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STUDIES AND RESEARCH ON EPIDEMIOLOGY, CLINICAL TABLE AND TREATMENT OF CLOSTRIDIUM DIFFICILE INFECTION IN SOUTH-EASTERN ROMANIA

- DOCTORAL THESIS SUMMARY -

PhD Coordinator:
Prof. univ. dr. Sorin RUGINĂ

PhD Student:
GHEORGHE (BAROIU) Liliana

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CONTENT

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Chapter 1

CURRENT STAGE ON EPIDEMIOLOGY, CLINICAL AND TREATMENT OF CLOSTRIDIUM DIFFICILE INFECTION

1.1. Introduction

The 21st century represents a challenge for the professionals in Infectious Diseases and Epidemiology regarding the use of antibiotics in the light of the increasing microbial resistance, the decrease of the number of new antibiotic molecules under study and of the exponential increase of the number of cases of *antibiotic*-associated *diarrhea* with Clostridium difficile.

Clostridium difficile (ICD) infection was directly correlated with the destruction of the microbiome after taking *antibiotics* (especially fluoroquinolones, cephalosporins, clindamycin). CDC (Centers of Disease Control and Prevention) declared in 2011, ICD a public health problem and the main post-medication adverse effect in hospitals in the United States of America [1]. This happened after the registration in 2011 in the United States of 500,000 cases of ICD, with 29,000 deaths in the first 30 days after diagnosis and with 83,000 cases that developed the recurrence. Also, the cost of an ICD patient was estimated in the United States at about $ 35,000, and the cost of ICD for the entire American health system at $ 3 trillion. At the same time, the CDC launches a disease surveillance program in 2013 [2].

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacillus, first identified in 1935 (Hall and O'Toole) in the colon of healthy newborns [3]. He was considered non-pathogenic until 1978 (Bartlett et al.) [4] when he was identified as a pathogen of pseudomembranous colitis in a patient pretreated with antibiotics. PCR (Polymerase Chain Reaction) detection of toxin B was first reported by Gumerlock in 1993 [5], and the complete genomic sequence of CD (Clostridium difficile) was first published in 2005 by Sanger, UK Institute [6].

The epidemiology of the disease has changed dramatically in recent years with significant increases in the incidence and severity of reported cases in the United States and Europe [7], [8], [9], [10]. This change was attributed to the emergence of a hypervirulent CD strain, associated with epidemics in Canada, USA, Europe [6], [11], [12] - (BI / NAP1 / 027), a rapidly spreading strain, with a mutation in - a negative regulator of the production of toxins, which led to a higher production of toxins. This strain also synthesizes an additional toxin, has an increased ability to generate spores and has increased resistance to fluoroquinolones compared to the historical strain [11], [13].

Galați County is, in 2018, in the top 5 counties with the highest number of ICD cases at 100 national discharges.

In conclusion, it is considered necessary to identify diagnostic and treatment algorithms adapted to the possibilities and needs of the South-East region, with the discovery of the most appropriate preventive measures (from the point of view of antibiotic abuse and of human transmission), which could contribute to the control of ICD extension.

1.2. Microbiological and epidemiological aspects of Clostridium difficile infection

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacteria in the Clostridiaceae family, the genus Clostridium,it grows well in enriched environments - blood and selective agar with cycloserine, cefoxitin, egg yolk agar, fructose (CCFA) or on chromogenic-chrom ID C. difficile agar media (bioMérieux). In the presence of oxygen, the vegetative form of CD can survive up to 24 hours on a non-living surface, while CD spores can survive for up to 2 years on non-living surfaces, which are exposed to oxygen. Clostridium difficile is a *commensal colonic bacteria* of more than 50% of children under one year of age, the numbers falling to about 3-5% in adults. This bacteria was found in the soil, lakes and from domestic animals: cattle, horses, donkeys, dogs, cats and rodents as well as from wild animals.

Clostridium difficile has a circular chromosome that has 4,290,252 base pairs and a circular plasmid with 7,881 base pairs. Conjunctival transposomes account for approximately 11% of the genome. Transpososomes are mobile fragments of DNA that can move from one location to another within the genome. These mobile elements are responsible for the acquisition by CD of an extensive series of genes involved in antimicrobial resistance, virulence, host interaction and the production of surface structures.

The factors involved in CD virulence are adhesins, extracellular enzymes, fibrils, paracrystalline capsule and three protein toxins: Toxin A, Toxin B, and Binary Toxin.

1.3. Clostridium difficile infection - epidemiological aspects

The epidemiological process

The source of the pathogen is represented by humans, the wild or domestic animals that house the Clostridium difficile in the gastrointestinal tract (sick or healthy carriers).

The transmission can be done in two ways: the direct mode is represented by the transmission of the Clostridium difficile strain between the patients and between them and the medical personnel and the indirect mode is represented by the objects, including the clothing, the medical-sanitary instrument, the thermometers and the furniture, which constitute an important way of transmission. The hands can be contaminated directly (including by self-contamination) or indirectly. Contamination of the community environment, of households, seems to be relatively common, CD was found on boots and shoes [44], which suggests an introduction of contaminated soil outside the house, on kitchen surfaces and refrigerators [45] which may indicate transfer from food, to production animals, to pets and to wild animals.

