.

tween methods was a χ^2 test, with 95% indicating statistical significance ($P < 0.05$).

Results

Comparison of the CV medium – PCR protocol and the conventional method for isolation of *V. parahaemolyticus*

The utility of CV medium – PCR protocol as a routine method for the isolation and identification of *V. parahaemolyticus* was compared with the conventional method (TCBS – biochemical tests – Wagatsuma agar), which is the current protocol used in laboratories. In this study, we tested a total of 131 samples, including samples of shrimp (n = 25), seawater (n = 29), sediment (n = 20), and stools (n = 57). They were plated on CV and TCBS media. From a total of 131 samples analyzed, 76 (58%) violet colonies presumptive to be *V. parahaemolyticus* were found on CV medium. In contrast, only 60 (45%) typical green colonies presumptive to be *V. parahaemolyticus* were isolated on TCBS (Table 2). The isolation rates of colonies from shrimp, seawater, sediment, and stool presumptive to be *V. parahaemolyticus* were 100%, 95%, 93%, and 9% for CV, and 80%, 90%, 72%, and 2% for TCBS, respectively (Table 2). These data indicate that CV medium is more efficient than TCBS agar for the isolation of colonies presumptive to be *V. parahaemolyticus* from shrimp, sediment, seawater, and clinical samples.

Once colonies presumptive to be *V. parahaemolyticus* were isolated on TCBS or CV, the bacterial strains were confirmed through biochemical tests and PCR, respectively. From 60 (45%) green colonies isolated on TCBS, only 52 (40%) were *V. parahaemolyticus* by biochemical tests, whereas from 76 (58%) violet colonies isolated on CV medium, 70 (53%) were *V. parahaemolyticus* by PCR (Table 2). These results show that from a total of 131 samples analyzed, *V. parahaemolyticus* was isolated and identified more frequently with the CV medium – PCR protocol (70) than the TCBS – biochemical tests protocol (52), a difference that was statistically significant ($P < 0.05$). Among environmental and clinical samples, only the shrimp samples showed a significant difference ($P < 0.05$) between the 2 protocols: *V. parahaemolyticus* having been detected in 92% of the samples with CV medium – PCR and 64% with TCBS – biochemical tests.

All the colonies from stool samples presumptive to be *V. parahaemolyticus* that were isolated by either media were confirmed to be so by biochemical tests or PCR, according to the case (Table 2). However, a low number of colonies from shrimp, seawater, and sediment samples presumptive to be *V. parahaemolyticus* were not in fact thus: 8 on TCBS (green colony) and 6 on CV (violet colony) media. These data show that not all green colonies on TCBS and violet colonies on CV were *V. parahaemolyticus* meaning that other bacteria developed these phenotypical features.

Growth characteristics of various bacterial species on CV and TCBS media

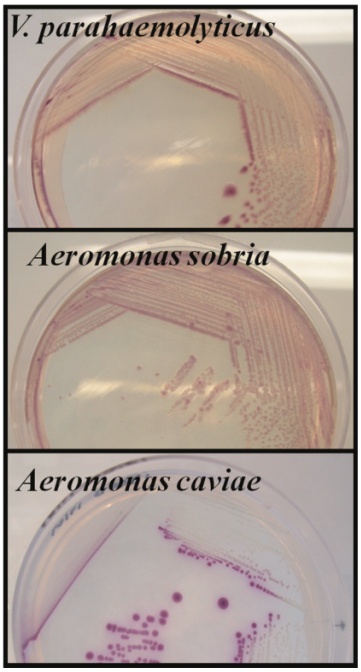
To evaluate the ability of different bacterial strains to develop violet colonies on CV and green colonies on TCBS, 84 bacterial strains were inoculated on CV as well as TCBS media (from the culture collection of the Public Health Laboratory). Five species other than *V. parahaemolyticus* were able to develop violet colonies on CV that could not easily be distinguished from those formed by the latter bacteria. These bacteria were *Vibrio fluvialis*, *Listeria monocytogenes*, *Aeromonas caviae* (clinical), *Aeromonas sobria* (shrimp), and *Pseudomonas* (Fig. 2). Contrarily, only the *Vibrio* strains were able to grow on TCBS (Fig. 2). These data indicate that CV medium is less selective for *Vibrio* spp. than TCBS medium, when the bacteria used were pure strains.

