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ENTERIC INFECTION WITH *CLOSTRIDIUM* BACTERIA IN ARGES COUNTY

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Abstract
One of the most important concerns of medical care is health care-associated infection. Certain bacterial species can be involved, but they produce different types of diseases. *Clostridium difficile* have been associated with intestinal diseases in humans, mostly after antibiotic treatments for a long period. These infections can vary from mild diarrhea to severe colitis. The aim of this paper was to establish the incidence of this kind of enteric infections in 2017-2018 in patients from Arges hospital. 294 patients were investigated, age one to 94 years, from villages and towns, both women and men. The most patients had age over 60 years. The presence of toxins was detected.
The incidence of infections was higher in men than in women; mostly toxin B was detected in patients.
The main attention in many infections is the ability of antibiotics to reduce both pathogenic and saprophytic bacteria, so the pathogens like *Clostridium difficile* can increase and cause enteric diseases.

Keywords: enteric infection, toxin, antibiotic.

1. INTRODUCTION

Clostridium difficile were described since 1935 in faeces of healthy children and afterwards were associated with colitis in antibiotic treatment. It can be found in soil, hay, sand, dung and sewage, too, and in faeces of animals (sporadic it can be a veterinary pathogen). Nowadays these bacteria seem to be the main reason of nosocomial diarrhea all over the world (Curry, 2010). It is not a pathogen for foodborne illness.
Clostridium difficile is a spore-forming Gram-positive pathogen and it could be the cause of some enteric infections in humans. It is an obligate anaerobe bacterium and the infections are started by vegetative cells in susceptible host, but the spores of *C. difficile* are responsible for the horizontal spread of disease, especially in hospitals (Paredes-Sabja et al., 2014).
The pathogenicity of this bacterium consist of synthesis of two exotoxins, named toxin A (enterotoxin) and toxin B (cytotoxin), responsible for patient's symptoms. Both toxins are large proteins and have two subunits, A and B; the subunit A is delivered into the target cell (Burnham and Carroll, 2013). These toxins can damage cytoskeletal structure and have strong cytopathic effects in tissue culture (especially on intestinal cells, but *in vitro* studies suggest that cardiac, renal and neurologic cells are affected, too) and due to cell death. The significant toxemia is correlated with fulminant disease and toxin B plays a major role in pathogenesis of *Clostridium difficile* infection (Di Bella et al., 2016), toxin B being an enterotoxin as toxin A, increasing mucosal

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permeability and an inflammatory response (Carter et al., 2010). Some strains of *C. difficile* can produce another toxin, named *C. difficile* transferase (CDT), known as binary toxin, because due to the synthesis of two separate polypeptides (Burnham and Carroll, 2013).
The health care-associated infections (HAI) are global concern in hospitals. Multiple factors can be responsible for these diseases, environmental factors and patient - related factors; nurse-to-patient ratio, some medical procedures, long-term intravenous treatments, and contact with infected objects or substances are considered relevant (Al-Tawfig, Tambyah, 2014). The patient's immunodeficiency or usage of immunosuppressive agents, chronically illness (like diabetes, leukaemia and HIV infection), prolonged hospitalization are a few risk factors, too.
Regarding to *Clostridium difficile*, the long term hospitalization and extended antibiotic treatment are the main factors (Ali et al., 2015), but other factors may be important for the spread of bacteria.
In hospitals, patients with symptomatic or asymptomatic infection produced by *Clostridium difficile* can disseminate the bacterial spores and can contaminate varied surfaces, making possible a horizontal transmission of disease. After that, the hand of patients, visitors, health care personnel can contaminate the objects and environment of susceptible subjects.
The major procedures of disinfection are required because the resistance of spores. The spores allows the bacteria to survive in environmental conditions, they are produced as a response to nutrient deprivation and are metabolically dormant (Kochan et al., 2018). The *C. difficile* spores are resistant to oxygen, radiation, desiccation, heat, low pH and some disinfectants (even to 70% ethanol), but are susceptible to other (such sodium hypochlorite), thus it is necessary to apply a severe disinfection in hospital rooms to prevent transmission to new patients (Curry, 2010).

