INVOLVEMENT OF CLOSTRIDIUM DIFFICILE INFECTION IN THE ETIOLOGY OF DIARRHEAL DISEASE

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Abstract: *Purpose: Evaluation of Clostridium difficile infection involvement in the etiology of diarrheal disease. Material and methods: A retrospective study was conducted in the Laboratory of Infectious Diseases Clinic I Tîrgu-Mureş, between 03.2009 and 12.2011. 6529 samples of feces were processed following the coproculture method. Rotavirus testing was performed by latex-agglutination. 1923 samples were processed following the ELISA technique, for Clostridium difficile, Campylobacter jejuni, and Giardialamblia. Results: Through the coproculture method, we reported a rate of 22.53% positive results. The highest percentage was found for Salmonella spp., E. coli and then Shigella spp. ELISA test was most frequently positive for Campylobacter spp. (4.47%), Giardia lamblia (2.65%), Clostridium difficile (1.25%). Conclusions: The most common positive diagnosis was for Campylobacter spp, followed by Giardialamblia, Salmonella spp., E. coli, and only then Rotavirus. Infection caused by Clostridium difficile was identified in a low percentage.*

Cuvinte cheie: diaree, etiologie, Clostridium difficile

Rezumat: *Scop: Evaluarea implicării infecţiei cu Clostridium difficile în etiologia bolii diareice. Material şi metodă: S-a efectuat un studiu retrospectiv în perioada 03.2009 – 12.2011 în Laboratorul Clinicii de Boli Infecţioase Tîrgu-Mureş. Au fost prelucrate 6529 probe de materii fecale, prin metoda coproculturii. Testarea pentru Rotavirus s-a efectuat prin latexaglutinare. Pentru Clostridium difficile, Campylobacter jejuni, Giardia lamblia s-a folosit tehnica ELISA, prelucrându-se 1923 probe. Rezultate: La coprocultură s-au raportat rezultate pozitive într-un procent de 22,53%. Procentul cel mai mare a fost întâlnit în cazul Salmonella spp, apoi E. coli şi Shigella spp. Pozitivarea cea mai frecventă a testărilor ELISA a fost în cazul Campylobacter spp. (4,47 %), Giardia lamblia (2,65 %), Clostridium difficile (1,25 %). Concluzii: Cel mai frecvent diagnostic pozitiv a fost pentru Campylobacter spp., urmat de Giardia lamblia, Salmonella spp., E. coli şi doar apoi de Rotavirus. Infecţia cauzată de Clostridium difficile a fost identificată într-un procent scăzut*

INTRODUCTION

The etiology of infectious diarrhoea is varied, involving many pathogens, bacterial, viral, parasitic and fungal, clinical manifestations and evolution being determined primarily by the etiologic agent. The higher frequency represented by intestinal bacterioses-shigellosis, salmonellosis – in the official statistics from our country in 1995-2004, does not represent real possible etiologies, but rather the concerns and the limits in the activity of specialized laboratories to determine especially these bacterial etiologies.(1)

Most studies in the literature also argue that in nosocomial diarrhoea, community enteric pathogens are not so often involved in the etiology, the leading cause for hospital-acquired diarrhoea being *Clostridium difficile*.(2-5)

PURPOSE

∨ Evaluation of acute diarrheal disease etiology;
∨ Evaluation of Clostridium difficile infection involvement in the etiology of diarrheal disease;
∨ Evaluation of the risk factors associated with Clostridium difficile infection.

METHODS

A retrospective study was conducted in the Laboratory of Infectious Diseases Clinic I Tîrgu-Mureş between 03.2009 - 12.2011. A total of 6529 samples of feces were processed following the coproculture protocol for the presence of enteric pathogens: *Salmonella* spp, *Shigella* spp, *E. coli* enterotoxigenic (ETEC), *E. coli* enteroinvasive (EIEC), O157/H7 *E. coli*, *Yersinia enterocolitica*. Children under 2 years old were tested for the presence of *E. coli* enteropathogenic (EPEC). In the summertime and in cases with suggestive clinical signs, we investigated the presence of *Vibrio cholerae*. Rotavirus testing was performed by latex-agglutination.

Testing for the presence of Clostridium difficile toxins A and B and for the antigens of *Campylobacter jejuni*, *Giardia lamblia*, *Cryptosporidium, Entamoeba, Echinococcus,* Rotavirus, Adenovirus was performed through the ELISA technique. A total number of 1923 samples of feces were processed through this method.

For the statistical analysis, we used the Graphpad software (QuickCalcs Online Calculators for scientist), available at http://www.graphpad.com/quickcalcs/.

