Evaluation of Risk Factors for *Clostridium difficile* Infection Based on Immunochromatography Testing and Toxigenic Culture Assay

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ABSTRACT In recent years, the diagnostic method of choice for *Clostridium difficile* infection (CDI) is a rapid enzyme immunoassay in which glutamate dehydrogenase (GDH) antigen and *C. difficile* toxin can be detected (*C. diff* Quik Chek Complete; Alere Inc.) (Quik Chek). However, the clinical significance remains unclear in cases that demonstrate a positive result for GDH antigen and are negative for toxin. In this study, we used the Quik Chek test kit on fecal samples, with an additional toxin detection step using a toxigenic culture assay for the aforementioned cases. CDI risk factors were assessed among the 3 groups divided by the Quik Chek test results. The study involved 1,565 fecal samples from patients suspected to have CDI who were hospitalized during the period of April 2012 to March 2014. The 3 groups were defined as follows: both GDH antigen positive and toxin positive (by Quik Chek test) (toxin-positive [TP] group, $n = 109$), both GDH antigen and toxin negative (toxin-negative [TN] group, $n = 111$), and positive only for GDH antigen but toxin positive with subsequent toxigenic culture (toxigenic culture [TC] group, $n = 72$). The gender, age, number of hospitalization days, white blood cell (WBC) counts, serum albumin levels, body mass index (BMI), fecal consistency, and use of antibacterials and proton pump inhibitors (PPIs) were analyzed. The positive rate for the fecal direct Quik Chek test was 7.0% (109/1,565 cases). However, toxigenic culture assays using the Quik Chek test for only the GDH-antigen-positive/toxin-negative samples were 35.3% positive (72/204 cases). As a result, the true positive rate for *C. difficile* toxin detection was estimated to be 11.6% (181/1,565 cases). Moreover, significant differences ($P < 0.05$) in the number of hospitalization days (>50 days), WBC counts ($>10,000$ WBCs/ μ l), and use of PPIs comparing the TN, TP, and TC groups, were observed. The odds ratios (ORs) for the development of CDI were 1.61 (95% confidence interval [CI], 0.94 to 2.74) and 2.98 (95% CI, 1.59 to 5.58) for numbers of hospitalization days, 2.16 (95% CI, 1.24 to 3.75) and 2.24 (95% CI, 1.21 to 4.14) for WBC counts, and 9.03 (95% CI, 4.9 to 16.6) and 9.15 (95% CI, 4.59 to 18.2) for use of PPIs in the TP and TC groups, respectively. These findings demonstrated that the use of PPIs was a significant risk factor for CDI development. Moreover, antibacterials such as carbapenems, cephalosporins, and fluoroquinolones were demonstrated to be risk factors. In conclusion, identification of the TC group of patients is thought to be important, as this study demonstrates that this group bears the same high risk of developing CDI as the TP group.

KEYWORDS *Clostridium difficile* infection, glutamate dehydrogenase, *C. diff* Quik Chek Complete test, risk factor, toxigenic culture assay

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Clostridium difficile is an obligatory, anaerobic, Gram-positive bacillus with an ability to form protective spores. It is known that *C. difficile* inhabits the intestinal tracts and feces of humans and animals (e.g., cows, horses, dogs, and cats) and is found in the natural environment in soil, hay, and sand (1). *C. difficile* infection (CDI) is known to spread through hand-to-hand or surface-to-hand contact of health care workers (2–4). Risk factors for CDI include the use of antibiotics or proton pump inhibitors (PPIs) during hospitalization, and CDI is more common and severe among elderly individuals and patients with multiple comorbidities (5). Hurley and Nguyen (6) reported a direct correlation between the *C. difficile* detection rate and the duration of patient hospitalization, with a high detection rate of about 50% when patients were hospitalized for ≥ 4 weeks. *C. difficile* is diagnosed by stool culture or by testing for glutamate dehydrogenase (GDH) or bacterial toxins. If a person without any symptoms tests positive for *C. difficile*, then the result is considered to indicate colonization rather than infection (7–9). Enzyme immunofluorescence (enzyme-linked immunofluorescence assay [ELIFA]), enzyme-linked immunoadsorption (enzyme-linked immunosorbent assay [ELISA]), and immunochromatography methods are used for *C. difficile* toxin detection. In this study, we used the *C. diff* Quik Chek Complete (Quik Chek) immunochromatography test kit, which is a rapid cassette assay that simultaneously detects GDH antigen and toxins A and B of *C. difficile* in fecal specimens from patients with suspected CDI. This test is widely used for the diagnosis of *C. difficile* disease, and this GDH antigen detection method is recommended by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) (5) as a screening test for CDI. In addition, detection of toxins by cell susceptibility testing or toxigenic culture is recommended for GDH-antigen-positive cases (1, 10–14). However, the CDI risk in cases with positive GDH antigen results and negative toxin assay results is currently unclear. In this study, results using the Quik Chek test and toxigenic culture were obtained and CDI risk factors were analyzed in the 3 groups (GDH-positive/toxin-positive group, GDH-positive/toxin-negative group, and GDH-negative/toxin-negative group), divided by Quik Chek test results. Patient gender, patient age, number of hospitalization days, white blood cell (WBC) count, serum albumin levels, BMI, feces consistency, antibacterial use, and PPI use were also included in the analysis (15).