Sensitivity and specificity of Wagatsuma agar for detection of pathogenic (TDH toxin) *V. parahaemolyticus*

Once colonies of *V. parahaemolyticus* are isolated and confirmed, the next step in biosurveillance is to determine the pathogenicity of these strains. The major virulence factor of *V. parahaemolyticus* is TDH, encoded by the *tdh* gene. TDH causes β-hemolysis of human erythrocytes on Wagatsuma agar, a reaction known as the Kanagawa phenomenon (Miyamoto et al. 1969). The association between a positive Kanagawa phenomenon by a strain and its ability to cause gastroenteritis has been well established (Chun et al. 1975) and, hence, is the reason for the routine use of the Kanagawa phenomenon to determine the pathogenicity of *V. parahaemolyticus* strains in developing countries. We determined the sensitivity and the specificity of this agar with 120 strains of *V. parahaemolyticus* (70 violet colonies iso-

Fig. 2. (A) Appearance of several bacterial species on CHROMagar Vibrio (CV) medium. (B) Colony morphologies of various bacteria grown on CV and TCBS (thiosulfate – citrate – bile salts – sucrose) media. The pure culture of several bacterial species from the culture collection of the Sinaloa State Public Health Laboratory were plated and incubated at 37 °C for 18 h.

A



B

Species	No. of strains tested	CHROMAgar Medium		TCBS Medium	
		Size of colony[a]	Color of colony	Size of colony[a]	Color of colony
V. parahaemolyticus	50	+++	Violet	+++	Green
Vibrio fluvialis	10	++	Violet	++	Yellow
Listeria monocytogenes	1	+	Violet	NG	
Aeromonas caviae	3	+++	Violet	NG	
Aeromonas sobria	2	+++	Violet	NG	
Pseudomonas 1883	1	++	Violet	NG	
E.coli EAEC042	1	+	Blue	NG	
E.coli ETEC	1	+	Blue	NG	
E.coli DAEC	1	+	Blue	NG	
E.coli EPEC	1	+	Blue	NG	
E.coli EHEC	1	+	Blue	NG	
E.coli ATCC	4	+	Blue	NG	
E.coli EIEC-C2	1	+	White	NG	
E.coli uropathogenic	1	NG		NG	
S.aureus	1	+	White	NG	
Pseudomonas	1	+++	Pale blue	NG	
V.metschnikovii	1	NG		++	Yellow
S.pyogenes	1	NG		NG	
Shigella flexnerii	1	NG		NG	
S.pneumoniae	1	NG		NG	

[a]+, 0.1-1.0 mm; ++, 1.1-3.0 mm; +++, 3.1-5.0 mm. NG, no growth.

Table 3. Hemolytic activity of Wagatsuma agar (Kanagawa phenomenon) inoculated with 120 strains of *Vibrio parahaemolyticus**.

	Kanagawa phenomenon			
	24 h	48 h	72 h	84 h
No. positive for *tdh*	54	79	88	113
No. negative for *tdh*	66	41	32	7
Sensitivity (%)	65.4	75.6	83.3	96.2
Specificity (%)	92.9	52.4	42.2	9.5
Positive predictive value (%)	9.4	74.7	73.9	66.4
Negative predictive value (%)	59.1	53.7	59.4	57.1

*By PCR, of 120 *V. parahaemolyticus* strains, 78 were positive for *tdh* and 42 were negative for *tdh*. These strains were then inoculated onto Wagatsuma agar.

lated in CV in this study and 50 strains from the culture collection of Public Health Laboratory). Of these, 78 (65%) strains were positive for the *tdh* gene (by PCR) and 42 (35%) strains were negative (all strains were *trh* negative, data not shown). These strains were inoculated on Wagatsuma agar, and the results were observed at 24, 48, 72, and 88 h.