RESULTS

Between 03.2009 and 12.2011, in the Laboratory of Infectious Diseases Clinic Tîrgu-Mureş, 6529 samples of faeces were processed following the coproculture protocol. Of all the cases, we reported positive results at a rate of 22.53%.

The distribution by age groups reveals a significant amount of positive cases in children, the average age of positive cases being 10.33 years, with a median of 2 (95% CI 9.1399-11.5235, aged 0-10.2 years). We reported the highest percent of positive cases for *Salmonella*spp, followed by *E. coli* and *Shigella* spp., the other species being reported in a smaller percentage. Of all the cases positive for *Salmonella* spp. (118), most of them were *Salmonella* gr. B (70 cases), followed by *Salmonella* gr. D (31 cases), the others species being reported in smaller percentages. *E. coli* was identified in 91 cases, most of them being EPEC (68 cases), followed by ETEC (22 cases), EHEC being reported in only one case. *Shigella* spp. was identified in 61 cases, with *S. flexneri*being the most frequent species (26 cases), followed by *S. sonnei* (18 cases), *S. dysenteriae* (13 cases), *S. boydii* (3 cases). *Yersinia* spp. was reported in 9 cases, all of them being *Yersinia enterocolitica*.

302 samples of feces were processed through latex-agglutination in order to test the presence of Rotavirus (representing a percent of 4.71% from all the samples that were processed in the laboratory during the study). There were 84 positive cases, representing a percentage of 27.81% from the 302 samples tested for Rotavirus (1.28% from all the samples that were processed).

In terms of age distribution, most cases positive for rotavirus came from the toddler age group (47cases), followed by infants (29 cases). The average age was 2.85 years, with the median 1.4 (95% CI, 1.5219-4.1953, aged 0.11 to 40 years).

Of the 1923 samples processed by ELISA technique, most of the positive cases were reported for *Campylobacter* spp (4.47%), *Giardia lamblia* (2.65%) and *Clostridium difficile* (1.25%). Testing for the others species (*Cryptosporidium, Entamoeba, Echinococcus,* Rotavirus, Adenovirus) were performed and reported positive in a much smaller percentage.

Of the 828 samples tested for *Campylobacter* spp., positive samples were reported in 10.39% cases. The distribution of the positive cases by age groups showed higher percentages for small children (26.74%), infants (24.42%) and preschoolers (17.44%). The average age was 12.67 years, with the median 2 (95% CI, 3973-21372, aged 0.1 to 82 years).

Testing for *Clostridium difficile* was performed in 380 cases (19.76% of all the processed samples), in 1543 cases this diagnosis was not requested. Positive results were reported in 6.32% cases of all the samples that were processed for *C. difficile*.

The distribution of the positive cases by age groups showed higher percentages for adults, followed by the elderly. The average age was 41.16 years, with median 40 (95% CI, 28.08 to 54.24, ages 1-81years). The hospitalization stay was variable, with values ranging from 1 day (outpatients) to 17 days, the average period of hospitalization was 4.67 days. In 45.83% cases, the patients were treated with proton pump inhibitors, H_2 receptor antagonists were administrated in only 16.67% cases. The patients were treated with antidiarrheal drugs in 41.67% cases and with antispasmodics in only 12.5% cases. Of all antibiotic treatments that were administered to our patients, the most frequently used were third generation cephalosporines (in 37.5% of cases), followed by fluoroquinolones (in 20.83% of cases), penicillins (in 16.67%% of cases).

Of the 775 samples tested for *Giardia lamblia*, positive results were reported in 51 cases, representing a percentage of 6.58%, negative results were reported in 92.38% of cases (716 cases). The percentage of inconclusive results was reported in 1.03% cases.

DISCUSSIONS

Most of the acute diarrheal diseases are self-limiting, even if their etiology is infectious or not. Data from specialised literature show that acute diarrheal diseases appears most frequently in children under 5 years old, presenting an average of 3.2 diarrheal episodes every year, but in some developing countries, the number of diarrheal episodes may reach up to 12/year.(6-9)

In our study, acute diarrheal disease was most frequently identified in the age groups of children less than 6 years old, with the highest number of cases in the small children age group (1.1 to 3 years old) and infants (1-12 months).

The percentage of positive samples that were processed through coproculture was variable depending on the species identified, from 0% (*V. cholerae*) and 1.81% (for *Salmonella* spp). In several studies conducted between 1980-1997, coproculture positivity was reported in percentages ranging from1.5 to 5.6%.(10,11) From the samples processed by ELISA, positive results were reported between 0% (for *Cryptosporidium* spp, *Entamoeba* spp and *Echinococcus*) and 4.47% for *Campylobacter* spp (table no. 1).

