THE IMPACT OF TOXIGENIC CULTURE ON ANTIMICROBIAL PRESCRIPTIONS FOR CLOSTRIDIOIDES DIFFICILE INFECTION THE ROLE OF DIAGNOSTIC STEWARDSHIP

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Abstract

Introduction : Toxigenic culture has been recommended as a sensitivity enhancement option for testing *Clostridioides* difficile infections (CDI). However, no studies have evaluated whether toxigenic culture impacts clinical decisions such as CDI treatment.

Methods : At Nara Medical University Hospital, simultaneous testing of glutamate dehydrogenase antigen (A) and toxin A/B (T) by immunochromatography has been conducted since November 2013. Furthermore, toxigenic culture (C) has been adopted since April 2018. Therefore, patients tested for CD were divided into two groups: pre-period from April 2014 to March 2018 and post-period from April 2018 to March 2021. Patient data were retrospectively examined.

Results : The study included 1262 and 1023 cases in the pre- and post-periods, respectively. A significant reduction in A+T+ cases could be observed with 64 (5.1 %) and 28 (2.7%) in the pre- and post-periods (P = 0.005), respectively. Of the 104 A+T- cases undergoing toxigenic culture in the post-period, 54 (51.9 %) were A+T-C+. The antimicrobial administration ratio for the A+T-C+ cases (68.5 %) was lower than that for the A+T+ patients (90.6 and 82.1 %, P = 0.014 and P = 0.417, in the pre- and post-periods, respectively), and was not significantly different from that of the A+T-patients (64.2 and 64.1 % in the pre- and post-periods, respectively) or from that of the A+ T-C-patients (64 %).

Conclusion : This study showed that toxigenic culture does not necessarily affect the antibiotic administration ratio or duration. A coordinated approach under diagnostic stewardship for improved reporting and interpretation of toxigenic cultures would be necessary.

Key words : *Clostridioides difficile* infection (CDI), toxigenic culture, diagnostic stewardship

Introduction

Clostridioides difficile infection (CDI) is a severe medical condition in humans caused by the toxin-producing bacterium *Clostridioides difficile* (CD). Therefore, in addition to the

characteristic clinical manifestations of CDI, such as diarrhea, CDI diagnosis could also be confirmed by verifying the presence of toxin or toxin-producing CDI in the feces [1].

The immunochromatography-based simultaneous detection of GDH antigen and toxin A/B is a commonly used CDI test in Japan. The test is characterized by its high sensitivity to the GDH antigen, although it displays low toxin sensitivity [2–4]. Another current method comprises anaerobic feces culture. Once CD grows in these cultures, a toxin test is performed using the grown colony. This approach is known as toxigenic culture, and evidently forms the most sensitive and reliable test for CDI diagnosis [1, 5]. Although toxigenic culture is highly sensitive in terms of CD toxin production, it is not cost- and work-efficient. In fact, it is expensive and laborious to culture stools anaerobically and repeat the toxin test using colonies.

A substantial number of antigen-positive toxin-negative cases were reportedly diagnosed by the toxigenic culture approach using simultaneous fecal GDH antigen and toxin testing of toxin-producing strains [1–4]. Therefore, it is expected that the simultaneous detection of fecal GDH antigen and toxin would improve the CDI diagnostic accuracy, increase the rate at which essential treatment strategies are implemented, and decrease any unnecessary treatment. However, no studies have evaluated yet how toxigenic cultures could impact clinical practice.

In this study, the hospital has adopted toxigenic culture since April 1, 2018, as a part of the measures against nosocomial infections. We compared the physicians' prescription of CDI antibiotics to see if performing toxigenic culture could change the prescription patterns.

Materials and methods

Subjects and study design

This study retrospectively examined patients who underwent the "C. DIFF QUIK CHECK COMPLETE" rapid membrane enzyme immunoassay (ABBOTT, Japan) between April 2014 and March 2021 at Nara Medical University Hospital. The subjects were at least 18 years old. If the same patient were tested multiple times, only tests performed at least 12 weeks apart were included and counted as a case. Furthermore, if oral metronidazole (oMNZ), oral vancomycin powder (VCM), or injected metronidazole (iMNZ) were initiated within 5 days before or after the noted test date, the prescribed medication would be considered a treatment related to the tested case. There were no cases wherein fidaxomicin was prescribed during this study.