MATERIALS AND METHODS

Fecal samples. This study was carried out in accordance with the recommendations of ethical guidelines for clinical research in Japan. All experiments were performed in accordance with the principles of the Declaration of Helsinki, with written informed consent. The study protocol (protocol 15R-196) was approved by the ethics committees of Tokai University School of Medicine. Feces samples from 1,565 patients with suspected CDI who were hospitalized in April 2012 to March 2014 at Tokai University Hospital were analyzed by the Quik Chek immunochromatography test, according to the manufacturer's instructions. Feces were inoculated onto *C. difficile*-selective culture medium (Kohjin Bio medium-cycloserine-cefoxitin-mannitol agar [KBMCCMA]; Kojin Bio. Co., Ltd.) and cultured under anaerobic conditions for 48 h at 37°C (TEHA system and anaerobic box; Hirasawa Works Inc.). Bacteria were stocked on growth-associated molecule (GAM) soft agar medium (Kyokuto Co., Ltd.) and verified by Gram staining; *C. difficile* was identified with Rap ID ANA II kits (Thermo Fisher Scientific) when required.

Statistical analysis of correlations among groups. The analyzed factors included gender, age, number of hospitalization days (at the time of toxin check), WBC count, serum albumin level, BMI, feces consistency, and the use of antibiotics and PPIs. Nonparametric analyses among the 3 groups were performed using the Kruskal-Wallis H test in SPSS (IBM Japan Corp.) statistical analysis software. Comparisons between 2 groups were performed using the Mann-Whitney U test and the chi-square test in Excel Statistics (SSRI Corp.). Odds ratios (ORs) and 95% confidence intervals (CIs) were determined for estimation of CDI risk.

Toxigenic culture. *C. difficile* bacterial GDH antigen and toxins were checked according to the SHEA and IDSA guidelines from 2010 (5). Fecal samples positive for GDH antigen and negative for toxins by the Quik Chek test were anaerobically cultivated using KBMCCMA, and colonies formed were directly tested again by the Quik Chek test after a 2- to 5-day-culture.

Group classification by test results. According to the Quik Chek test results, the cases were divided into the following 3 groups. (i) Of the 1,565 cases, 1,252 were negative for both GDH and toxin; 111 of those 1,252 cases were selected as the Quik Chek toxin-negative (TN) group. Because the cases in the TN group without toxin production were excluded from CDI diagnosis, the number of cases for which all microbiological and clinical data were complete was 111. There were no significant differences in age and gender, compared to the other 2 groups. (ii) Of the 1,565 cases, 109 were both GDH antigen positive and toxin positive and were designated the Quik Chek toxin-positive (TP) group. (iii) A total of 204 cases

TABLE 1 Primers used in this study

Primer	Sequence (5' to 3')	Target	Size (bp)
NK2	CCCAATAGAAGATTCAATATTAAGCTT	<i>tcdA</i> gene unique sequence	251
NK3	GGAAGAAAAGAACTTCTGGCTCACTCAGGT	<i>tcdA</i> gene repetitive sequence	1,265
NK9	CCACCAGCTGCAGCCATA		
NK11	TGATGCTAATAATGAATCTAAATGGTAAC		
NK104	GTGTAGCAATGAAAGTCCAAGTTTACGC	<i>tcdB</i> gene unique sequence	203
NK105	CACTTAGCTCTTTGATTGCTGCACCT		

were GDH antigen positive but toxin negative, and those fecal samples were additionally subjected to toxigenic culture analysis (toxigenic culture [TC] group [72 cases]).