Table no. 1. The percentage of the aethiological agents of the acute diarrheal disease

Pathogenic agent	Coproculture	Latex-agglutination	ELISA
	% from all the processed samples		
Salmonella **spp.**	**1,81 %**	-	-
E. coli	**1,39 %**	-	-
Shigella **spp.**	**0,93 %**	-	-
Yersinia **spp.**	**0,14 %**	-	-
Campylobacter **spp.**	-	-	**4,47%**
Clostridium difficile	-	-	**1,25%**
Giardia lamblia	-	-	**2,65%**
Rotavirus	-	**1,28%**	**1,2%**
Adenovirus	-	**0%**	**0,16%**

It was noted that the most frequent positive diagnosis was for *Campylobacter* spp., followed by *Giardia lamblia*, *Salmonella* spp, *E. coli*, and only then by Rotavirus.

Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe, published in 2008, (12) shows that Rotavirus is considered the most common etiological agent of acute gastroenteritis in children. Our study identified the most frequent cases of Rotavirus in the age groups of children less than 6 years old, especially in small children (55.95%) and infants (34.52%), but it was not identified as the main etiological agent of acute diarrhoea.

The same guidelines show that across countries, the main etiologic agent of acute bacterial diarrhoea is *Campylobacter* spp (especially in Northern European countries), or *Salmonella* spp. (in Southern Europe). Similar data have been identified in our study, the main bacterial etiological agent of acute diarrhoea was *Campylobacter* spp (4.47%), followed by *Salmonella* spp. (1.81%).

Clostridium difficile is recognized as the most common cause of colitis in hospitalized patients treated with antibiotics, chemotherapy or other treatments that alter the intestinal flora.(13) In a study published in 1996, the authors

reported positive results attesting the feces for cytotoxins produced by *C. difficile* with a frequency of 14%.(13)

At the European Congress of Clinical Microbiology and Infectious Diseases from Vienna (2010), CDI (*C. difficile* infection) was frequency present with values ranging from 0 to 1.9 in 2007-2009.(14) CDI rate of 1.25% that our study identified correlates with the data from the literature, being close to those reported in Europe. However, we believe that the frequency of this infection in our country is much higher. The lack of a national protocol for diagnosis and the fact that the national data about the frequency of this infection are sporadic makes the correct reporting of the results impossible. A study published in 2009 reported that the period of hospitalization for patients with CDI was more than double than that of undiagnosed patients with this infection (13.5- 14.9 days to 5.4-5.6 days). The length of hospitalization was higher in the patients older than 65 years old and the prevalence was increasing in those admitted in units where hospitalization is usually prolonged.(15)

Another study published in 2011 shows that the older age, the use of antibiotics and proton pump inhibitors were significant risk factors for the nosocomial infections with *C. difficile*. Hospitalization with 2 months before the infection, the treatment with chemotherapy, proton pump inhibitors, H_2 receptor antagonists and antitoxin B antibodies were significant risk factors for the colonization of hospitalized patients.(16)

The data from our study correlate with those from the literature, most patients diagnosed with CDI presented risk factors (prolonged hospitalization, antibiotics, antacids). In 45.83% of cases, the patients were treated with proton pump inhibitors, H_2 receptor antagonists being administered only in 16.67% of cases. Antidiarrheal drugs were administered in 41.67% cases, antispasmodics in only 12.5% of cases. Of all antibiotic treatments that were administered to our patients, the most frequently used were the third generation cephalosporins (in 37.5% of cases), followed by fluoroquinolones (in 20.83% of cases) and penicillins (in 16.67% of cases). Rarely, the patients were treated with aminoglycosides and macrolides (in 8.33% of cases), carbapenems and oxazolidinones being administered in a single case each (4.17%).

The multiple risk factors in the patients diagnosed with CDI in our study also argue that the frequency of infection is probably underdiagnosed, especially by not selecting samples according to the testing criteria for CDI.

CONCLUSIONS

1. Acute diarrheal disease was most frequently identified in the age group of children under six years old, with the largest number of cases in the small children age group (1.1 to 3 years old) and infants (1 - 12 months old).
2. The most common positive diagnosis was for Campylobacter spp., followed by Giardia lamblia, Salmonella spp., E. coli, and only then by Rotavirus.
3. Viral etiology of acute diarrheal disease was most commonly represented by Rotavirus.
4. CDI was identified in a low percentage.
5. Most patients diagnosed with CDI had risk factors (prolonged hospitalization, antibiotics and antacids).

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