From April 1, 2014, to March 31, 2018 (pre-period), the CD test was conducted using only C. DIFF QUIK CHEK COMPLETE. From April 1, 2018, to March 31, 2021 (post-period), in addition to the "C. DIFF QUIK CHEK COMPLETE", the CD test was performed with the CD toxin test (toxigenic culture) using cultured and grown colonies. However, toxigenic culture was not performed in some cases during long-term holidays, such as weekends and New Year's Holidays.

Tests

The "C. DIFF QUIK CHECK" was performed in accordance with the manufacturer's protocols. Toxigenic cultures were anaerobically cultured in pre-reduced CCFA medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for 24–48 h, and the presence or absence of toxigenicity of the developed colonies was determined using C. DIFF QUIK CHECK.

Statistical analysis

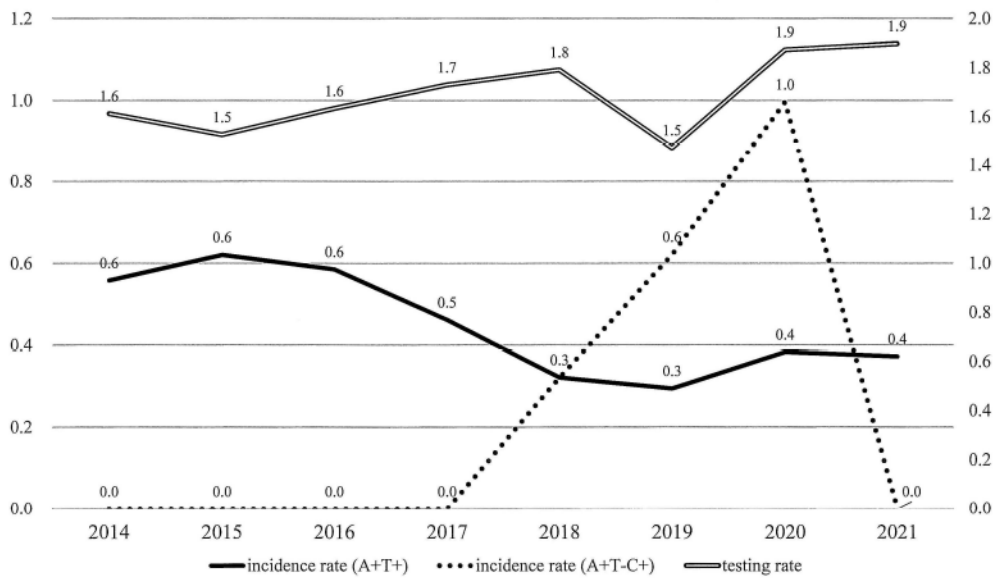
The CDI incidence rate (antigen-positive cases or antigen-positive, toxin-negative, and culture-positive cases) was expressed in terms of "per 10,000 patient-days (PD)". The CD testing rate was calculated using the number of "C. DIFF QUIK CHECK" per 1,000 PD. We used Fisher's exact test to compare antimicrobial administration ratios between patient sub-groups. A result with a two-sided p-value of less than or equal to 0.05 was considered statistically significant. No adjustments were made for the multiple comparison testing. All statistical analyses were conducted using R, version 4.0.5 (R Core Team).

This project was approved by the Ethics Committee of Nara Medical University (Project identification code No. 1465).

Results

Figure 1 shows the CDI incidence rate (per 10,000 PD) and CD testing rate (per 1,000 PD) from April 1, 2014, to March 31, 2021. The presented data indicate if antigen- and toxin-positive (A+T+) cases and antigen-positive, toxin-negative, and toxigenic culture-positive (A+T-C+) cases were confirmed as CDI. The incidence rate of A+T+ gradually decreased during this period but the incidence rate of the A+T-C+ cases increased with the start of the toxigenic culture from April 1, 2018. The testing rate gradually increased during this period. The total number of tests performed during the study period was 3391 for 2210 people (1.5 times per person on average).