Patients' receptivity to infections caused by Clostridium difficile occurs when the host-bacterial relationship is imbalanced [86]. The body is susceptible to infection with these microorganisms when there are gaps in the commensal microflora of the colon (antibiotic, immunodepression) or mucosal barriers or the teguments are compromised by surgery, trauma, tumor, ischemia or necrosis, decreasing the potential for local oxide reduction. In addition, the decrease of the general resistance of the organism by age, alcoholism, diabetes, neoplasia, immunosuppression, angiopathies, causes an increased receptivity to infections with Clostridium difficile.

Community / nosocomial cases

The infection with Clostridium difficile was initially considered nosocomial, afterwards there were cases in which an epidemiological link with any medical services unit could not be established and the community infection was defined.

The definition of the case with exposure to a health care center implies that the symptoms appear within 4 weeks after discharge or at least 48 hours from the beginning of hospitalization [88], [89], and the community infection implies the discharge from a medical unit for at least 8 weeks or without proven contact with the healthcare system.

1.4. Diagnosis and treatment in Clostridium difficile infection

1.4.1. ICD diagnosis

Clinical manifestations of C. difficile infection can range from asymptomatic colonization to life-threatening infection. The clinical diagnosis of colitis with CD involves a new onset, at least 3 stools per 24 hours, unformed, grade 6, 7 Bristol scale [130], [131]. The most common clinical symptoms are: aqueous diarrhea with mucus and sometimes blood, abdominal pain in cramps or continuous. They may sometimes be accompanied by fever, dehydration syndrome, nausea, vomiting, lack of appetite, general influenced state [132].

In mild form, C. difficile produces abdominal discomfort and presents only colitis without pseudomembranes, manifested by soft mucus stools, in afebrility.

Severe forms can be accompanied by acute abdomen or toxic megacolon, in which there is decreased muscle tone of the smooth muscle in the colon wall, with significant dilation and possibly perforation. The development of a paralytic ileus with dilation of the colon leads to a paradoxical decrease of diarrhea, accompanied by signs of hypovolemic shock, possibly signs of acute abdomen. The diagnosis of fulminant ICD involves onset with severe or complicated clinical form, characterized by hypotension or shock, ileus or megacolon.

Toxic megacolon (TM) is the total or segmental dilation of the colon, non-obstructive, over 6 cm in diameter, associated with signs of systemic toxicity. TM was initially described in 1950 by Marshak and Lester [263], and Jalan, in 1967 [264], defines the three mandatory diagnostic criteria:

A - radiological - transverse or ascending colon dilation over 6 cm in diameter;

B - three criteria of the following - fever over 38.6 degrees Celsius, heart rate over 120 / min, leukocytosis over 10 500 / ul, anemia;

C - a criterion between - dehydration, confusion, electrolyte abnormalities, hypotension.

Uncommon clinical forms of ICD include enteropathy, which loses protein and extracolonic involvement.

Enteropathy that loses protein

Enteropathy with hypoalbuminemia has been described in association with acute CD infection in absense of fulminant colitis [265, 266]. Inflammation of the intestinal wall allows albumin to leak into the lumen, causing colonic loss of albumin with inadequate compensatory hepatic synthesis. As a result, serum albumin levels may fall below 2.0 g / dL. Ascites and peripheral edema can be observed clinically. Protein-losing enteropathy responds to appropriate medical therapy for CD infection.

Extracolonic affectation

Rare cases of appendicitis with CD, enteritis of the small intestine and extraintestinal disorders (CD cellulitis, soft tissue infection, bacteremia and reactive arthritis [269], [272], [273]) have been described.

1.4.2. Paraclinic diagnosis

All patients with new onset, with at least 3 unformed seats (Bristol scale 6, 7), with risk factors for ICD or with a long evolution and without any other etiological suspicion, are tested for CD. The laboratory may exclude normal stool samples from testing, except for epidemiological studies.

Laboratory tests

It is recommended to use an algorithm for CD testing (GDH and toxins, GDH and toxins arbitrated by NAAT or NAAT and toxins) and sample selection (laboratories will only work on soft stool samples, taking the shape of the container) and clinical correlation (testing after establishment diagnosis of diarrhea and after raising the suspicion for the etiology with CD).

Children under 2 years of age should be tested for CD only if they have evidence of pseudomembranous colitis or toxic megacolon or if they have clinically significant diarrhea and other causes of diarrhea have been excluded. In children over 2 years of age, CD testing is recommended for patients with prolonged or aggravating diarrhea and risk factors (for example, inflammatory bowel disease, immunodepression, contact with the health system or recent antibiotic therapy).

1.4.3. Imaging and anatomopathological diagnosis

The anatomopathological aspect of colonoscopy or necropsy is typical, with yellow and pseudomembrane deposits. Microscopically, it describes destroyed epithelium, with focal areas of necrosis, neutrophilic infiltrate in the mucosa, pseudomembranes containing necrotic leukocytes, fibrin, mucus and cellular debris. Abdominal computed tomography, as an imaging method of choice for ICD, when it is in the stage of pseudomembranous colitis or with other complications, or the association of another intra-abdominal pathology is suspected, reveals the thickening of the colonic wall, ascites and irregularity of the intestinal wall.