Detection of toxin genes in *C. difficile* strains isolated from the TC group. Eighty cases that were positive for GDH antigen and negative for toxins by the Quik Chek test were analyzed for toxin A and B genes by PCR. DNA was extracted from the cultured colonies using a nucleic acid extraction kit (EIDIA Co., Ltd.). The PCR was performed using TaKaRa *Ex Taq* (TaKaRa Bio Inc.). The primers NK2 (5'-CCCAATAGAAGATTCAATATTAAGCTT-3'; positions 2479 to 2505) and NK3 (5'-GGAAGAAAAGAACTTCTGGCTCAC TCAGGT-3'; positions 2254 to 2283) were derived from the nonrepeating portion of the *C. difficile* toxin A gene, and the primers NK9 (5'-CCACCAGCTGCAGCCATA-3'; positions 8043 to 8060) and NK11 (5'-TGATGCTAATAATGAATCTAAATGGTAAC-3'; positions 6795 to 6824) were derived from the repeating portion of the *C. difficile* toxin A gene (13) (Table 1). A segment of the toxin B gene was amplified by using primers NK104 (5'-GTGTAGCAATGAAAGTCCAAGTTTACGC-3'; positions 2945 to 2972) and NK105 (5'-CACTTAGCTCTTTGATTGCTGCACCT-3'; positions 3123 to 3148), which targeted the nonrepeating sequence of the *C. difficile* toxin B gene (13) (Table 1). PCR using the NK3-NK2 and NK104-NK105 primer sets was performed under the following cycling conditions: an initial denaturation step at 95°C for 2 min, followed by 35 cycles of amplification at 95°C for 20 s and 55°C for 120 s. PCR with the NK11-NK9 primer set was similarly performed with 35 cycles of 95°C for 20 s and 62°C for 120 s. After completion of the PCR cycles, the tubes were incubated at 74°C for 5 min.

RESULTS

Rates of toxin detection by the Quik Chek test. The rate of toxin detection directly from feces by the Quik Chek test was 7.0% (109/1,565 cases) (Table 2). Of 1,565 cases, 204 (13.0%) were GDH positive and toxin negative. After cultivation by toxigenic culture of the fecal samples, GDH antigen and toxin were checked again using colony samples (3 to 5 colonies from each plate were tested). This testing showed that 102 cases were negative for both GDH antigen and toxin, 72 cases (35.3%) were positive for both GDH antigen and toxin, and 30 cases (14.7%) failed to be cultivated. The final rate of toxin detection was 11.6% (181/1,565 cases).

Analysis of risk factors for the 3 groups. Characteristics of patients in the 3 groups were compared to evaluate risk factors for CDI (Table 3).

(i) Hospital stays. The average lengths of hospitalization (mean $\pm$ standard deviation [SD]) were 60.7 $\pm$ 58.7 days (range, 2 to 357 days), 80.9 $\pm$ 69.4 days (range, 11 to 492 days), and 110.9 $\pm$ 272.4 days (range, 8 to 2,370 days) for the TN, TP, and TC groups, respectively ($P < 0.001$). A significant difference between the TN group and the TP group was confirmed by the Kruskal-Wallis H test ($P < 0.001$), with patients from the TP group spending more days in the hospital than those from the TN group; however,

TABLE 2 Direct fecal assays and toxigenic culture assays using Quik Chek testing for detection of *C. difficile*

Method	Assay result ^a		No. of cases (%)
	GDH	Toxin	
Direct assay (direct testing of fecal samples)	+	+	109 (7.0) ^b
	+	—	204 (13.0)
	—	+	0
	—	—	1,252 (80.0)
Culture assay (testing after toxigenic culture) (204 samples) ^c	+	+	72 (35.3) ^b
	+	—	102 (50.0)
	No growth		30 (14.7)

^a+, positive; —, negative.

