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BRIEF COMMUNICATION

The importance of matrix-assisted laser desorption ionization–time of flight mass spectrometry for correct identification of *Clostridium difficile* isolated from chromID *C. difficile* chromogenic agar

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Abstract The clinical workflow of using chromogenic agar and matrix-assisted laser desorption ionization time-of-fight mass spectrometry (MALDI-TOF MS) for *Clostridium difficile* identification was evaluated. The addition of MALDI-TOF MS identification after the chromID *C. difficile* chromogenic agar culture could significantly improve the diagnostic accuracy of *C. difficile*.

Clostridium difficile is the main pathogen causing nosocomial antibiotic-associated diarrhea,[1–4] whereas some other *Clostridium* species are treated as normal flora in human fecal specimens. According to recent studies, prompt identification of *C. difficile* from hospitalized patients is key for the infection control of a nosocomial *C. difficile* outbreak.[5,6] Because of the high similarities of phenotypic

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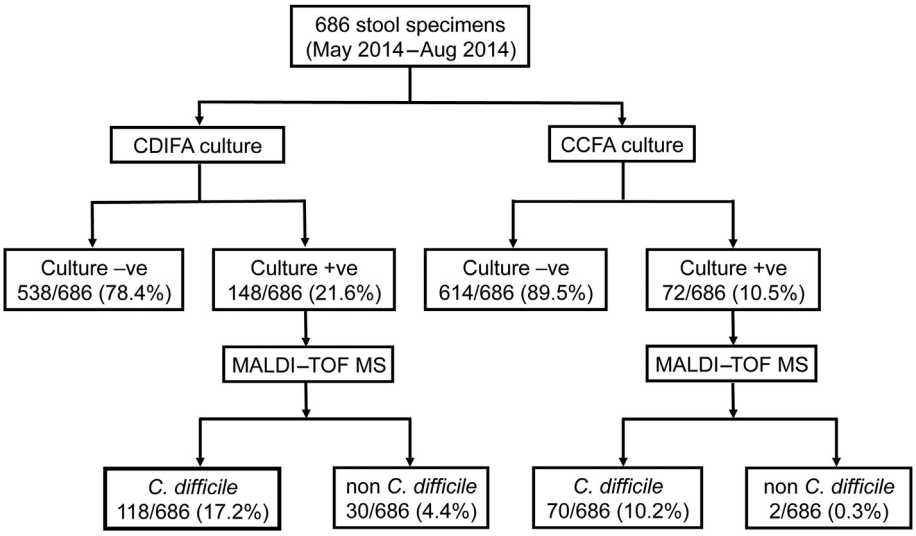

Figure 1. Comparison of the diagnostic accuracy between CDIFA or CCFA culture alone and MALDI-TOF MS after culture. *C. difficile*, *Clostridium difficile*, CCFA, Oxoid *Clostridium difficile* selective agar, CDIFA, *Clostridium difficile* chromogenic agar, MALDI-TOF MS.

characteristics among *Clostridium* species, reliable species identification can only be determined by *16S* rRNA gene sequencing from colonies isolated from cycloserine cefoxitin fructose agar.[7,8] However, the procedures are complicated and time-consuming (8–12 hours). Starting in 2013, the implementation of chromogenic agar for *C. difficile* identification has been shown to improve the recovery rate and turn-around time.[9–13] However, the diagnostic specificity of the chromogenic agar for *C. difficile* was not clearly defined in the reports.

The objective of this study was to define the specificity of the commercial chromID *C. difficile* chromogenic agar (CDIFA; bioMérieux, Marcyl'E'toile, France) for *C. difficile*. We also tried to evaluate the practical importance of incorporating the matrix-assisted laser desorption ionization time-of-fight mass spectrometry (MALDI-TOF MS) for species confirmation after the chromogenic agar culture.