2. MATERIALS AND METHODS

In this paper the frequency of intestinal infections with *Clostridium difficile* in patients from Arges hospital was established. The stool samples were collected from 141 women and 153 men with intestinal diseases (294 patients between 1 to 94 years old). The patients came from urban or rural residence. More than 70% of these patients were over 50 years old (Table 1), both in female and male subjects. The samples were processed in hospital laboratory and the immunochromatography assay was applied - Trio toxin A/B/GDH tests. This is a immunochromatographic rapid test used to detect both toxins (A and B toxins) of *Clostridium difficile*, and glutamate dehydrogenase (GDH), a metabolic enzyme recognized as *C. difficile* antigen, elaborated in large amount by all strains of these bacteria, toxigenic or non-toxigenic type (Calderaro et al., 2013). This test is more beneficial than bacterial culture in anaerobiosis, because approx. 2% of healthy adults and more than 50% of young children can have *C. difficile* in enteric microbiota and it is appropriately for those patients with persistent diarrhea, which were treated with antibiotics for a long period.

Table 1. The distribution of studied cases

Patient gender/ Age groups	0-9 years	10-19 years	20-29 years	30-39 years	40-49 years	50-59 years	Over 60 years	**Total**
Male	4	1	5	10	11	22	100	**153**
Female	2	3	6	10	16	11	93	**141**
Total	**6**	**4**	**11**	**20**	**27**	**33**	**193**	**294**

The stool samples were solved in extraction solution and a few drops were put into device well. If the sample was positive for wanted marker, a rose-pink coloured band appeared in the positive

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reaction zone because of antibody - antigen complex formation. The same coloured band has to appear in the control zone if the reagents are correctly.

3. RESULTS AND DISCUSSIONS

The aim of this study was to relieve an incidence of intestinal infections with *Clostridium difficile* in hospitalized subjects. Among 294 studied cases, 21.08% (62 cases) were confirmed as infections produced by anaerobic bacteria *C. difficile* (Figure 1). Nowadays *Clostridium difficile* seems to become the main reason of nosocomial diarrheas world-wide, while approx. 15% of patients with antibiotic treatment develop symptoms as diarrhea, among those 20 - 30% having infections with *C. difficile* (Paredes-Sabja et al., 2014).

The most cases were in male patients (62.9%), so the incidence of *C. difficile* infections in male was 25.49%, while the incidence in female was 16.31% (Figure 2).