In the pre-period, 1,262 cases from 1,192 patients were evaluated. Among the post-period cases, 1,023 cases from 981 patients were evaluated. Table 1 shows the number and proportion of antigens, toxins, and toxigenic culture cases in each group, and the type and duration of the applied antibiotics. The number of A+T+ cases was 64 (5.1 %) in the pre-period, which



A+T+: antigenpositive, toxin-positive
A+T-C+: antigen-positive, toxin-negative, and toxigenic culture-positive

Fig. 1. Annual trends of incidence and testing rates for CDI A+T+ and A+T-C+ cases.

decreased to 28 (2.7 %) in the post-period (P = 0.005). Furthermore, we could detect 172 (13.1 %) and 130 (12.3 %) A+T- cases in the in the pre- and post-periods, respectively. Of the 128 A+T- cases in the post-period, 104 cases underwent toxigenic culture, of which 54 (51.9 %) were antigen-positive, toxin-negative, and toxigenic culture-negative (A+T-C+).

Table 2 shows the treatment details for the respective groups. The antimicrobial administration ratio of the A+T-C+ cases (68.5 %) was lower than that of the A+T+ cases (90.6 and 82.1%, P = 0.014 and P = 0.417, in the pre- and post-periods, respectively), and was not significantly different from that of the A+T-patients (64.2 and 64.1 % in the pre- and post-periods, respectively) or from that of the A+ T-C-patients (64 %) and was higher than that for A-T- cases (9.5% in the pre-period and 4.7% in the post-period) (P<0.001 for both compared to A+T-C+ cases).

In terms of treatment, in A+T+ cases, VCM was used more often (12 cases, 42.9 %) than oMNZ (25 cases, 39.1 %) during the pre-period. However, during the post-period, oMNZ was applied more often (12 cases, 42.9 %) than VCM (6 cases, 21.4 %). For both the A+T-C+ and A+T-C- post-period cases, VCM was used more often than oMNZ. The treatment period tended to be shorter during the pre- and post-periods (8.1 and 8.0 days, respectively) for the A-T-cases. However, in other cases, no noticeable difference could be detected between approximately 10 to -14 days.

Table 1. Consequences of antigens, toxins, and toxigenic cultures in the pre- and post-periods.

	Pre-period (n = 1262)	Post-period (n = 1023)
A+T+	64 (5.1 %)	28 (2.7 %)
Male	39 (60.9 %)	18 (64.3 %)
Age (mean, years)	70.2	71.3
A+T-	162 (12.8 %)	128 (12.5 %)
Male	93 (57.4 %)	85 (66.4 %)
Age (mean, years)	68.3	69.5
A+T-C+		54 (5.3 %)
Male		38 (70.4 %)
Age (mean, years)		70.8
A+T-C-		50 (4.9 %)
Male		31 (62 %)
Age (mean, years)		67.5
A+T-Cn/a		24 (2.3 %)
Male		16 (66.7 %)
Age (mean, years)		70.5
A-T-	1036 (82.1 %)	867 (84.8 %)
Male	614 (59.3 %)	507 (58.5 %)
Age (mean, years)	67.5	68.2

A+T+: antigen-positive, toxin-positive
A+T-: antigen-positive, toxin-negative
A+T-C+: antigen-positive, toxin-negative, and toxigenic culture-positive
A+T-C-: antigen-positive, toxin-negative, and toxigenic culture-negative
A+T-Cn/a: Antigen-positive, toxin-negative, no toxigenic culture outcome
A-T-: Antigen-negative, toxin-negative

Table 2. Administered antibacterial drugs and duration of administration by CDI test results in the pre and post-periods.

