Is *Clostridioides difficile* toxins detection necessary when the glutamate dehydrogenase enzyme is detected?

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Abstract

Introduction: *Clostridioides difficile* causes diarrhea and pseudomembranous colitis. Its diagnosis is made with glutamate dehydrogenase (GDH) or toxins A and B detection and is confirmed with nucleic acid amplification tests. **Objective:** To define if GDH determination is redundant to that of toxins. **Methods:** Retrospective, observational study in diarrheal stools of patients with suspected *Clostridioides difficile* infection. Toxins and GDH were determined by immunochromatography. Bayesian simulation was performed with likelihood ratios; a p -value < 0.05 was regarded as significant. **Results:** 329 GDH and toxin A and B results were analyzed. *Clostridioides difficile* infection prevalence was 18.2 %. Sensitivity and specificity of the GDH test were 0.90 and 0.89, respectively. Positive likelihood ratio was 8.9, and negative was 0.11. **Conclusions:** A negative GDH result considerably reduces the probability of infection but does not rule it out. *Clostridioides difficile* toxins detection may be necessary in institutions where nucleic acid amplification is not affordable or accessible.

KEY WORDS: *Clostridioides difficile*. Glutamate dehydrogenase. Toxin A. Toxin B.

¿La detección de toxinas de *Clostridioides difficile* es necesaria cuando se detecta la enzima glutamato deshidrogenasa?

Resumen

Introducción: *Clostridioides difficile* causa diarrea y colitis pseudomembranosa. Su diagnóstico se realiza con la detección de glutamato-deshidrogenasa (GDH) o las toxinas A y B y se confirma con pruebas de amplificación de ácidos nucleicos. **Objetivo:** Definir si la determinación de GDH es redundante a la de las toxinas. **Métodos:** Estudio observacional retrospectivo de muestras fecales de pacientes con sospecha de infección por *Clostridioides difficile*. Las toxinas y GDH se determinaron mediante inmunocromatografía. Se realizó una simulación bayesiana con los cocientes de probabilidad; se consideró significativo un valor de $p < 0.05$. **Resultados:** Se analizaron 329 resultados de GDH y toxinas A y B. Se encontró una prevalencia de infección de *Clostridioides difficile* de 18.2 %. La sensibilidad y especificidad de la prueba de GDH fue de 0.90 y 0.89, respectivamente. El cociente de probabilidad positivo fue de 8.9 y el negativo, de 0.11. **Conclusiones:** Un resultado negativo de GDH disminuye considerablemente la probabilidad de infección, pero no la descarta. La detección de toxinas de *Clostridioides difficile* puede ser necesaria en instituciones donde la amplificación de ácidos nucleicos no es económica o accesible.

PALABRAS CLAVE: *Clostridioides difficile*. Glutamato deshidrogenasa. Toxina A. Toxina B.

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Introduction

Clostridioides difficile is responsible for infectious diarrhea and pseudomembranous colitis, the incidence and prevalence of which has increased due to the use and abuse of antibiotics.¹ Clinical presentation can range from an asymptomatic to a toxic megacolon-generating condition. In 2011, mortality from this infection increased to 6.4 % in the United States.² Currently, it occurs not only in hospitals, but also in the community.³ Diagnosis is made on stool samples: in two steps, with an immunoassay to detect toxins A and B and the glutamate dehydrogenase (GDH) enzyme, and confirmation with nucleic acid amplification; or in one step, with the performance only of the latter (a test that is costly and difficult to access to).^{1,2,4}

According to the two-step procedure, in a study carried out by Goldenberg et al. in 2010, 500 stool samples were reported; toxin detection was considered redundant when GDH was observed, since GDH is considered highly sensitive, although it is poorly specific.^{3,5} The purpose of the present study was to corroborate whether *Clostridioides difficile* toxins detection is redundant when GDH is determined..

Methods

A retrospective, observational study was carried out in a private hospital of Mexico, which has 95 beds. All stool samples with suspected *Clostridioides difficile* infection from hospitalized patients or outpatients of any age were analyzed from December 2016 to October 2018. Toxins A and B and GDH determination was carried out by qualitative immunochromatography with the CerTest® kit (Biotec SL, Zaragoza, Spain), whose sensitivity and specificity are higher than 0.99, according to the manufacturer's specifications. With the collected information, data were analyzed in rates and proportions. In addition, GDH detection diagnostic performance relative to toxin detection was determined. A Bayesian analysis was carried out to define the change in pre-test probability according to the GDH detection result. The proportions of GDH and toxin A and B detection were compared using the chi-square test with the VassarStats.net online statistical program. A p-value < 0.05 was considered to be statistically significant.

Results

Three-hundred and twenty-nine toxin A and B, as well as GDH analysis results were evaluated; 176 corresponded to women (53.4 %) and only 47 (14.2 %) to hospitalized

Table 1. Glutamate dehydrogenase (GDH) enzyme diagnostic performance evaluation

GDH	Toxin A or B-positive		Toxin A and B-negative		Total	
Positive	54	(66.66%)	27	(33.33 %)	81	(100%)
Negative	6	(2.41%)	242	(97.58 %)	248	(100%)
Total	60	(18.23%)	269	(81.76 %)	329	(100%)

$\chi^2 = 164.7$, $p < 0.001$. Prevalence: 18.2 % (95 % CI = 19.3-22.9); sensitivity: 0.90; specificity: 0.89; positive predictive value: 0.67 (95 % CI = 0.55-0.77); negative predictive value: 0.97 (95 % CI = 0.95-0.99); positive likelihood ratio: 8.9 (95 % CI = 6.2-12.9; negative likelihood ratio: 0.11 (95 % CI = 0.05-0.24).

patients. Median age was 56 years (interquartile range [IQR] = 32-71). Toxin A was detected in 52 specimens (15.8 %), toxin B in 41 (12.4 %) and GDH in 81 (24.6 %).

In 60 patient samples, at least one toxin was detected; therefore, prevalence was 18.2 %. GDH was determined to have a sensitivity and specificity of 0.90 and 0.89, respectively. Positive predictive value was 0.67, and negative predictive value was 0.97. Positive likelihood ratio was 8.9, whereas negative likelihood ratio was 0.11 (Table 1). The Bayesian analysis determined the probability of having *Clostridioides difficile* infection with GDH detection. Based on the above, if GDH result is positive, the probability goes from 0.182 to 0.66; when the result is negative, the probability of infection goes from 0.182 to 0.023.

Discussion

In the institution where the present study was carried out, the two-step algorithm is employed; however, nucleic acid amplification implementation is carried out sporadically due to its elevated cost; therefore, diagnosis is generally established based on clinical presentation and screening tests.

The prevalence of *Clostridioides difficile* determined by the detection of any of both toxins was 18.2 % in the study sample. This proportion may be underrepresented because toxin identification is not considered the gold standard. The presence of GDH increases post-test probability of *Clostridioides difficile* infection to 66 % and, when not detected, it decreases to 2.3 %. This suggests an adequate performance to rule out infection when GDH detection is negative.

These results confirm the observation of Goldenberg et al. regarding the redundancy of detecting toxins together with GDH.^{3,5} We consider that this measure is necessary in institutions where nucleic acid amplification is not accessible, given that six

cases (10 %) were found with toxins positive and GDH negative detection.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that they followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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