In patients with sepsis due to megacolon, abdominal radiography may be performed instead of CT scan to determine the presence of the megacolon in a timely manner.

1.4.4. Positive diagnosis

Criteria:

• Clinical picture compatible with ICD and highlighting CD toxin in fecal matter or

• Pseudomembranous colitis (endoscopic, intraoperative or necroptic) or

• Suggested histopathological aspect when examining the operative part or at the autopsy.

The Romanian ICD diagnostic guide defines the clinical picture compatible with ICD as follows:

1. diarrhea: decreased consistency (Bristol scale 5-7) and increased frequency;

2. ileus: vomiting and constipation associated with a radiological image suggestive of enteral distension;

3. Toxic megacolon: radiological highlighting of colonic distension and signs of severe SIRS.

1.4.5. Differential diagnosis

1. Clostridium difficile infection must be differentiated from other infectious and non-infectious causes of diarrhea. Most diarrheal episodes associated with antibiotic therapy are not attributed to C. difficile infection (but rather to osmotic mechanisms), while antibiotic-associated diarrhea associated with colitis is predominantly caused by Clostridium difficile.

Infectious diarrhea - other microorganisms that have been causally involved in antibiotic-associated diarrhea are described, namely: Staphylococcus aureus, Klebsiella oxytoca, Clostridium perfringens and Salmonella [275], [276], [277], [278], [279] . Clinical manifestations are similar to those of C. difficile infection; the diagnosis is made through coproculture.

Non-infectious diarrhea - Causes of non-infectious diarrhea that can mimic C. difficile infection include post-infectious irritable bowel syndrome, chronic inflammatory bowel disease, celiac disease, and microscopic colitis. The cessation of diarrhea symptoms upon discontinuation of the antibiotic is a distinguishing feature of osmotic diarrhea. The presence of fever and leukocytosis leads to an infectious etiology, with CD or other germs.

2. Acute abdomen - ICD may present, with abdominal distension imitating, the small intestine ileus, Ogilvie syndrome (colonic pseudo-obstruction), volvulus or ischemia [274].

The approach of the diagnosis varies according to age, sex and comorbidities; data from medical history, physical examination, surgical and imaging consultation (empty abdominal x-ray or abdominopelvic CTscan) are useful.

3. Shock - severe hypotension may occur in fulminant infection with Clostridium difficile and / or in toxic megacolon when intestinal perforation with peritonitis occurs. In addition, CD infection may develop during antibiotic treatment for septic shock caused by a bacterial infection separate. Shock from other causes (such as septic shock or cardiogenic shock) must be differentiated from severe hypotension due to CD infection by cardiac and hemodynamic evaluation.

4. Post-infectious irritable bowel syndrome - Post-infectious irritable bowel syndrome occurs in approximately 10% of patients who have been successfully treated for an initial episode of C. difficile. These patients can have up to 10 aqueous stools per day; this clinical picture should be differentiated by a relapse of CD infection based on the positive diagnostic criteria of ICD.

5. Chronic inflammatory bowel disease - C. difficile infection can complicate the course of chronic inflammatory bowel disease (IBD) [280], [281].

6. Benign or malignant tumors of the colon - the diagnosis is established by colonoscopy with biopsy.

7. Acceleration of reactive intestinal transit to medication or associated pathology - chemotherapy of cancer disorders is most frequently incriminated, immunochromatography for Clostridium difficile of all episodes meeting the clinical criteria of diarrhea is recommended.

1.5. ICD treatment

1.5.1. Prevention

ICD prevention measures include: isolation measures, hand hygiene, environmental disinfection and a protocol for antibiotic administration.

Isolation of patients with suspected ICD or ICD is a preventive measure to be used in all medical institutions, regardless of specialty or local epidemiology. It is recommended that patients with ICD be admitted to a salon with a bed and bathroom. If there is a limited number of lounges with a bed, priority is given to patients with multiple stools or fecal incontinence. If there are multi-bed rooms, only ICD patients will be admitted to them, they will be provided with their own bathroom, separate from patients with other conditions. When single-bed lounges are not available and patients are admitted to multi-bed lounges, the risk of recurrence increases.

Patients with suspected ICD should be isolated from patients with diagnosed ICD and other diseases and treated with all precautionary measures from ICD until the results of C. difficile tests are reached.

Hospital partitioning and accessibility to hand washing are essential elements in the prevention and control of ICD.

Contact precautions: use of gloves and long robes

The recommendation is for medical staff to wear long uniforms and gloves when entering a patient's room with ICD and while caring for patients with ICD.

Hand hygiene

Hand hygiene is performed before and after contact with a patient with ICD and after gloves removal, by washing with soap and water. Hand hygiene is considered one of the corner stones of preventing CD transmission. Encouraging patients to wash their hands and shower might be a useful strategy to reduce the amount of spores on the skin.

Use of disposable equipment

Disposable equipment should be used for the patient with ICD, when possible, and reusable equipment should be cleaned and disinfected, especially with a sporicide disinfectant compatible with the equipment.

Disinfection with sporocidal substances

Terminal disinfection of the salon with a sporicidal agent should be used during endemic periods or in the rooms where ICD cases are repeatedly admitted.

Daily surface cleaning with a sporocidal agent, a hypochlorite solution or bleaching wipes containing 0.55% active chlorine should be used.

