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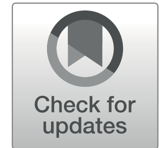

CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Evaluation of glutamate dehydrogenase (GDH) and toxin A/B rapid tests for *Clostridioides* (prev. *Clostridium*) *difficile* diagnosis in a university hospital in Minas Gerais, Brazil

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Abstract

Clostridioides (*Clostridium*) *difficile* is responsible for most cases of nosocomial diarrhea and, despite the high prevalence of the disease worldwide, the best laboratory diagnostic approach to diagnose *C. difficile* infection (CDI) is a subject of ongoing debate. Although the use of multiple tests is recommended, the cost of these algorithms commonly exceeds the affordability in some countries. Thus, to improve CDI diagnosis in a university hospital in Brazil, this study analyzed two immunochromatographic tests and one enzyme immunoassay (ELISA) to evaluate the detection of glutamate dehydrogenase (GDH) and A/B toxins of *C. difficile*. Stool samples of 89 adult patients presenting nosocomial diarrhea during hospitalization were included. The toxigenic culture was used as the reference method. GDH detection by both commercial tests showed high sensitivity (100%) and specificity (92.1%). On the other hand, toxin-based methods showed a sensitivity between 19.2 and 57.7%. In conclusion, the results suggest that rapid tests for GDH detection are not only suitable for CDI diagnosis as screening tests but also as a single method.

Keywords Pseudomembranous colitis · Nosocomial diarrhea

Clostridioides (*Clostridium*) *difficile* is responsible for most cases of antibiotic-associated diarrhea worldwide [1]. Despite the severity of the disease, the best laboratory diagnostic approach to diagnose *C. difficile* infection (CDI) is a subject of ongoing debate [2]. The diagnosis of CDI is frequently based on the clinical history and the detection of A/B toxins, and/or toxigenic isolates, by a combination of laborious methods [3]. In this context, the use of rapid tests for the detection of *C. difficile* glutamate dehydrogenase (GDH) is increasing as a screening method due to its low cost, high sensitivity, and fast results [4, 5]. However, if this method is not capable of differentiating non-toxigenic from toxigenic *C. difficile* strains, a stool sample positive for GDH is usually subjected to a second test involving a toxin-detecting assay, such as enzyme immunoassays (ELISA) and lateral flow tests, or a DNA-based test, commonly nucleic acid amplification-based tests [3].

Although the use of multiple tests is highly recommended to diagnose CDI [3], the cost of these algorithms commonly exceeds the affordability in developing countries [5]. In Brazil, despite the low sensitivity of ELISAs for the detection of A/B toxins, this method is still largely used as a single test in several hospitals [6, 7]. The use of a low sensitivity test can jeopardize the efficacy of control measures if infected individuals are kept without the necessary isolation and are not properly treated [6, 8]. Thus, to improve CDI diagnosis, this study aimed to evaluate two commercial assays for CDI diagnosis through detection of the GDH component and A/B toxins in hospitalized adults with diarrhea in a university hospital in Brazil.

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The study was performed at the Clinical Hospital of the Federal University of Minas Gerais (UFMG), a 500-bed quaternary care hospital in Belo Horizonte, Minas Gerais state, in southeastern Brazil. A total of 89 adult patients (aged over 18 years), who had received antimicrobials in the last 3 months and presented diarrhea symptoms for a minimum of 72 h during hospitalization, were included in the study [3]. Similar to previous studies [7, 9], diarrhea was defined as three or more discharges of loose stools a day corresponding to Bristol stool chart types 5 to 7 (as defined by Lewis et al. [10]), for more than 48 h. Unformed stool samples were obtained in sterile containers and aliquots were stored at −20 °C until all tests were performed. All procedures were approved by the Research Ethics Committee of the Faculty of Medicine of the Federal University of Minas Gerais (CAAE - 0710.0.203.0000.11).