In brief, 686 soft stool or liquid stool specimens from 508 patients hospitalized in a healthcare network in Hong Kong, which included a university-affiliated hospital and three extended-care hospitals, were collected between February 28, 2014 and May 5, 2014. The specimens were plated directly onto CDIFA and onto Oxoid Clostridium difficile Selective Agar (CCFA) (Oxoid Ltd., Hamsphire, UK) for the clostridia culture. The media were incubated under an anaerobic condition for at most 72 hours. The cultures were examined after incubating for 24 hours, 48 hours, and 72 hours. Cultures showing no growth after 72 hours were confirmed as a negative culture. Once suspected flat and irregular gray-black colonies were found growing on the culture media within 72 hours, the incubation was stopped and further identification was followed up. The suspected colonies were identified by the Bruker Microflex LT MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) by using the direct transfer method. Bacterial identification was matched with the Bruker spectra library program (version 4.0.0.1) that was preinstalled in the Bruker Biotyper device (version 3.1; Bruker.1). Cultures with ambiguous identification were further confirmed by a 1026 bp partial *16S* rRNA gene sequencing.[14,15] The diagnostic performance of the two media was analyzed statistically using MedCalc version 14.12.0 statistical software (MedCalc Software, Ostend, Belgium). Statistical analyses were performed using the Chi-square test and McNemar's test.

In general, gray-black irregular suspected *C. difficile* colonies could be observed in 21.6% (148/686 specimens) and 10.5% (72/686 specimens) of the CDIFA and CCFA cultures, respectively. On average, positive cultures from CDIFA could be observed at approximately 48 hours. By contrast, cultures from CCFA required 64 hours on average to grow. For species identification, we further identified the colonies by MALDI-TOF MS. Among the positive cultures, only 118 of the CDIFA cultures and 70 of the CCFA cultures were identified as *C. difficile* with a score > 2.0. The actual *C. difficile* recovery rate for CDIFA and CCFA was 17.2% (118/686 specimens) and 10.2% (70/686 specimens), respectively ($p < 0.001$; Figure 1 and Table 1).

For the 30 non-*Clostridium difficile* cultures growing on CDIFA, 28 cultures were identified as *Clostridium hathewayi* and the other two cultures were identified as *Clostridium tertium* and *Clostridium disporicum*. All non-

Table 1 Identification of positive cultures of the chromID Clostridium difficile agar and Oxoid *C. difficile* Selective Agar.

Identification	Isolates recovered, *n* (%)	
	CDIFA	CCFA
C. difficile	118 (17.20)	69 (10.06)
Clostridium species other than *C. difficile*		
C. hathewayi	28 (4.08)	2 (0.29)
C. tertium	1 (0.15)	0
C. disporicum	1 (0.15)	0
No growth	538 (78.43)	615 (89.65)
Total	686 (100)	686 (100)

CCFA, Oxoid Clostridium difficile Selective Agar (CCFA); CDIFA, chromID *C. difficile* agar (CDIFA).

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Table 2 Identification results of 30 non-Clostridium difficile isolates growing on chromID C difficile agar.

Sample no.	Culture		Identification		
	CDIFA	CCFA	MALDI-TOF ID	Score	16S rRNA gene sequencing
1	Growth	No growth	*Clostridium hathewayi*	2.293	*C. hathewayi*
3	Scanty growth	No growth	*C. hathewayi*	2.186	*C. hathewayi*
10	Growth	No growth	*C. hathewayi*	2.041	*C. hathewayi*
11	Growth	No growth	*C. hathewayi*	1.981	*C. hathewayi*
12	Growth	No growth	*C. hathewayi*	1.887	*C. hathewayi*
16	Growth	No growth	*C. hathewayi*	1.976	*C. hathewayi*
61	Growth	No growth	*C. hathewayi*	2.245	*C. hathewayi*
63	Scanty growth	No growth	*C. hathewayi*	2.080	*C. hathewayi*
101	Heavy growth	No growth	*C. hathewayi*	2.064	*C. hathewayi*
128	Heavy growth	No growth	*C. hathewayi*	2.054	*C. hathewayi*
167	Heavy growth	No growth	*C. hathewayi*	2.087	*C. hathewayi*
236	Growth	No growth	*C. hathewayi*	2.000	*C. hathewayi*
294	Growth	No growth	*C. hathewayi*	2.162	*C. hathewayi*
378	Growth	Growth	*C. hathewayi*	2.020	*C. hathewayi*
429	Scanty growth	No growth	*Clostridium disporicum*	1.868	*C. disporicum*
451	Heavy growth	No growth	*C. hathewayi*	2.015	*C. hathewayi*
454	Growth	No growth	*C. hathewayi*	2.059	*C. hathewayi*
494	Growth	No growth	*C. hathewayi*	2.056	*C. hathewayi*
544	Growth	No growth	*C. hathewayi*	2.010	*C. hathewayi*
569	Scanty growth	No growth	*C. hathewayi*	2.150	*C. hathewayi*
578	Growth	No growth	*C. hathewayi*	2.034	*C. hathewayi*
583	Scanty growth	Growth	*C. hathewayi*	2.147	*C. hathewayi*
621	Scanty growth	No growth	*C. hathewayi*	2.142	*C. hathewayi*
624	Growth	No growth	*C. hathewayi*	1.901	*C. hathewayi*
626	Growth	No growth	*C. hathewayi*	1.976	*C. hathewayi*
630	Growth	No growth	*Clostridium tertium*	2.336	*C. tertium*
632	Growth	No growth	*C. hathewayi*	2.228	*C. hathewayi*
634	Scanty growth	No growth	*C. hathewayi*	2.039	*C. hathewayi*
651	Scanty growth	No growth	*C. hathewayi*	2.057	*C. hathewayi*
655	Growth	No growth	*C. hathewayi*	2.020	*C. hathewayi*