Non-touch disinfection technologies that use ultraviolet radiation or hydrogen peroxide vapor to disinfect the environment are effective in reducing the amount of viable CD spores in salons [183], [184], [185]. No single methodology (without prejudice or otherwise) is superior to the combination of methods in reducing the incidence of ICD.

Duration of precautionary measures in ICD

Currently, the CDC recommends that contact precautions be continued throughout the disease [188].

Identification of asymptomatic Clostridium difficile carriers

There is insufficient data to recommend the examination for asymptomatic CD transport and the application of contact precautions to asymptomatic carriers.

The role of antibiotic administration in controlling ICD rates

The frequency and duration of high-risk antibiotics, as well as the combination of antibiotics, must be minimized by implementing an antibiotic administration program.

The antibiotics that are used should be based on the local epidemiology and CD strains present. Restriction should be considered for: fluoroquinolones, clindamycin and cephalosporins (excluding antibiotic surgical prophylaxis), carbapenems.

Many hospitals have implemented antibiotic administration policies. Benefits of these programs include improved patient therapy outcomes, reduced adverse events (including ICD), improved antibiotic susceptibility rates, and optimized resource utilization [195].

The role of proton pump inhibitor restriction in controlling ICD rates

Although there is an epidemiological association between the use of PPIs and ICDs and unnecessary PPIs should always be discontinued, there is insufficient evidence to discontinue treatment with PPIs as a preventive measure of PCI. The use of type 2 histamine receptor antagonists had a lower risk of ICD compared to the use of PPIs.

The role of probiotics in primary prevention in ICD

There is insufficient data at this time to recommend the administration of probiotics for primary prevention of ICD. Several meta-analyzes indicate that probiotics may be effective in preventing ICD when administered to patients with antibiotics without a history of ICD [196], [197], [198], [199].

1.5.2. Treatment

Auxiliary treatment for ICD

1. It is recommended to discontinue antibiotic treatment as soon as possible, knowing that their continued use reduces the clinical response and increases the recurrence rate of ICD [203], [204].

2. The administration of digestive antimotility agents in patients with diarrhea, such as loperamide, is considered to be favorable for intestinal paresis and the occurrence of toxic megacolon, so it is recommended to avoid them in therapy [205], [206].

3. Antibiotic therapy for ICD should be started empirically only in cases where a substantial delay (over 48 hours) is expected in laboratory confirmation or for fulminant ICD.

Treatment of the initial episode of ICD

The objectives of the treatment of the initial episode of ICD are symptom resolution and sustained resolution for one month.

The recommendations of the IDSA 2018 guide are: either vancomycin or fidaxomycin for an initial episode of ICD. The dose is: vancomycin 125 mg, by mouth, 4 times daily or fidaxomycin 200 mg twice daily, 10 days. If access to vancomycin or fidaxomycin is limited, metronidazole is used only for an initial episode of not severe ICD. The recommended dose of metronidazole is 500 mg by mouth, 3 times daily for 10 days. Repeat treatment or prolonged treatment with metronidazole should be avoided because of the risk of cumulation and potentially irreversible neurotoxicity. If patients improved their symptoms but did not have symptom resolution within 10 days, treatment may be extended up to 14 days [1].

The recommendations of the Romanian treatment guide are: metronidazole 500 mg at 8 hours, by mouth; 10 days (does not extend beyond 14 days due to the increased cumulative risk of neurotoxicity); not more than 3-4 days after the remission of the symptoms, it is indicated in the ICD of reduced or medium severity; vancomycin 125 mg at 6 hours, by mouth , 10-14 days, is indicated in high severity ICD.

Treatment of fulminant ICD

1. For fulminant ICD (severe or complicated ICD, characterized by hypotension or shock, ileus or megacolon), vancomycin administered orally is the regimen of choice. If ileus is present, add vancomycin administered to the rectum. The dose of vancomycin is 500 mg by mouth, 4 times daily and 500 mg in approximately 100 ml of saline solution, per rectum, every 6 hours, as a retention enema, associated with metronidazole 500 mg intravenously every 8 hours.

2. If surgical management is required for severe patients (patients with megacolon, perforation of the colon, acute abdomen, or for patients with septic shock and organ failure), subtotal colectomy will be performed with preservation of the rectum.

Deviation loops, ileostome with colonic lavage, followed by enema with vancomycin dissolved in saline solution is an alternative approach that may lead to symptom improvement. Increased total leukocyte counts above 25 000 IU / ml or increased lactate level over 5 mmol / l are associated with high mortality and may be useful in identifying patients whose survival expectancy is limited and for whom surgery is the best option. [222].

In patients with suspected vancomycin and metronidazole resistance, intravenous administration of tigecycline (100 mg loading dose followed by 50 mg twice daily) or passive immunoglobulin intravenous (150-400 mg / kg) is indicated, but do not have sufficient studies to support their efficacy [216], [217], [218], [219], [220], [221].

Recurrent ICD treatment

The first recurrence - the recommendations of the American guide

- a first recurrence of ICD is treated with oral vancomycin in the form of a conical or pulse cure, preferably a standard 10-day treatment of vancomycin, or
- a first recurrence of ICD is treated with a 10-day course of fidaxomycin, preferably a standard 10-day treatment of vancomycin; or
- a first recurrence of ICD is treated with a standard 10-day course of vancomycin, preferably a second course of metronidazole, if metronidazole was used for the primary episode.

