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A bedside test for *Clostridium Difficile* infection: an Emergency Department use. Preliminary results

D. MARSILIANI, G. DE MARCO, C. PETRUZZIELLO, G. MERRA, F. FRANCESCHI, V. OJETTI

Department of Emergency Medicine, Catholic University of the Sacred Heart, "A. Gemelli" Hospital, Rome, Italy

Abstract. – OBJECTIVE: Clostridium Difficile (CD) infection is a severe cause of diarrhea in patients with prolonged hospitalization and/or previously treated with antibiotics. CD's A and B toxins are responsible for either diarrhea or septical status as well as other complications including toxic megacolon. Toxins isolation, usually performed by a central microbiological laboratory (CML), is mandatory for the final diagnosis of the disease. The clinical suspect of CD infection (CDI) results in the isolation of the patients, until the fecal test does not exclude the disease. Positive patients need to maintain isolation and start a specific antibiotic therapy. The aim of this study was to verify the sensitivity and specificity of a rapid test for the diagnosis of CDI.

PATIENTS AND METHODS: We enrolled 20 (13F/7M, mean age 70 ± 12 yrs) consecutive pts who accessed the Emergency Department (ED) with diarrhea and a clinical suspect of CDI. An immune-enzymatic bedside test (Beta Diagnostici, Messina, Italy) for the detection of GDH, toxin A and B of CD was used. The results of this test were then compared to the CML one's, on the same patient.

RESULTS: 6 patients resulted positive to the bedside test compared to 7 of CML test (86% of concordance). In this patient, the bedside test showed a strong positivity for GDH without signs of toxin, meanwhile the CML test revealed the toxins. Possibly, the lower toxins concentration in this patient was responsible for such discordance. Both tests showed a full concordance for negative patients. Another interesting finding is that the bedside test provides results in only 5 minutes, compared to several hours (even 48) of CML test.

CONCLUSIONS: The bedside test is a rapid and affordable tool for rapid diagnosis of CD infection especially in a ED where the positivity of the test affects either hospitalization or treatment.

Key Words:
Clostridium difficile, Rapid test, Emergency department, Antibiotic treatment, Diarrhea, Isolation.

Introduction

Clostridium difficile (*C. difficile*) was originally described by Hall and O'Toole in 1935[1], which wrongly thought it was part of the normal gut flora. Only 40 years later it was identified as the primary cause of pseudomembranous colitis[2-3], and demonstrated it was the main organism isolated from the feces of patients with diarrhea treated with antibiotics[4].

C. difficile is a Gram-positive spore-forming anaerobic bacillus[5], able to produce enteric symptoms; the so called *Clostridium difficile Infection (CDI)* may produce different clinical syndromes, ranging from asymptomatic intestinal colonization to diarrhea, colitis, pseudomembranous colitis and death.

C. difficile spores are transmitted via the fecal/oral pathway. These spores are resistant to commonly-used decontaminants, may resist on inanimate objects and can persist for long periods of time without losing pathogenicity[6]. The normal Gut Microbiota does not allow the germination of the spores, but when the body is under antibiotic treatments, *C. difficile* can germinate sufficiently to establish an infection. A critical step in the pathogenic process is the attachment to intestinal epithelial cells. The pathogenicity of *C. difficile* is mediated primarily through the release of two toxins[7], A and B[8], acting as glucosyltransferases able to inactivate small GTPases such as Rho, Rac and Cdc42 within eukaryotic target cells, leading to actin polymerization, opening of tight junctions and ultimately causing cell death[9].

Recent studies using Toxin A-negative *C. difficile* mutants demonstrate the importance of Toxin B in a CDI animal model[10], while several studies reported the isolation of Toxin A-negative/Toxin B-positive strains of *C. difficile* in patients with CDI and colitis[11].

Corresponding Author: Veronica Ojetti, MD, Ph.D; e-mail: veronica.ojetti@tin.it

Figure 1. A bedside test with a strong positivity for GDH.

Glutamate dehydrogenase (GDH) of *C. Difficile* is an enzyme expressed on the surface of the bacterium, produced in large quantities both by toxigenic and not toxigenic strains. Therefore, GDH is an excellent marker for the detection of this microorganism in the feces of infected patients.