At 24 h, just 54 strains of *V. parahaemolyticus* were positive for the Kanagawa phenomenon, with 65% sensitivity and 92.9% of specificity. However, at 48, 72, and 88 h the number of strains increased to 79, 88, and 113, respectively, with an increased sensitivity but decreased specificity over time (Table 3). These data suggest that the longer the interpretation time, the more strains were positive and the greater the sensitivity of the methodology. Nevertheless, this increase in the sensitivity came at the cost of a decrease in specificity, as there were more false-positive strains. The best interpretation time for the Kanagawa phenomenon was at 48 h because the positive and negative predictive values were 74.7% and 53.7%, respectively (Table 3). These data indicate that the Kanagawa phenomenon is not the best tool to detect positive *tdh* in *V. parahaemolyticus.*

Discussion

This study demonstrates the utility of the CV medium – PCR protocol as a reliable and time-saving procedure for the isolation and identification of *V. parahaemolyticus* in environmental and clinical stool samples and demonstrates its application to biosurveillance. Because of the increase in the incidence of *V. parahaemolyticus* infections, several methods have been developed to identify these strains rapidly (Hara-Kudo et al. 2001) owing to the time employed with the conventional methods. In an outbreak, time is of essence in making public health decisions, as is the ability to identify the causal agent and source of infection.

In the majority of public health laboratories in the world, and particularly in developing countries, the selective TCBS medium for *V. parahaemolyticus* isolation is used, in which it is difficult to visually distinguish *V. parahaemolyticus* (forms green colonies) from other *Vibrio* species like *Vibrio vulnificus* or *Vibrio mimicus* (either form green colonies or are covered by a yellow color produced by sucrose-fermenting bacteria, such as *Vibrio alginolyticus*).

This study demonstrated the ability of a wide variety of bacterial species, such as *Vibrio fluvialis*, *Listeria monocytogenes*, *Aeromonas caviae*, *Aeromonas sobria*, and *Pseudomonas*, to grow adequately on CV, developing violet colonies. This contrasts with the findings obtained by Hara-Kudo et al. (2001), who previously reported that on this growth medium, *V. parahaemolyticus* colonies develop a purple color that distinguish them from other related bacterial strains. Therefore, upon observing the growth of organisms that form violet colonies on CV, an advanced identification tool like PCR (*R72H* and *tl* genes) must be used to confirm *V. parahaemolyticus* and avoid misinterpretation of the results.

In spite of the reduced selectivity for *Vibrio* spp. and the possible presence of false-positive colonies on CV medium in comparison with TCBS medium, we were able to detect a superior number of samples with violet colonies that corresponded to *V. parahaemolyticus*. These results are similar to a study reported by Hara-Kudo et al. (2001), who tested this CV medium for detecting *V. parahaemolyticus* only from seafood, and not from other sources like seawater, sediment, and stool samples. In general, by using environmental (shrimp, seawater, and sediment) and clinical (stool) samples taken from routine biosurveillance, this study demonstrated that the total detection rate was significantly higher with the CV medium – PCR protocol than the TCBS – biochemical tests protocol.

On other hand, owing to the ability of clinical and some environmental isolates of *V. parahaemolyticus* to hemolyse human or rabbit blood on a special agar medium (Wagatsuma agar), the so-called Kanagawa phenomenon (Miyamoto et al. 1969) continues to be a reliable marker of the virulence of the organism in laboratories of some developing countries. However, we found false-positive and doubtful results associated with the low sensitivity and specificity for the identification of *tdh*-positive *V. parahaemolyticus* when the Wagatsuma agar was used. False-positive hemolytic reactions occasionally occur owing to pH changes around the colonies, fragility of erythrocytes, or hemolysis caused by hemolysins other than the thermostable direct hemolysin (Chun et al. 1975). Although our results are consistent with the recommended interpretation time (48 h) (Miyamoto et al. 1969), we suggest the PCR technique for identifying pathogenic *V. parahaemolyticus* (*tdh* gene) to eliminate doubtful results and save time.

Importantly, the use of the CV medium – PCR protocol reduced the time for isolation and identification of *V. parahaemolyticus* from 96 to 48 h. In summary, the CV medium – PCR protocol is more efficient and accurate for identifying *V. parahaemolyticus* and pathogenic strains from clinical and environmental samples than the conventional method (TCBS – biochemical tests – Wagatsuma agar). The CV medium – PCR protocol could be a powerful tool in public health laboratories for monitoring *V. parahaemolyticus* strains in clinical and environmental samples.

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