	Pre-period	Treatment period (days)	Post-period	Treatment period (days)
A+T+	(n = 64)		(n = 28)	
Any Tx	58 (90.6 %)	11.5	23 (82.1 %)	
oMNZ	25 (39.1 %)	12.9	12 (42.9 %)	11.1
VCM	30 (46.9 %)	11.1	6 (21.4 %)	12.8
iMNZ	0 (0 %)		3 (10.7 %)	9.3
Mixed	3 (4.7 %)	16.0	2 (7.1 %)	9.5
A+T-	(n = 162)		(n = 128)	
Any Tx	104 (64.2 %)	11.9	82 (64.1 %)	12.0
oMNZ	52 (32.1 %)	11.3	31 (24.2 %)	9.8
VCM	42 (25.9 %)	12.1	40 (31.3 %)	12.7
iMNZ	0 (0 %)		1 (0.8 %)	1
Mixed	10 (6.2 %)	13.5	10 (7.8 %)	16.9
A+T-C+	(n = 0)		(n = 54)	
Any Tx			37 (68.5 %)	13.2
oMNZ			12 (22.2 %)	10.0
VCM			20 (37 %)	14.8
iMNZ			1 (1.9 %)	1
Mixed			4 (7.4 %)	18.3
A+T-C-	(n = 0)		(n = 50)	
Any Tx			32 (64 %)	11.1
oMNZ			12 (24 %)	9.4
VCM			15 (30 %)	10.9
iMNZ			0 (0%)	
Mixed			5 (10 %)	15.4
A+T-Cn/a	(n = 0)		(n = 24)	
Any Tx			13 (54.2 %)	11.2
oMNZ			7 (29.2 %)	10.0
VCM			5 (20.8 %)	11.4
iMNZ			0 (0 %)	
Mixed			1 (4.2 %)	19.0
A-T-	(n = 1036)		(n = 867)	
Any Tx	98 (9.5 %)	8.2	41 (4.7 %)	8.0
oMNZ	18 (1.7 %)	9.8	7 (0.8 %)	7.1
VCM	77 (7.4 %)	7.7	30 (3.5 %)	7.9
iMNZ	1 (0.1 %)	9.0	4 (0.5 %)	10.8
Mixed	2 (0.2 %)	8.5	0 (0 %)	

A+T+ : antigen-positive, toxin-positive
A+T-: antigen-positive, toxin-negative
A+T-C+: antigen-positive, toxin-negative, and toxigenic culture-positive
A+T-C-: antigen-positive, toxin- negative, and toxigenic culture-negative
A+T-Cn/a: antigen-positive, toxin-negative, no toxigenic culture outcome
A-T-: antigen-negative, toxin-negative

Discussion

CDI incidence rate

When CDI was defined by A+T+ or A+T-C+ cases, the CDI incidence rate in our hospital ranged between 0.4 and –1.4. In particular, in 2020, the incidence rates of A+T+ and A+T-C+ cases were 0.4 and 1.0, respectively, for a total of 1.4. The CDI incidence rates in Japanese hospitals were reportedly 0.8, 1.6, and 3.4 according to Hikone et al. [6], Mori et al. [7], and Honda et al. [8], respectively. However, a recent study using stricter criteria reported a value of 7.4, suggesting that our CDI diagnosis might be underestimated [9]. In this study, the stool was collected in cases of clinically significant diarrhea (CSD) (defined by one of the following conditions: 1) at least three diarrheal bowel movements (Bristol stool chart grade 6–7) in the previous 24 h, or a diarrheal bowel movement with abdominal pain and/or cramping; 2) among

patients with pre-existing chronic diarrhea, an increase of ≥ 3 diarrheal stools compared with the usual diarrheal frequency; 3) the same frequency of diarrhea with new or worsening abdominal pain and/or cramping). It uses the diagnostic criteria of a toxigenic culture or nucleic acid amplification test to diagnose toxin positivity [9]. We need to focus on suspecting CDI and obtaining specimens appropriately, as well as testing specimens as a part of the diagnostic stewardship program.

CDI testing rate

Regarding the testing rate, Kato et al. reported that the average value was 30.4/10,000 PD, varying between 3.6 and –256.8 per 10,000 PD in Japanese hospitals [9]. Our testing rate ranged between 15 and –19/10,000 PD. The fact that our CDI incidence rate was approximately 1/5 despite the testing rate being approximately half that described by Kato et al. suggests that our hospital is testing patients with a lower CDI possibility or is testing the same patients repeatedly. Indeed, an average of 1.5 tests were performed per person in our study. Regarding the CDI test, negative confirmation after the start of the treatment is not required, and clinicians need to be informed to avoid any unnecessary tests.