Vancomycin as a conical and pulsed dose regimen has the following regimen: after regular administration of 125 mg 4 times daily for 10-14 days, vancomycin 125 mg 2 times daily for one week, 125 mg a given daily for one week; then 125 mg every 2 or 3 days for 2-8 weeks, in the hope that C. difficile vegetative forms will be kept under control while restoring the normal microbiota.

The first relapse - the recommendations of the Romanian guide

- for the first relapse: the same antibiotics and doses as in the initial episode.

Multiple relapses

- oral vancomycin therapy, using conical or pulse therapy (joint American and Romanian guideline recommendation),
- a standard oral vancomycin treatment, followed by rifaximin - 400 mg 3 times daily for 20 days, immediately after completion of standard therapy (US guideline recommendation);
- fidaxomycin (US guideline recommendation);
- fecal microbiological transplantation in patients with multiple relapses of ICD who have failed appropriate antibiotic treatments.

Several probiotics, including Saccharomyces boulardii and Lactobacillus species, have been successful in preventing ICD recurrences [233], [234], [235]. However, no one has yet demonstrated significant and reproducible efficacy in the controlled clinical setting.

Patients requiring systemic antibiotic therapy for conditions other than ICD

At this time there is not enough data to recommend extending the treatment period or resuming anti-C. difficile treatment for patients who require continuous antibiotic treatment directed against the underlying infection or who need to repeat antibiotic therapy shortly after completion of treatment with ICD. Patients requiring systemic antibiotic therapy concomitantly with treatment for ICD are at increased risk of relapse and its associated complications [204], [228], [229]. Many clinicians extend the duration of ICD treatment in such cases until systemic antibiotic therapy is completed. Low doses may be sufficient to prevent recurrence, for example, 125 mg vancomycin once daily.

Factors that may influence the decision to start secondary prophylaxis include the length of time since previous ICD treatment and patient characteristics (number of previous ICD episodes, severity of previous episodes, and patient fragility).

Fecal Transplantation (TMF)

Clinical investigations in patients with recurrent ICD have shown a significant disturbance of the intestinal microbial diversity, as well as the number of relative bacterial populations. Processed stool instillation, collected from a healthy donor in the intestinal tract of patients with recurrent ICD, has been used with a high degree of success for correcting intestinal dysbiosis caused by repeated antibiotic treatments [239], [240], [241], [242].

The success rate differs with the mode of administration and varies between 77% and 94% at administration through the proximal small intestine [239], [243] and 80% -100% at fecal instillation through the colon [241], [244], [245], [246], [247].

Treatment of ICD in children

Treatment of an initial episode or first recurrence of non-severe ICD in children

It is recommended either metronidazole or vancomycin in the treatment of children with an initial episode or the first recurrence of ICD, nonsevere. Dosages: Metronidazole, 10 days, 7.5 mg / kg / dose, by mouth, at 8 hours, maximum dose - 500 mg at 8 hours; Vancomycin, 10 mg / kg / dose, by mouth at 6 hours, maximum dose - 125 mg at 6 hours, 10 days.

Treatment of severe initial episode of ICD in children

For children with an initial episode of severe ICD, vancomycin is recommended, 10 days, by mouth and per rectum, 10 mg / kg / dose at 6 hours, maximum dose - 500 mg at 6 hours, with or without metronidazole 10 days, intravenous, 10 mg / kg / dose, at 8 hours, maximum dose - 500 mg at 8 hours.

Treatment of second or greater episode, recurrent ICD in children

For children with a second or greater episode of recurrent ICD, oral vancomycin - preferably or metronidazole is recommended. Dosages: Metronidazole, 10 days, by mouth, 7.5 mg / kg / dose, at 8 hours, maximum dose -500 mg at 8 hours; Vancomycin, 10 mg / kg / dose, at 6 hours, maximum dose - 125 mg orally at 6 hours, 10 days. For children with a second recurrence, who have been treated exclusively with metronidazole, they will be treated with oral vancomycin, the standard 10 day cure.

For children with multiple relapses of ICD:

- Vancomycin in conical or pulse regimen -10 mg / kg / dose with maximum 125 mg at 6 hours, for 10-14 days, then 10 mg / kg / dose with maximum 125 mg at 12 hours, 7 days, then 10 mg / kg / dose with maximum 125 mg at 24 hours, 7 days, then 10 mg / kg / dose with maximum 125 mg at 2-3 days, 2-8 weeks, or
- Vancomycin 10 days, followed by rifaximin 20 days (rifaximin is not approved under 12 years, maximum dose 400 mg at 8 hours), or
- Fecal microbiota transplant. Fecal microbial transplantation for children and adolescents with multiple relapses of ICD should be considered following standard antibiotic treatment.

Chapter 2

STUDIES AND RESEARCH ON EPIDEMIOLOGY, CLINICAL AND THE TREATMENT OF CLOSTRIDIUM DIFFICILE INFECTION

2.1. Purpose and objectives of the doctoral thesis

Purpose of the doctoral thesis

The present study aims to identify the circulating strain in the Galati area, comparing the number of cases with the other counties in the southeastern area of Romania and identifying the particularities of the infection in this area and, subsequently, the possibility of identifying an optimal prophylaxis and treatment algorithm.

