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Evaluation of the Selective Enrichment Culture to Recover *Clostridium difficile*

Byoungrak An[1], Heejung Kim[1,2] and Kyungwon Lee[2]

[1]Department of Laboratory Medicine, Yongin Severance Hospital, Yongin, 449-930, Korea
[2]Departments of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul 120-752, Korea

To evaluate the recovery rates to increase toxigenic *C. difficile*, the selective enrichment broth culture methods were compared with commonly used cytotoxin assays and toxigenic culture. First, the enrichment culture, using the selective medium broth for 2 to 5 days, was performed and then, toxigenic *C. difficile* was confirmed by *C. difficile* toxin gene-specific PCR after being cultured on *C. difficile* selective agar. The sensitivity of *C. difficile* from the enrichment culture (100%) was higher than that of *C. difficile* selective agar culture (93.8%), while positive predictive values (PPV) were low; 72.7% (16/22) and 88.2% (15/17). PPV of the enrichment culture are not high. Recently, combinations of *C. difficile* selective agar culture, *C. difficile* A & B assays, glutamate dehydrogenase, and nucleic acid amplification method are widely used. The enrichment culture was disadvantageous in PPV, turn-around time, and cost. So, what we performed is not considered as a common method of diagnosis of *C. difficile*-associated diarrhea.

Keywords: *C. difficile*, Enrichment culture, Toxigenic culture

Corresponding author: Heejung Kim
Departments of Laboratory Medicine, Yonsei University Yongin Severance Hospital 225 Geumhak-ro, Cheoin-gu, Yongin 449-930, Korea
Tel: 82-31-331-8755
E-mail: hjkim12@yuhs.ac

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Introduction

Clostridium difficile produce toxin A and B, which is the main causative organism of antibiotic-associated diarrhea and pseudomembranous colitis (Bartlett, 2002; Kyne, 2002). Recently, to increase diagnosis for patients with suspicious *C. difficile* infection, combination methods such as cytotoxicity assay, toxigenic culture, and toxin gene-specific PCR are commonly used and these methods have the efficacy in terms of the turn-around time, sensitivity, and specificity. *C. difficile* culture has inherent difficulties, thus efforts to increase toxigenic culture for antimicrobial susceptibility testing, molecular typing, and/or identification of virulence factors are required.

The recovery of the *C. difficile* and toxigenic *C. difficile* has been improved through selective enrichment culture method (Buchanan, 1984; Levett, 1984). For common method to increase the toxigenic *C. difficile* culture rate, more evaluation data are still needed. In this study, the current method, *C. difficile* selective agar culture, was compared with selective enrichment culture for the efficacy of the latter.

Materials and Methods

1. Materials

A total of 203 loose specimens, submitted to the clinical microbiology laboratory of the Severance Hospital for *C. difficile* culture from January to February 2013, were collected from patients showing clinical signs of *C. difficile*-associated diarrhea (CDAD).

2. Methods

1) *C. difficile* toxin test

VIDAS *C. difficile* Toxin A/B (VIDAS-CDAB; bioMérieux)

Table 1. Comparison of VIDAS, CDSA, and enrichment culture methods to the toxigenic culture for the detection of *Clostridium difficile*

Tests	N of samples	Results	Toxigenic culture (N)		Sensitivity	Specificity	PPV	NPV
			Positive	Negative	(%, 95% confidence interval)			
VIDAS*	203	Positive	6	7	37.5	96.3	46.2	94.7
		Negative	10	180	(14.5~60.5)	(93.5~99.0)	(19.1~73.3)	(91.6~97.9)
CDSA	203	Positive	15	2	93.8	98.9	88.2	99.5
		Negative	1	185	(81.9~100)	(97.5~100)	(72.9~100)	(98.4~100)
Enrichment culture	203	Positive	16	6	100	96.8	72.7	100
		Negative	0	181		(94.3~99.3)	(54.1~91.3)	

VIDAS, VIDAS *Clostridium difficile* A & B assay; CDSA, *Clostridium difficile* Selective Agar; PPV, Positive predictive value; NPV, Negative predictive value. *Excluded with 3 of equivocal results.

test were performed on the day of specimen reception.