Toxigenic culture effect and impact

Of the 104 cases that underwent toxigenic culture, 54 (51.9 %) were positive. As there were 28 A+T+ cases, approximately 1.9 times as many cases could be diagnosed by toxigenic culture. Mori et al. also reported that 127 (71.8 %) of 177 of toxigenic culture cases were positive [7]. The present immunochromatography method for the simultaneous detection of GDH antigens and toxins exhibits a major sensitivity problem, suggesting the usefulness of toxigenic culture. In this study, toxigenic culture was also performed on 867 antigen-negative cases using immunochromatography, 17 of them yielding positive cultures, while six of these cases were toxin-positive (data not shown). Toxigenic culture is a costly and labor-intensive test. As such, although antigen-positive and toxin-negative cases are appropriate targets for the toxigenic culture, from a financial point of view, it is necessary to decide whether toxigenic culture should be performed even on antigen-negative cases.

The impact of toxigenic cultures on clinical practice (antibiotic administration)

The primary purpose of this study was to examine how toxigenic culture could impact CDI clinical management, especially treatment. The greatest merit of toxigenic culture lies in its ability to distinguish between toxin- and non-toxin-producing strains among the bacterial strains in the feces of A+T- patients. Once an A+T-C+ patient has been identified, the patient would likely receive a treatment similar to A+T+ case-related treatments, and in the case of A+T-C- would likely receive treatment similar to A-T- case-related treatments. In this study, the treatment ratio for A+T-C+ cases was 68.5 %, which was significantly lower than the treatment ratio of 90.6 % (pre-period) or 82.1 % (post-period). However, the treatment ratio of A+T-C- cases was 64 %, which was equivalent to the treatment ratio of A+T-C + cases and was higher than the treatment ratio of A+T-cases, which was 9.5 % (pre) or 4.7 % (post). Since the toxigenic culture-related results are released several days after the immunochromatography-related

results, it is unclear whether physicians have reliably confirmed the results of the toxigenic culture at that time. Even if the results are confirmed, physicians could potentially lack the knowledge to optimally use and interpret the results in clinical judgment. When conducting the toxigenic culture tests, we believe that it would be necessary to meticulously explain the procedure and the relevant interpretations to the physicians.

Limitations

Our study had several limitations. First, this is a retrospective study limited to cases that underwent the CD test and might not include those that did in fact require a CD test. Therefore, the incidence rate might not accurately reflect the CDI incidence in the hospital. However, this study aimed to grasp the current clinical situation and eliminate the existing issues, and we believe that the study design served this purpose.

Second, there were times and situations where toxigenic culture was difficult to perform in a clinical setup and hence was not carried out in certain cases from the post-period group. This might have affected the results of this study, depending on the details of cases that did not undergo toxigenic culture.

Third, the treatment of CDI is not limited to the administration of antibacterial drugs against CD; there is also a method involving the discontinuation of the antibacterial drug that may have caused the CDI. However, the antibacterial drugs used in this study were not evaluated. As a result, in cases that did not receive CDI treatment, it was unclear whether the CDI was cured because the appropriate antibacterial drug was not administered or simply due to discontinuing the previously prescribed antibacterial drug.

Conclusions

In conclusion, the toxigenic culture increased the number of cases in which CDI could actually be diagnosed by identifying the toxin-producing strain, especially in cases where the conventional test result was A+T-. However, there was no significant change in the antibiotic administration pattern followed by the clinicians for CDI.

Even if the toxigenic culture test is performed, it is necessary to report the results at an appropriate time and inform and educate clinicians so that they can make appropriate evaluations before writing prescriptions. In recent years, the importance of diagnostic stewardship, which promotes the proper use of antibacterial agents and improves patient prognosis through appropriate diagnosis, has been emphasized in the field of infectious disease medical care. The aforementioned efforts contribute toward diagnostic stewardship in cases of CDI.

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Conflict of interest

The authors declare that they have no conflict of interest.

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