^bThe true-positive rate was 11.6% (181/1,565 samples).

^cA total of 204 samples with GDH-positive and toxin-negative results in direct assays were used for toxigenic culture assays.

TABLE 3 Characteristics of patients in the TN, TP, and TC groups

Characteristic	TN group (n = 111)	TP group (n = 109)	TC group (n = 72)
Age (mean $\pm$ SD) (yr)	72.0 $\pm$ 10.9	72.8 $\pm$ 15.7	68.3 $\pm$ 17.4
Male (no. [%])	79 (71.2)	61 (56.0)	42 (58.3)
Female (no. [%])	32 (28.8)	48 (44.0)	30 (41.7)
Hospitalization (mean $\pm$ SD) (days)	60.7 $\pm$ 59.3 ^a	80.9 $\pm$ 69.7 ^a	110.1 $\pm$ 272.4 ^a
Diarrhea (no. [%])	53 (47.7)	75 (68.8)	54 (75.0)
WBC count (mean $\pm$ SD) (WBCs/ μ l)	9,155 $\pm$ 6,268 ^a	11,222 $\pm$ 5,881 ^a	9,644 $\pm$ 4,462 ^a
Albumin level (mean $\pm$ SD) (g/dl)	2.57 $\pm$ 0.67	2.42 $\pm$ 0.54	2.48 $\pm$ 0.67
BMI (mean $\pm$ SD) (kg/m ²)	19.8 $\pm$ 4.14	20.0 $\pm$ 3.90	19.7 $\pm$ 4.4

^aSignificant difference between groups ($P < 0.05$).

there was no significant difference (Bonferroni significance level, $P = 0.016$) between the TN group and the TC group ($P = 0.192$). The OR for the TP group (60/109 cases) and the TC group (50/72 cases), compared to the TN group (48/111 cases), was 1.61 (95% CI, 0.94 to 2.74), comparable to the OR for the number of cases exceeding 50 days of hospitalization (OR, 2.98 [95% CI, 1.59 to 5.58]). The TC and TP groups demonstrated a tendency for long hospital stays (Fig. 1 and Table 4).

(ii) WBC counts. The average leukocyte counts were 9,155 $\pm$ 6,268 WBCs/ μ l (range, 200 to 32,000 WBCs/ μ l), 11,222 $\pm$ 5,881 WBCs/ μ l (range, 1,500 to 33,800 WBCs/ μ l), and 9,644 $\pm$ 4,462 WBCs/ μ l (range, 2,400 to 25,500 WBCs/ μ l) for the TN, TP, and TC groups, respectively (Fig. 2 and Table 3). There was a significant difference ($\chi^2 = 11.648$, $P = 0.003$) between the 3 groups. In addition, multiple comparisons were conducted for each pair, and a significant difference ($P = 0.001$) was observed for the WBC counts in the TN and the TP groups. In contrast, there were no significant differences in mean WBC counts between the TN group and the TC group or between the TP group and the TC group (Table 3). The percentage of patients with WBC counts of $\geq 10,000$ WBCs/ μ l was significantly greater in the TP group ($P = 0.0084$) and the TC group ($P = 0.0123$) than in the TN group (Table 4). Furthermore, patients were more likely to have a WBC count of $\geq 10,000$ WBCs/ μ l in the TP group (52/109 cases; OR, 2.16 [95% CI, 1.24 to 3.75]) and the TC group (35/72 cases; OR, 2.24 [95% CI, 1.21 to 4.41]) than in the TN group (33/111 cases) (Table 4).