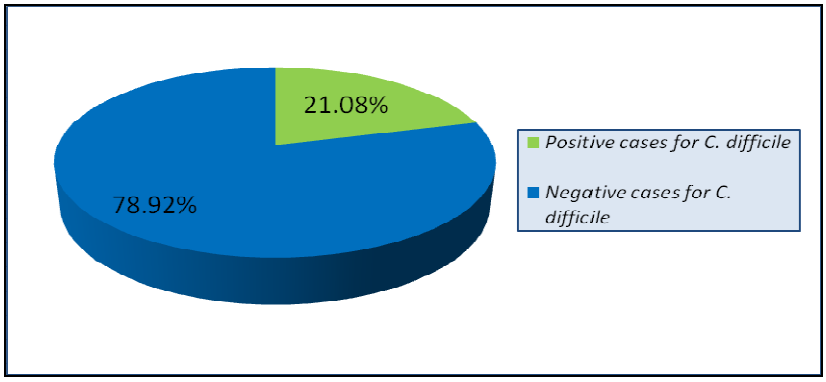

Figure 1. The incidence of C. difficile infections in studied cases group

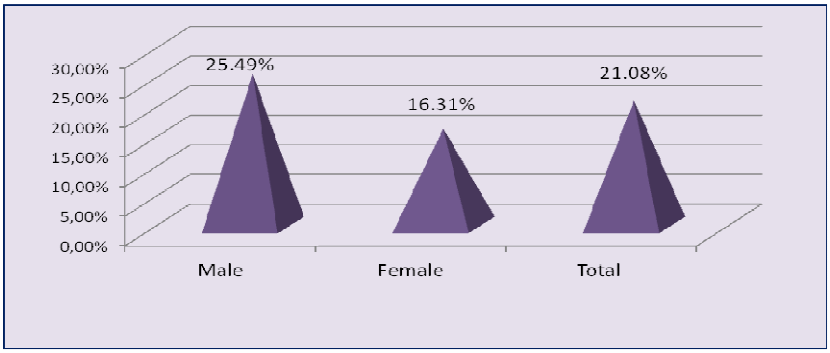

Figure 2. The incidence of C. difficile infections according to patient's gender

Regarding to distribution according to patient age, the *C. difficile* infections were more frequent in patients over 60 years, both in male and female subjects (Figure 3), because of the chronically illness and repeated hospitalizations. Persons older than 60 years have a tenfold greater risk of *C. difficile* infections than younger adults and the risk of death due to infections is also increased.

There were no *C. difficile* infections in children (0 - 9 years old or 10 - 19 years old). The most cases of infections were in male patients over 60 years.

According to patient's residence, 58% from all patients with *C. difficile* infections were from urban residences, but this percentage was no relevant for incidence of disease.

According to tests results, just 6 from 62 cases presented both toxin A and toxin B and just for one patient were detected toxin A, toxin B and GDH. The immunochromatographic tests were positive

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for toxin B in 21 samples, but the most important was GDH, the enzyme was detected in 27 samples.

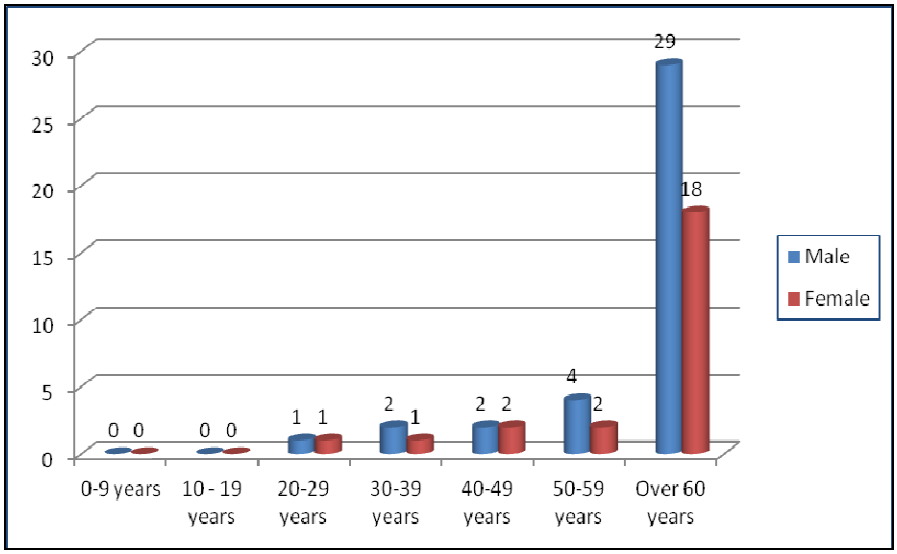

Figure 3. The number of C. difficile infections according to patient's ages and gender

4. CONCLUSIONS

The incidence of *Clostridium difficile* infections in patients in Arges County was similar to values of incidence from other studies. The well represented in studied cases group and the most affected age group was the over 60 years.

Regarding to *C. difficile* infections, the male patients and the patients from urban residences were more than female patients, respective the patients from rural residences.

Mostly glutamate dehydrogenase (GDH) and toxin B of *Clostridium difficile* were detected in stool samples.

Among all the health care-associated infections, the *C. difficile* infections are a major concern in hospitals and the diagnostic methods are more reliable.

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