CCFA, Oxoid *Clostridium difficile* Selective Agar; CDIFA, *Clostridium difficile* chromogenic agar; MALDI-TOF ID, matrix-assisted laser desorption ionization—time of flight mass spectrometry identification.

Clostridium difficile specimens, except two *C. hathewayi* specimens, did not grow on CCFA. The morphology of the non-*Clostridium difficile* colonies growing on CDIFA was also gray-black and irregular, which was highly similar to the morphology of the *C. difficile* colonies. It was difficult to differentiate the two species by morphology and color change. The identities of these 30 non-*Clostridium difficile* specimens were further confirmed by *16S* rRNA gene sequencing (Table 2).

Our study showed that the CDIFA had a much shorter average recovery time for *C. difficile,* compared to the CCFA (48 hours vs. 64 hours). This would allow the laboratory staff to alert the clinical microbiologists earlier that the clinical symptoms of the patients may be caused by *C. difficile* infection. The CDIFA also demonstrated a significantly higher *C. difficile* recovery rate than CCFA (21.6% vs. 10.5%; $p < 0.001$). However, the use of CDIFA alone for *C. difficile* direct culture could result in a 20.27% (30/148) false-positive rate. False-positive for non-*Clostridium* species growing on CDIFA has been reported in some studies,[10,13,16] although we found that all false-positive cultures isolated in this study were *Clostridium* species (e.g., *C. hathewayi*, *C. disporicum*, and *C. tertium*). These *Clostridium species* were all classified as commensal flora in healthy human feces. *Clostridium hathewayi*, which can cause disease once it gets into the bloodstream,[8,17] was first recovered by CDIFA. However, this may be because of the prolonged incubation of CDIFA. Further investigation should be followed up.

By adding the MALDI-TOF MS after the CDIFA culture, the specificity and positive predictive value could be significantly improved from 94.72% to 100% and from 79.73% to 100%, respectively ($p < 0.001$). The false-positive culture on CDIFA could be solved by using MALDI-TOF MS as the confirmation tool. Because the MALDI-TOF MS step is simple and cheap, which the processing time and running cost is around 10 minutes and US$1, the MALDI-TOF MS is a more affordable confirmation test than the *16S* rRNA gene sequencing for *C. difficile* identification. This rapid and reliable workflow for *C. difficile* identification would benefit the infection control of nosocomial antibiotic-associated diarrhea.

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In conclusion, the CDIFA required short recovery time and showed high sensitivity to *C. difficile*. The CDIFA could also culture *Clostridium* species other than *C. difficile*; therefore, the addition of rapid MALDI-TOF MS after the CDIFA culture could significantly improve the diagnostic accuracy for *C. difficile*. We highlighted the importance of MALDI-TOF MS for correct species identification of *C. difficile* from clinical specimens and this workflow was highly recommended.

Conflicts of interest

None declared.

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