The objectives of the doctoral thesis

1. Identification of circulating strains in the Galați region.
2. Demonstration of the stability of vancomycin powder from a vial for intravenous use in artificial gastric juice and artificial gastrointestinal juice using UV-Vis spectra, given that the clinical study used this pharmaceutical form in oral treatment.
3. Comparison of the number of cases in the host hospital of the clinical study with those of the other hospitals in the region that report cases of ICD.
4. Identification of hospitals and sections of the source of infection of the patients in the study, the percentage of community cases and of indefinite source in order to be able to identify adapted prophylactic measures and to define the spread of the infection in the community.
5. Clinical-biological characterization of the studied lots and their comparative statistical analysis.
6. Quantifying the response to the treatment and the prognosis of the studied groups, identifying the demographic, clinical and paraclinical parameters that influence the duration of the treatment, the risk of death and recurrence.
7. Developing an ICD treatment protocol for the Galaţi Infectious Diseases Hospital, a course support for nurses, scientific communications for doctors and a brochure with patient information.

In order to reach these objectives, the following studies have been carried out:

Study 1 - Complex etiological diagnosis of Clostridium difficile infection in 50 patients for determination of circulating strain in Galați region.

Study 2 - Demonstration of the stability of vancomycin and metronidazole in gastric and gastrointestinal juices using UV-Vis spectra.

Study 3 - Epidemiology of Clostridium difficile infection in southeastern Romania.

Study 4 - Clinico-epidemiological study of Clostridium difficile infection in a group of 706 patients admitted to the Clinical Hospital of Infectious Diseases “Sf. Cuv. Parascheva ”Galați from 1.01.2017 to 31.12.2018.

The agreement for the clinical study was obtained from the Ethics Commission of the Clinical Hospital of Infectious Diseases „Sf. Cuv. Parascheva ”Galați and the Bioethics Commission of the“ Ovidius ”University of Constanța and also the informed consent of each patient enrolled in the study.

The agreement of the manager and the legal commission of each hospital was obtained for the supply of the number of cases of ICD, treated in those units, between 1.01.2017 ÷ 31.12.2018 and the percentage of cases associated with the medical assistance, of the hospitals in the south-east region of Romania, who reported cases of ICD, namely: "Sf. Apostol Andrei County Emergency Hospital" Galati, Emergency Clinical Hospital for Children" Sf. Ioan "Galati, Pneumophysiology Hospital Galati, Emergency County Clinical Hospital" Sf. Apostol Andrei ” Constanța, Clinical Hospital for Infectious Diseases Constanța, Clinical Hospital of Pneumophysiology Constanta, Emergency County Hospital Tulcea, Emergency County Hospital Buzău, Emergency County Hospital Brăila and ”Sf. Pantelimon ” Emergency County Hospital Focșani.

2.2. Study 1 - Complex etiological diagnosis of Clostridium difficile infection

2.2.1. Objectives

1. Comparison of the method of identification by immunochromatography with the reference method with the highest sensitivity - culture in anaerobiosis and with a fast method with high sensitivity - detection of CD genes in the stools by PCR, as well as obtaining preparations for analysis in optical and electron microscopy.

2. Identification of the circulating strain in the Galati area, being known that the type of the strain influences the severity of the disease episode and the death rate through ICD, as well as the survival of the strain in the external environment and consequently the possibility of spreading the infection.

2.2.2. Material and method

2.2.2.1. Immunoassay

CD immunostaining on the stool sample was performed on CERTEST BIOTECH S.L., SPAIN [96] tests.

2.2.2.2. Anaerobic culture of Clostridium difficile

Fecal matter, whether or not treated with ethanol, was seeded into plates with chromogenic medium (chromID ™ C. difficile agar, bioMerieux), plates inserted into anaerobic bags (anabag anaerobic bioMerieux) and incubated in anaerobic atmosphere, 24 ÷ 48 hours at 37 ºC. After 24 to 48 hours of incubation the plates are removed and examined. The appearance of suspected colonies of Clostridium difficile is from gray to black, smooth or irregular colonies, figure 1.

Fig. 1. *Plate with chromogenic medium and blood culture medium*

2.2.2.3. Optical microscopy analysis of germs obtained by culture

Gram colored smears were performed for microscopic verification and Gram positive bacilli were observed with subterminal spore, figure 2.

