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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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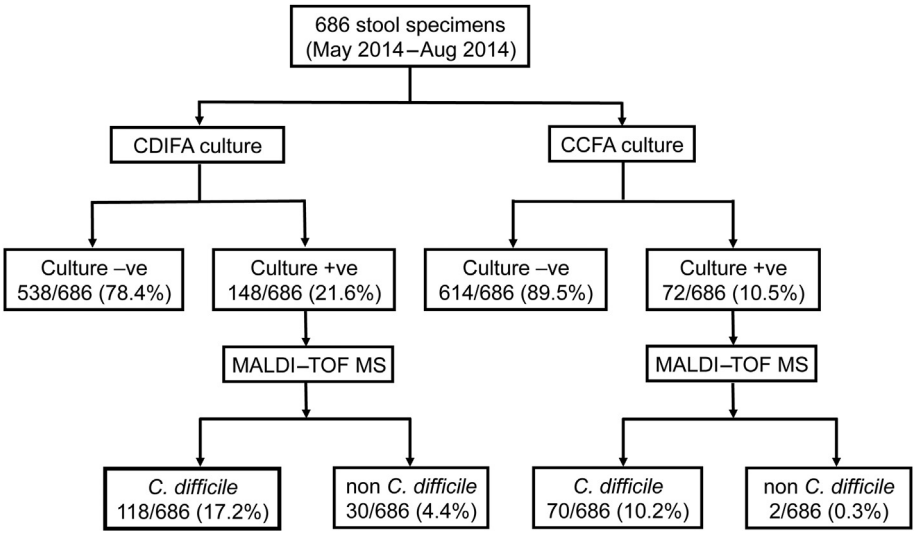

Figure 1. Comparison of the diagnostic accuracy between CDIFA or CCFA culture alone and MALDI-TOF MS after culture. *C. difficile*, *Clostridium difficile*, CCFA, Oxoid *Clostridium difficile* selective agar, CDIFA, *Clostridium difficile* chromogenic agar, MALDI-TOF MS.

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).