A TWO STEP ALGORITHM FOR THE DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFECTION: SCREENING WITH A RAPID IMMUNOASSAY FOR THE DETECTION OF GLUTAMATE-DEHYDROGENASE AND TOXINS A AND B FOLLOWED BY A REAL-TIME PCR FOR CLOSTRIDIUM DIFFICILE 

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Introduction
Since 1996, the diagnostic scheme for Clostridium difficile infection (CDI) in our laboratory (fig.1a) has been based on both direct faecal-cytotoxicity assay (CTA) and toxicogenic culture (TC). The latter consists of culture of faeces on selective medium and detection of toxin production on colonies by enzyme immuno-assay (EIA); it has demonstrated a much better sensitivity than CTA alone and a better specificity than culture alone (Delmée et al. 2005). It is however a very slow procedure : it takes at least an overnight incubation to get a result.

Aiming at providing more rapid and accurate results, we evaluated an algorithm testing glutamate dehydrogenase (GDH) and Tox A&B on all samples followed by a RT-PCR on GDH+ Tox A&B- samples (fig.1b). We compared this algorithm with RT-PCR on all samples (fig1a).

Materials and methods

Stools: were from inpatients (>2y) suffering from antimicrobial- or chemo-therapy associated diarrhoea. 270 stools were tested : 336 (12.6%) were fresh stool samples collected in 2010 and 34 (12.6%) were stool samples collected over a 24 months period and kept at -40°C. The prevalence of positive culture samples was 9.75% for the prospective samples and 20% for all samples.

Cultures: on CCFA (twice overnight anaerobic incubation) - CTA: sterile faecal filtrate on MRC-5 cells - CCFAT: CCFA + sodium taurocholate.

Toxicogenic culture (TC): colonies on CCFA are picked up, mixed in the Elisa sample diluent and tested for toxA&B as for faecal specimens - C. Diff Quik Check Complete™ (Techlab, Blacksburg, VA, USA)

Two tests were evaluated :

- Immunoassay
  C. Diff Quik Check Complete™
  real-time PCR
  GeneXpert
  C. diff (diff) GDH and Tox A&B

- C. Diff Quik Check Complete™
  CTA: in-house cell cytotoxicity
  CTA: tox A&B test

TC was considered as the Gold Standard.

Diagnostic algorithms

The performances of a two-step algorithm (GDH and Tox A&B), followed by RT-PCR on GDH+ Tox A&B- stools, was compared with the one step RT-PCR algorithm and the two-step algorithm (GDH and Tox A&B) followed by TC on GDH+ Tox A&B- stools with TC on all samples as Gold Standard.

Results

Table 1a shows results of cellcytotoxicity on prospective stool samples whereas table 1b shows results of cellcytotoxicity on prospective and retrospective stool samples. Table 2a shows the performances of RT-PCR on prospective stool samples and table 2b on all samples. Table 3a shows the results of the two-step algorithm (GDH and Tox A&B), followed by RT-PCR on GDH+ Tox A&B- stools, on prospective stool samples. Table 3b gives the results of the two-step algorithm (GDH and Tox A&B), followed by RT-PCR on all samples.

Discussion

Screening fresh stools with GDH and ToxA&B, followed by a RT-PCR on GDH+ and ToxA&B- samples, showed a good NPV (91.1%). With a sensitivity of 92.6%, against 86,96% for RT-PCR on all samples; and a specificity of 96% for CTA, this algorithm can be envisaged as a good method for the detection of C. difficile in faecal specimens, in less than 2 hours.

With such algorithm, one missed 4/23 (17%) positive specimens, 1 due to the GDH and Tox A&B screening and 3 due to the RT-PCR. We obtained 4 false positive results, all became positive on CCA plates added with sodiumtaurocholate.

The algorithm required less RT-PCRs (19) than performing RT-PCR on all samples (236), which is financially reasonable, although 236 rapid tests, (GDH and Tox A&B), need to be performed.

In frozen (-80° C) stool samples, the sensitivity of both GDH+Tox A&B and RT-PCR was lower, whereas toxigenic culture gave the best results.

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