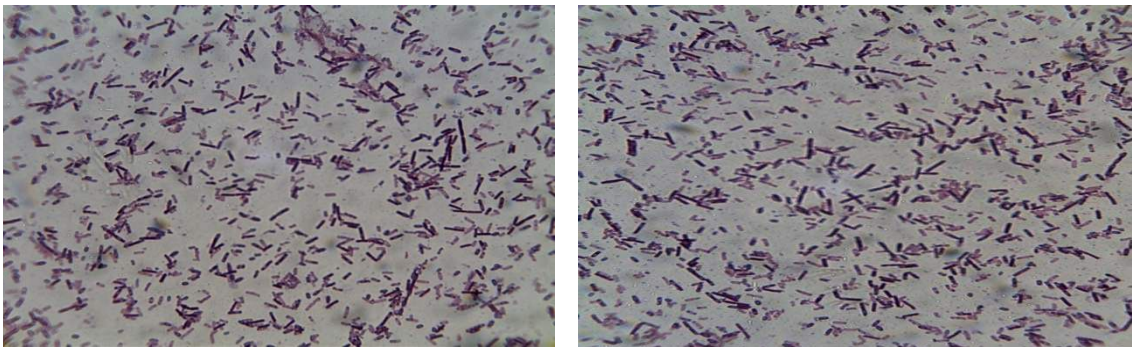

Fig. 2. *Sporulated Gram positive bacilli from culture (optical microscopy)*

2.2.2.4. Identification of germs obtained through culture

From the sector obtained on the plate with blood medium, anaerobically incubated, the VITEK 2 ANC cards are identified, figure 3.

2.2.2.5. Preservation of Clostridium difficile strains

Bacilli obtained by culture on chromogenic media and identified with the VITEK apparatus is maintained in thioglycolated broth with resazurin (THIO-T), figure 4.

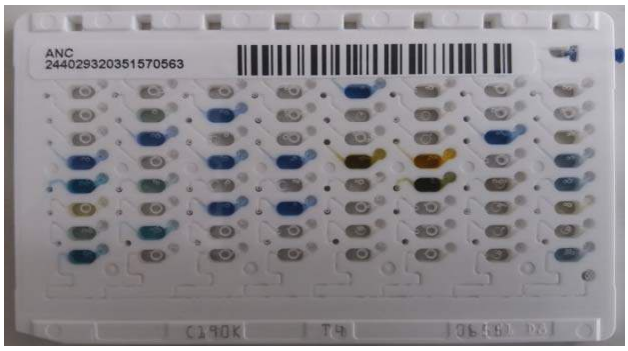

Fig. 3. *Vitek CD identification test*

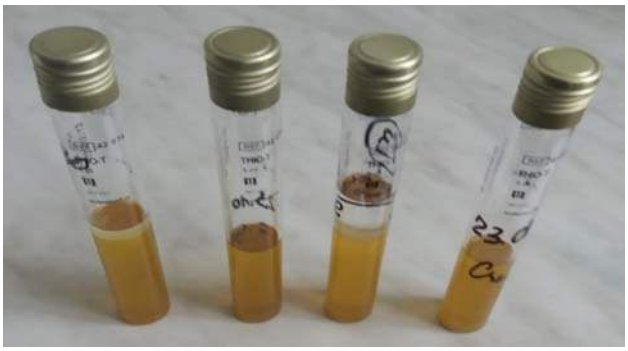

Fig. 4. *Thioglycolate tubes for CD staining*

Electron microscopy analysis of the bacilli identified VITEK

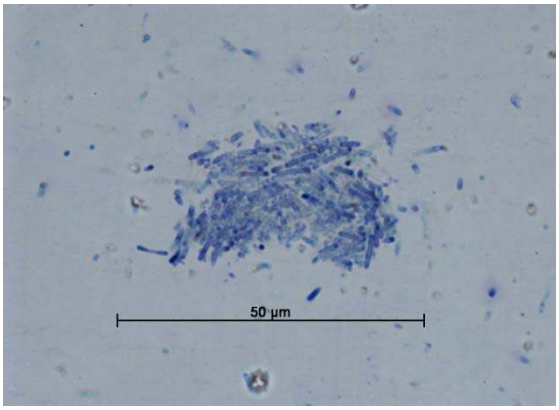

Fig. 5. *Bacteria sporulated by Clostridium difficile - electron microscopy image*

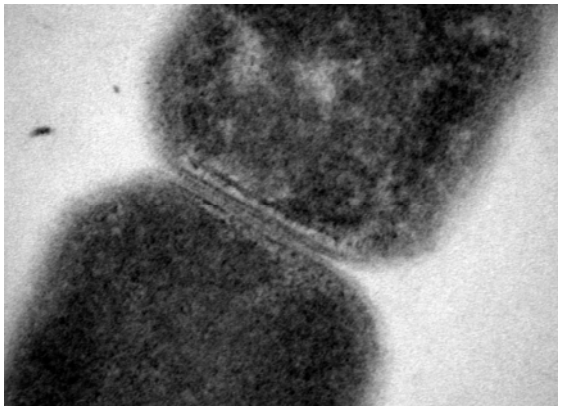

Fig. 6. *Two CD bacilli resulting from binary division - electron microscopy*

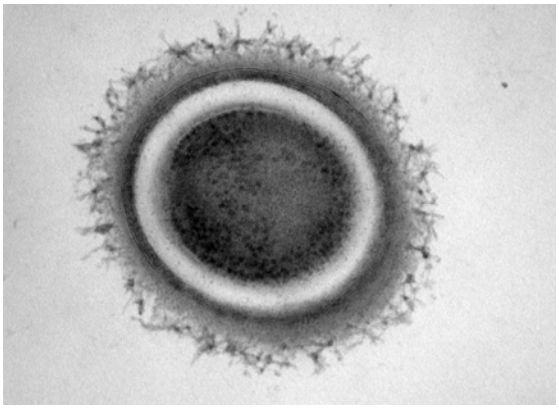

Fig. 7. *Cross section bacillus Clostridium difficile - electron microscopy image; structural details: cell membrane, cytoplasm, cellular organelles*