Nevertheless, diagnosis is confirmed only after toxins isolation that is usually performed by a central microbiological laboratory (CML), within the open time of the laboratory and with a processing time of several hours. Normally patients needed to be isolated until the test result arrive and start specific antibiotic therapy By guidelines, patients need to be isolated until the results of those test exclude the infection. Therefore, a fast identification of *C. difficile* infection is crucial in the Emergency Department (ED), since the early isolation of the patient and the starting of an appropriate antibiotic treatment is a fundamental step to prevent the spread of the disease[12].

Since timing of diagnosis of *C. difficile* infection is crucial, we have designed a study aimed at evaluating the accuracy of a new kit for the fecal detection of GDH, A and B toxin in only 5 minutes, compared to standard diagnosis performed by CML.

Patients and Methods

We enrolled 20 consecutive patients (13F/7M; mean age 70 ± 12 yrs) accessing the ER for diarrhea with a clinical suspect of *C. Difficile* infection. No exclusion criteria were adopted.

At enrollment, medical history, physical examination, laboratory tests (blood cell count, hepatic and renal function, electrolytes) were collected.

Fecal samples were obtained from all patients, while simultaneous qualitative detection of *C. difficile* GDH, Toxin A and Toxin B has been performed either using the rapid enzyme-linked immunosorbent assay (ELISA) kit (Beta Diagnostici, Messina, Italy) or the standard test performed by CML.

This rapid kit was stored either at refrigerated or room temperature, as indicated by the manu-

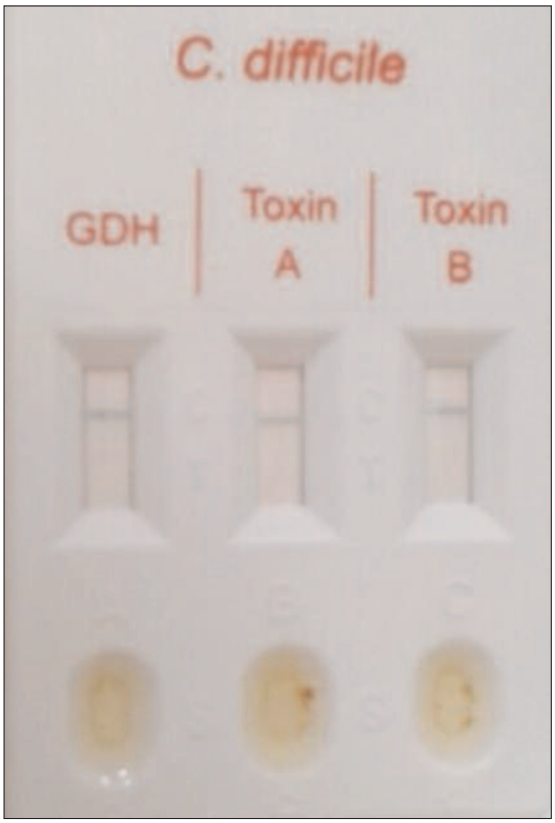

Figure 2. A negative bedside test.

facturer. For each patient, we collected sufficient quantity of feces (1-2 g or mL for liquid sample) inside clean and dry containers (no preservatives or transport media), which were stored at a temperature of 2-8ºC/36-46.4ºF for 24h prior to testing. Fecal specimens were then analyzed by the rapid kit; briefly, feces were introduced inside the vial and after shaking the sample, in order to assure good sample dispersion, 4 drops of the solution were introduced inside the spot marked with A (Toxin A), B (Toxin B) and C (GDH). Five minutes later the results of the test were collected: if the test displays only one line, the result is negative, while the presence of two or more lines (one for toxin A, one for toxin B and one for GDH) suggests a positive result. Simultaneously, another fecal sample isolated from each patient were analyzed by the CML.

Samples have been analyzed with no drop outs; the study was approved by the Independent Ethics Committee of the Catholic University of Rome and conducted in accordance with the Declaration of Helsinki. None of the patients received any honorary or economical benefits for the participation in the study.

The primary endpoint of this study was to assess the feasibility of the use of the new Beta Diognistici kit in the ED setting; the secondary endpoint was to assess the accuracy of this test compared to the standard procedure performed by the CML.

