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Short Communication

Establishment of campylobacter infection with immunochromatographic test

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Abstract

***Campylobacter spp.* are the most commonly reported bacterial causes of food toxic infections in the world. Traditionally, microorganisms of the genus *Campylobacter* are difficult to detect because they require a special incubation medium under micro-aerophilic conditions. To introduce mass immunochromatographic tests in diarrheal patients, verifying their diagnostic value with that of a molecular-biological method optimized by us. Fecal samples of 520 children with diarrhea syndrome we retested by immunochromatography (ICT) and Eva Green Real-time PCR for simultaneous differentiation of *C. jejuni/coli* directly from feces. We found a statistically significant difference between the two diagnostic tests at Sig. <0,05 and t >3. Using calculator for value of the Cohen's *d (reflecting the magnitude of the difference between two variables)* we calculated Cohen's *d* = 0.346, which in fact suggests that the difference between the diagnostic capabilities of the ICT and the Eva Green Real-time PCR methodis very small. The diagnostic capabilities of modern ICT are significant and the work with them can significantly ease the activity of the clinician in the necessity to make quick decisions about the therapeutic behavior.**

Key words: *Campylobacter, ICT, PCR*

INTRODUCTION

Campylobacter spp. are the most commonly reported causative agents of bacterial gastroenteritis in developed countries, respectively Campylobacteriosis is the most common cause of acute diarrhea of bacterial origin (Butzler, 2004; Vila et al., 2016).

The most common symptoms of the infection are watery diarrhea with blood impurities, fever and abdominal pains (Grzybowska-Chlebowczyk et al., 2013). Bulgaria is still not routinely tested for *Campylobacter* spp. in diarrheal patients, and when doing so, classic culture methods are used. They are slow, labor-intensive and capricious. This leads to a delay in the etiological diagnosis, the lack of antibiotic therapy or, on the contrary, the use of unnecessary antibiotics (Boyanova et al., 2004).

Purpose

With the present work we aim to introduce massive application of immunochromatographic tests in diarrheal patients, verifying their diagnostic value with that of optimized by us molecular-biological methods.

MATERIALS AND METHODS

520 patients, aged 0-14 years, hospitalized at the "Prof. Iv. Kirov" Children's Clinic of University Hospital for Infectious Diseases for a period of 3 years were examined. Fecal samples from 520 hospitalized children, each of which had diarrhoea with blood and blood impurities and fever contamination, were examined by immunochromatographic tests. Immunohromatographic tests (ICT) of *CerTestBiotec*, Spain, labeled with monoclonal antibody against *Campylobacter*generic antigen were used. Of patients positive for *Campylobacter* spp. under ICT, repository feces for a PCR assay were sent to the National Centre for Infectious and Parasitic Diseases – Sofia. The PCR

method approved by us and used for the simultaneous detection and differentiation of *C. jejuni/coli* directly from a faecal sample is based on three pairs of primers in a common reaction. The AB2/R2 primers amplifying a specific region (74bp) of the *C.jejuni*ceu EF/R gene, amplifying a specific region (72bp) of the *ceuE* gene for *C. coli* and campF2/R2, amplifying a specific region (108bp) of a generic gene for *Campylobacter* spp.

RESULTS

We found 182 patients positive for campylobacteriosis by ICT, with gender equality almost equal – 88 (48.1%) men and 94 (51.9%) women (Figure 1).

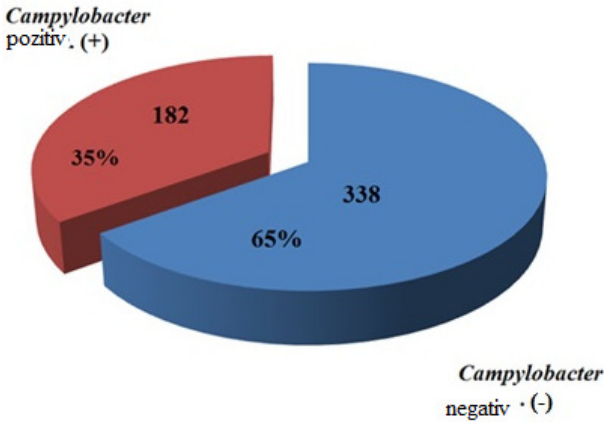

Figure 1. Distribution of positive and negative ICTs for campylobacteriosis.

Using the Eva Green Real-time PCR method optimized by us, we tested 112 of 182 feces, positive for ICT. We confirmed the identification of 98 clinical isolates, 73 (73/98) of *C. jejuni*, 19 (19/98) *C. Coli* and 6 (6/98) *Campylobacter spp*. (Figure 2).

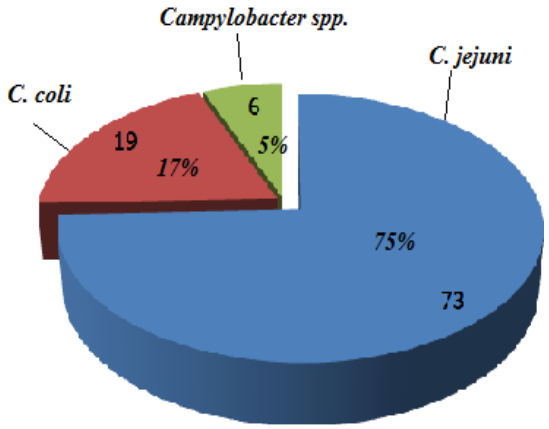

Figure 2. Results from Eva Green Real-time PCR for 112 samples of hospitalized patients with diarrhea syndrome.