(iii) Antibiotic agents. In the TN group, the rates of usage of penicillins, carbapenems, cepheems, and other antibiotics (trimethoprim-sulfamethoxazole or rifampin) were 46.8%, 7.2%, 25.2%, and 31.5%, respectively; 10.8% of patients recorded no antibiotic use. Similar results were obtained for the TP group (40.4%, 11.0%, 36.7%, and 10.1%, respectively) and the TC group (44.4%, 15.3%, 33.3%, 29.2%, and 8.3%, respectively), with no significant difference between the groups ($\chi^2 = 0.3047$, $df = 2$; $P = 0.8587$). Comparing the TP group and the TN group, the use of cephalosporins (TP group, 40/109 cases; TN group, 28/111 cases; OR, 1.72 [95% CI, 0.96

FIG 1 Comparison of hospitalization durations among the 3 groups. TN, the group of cases negative by *Clostridium difficile* toxin direct fecal assay; TP, the group of cases positive by direct fecal assay; TC, the group of cases positive by toxigenic culture assay. The median hospitalization duration was 55.50 days; $\chi^2 = 15.448$, $P < 0.05$ (Kruskal-Wallis test).

TABLE 4 Comparison of risk factors among the TN, TP, and TC groups

Characteristic	<i>P</i> ^a	OR (95% CI) ^b	
		TN group vs TP group	TN group vs TC group
Age of >60 yr	0.171	0.90 (0.39–2.06)	0.46 (0.20–1.05)
Hospitalization for >50 days	<0.05	1.61 (0.94–2.74)	2.98 (1.59–5.58)
Characteristics of feces	0.517	NT ^c	NT
WBC count of >10,000 WBCs/ μ l	<0.05	2.16 (1.24–3.75)	2.24 (1.21–4.14)
Albumin level of >2.0 g/dl	0.163	0.95 (0.50–1.81)	0.84 (0.42–1.72)
BMI of >15.0 kg/m ²	0.480	0.85 (0.34–2.14)	0.99 (0.34–2.89)
Gender	0.071	1.94 (1.11–3.40)	1.76 (0.95–3.29)
Use of PPIs	<0.05	9.03 (4.9–16.6)	9.15 (4.59–18.2)

^aStatistical analysis was performed and *P* values were determined with the Kruskal-Wallis H test.

^bORs were determined between 2 groups (TN group versus TP group or TN group versus TC group).

^cNT, not tested.

to 3.07]), carbapenems (TP group, 12/109 cases; TN group, 8/111 cases; OR, 1.59 [95% CI, 0.62 to 4.06]), or other antibiotics (trimethoprim-sulfamethoxazole or rifampin) (TP group, 12/109 cases; TN group, 8/111 cases; OR, 1.59 [95% CI, 0.62 to 4.06]) increased the odds of CDI. Comparing the TC group and the TN group, the use of cephalosporins (TC group, 24/72 cases; TN group, 28/111 cases; OR, 1.48 [95% CI, 0.77 to 2.84]), carbapenems (TC group, 11/72 cases; TN group, 8/111 cases; OR, 2.32 [95% CI, 0.89 to 6.09]), lincomycin (TC group, 7/72 cases; TN group, 7/111 cases; OR, 1.60 [95% CI, 0.54 to 4.77]), or fluoroquinolones (TC group, 8/72 cases; TN group, 9/111 cases; OR, 1.42 [95% CI, 0.52 to 3.86]) increased the odds of CDI (Table 5).

(iv) PPIs. PPI utilization rates in the TN, TP, and TC groups were 26.1%, 76.1%, and 76.4%, respectively (Fig. 3). The relative PPI use was significantly higher in the TP and TC groups than in the TN group ($\chi^2 = 70.588$, *df* = 2; *P* < 0.001). Furthermore, comparing the TP group (83/109 cases) and the TC group (55/72 cases) to the TN group (29/111 cases), PPI use increased the relative risk of CDI by 9.03 times (95% CI, 4.9 to 16.6 times) and 9.15 times (95% CI, 4.59 to 18.1 times), respectively (Table 4).

(v) Other characteristics. Gender, age, clinical outcomes, serum albumin levels, BMIs, and fecal consistency showed no significant differences between the groups (Table 4).

Detection of toxin A and B genes in isolated strains. The presence of toxin A and B genes was analyzed by PCR for 80 of the 102 specimens that were toxin negative even after toxigenic culture (Fig. 4). As a result, 59 strains (73.8%) were positive for both toxins, 2 strains (2.5%) were toxin A positive and toxin B negative, and 19 strains (23.8%) were negative for both toxins (Table 6). No strains that were toxin A negative and toxin B positive were detected. These results showed that *C. difficile* strains containing toxin genes could be detected despite negative toxin results by toxigenic culture.