All fecal samples were subjected to the following tests: two lateral flow tests (immunochromatographic tests), both of which simultaneously detect GDH and A/B toxins (Toxin/GDH ECO Teste, Ecodiagnostica, Brazil, and C. DIFF QUIK CHEK COMPLETE, Techlab Inc., USA), an enzyme-linked immunosorbent assay (ELISA) kit for detecting A/B toxins (*C. DIFFICILE* TOX A/B II, Techlab Inc., USA), and toxigenic culture (TC) as the reference method. TC was performed as previously reported [7]. Briefly, equal volumes of stool samples and 96% ethanol (v/v) were mixed and incubated for 30 min at room temperature. Subsequently, 20 μL was streaked onto cycloserine-cefoxitin fructose agar (Sigma-Aldrich Co., USA) and Mueller-Hinton agar (Difco Inc., USA), both supplemented with 7% horse blood and 0.1% sodium taurocholate. After incubation at 37 °C for at least 72 h under anaerobic conditions, colonies on both media were analyzed by a previously described in-house PCR to detect the following genes: *tpi* (triose phosphate isomerase, a housekeeping gene), *tcdA* (toxin A), *tcdB* (toxin B), and *cdtB* (binary toxin gene) [11]. ELISA and lateral flow tests were performed according to the manufacturers' instructions.

The sensitivity, specificity, positive predictive, negative predictive, and 95% confidence interval values for the lateral flow tests and the ELISA were analyzed against the TC results (StataCorp 12, StataCorp USA) [12]. The concordance between the two commercial lateral flow tests was analyzed using Cohen's kappa coefficient ("irr" package in R 3.6.3) [13].

Both tests for the detection of GDH showed a sensitivity of 100%, without any false-negative results (Table 1). The specificity (92.1%) was also very high: only five samples (6% of the total) received a false-positive result, mostly due to the presence of non-toxigenic *C. difficile* strains in the stool sample. Interestingly, one sample was positive for GDH in both tests and also for A/B toxins in the ELISA, but negative in the TC. Although this sample was included as a false-positive result for the ELISA and GDH tests, it is more likely to be a failed growth of the isolate in the medium used in the TC protocol [9, 14].

Table 1 Comparison of diagnostic kits for detection of *C. difficile* infection against toxigenic culture as a "gold standard" method in a university hospital in Minas Gerais, Brazil ($n = 89$)

Method	Results	Toxigenic culture (TC)		% (95% CI)			
		Positive	Negative	Sensitivity	Specificity	PPV	NPV
C. difficile Tox A/B II ELISA	Positive	15	1	57.7 (36.9–76.7)	98.4 (91.5–100)	93.8 (67.6–99.1)	84.9 (78.3–89.8)
	Negative	11	62				
GDH Eco Teste (Ecodiagnostica)	Positive	26	5	100 (86.8–100)	92.1 (82.4–97.4)	83.9 (69.2–92.3)	100 (-)
	Negative	0	58				
A/B Toxin Eco Teste (Ecodiagnostica)	Positive	6	1	23.1 (9–43.7)	98.4 (91.5–100.0)	85.7 (43.2–98.0)	75.6 (71.5–79.3)
	Negative	20	62				
C. Diff Quik Chek Complete (GDH only)	Positive	26	5	100 (86.8–100)	92.1 (82.4–97.4)	83.9 (69.2–92.3)	100 (-)
	Negative	0	58				
C. Diff Quik Chek Complete (A/B toxin only)	Positive	5	1	19.2 (6.6–39.3)	98.4 (91.5–100)	83.3 (38.0–97.6)	74.7 (70.9–78.1f
	Negative	21	62				

Sens, sensitivity; *Spec*, specificity; *PPV*, positive predictive value; *NPV*, negative predictive value; *ELISA*, enzyme immunoassay; *GDH*, glutamate dehydrogenase; *CI*, confidence interval

The performance of the GDH detection seen in the two commercial tests corroborates previous studies that reported the usefulness of this method as a screening test for the diagnosis of CDI [2, 15–18]. Interestingly, the similarities between the results obtained by these lateral flow tests demonstrate a very high concordance between the tests, with a K value of 0.975 (95% CI 0.926–1.000) [13]. Additionally, the high sensitivity and specificity of the GDH tests in the present study suggest that this method may be a promising tool for use as a single method in countries where the full algorithm for diagnosis of CDI is not affordable or available, in line with a previous proposal from a study in Chinese hospitals [5].

