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Clostridium difficile Testing: Have We Got It Right?

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We read with interest the recent article by Kaltsas et al. which retrospectively evaluated the impact of converting to a nucleic acid amplification test (NAAT)-based assay for *Clostridium difficile* detection (1). The authors described several possible consequences of such an approach as a result of the increased sensitivity associated with NAAT-based testing, namely, detecting patients with *C. difficile* colonization and mild *C. difficile* infection (CDI). This increased detection in turn might result in increased and unnecessary antimicrobial treatment. To investigate these assertions, we undertook a prospective clinical review during an evaluation of the Illumigene *C. difficile* loop amplification (LAMP) assay (Meridian Bioscience, Inc.). Clinicians were blinded to the results of the NAAT assay but were provided the results according to our existing *C. difficile* laboratory algorithm: a glutamate dehydrogenase enzyme immunoassay (EIA) screening test (C.DIFF CHEK-60 [Wampole]) followed by a *C. difficile* A/B II (Wampole) toxin EIA. All stool samples were cultured for *C. difficile* using Clostridium difficile agar (bioMérieux, Australia) and alcohol shock and toxigenic culture performed on positive isolates. PCR ribotyping (2) was performed using a previously published method. The Hospital Human Research Ethics Committee approved the study. Categorical data were analyzed using SPSS version 18.

C. difficile testing was limited to single hospital patient samples ($n = 98$) that took the form of the container. The majority of patients were female (70%; 69/98), with ages ranging from 6 months to 97 years (median, 75 years). Of note, at review, 21% of the patients no longer had diarrhea (≥3 loose stools in the 24 h prior to sample collection) (3). In contrast to NAAT testing, where symptoms did not correlate with positivity (diarrhea was present in 83% and 76% of NAAT-positive and -negative episodes, respectively; P not significant), EIA toxin-positive episodes were significantly more likely than EIA-negative episodes to still be symptomatic (100% versus 74%; $P < 0.01$) (Table 1).

TABLE 1 Comparison of clinical features and patient outcomes stratified by EIA and Illumigene test result[a]

Clinical characteristic	No. (%) with indicated Illumigene *C. difficile* LAMP assay result			
	EIA toxin positive ($n = 16$)		EIA toxin negative ($n = 82$)	
	Pos ($n = 15$)	Neg ($n = 1$)	Pos ($n = 15$)	Neg ($n = 67$)
Diarrhea[b]	15 (100)	1 (100)	10 (67)	51 (76)
Non-CDI antibiotic treatment ceased	13/14 (93)	1 (100)	9/12 (75)	27/52 (52)
CDI antibiotic treatment	13 (87)	1 (100)	5 (33)	8 (12)
Symptom improvement				
Day 3	11 (73)	0 (0)	13 (87)	54 (81)
Day 7	12 (80)	0 (0)	14 (93)	61 (91)
Outcomes				
Relapse[c]	1 (7)	0 (0)	2 (13)[d]	0 (0)
Mortality by day 30[e]	2 (13)	0 (0)	1 (7)	6 (9)

[a] CDI, *C. difficile* infection; Pos, positive; Neg, negative.
[b] At least 3 loose stools in the 24 h prior to sample collection.
[c] Within 30 days.
[d] Clinical relapse at 2 weeks in 1 nontreated LAMP-positive patient with repeat EIA-negative stool sample results.
[e] Death not attributed to CDI in any of the cases.

Not surprisingly, clinicians predominantly treated symptomatic patients with a positive EIA toxin result (88%; 14/16 treated). In contrast, specific CDI treatment was rarely administered (15%; 13/82) when EIA results were negative, despite ongoing symptoms. Symptoms improved (a decrease in stool frequency or improvement in stool consistency) (4) in the majority of patients at days 3 (80%) and 7 (89%), with no significant difference detected between EIA toxin-positive and EIA-negative episodes irrespective of NAAT result or specific treatment. This suggests that specific treatment would unlikely benefit EIA toxin-negative, NAAT-positive patients (as 87% and 93% were symptom free at days 3 and 7, respectively) despite all but two (13/15) of these episodes also being positive by toxigenic culture. This assertion is further supported by similar 30-day mortality and relapse rates observed between the two groups.

Although clinicians were blinded to the results of NAAT-based testing, our data suggest that clinicians are likely to treat NAAT-positive patients, which may result in overtreatment of mild CDI and *C. difficile* carriage. Conversely, EIA toxin positivity probably reflects a greater burden of infection, which correlates with the need for therapy and with outcomes (5, 6). Whether these results reflect all *C. difficile* ribotypes is unknown, with no hypervirulent NAP1 isolates identified in our study by PCR ribotyping (2). A possible explanation for the observed "oversensitivity" of NAAT testing in our study is that 21% of testing was performed on patients whose disease status did not meet the clinical definition of diarrhea (3) at the time of testing. Similarly, in the study by Kaltsas et al., 16% of episodes had nonspecific abdominal symptoms with no diarrhea. These results highlight the need for appropriate patient selection when performing testing and the real possibility of CDI overdiagnosis leading to unnecessary antibiotic usage.

In conclusion, NAAT-based *C. difficile* detection may not result in improved patient outcomes but may lead to increased antibiotic treatment for possible colonized states or self-limited infection (7). Further research using appropriately powered studies is needed to determine which patients benefit from specific CDI

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treatment and whether identification of patients with mild disease or carriage of toxigenic *C. difficile* (NAAT positive, EIA toxin negative) should continue for infection control purposes in an attempt to prevent transmission (7, 8, 9).

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