FIG 2 Comparison of WBC counts among the 3 groups. TN, the group of cases negative by *Clostridium difficile* toxin direct fecal assay; TP, the group of cases positive by direct fecal assay; TC, the group of cases positive by toxigenic culture assay. The median WBC count was 9,000/ μ l; $\chi^2 = 11.648$, *P* < 0.05 (Kruskal-Wallis test).

TABLE 5 Comparison of antibiotic use among the TN, TP, and TC groups

Antibiotic(s)	No. in TN group (n = 111)	No. in TP group (n = 109)	OR (95% CI) for TN group vs TP group ^a	No. in TC group (n = 72)	OR (95% CI) for TN group vs TC group ^a
Cephalosporins	28	40	1.72 (0.96–3.07)	24	1.48 (0.77–2.84)
Carbapenems	8	12	1.59 (0.62–4.06)	11	2.32 (0.89–6.09)
Monobactams	1	1	1.01 (0.06–16.34)		
Penicillins and penicillin combinations ^b	52	44	0.77 (0.45–1.31)	32	0.91 (0.50–1.65)
Aminoglycosides	6	5	0.84 (0.25–2.82)	1	0.25 (0.03–2.09)
Macrolides	4	5	1.27 (0.34–4.92)	2	0.76 (0.14–4.29)
Lincomycin	7	6	0.87 (0.28–2.66)	7	1.60 (0.54–4.77)
Fluoroquinolones	9	11	1.27 (0.51–3.20)	8	1.42 (0.52–3.86)
Other ^c	8	12	1.59 (0.62–4.06)	3	0.56 (0.14–2.18)
Unused	12	11	0.93 (0.39–2.20)	6	0.75 (0.27–2.10)

^aORs were determined between 2 groups (TN group versus TP group or TN group versus TC group) according to statistical analysis using the Kruskal-Wallis H test.

^bPenicillin combinations, tazobactam-piperacillin.

^cOther, trimethoprim-sulfamethoxazole or rifampin.

DISCUSSION

Our results demonstrated that additional toxin-positive cases could be discovered, despite an initial negative fecal Quik Chek immunochromatography test result, by repetition of Quik Chek testing after colony isolation and PCR analysis of the toxin-negative cases after retesting. As a result, 73.8% of the GDH-assay-proven cases had the presence of both toxin A and toxin B, 2.5% had the presence of only toxin A, and 23.8% had an absence of both toxins (nontoxic strains). These results confirm that using the direct fecal test without additional assays may result in underdiagnosis of the carriage of toxin-containing *C. difficile*. Furthermore, this finding implies that, among cases that are initially GDH positive and toxin negative by fecal testing, many toxin-positive CDI cases may be missed (14). For such cases, an additional toxigenic culture assay step using the Quik Chek test is important to increase test reliability; this was underlined in the joint guidelines of the IDSA and the SHEA published in 2010 (5). The toxin gene ownership ratios of isolated strains seem to vary, depending on the site and the test method. Plaza-Garrido et al. (16) reported a toxin detection rate of 20.6% with a PCR method. In our study, it was confirmed that the rate of detection of toxin genes in antigen-positive/toxin-negative strains was high (76.3%), but those strains evidently produced less toxin even after toxigenic culture, resulting in negative immunochromatography test results. Therefore, it was necessary to clarify whether those strains could become causative organisms of CDI.

In this study, based on Quik Chek test results, we classified the clinical cases into TN (*C. difficile* toxin negative), TP (*C. difficile* toxin positive), and TC (*C. difficile* toxin positive) groups and examined the risk factors among the groups. Significant differences in CDI risk were observed when comparing WBC counts ($P = 0.003$), hospital stays ($P < 0.001$),

FIG 3 Comparison of PPI use among the 3 groups. TN, the group of cases negative by *Clostridium difficile* toxin direct fecal assay; TP, the group of cases positive by direct fecal assay; TC, the group of cases positive by toxigenic culture assay; PPIs, use of proton pump inhibitors. $\chi^2_{\text{Yates}} = 70.588$, 2 df, $P < 0.05$ (Kruskal-Wallis test).