In addition to all feces, the reliability of the Eva Green Real-time PCR analysis was tested with 18 reference strains, 9 of which *C. coli* and 9 strains of *C. jejuni*. The visualization was performed in real time by tracking the specific product temperatures (Figure 3).

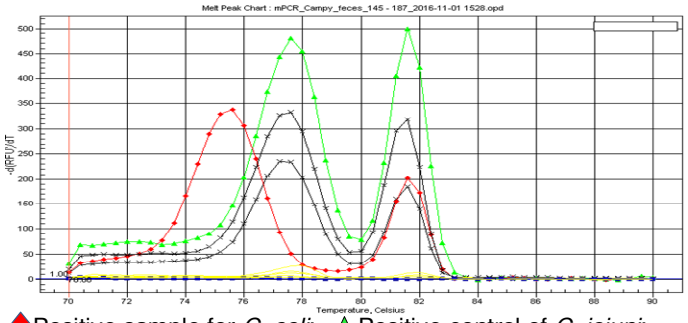

Figure 3. Results from Real-time PCR for detection of *Campylobacter* in feces samples.
Positive samples for *C. jejuni* as well as negative samples for *Campylobacterspp*:

For comparison of mean values between the ICT and Eva Green Real-time PCR variables, we used the T-test statistical method.

As can be seen from Table 1 (a) and (b), there is a statistically significant difference between the two variables – between the two diagnostic tests at Sig. <0,05 and t>3.

Using the Cohen's value calculator *d(reflecting the magnitude of the difference between two variables)* we calculated Cohen's *d* = 0.346, which in fact indicates that the difference between the diagnostic capabilities of IHT and the Eva Green Real-time PCR method is very small.

DISCUSSION

The results obtained showed a relatively high incidence of campylobacteriosis among hospitalized children with enterocolitis. Nearly ¼ of the patients we tested were positive for the intestinal infection.

The relatively higher incidence of *C. coli* (17%) among our patients compared to the EU-isolated average (about 10%) is of interest (Boyanova et al., 2004; Butzler, 2004; Grzybowska-Chlebowczyk et al., 2013). These data, however, are in line with the data provided by the Bulgarian authors about the prevalence of *C. coli* infection in broilers and broiler flocks in Bulgaria (Gurov et al., 2011).

The data we get are correlated with the statements of other authors (Al AmriAbiola et al., 2007; Regnath and Ignatius, 2014), according to which the diagnostic capabilities of modern ICT are significant and the work with them can significantly relieve the clinician's activity in the need to make quick decisions about therapeutic behavior.

Table 1. Comparison of the values of ICT and Evagreen Real-time PCR by T-test.

Statisticsfor a pair of variables					
		Mean	**N**	**Std. Deviation**	**Std. ErrorMean**
variables	**ICT**	,1	112	,09949	,00893
	PCR	,8661	112	,34211	,03233

(a)

Correlationsforpair of variables				
		N	**Correlation**	**Sig.**
variables	**ICT& PCR**	112	-,037	,696

Testfor a pair of variables

		Paired Differences							
					95% ConfidenceInterval of theDifference				
		Mean	**Std. Deviation**	**Std. ErrorMean**	**Lower**	**Upper**	**t**	**df**	**Sig. (2-tailed)**
variables	**ICT - PCR**	,12500	,35830	,03386	,05791	,19209	**3,692**	111	,000

(b)

REFERENCE

Al AmriAbiola A, Senok C, Abdulrahman Y (2007). Multiplex PCR for direct identification of Campylobacter spp. in human and chicken stools. J Med Microbio, 10, 1350-1355.

Boyanova L, Gergova G, Spassova Z, Coumanova R,Yaneva P, Mitov I, Derejan S, Krastev Z (2004). Campylobacter infection in 682 bulgarian patients with acute enterocolitis, inflammatory bowel disease, and other chronic intestinal diseases. Diagnostic Microbiology and Infectious Disease, 49: 71-74

Butzler J (2004). Campylobacter, from obscurity to celebrity. ClinMicrobiol Infect.;10(10):868-76.

Grzybowska-Chlebowczyk U, Kalita B, Flak-Wancerz A, Jasielska M, Wiecek S, Wojcieszyn M, Horowska S, Wojciech C, Wos H (2013). Clinical course of *Campylobacter* infections in children. Pediatria Polska, 88: 329-334.

Gurov B, Daskalov H, Naidenov V, Marmarski A (2011). Spread of *Campylobacter* spp. in broiler flocks and broilers. Collection of reports on veterinary medicine, 74-80.

Regnath T, Ignatius R (2014). Accurate detection of *Campylobacter* spp. antigens by immunochromatography and enzyme immunoassay in routine microbiological laboratory. Eur J MicrobiolImmunol (Bp). 156-8.

Vila J, Oliveira I, Zboromyrska Y, Gascon J (2016). Traveller's diarrhea. EnfermInfeccMicrobiolClin. 34(9):579-584.

Williams D, Schorling J, Barrett L, Dudley S, Orgel I, Koch W, Shields D, Thorson S, Lohr J, Guerrant R (1989). Early Treatment of Campylobacter jejuni Enteritis. Antimicrobal agents and Hemotherapy, 248-250.