2) Conventional CDSA culture

All stool specimens were subjected to alcohol shock. For conventional culture, *C. difficile* selective agar (CDSA; Becton Dickinson) were used. They were cultured anaerobically at 37°C for 48 h.

3) Enrichment culture

For selective enrichment culture, selective enrichment broth culture using heart infusion broth supplemented with 5% laked sheep blood, 0.1% taurocholate, 250 μg/mL cycloserine, 10 μg/mL cefoxitin, and 1% neutral red were used. The enrichment broth into which specimens had been inoculated was cultured under the anaerobic condition at 37°C for two to five days. When the color of the enrichment broth turned to yellow, *C. difficile* was identified through the subculture of the selective enrichment agar (Enrichment broth containing agar) and incubated anaerobically 37°C for 48 hrs.

4) Identification of toxigenic *C. difficile*

Species identification was performed on the basis of typical colony morphology and characteristic odor on agar plates and ATB 32A system (bioMérieux) results. PCR to detect *C. difficile* toxin genes were performed as described in previous studies (Spigaglia & Mastrantonio, 2004; Terhes *et al.*, 2004).

Results

At the VIDAS-CDAB test, 13 out of the 203 samples were positive (prevalence rate: 6.4%). The sensitivity, specificity, and positive and negative predictive values (PPV, NPV) in VIDAS-CDAB test were 37.5%, 96.2%, 46.2%, and 94.7%, respectively (Table 1).

A total of 17 *C. difficile* isolates were recovered from 203 specimens in the CDSA culture (prevalence rate: 8.3%), and two isolates were non- toxigenic. The sensitivity, specificity, PPV, and NPV in CDSA culture were 93.8%, 98.9%, 88.2%, and 99.5%, respectively.

A total of 22 *C. difficile* isolates were recovered from 203 specimens in the enrichment culture, and six isolates among them were non-toxigenic. The sensitivity, specificity, PPV, and NPV of enrichment culture were 100%, 96.8%, 72.7%, and 100%, respectively.

Discussion

The sensitivity (37.5%) and PPV (46.2%) in the VIDAS-CDAB test were much lower than those of conventional culture and enrichment culture. Compared with the sensitivity in the previous data (40.8~63.6%), our result was somewhat low, however, the PPV (100%) showed a significant gap (Kim *et al.*, 2012; Shin *et al.*, 2012). Among three equivocal result with VIDAS-CDAB, two samples showed minimal growth and one sample was not cultured at all in the enrichment culture. Also, the *C. difficile* were not cultured in 7 positive samples. Thus, additional studies are needed to figure out factors which affect culture condition. When the test shows low sensitivity and clinical CDI is strongly suspected, additional test methods are required to detect toxigenic *C. difficile* (Luis *et al.*, 2005; Reller *et al.*,

2007; Kim *et al.*, 2012).

Even though one sample was not isolated *C. difficile* in the CDSA culture, this sample was detected as toxigenic isolate in the enrichment culture, showing sensitivity and PPV (93.8% and 88.2%, respectively). The sensitivity in CDSA culture was slightly higher than that from the previous data (83.3~86.1%) (Han *et al.*, 2014). Twenty two isolates of *C. difficile* were cultured via selective enrichment culture process. However, six isolates were negative in the toxin gene by PCR and had high sensitivity (100%). The PPV was evaluated to be lower than that of CDSA culture (72.7%). Moreover, the 95% confidence interval of PPV had a wide range (from 54.1 to 91.3%). Phenol red was used in our enrichment broth, making it easy to detect *C. difficile* growth. When color change of broth from red to yellow occurred, we made subculture on the selective enrichment agar plate. But, the growth rate did not seem to be fast.

The enrichment culture methods is used to detect *C. difficile* toxin A/B gene by PCR after initial enrichment culture, or to increase the recovery rate of the specimen under various temperature and time conditions (Luis *et al.*, 2005).

In conclusion, the selective enrichment protocol in this study may be somewhat inadequate for common method in CDAD diagnosis.

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