FIG 4 Detection of toxin genes in *C. difficile* strains with GDH-antigen-positive and toxin-negative samples. M, molecule size markers; S1, S2, and S3, samples 1, 2, and 3, respectively; P, positive control; N, negative control; A1, toxin A (251 bp); A2, toxin A (1,265 bp); B1, toxin B (203 bp).

and the use of PPIs ($P < 0.001$) (Fig. 1, 2, and 3). The increased risk of CDI with increased WBC counts suggests a relationship between gastrointestinal inflammatory responses and disease. Henrich et al. (17) and Hookman and Barkin (18) reported that the OR of developing CDI with a WBC count of $\geq 20,000$ WBCs/ μl was 2.77. In this study, we showed that the TP and TC groups had increased WBC counts but also increased numbers of hospitalization days. This finding is consistent with the report by Hurley and Nguyen (6) linking the hospitalization period and *C. difficile* detection rate. In addition, Cadle et al. (15) reported that PPI use was a major inducer of CDI (OR, 4.17), which was borne out in this study, with an OR of 9.03 (95% CI, 4.9 to 16.6) for the TP group and an OR of 9.15 (95% CI, 4.59 to 18.2) for the TC group. Although the mechanism by which PPI use induces CDI has not been completely elucidated, PPIs suppress gastric acid secretion, facilitating invasion of exogenous pathogens. In the meta-analysis by Kwok et al. (19), the relationship between the use of PPIs and the onset of CDI was statistically significant (OR, 1.74 [95% CI, 1.47 to 2.85]), which is similar to our result. There were no significant differences (using the Kruskal-Wallis H test) among the 3 groups concerning antibiotic use, but the use of carbapenems, cephalosporins, and fluoroquinolones demonstrated increased relative risks of developing CDI (Table 5). It is possible that the control TN group had an excess of elderly people with multiple comorbidities and increased antimicrobial use, which might explain the lack of statistical significance. A prospective cohort study by Bartlett and Gerding (20) reported relative risks of 36.2 for cefotaxime, 9.0 for clindamycin, 7.8 for cefaclor, and 2.0 to 22.1 for β -lactams for the onset of CDI. Furthermore, in the meta-analysis by Vardakas et al. (21), administration of fluoroquinolones was shown to induce CDI (OR, 1.96). In this study, the relative risk of developing CDI with the use of carbapenem drugs with strong antimicrobial effects against intestinal bacteria was high. Tazobactam-piperacillin was the most frequently used antibiotic (43.2% in the TN group, 35.8% in the TP group, and 44.4% in the TC group), but the ORs for developing CDI were 0.77 (95% CI, 0.45 to 1.31) in the TP group and 0.91 (95% CI, 0.50 to 1.65) in the TC group, suggesting that tazobactam-piperacillin is not a risk factor for CDI induction, likely due to low antimicrobial activity against the

TABLE 6 Detection of toxin A and B genes by PCR for 80 strains that were GDH positive and toxin negative in toxigenic culture assays

Toxin-related gene results	No. (%) of strains
Toxin A positive, toxin B positive	59 (73.8)
Toxin A positive, toxin B negative	2 (2.5)
Toxin A negative, toxin B positive	0 (0)
Toxin A negative, toxin B negative	19 (23.8)

intestinal microbiota. With the results described above, it was demonstrated that the TC group (cases that were toxin negative in the initial Quik Chek tests) showed CDI risk factors similar to those for the TP group; therefore, similar measures to prevent nosocomial infection should be carried out.

In this study, 111 of 1,252 cases that were negative for both GDH and toxin were selected as the TN group. For the remaining 1,141 cases, microbiological and clinical data were not complete for the analysis of CDI risk factors, because in those cases CDI was excluded from the clinical point of view. A possible limitation of the present study is that all of the cases that were negative for GDH and toxin were not included in the TN group. It is possible that there might be an association between particular antibiotics and CDI risk if the number of cases in the TN group was increased to 1,252.

The results of this study suggest that cases that test toxin positive after toxigenic culture (the TC group in this study) need to be considered for potential CDI treatment if WBC counts are high, there is recorded use of antibiotics or PPIs, and/or the patient has been hospitalized for a long time. It is possible that this strategy will reduce and prevent hospital CDIs, potentially reducing treatment costs.

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