Statistical Analysis

Statistical analysis was performed using Person's Chi-squared test. $p < 0.05$ was considered statistically significant.

Results

For each patient we performed an immediate bedside rapid test, and requested the CML standard test. The rapid test gave a final response in 5 minutes compared to 2.880 minutes of standard CML test ($p < 0.0001$). Six patients resulted positive to the bedside test compared to 7 of CML test (86% of concordance); therefore, those tests provided discordant results in only one subject. In this patient, the bedside test showed a strong positivity for GDH without presence of Toxins A and B, meanwhile the CML test also revealed both the toxins (Figure 1). Possibly, a poor toxin concentration in this patient was responsible for such discordance[13]. On the other hand, both tests showed a full concordance for negative patients (100% of concordance) (Figure 2).

Interestingly, among 3 of 20 patients reporting a previous *C. difficile* infection, 2 resulted again positive to CD; in particular one patient resulted positive to both tests (bedside and CML), while another showed positivity to GdH at the bedside test and to toxin A, B and GDH at CML test.

Positive patients were finally isolated and started specific therapy, using Vancomicine (125 mg/4 times daily for 10 days). The final results of the different tests are summarized, for each patient, in the Table I.

Discussion

C. difficile infection represents an increasing and life threatening health care problem in Western Countries. During the past years, there has been an evolution in *C. difficile* testing. According to data coming from a survey performed in 2004 by the College of American Pathology, 42% of laboratories in the U.S. were using a solid phase enzyme immunoassay (EIA) method for the detection of *C. difficile* toxins A and B, while 26% used rapid immunochromatographic devices

Table I. A comparison between the bedside test (Toxin A, B and GDH) and CML results for each patient

Patient	Bedside Tox a	bedside Tox b	bedside GDH	LAB result
1 F	0	0	0	0
2 F	0	0	0	0
3 M	0	0	0	0
4 M	0	0	0	0
5 M	0	0	0	0
6 F	1	0	1	1
7 F	1	1	0	1
8 F	0	0	0	0
9 F	0	0	0	0
10 M	1	0	1	1
11 F	0	0	0	0
12 F	0	0	0	0
13 F	0	0	1	1
14 F	0	0	0	0
15 M	0	0	0	0
16 F	0	0	0	0
17 M	0	0	0	0
18 M	1	1	1	1
19 F	1	1	0	1
20 F	0	0	0	0

(ICDs) for toxins A/B and/or glutamate dehydrogenase (GDH) detection. Due to further improvement of rapid ICD assays, 46% of laboratories adopted these methods by 2008 while 43% still continued to use EIA methods[14].

At this time, there are several test for the detection of *C. difficile* infection: toxin A and A&B EIAs, GDH EIAs, ICDs for detection of toxins A/B or GDH, tissue culture neutralization (CTN), toxigenic culture and PCR for toxin genes. Nevertheless, none of them are able to provide immediate and accurate results.

Our study has demonstrated for the first time the feasibility and accuracy of a new bedside rapid test for the detection of *C. difficile* infection, with very good results (86% of correspondence). Concerning feasibility, performing the test is very easy even by nurses and only a small amount of stool is needed to get a reliable result. This makes the test suitable for patients with a clinical suspect of *C. difficile* infection, even in crowded areas, such as the ED.

Another important aspect of this test is the rapidity of the response, compared to standard CML, usually closed during the night and holidays. This aspect is crucial, as allows to provide in only 5 minutes a prompt isolation of the positive patient, to prevent the spread and evolution of the infection and to start an appropriate treatment for the disease[15]. While patients positive for both toxins need immediate isolation and treatment, those positive only for GDH need just isolation, before results of CML test give a definite response[15].

Finally, the cost of the rapid test was significantly lower compared to the standard CML test (96 rapid tests for €435), thus encouraging its use in patients accessing the ED for diarrhea possibly due to *C. difficile* infection.

Conclusions

The Beta Diagnostici kit represents a reliable tool for the detection of patients with *C. difficile* infection in the ED, where quickness is mandatory. Both low cost and speed of execution represents a valid reason to recommend its use in the emergency setting, especially when CML is not able to provide a fast response.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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