Recently, Cançado et al. [7] showed that, in hospitals where only one method is used for the diagnosis of CDI, the replacement of ELISA assays by GDH detection considerably increases the number of patients with CDI that are correctly treated, directly affecting the control of the disease. In fact, this is the case in most Brazilian hospitals where the diagnosis of CDI is still based only on the detection of A/B toxins by ELISA [6, 7]. It is also important to note that, compared with other known high-sensitivity methods, including TC and nucleic acid amplification tests, rapid tests for GDH detection are an easy-to-perform method, dispensing with the need for highly trained staff. It is also faster than toxigenic culture, which requires between 2 and 5 days for its results and is also at least five times cheaper than molecular assays [4, 5, 7].

One of the known limitations of rapid tests based on GDH detection is their inability to differentiate the presence of toxigenic from non-toxigenic *C. difficile* strains, leading to false-positive results [9]. In the present study, non-toxigenic strains accounted for 16% of the samples positive in the GDH tests (4.9% of all tested stool samples). This rate had a mild influence on the specificity of both GDH tests in this study, which was kept above 92%. Although the rates of patients with non-toxigenic strains are generally similar to that seen in this study [5, 19], there are reports of rates higher than 50% in some other institutions [18, 20–22]. Thus, it should be considered that the use of GDH as a single method should be preceded by an evaluation of its specificity and PPV in the institution, thus avoiding unnecessary antimicrobial treatment for CDI in asymptomatic carriers [9].

In contrast to the high sensitivity of GDH detection, lateral flow tests for the identification of the A/B toxin showed a markedly low sensitivity (19.2 to 23.1%), also found for the results of the ELISA kit, corroborating previous studies [6, 19, 23]. The limitations are related to the detection of free A/B toxins, which are susceptible to degradation and, more importantly, maybe present at levels below the threshold of detection [12, 24]. Therefore, studies have suggested that toxin-based methods should be used after or concomitantly with high-sensitivity tests, which include GDH detection [25]. However, due to the low sensitivity related to the detection of free A/B toxins, a third method, mostly nucleic acid amplification tests or toxigenic culture, would be necessary for samples GDH positive but negative for A/B toxins [9]. Thus, to avoid this three-step algorithm, molecular assays are commonly replacing ELISA or rapid tests for toxins in this second step [3, 26].

Accurate and rapid laboratory diagnosis is one of the critical bases for the control of CDI in hospital settings. Despite this, the best approach for the diagnosis of CDI remains controversial [9]. In Brazil, the diagnosis of CDI is still mostly based on the detection of A/B toxins by ELISA. In the present work, the high sensitivity and specificity of rapid tests for GDH detection suggest that this method can be useful not only as a screening test for the diagnosis of CDI, but also as a single test in situations where the algorithm for diagnosis of CDI is not available. Due to this result, the Clinical Hospital of the Federal University of Minas Gerais (UFMG) is changing its one-step protocol for CDI diagnosis from ELISA to GDH detection. During the next 2 years, a study monitoring the specificity of GDH will be conducted in light of this, to clarify the impact of this change on disease prevalence and antimicrobial prescribing practices in this institution.

Author's contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by Emily Oliveira Lopes and Amanda Nádia Diniz. The analysis was performed by Francisco Carlos Faria Lobato, Eduardo Garcia Vilela, Rodrigo Otávio Silveira Silva, and Carolina Pantuzza Ramos. The first draft of the manuscript was written by Carolina Pantuzza Ramos and Rodrigo Otávio Silveira Silva. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on a reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval This study was approved by the Research Ethics Committee of the Faculty of Medicine of the Federal University of Minas Gerais (CAAE – 0710.0.203.0000.11).

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