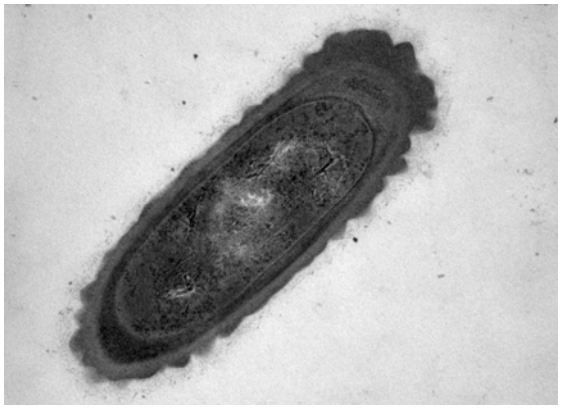

Fig. 8. *Longitudinal section of bacillus Clostridium difficile - electron microscopy image; structural details: cell membrane, cytoplasm, cellular organelles*

From the thioglycolate broth, after fixation with glutaraldehyde, preparations were performed that were examined by transmission electron microscopy at the National Institute of Research and Development in the Field of Pathology and Biomedical Sciences "Victor Babeș" Bucharest, and

were observed terminal or subterminal spores, details of bacterial structure (cell membrane, cytoplasm, nucleus, cellular organelles, spore) and the mode of multiplication by binary division, figure 5-8.

2.2.2.6. Identification of Clostridium difficile by PCR

Cepheid Xpert C. difficile BT tests were performed on Cepheid GeneXpert® instrument systems, diagnostic tests for the rapid detection of C. difficile tcdB (CD toxin B encoding gene), cdt (binary toxin gene) and deletion of a nucleotide at position 117 of the tcdC gene, from unformed (liquid or soft) stool samples collected from patients suspected of ICD. The assay uses the automatic real-time polymerase chain reaction (PCR) procedure to detect tcdB, cdt and tcdC deletion at base 117 associated with ribotype 027 strain [108]. Binary toxin is produced by a limited number of C. difficile strains, including strain 027. Binary toxin together with tcdB detection is often an indicator of more severe disease or disease recurrence.

Fig. 9. *GeneXpert Instrument Systems (GeneXpert apparatus, barcode scanner, GeneXpert computer software, user manual, stick and mixer set, pipette)*

In the immunochromatographic test, of the 720 strains studied, 611 (84.86%) were positive for Toxin A, 552 (76.66%) were positive for Toxin B, 523 (72.23%) positive for Toxin A and B, 720 were positive for GDH.

Anaerobiosis culture was performed on a batch of 60 strains that had immunochromatographic test with GDH-positive and at least one toxin A or B. The results were:

- specific growth on chromogenic environment, growth present on blood Colombia in anaerobic and absent growth on blood Colombia in aerobic - 51 strains - 85% of total cultivated strains;
- specific growth on chromogenic environment and growth present on anaerobic blood Colombia and aerobic blood columbia - 2 strains;
- mixed culture - 3 stems;
- non-specific growth on the chromogenic environment - 2 stems;
- without growth on the chromogenic environment - 2 stems.

The identification of VITEK was performed for the 51 strains with specific growth on chromogenic medium and growth present on blood Colombia, in anaerobic and absent growth on blood Columbia, in aerobic, and the following results were obtained:

- positive identification Clostridium difficile - 45 strains - 88.23% of total strains introduced for identification, 93.75% of total strains identified;
- positive identification Clostridium group - 2 strains;
- positive identification of Clostridium sporogenes - 1 strain;
- unidentified - 3 stems.

The 45 strains with VITEK positive identification for Clostridium difficile were analyzed by optical or electron microscopy. Gram positive bacilli were seen sporulated, with terminal or subterminal growth, with pili on the entire membrane surface and binary division.

The PCR test benefited a batch of 50 patients, in which the immunochromatography was performed and tested positive for GDH and at least one toxin A or B, were frozen at -70 ° C and worked in March 2019 with a GenExpert tool, for determine the ribotype of the strain (027 or not). The results were: 48 valid tests (2 errors), of which one test with non-oxygen strain, 3 tests with toxigenic strains other than 027, 44 tests with 027 strains (93.61% of the toxigenic strains).

2.2.3. Discussions

The results of the immunochromatographic, rapid, color test, should be interpreted with caution. Although the manufacturer claims a sensitivity> 95% and a specificity> 99% and recommends repeating samples with negative GDH and positive toxins, about the weak positive tests does not specify anything and possible here, the error coefficient to be higher. Performing the culture in anaerobiosis was performed on both positive and weak positive strains on the immunochromatographic test. Culture in anaerobiosis is the reference method, rated with the highest sensitivity but with low specificity, with limits due to the composition of the environment which allows the growth of microorganisms other than CD, namely: C. tertium, C. clostridioforme, Bacteroïdes, Lactobacillus, which can develop colonies similar to those of CDs, and some strains of CDs with particular nutritional requirements may not grow. The specificity of the media after 24 hours incubation is 92-96% and after 48 hours 96-98% [105]. Thus the study of our strains revealed only for 85% of the total cultivated strains, specific growth on chromogenic environment, increases present in Colombia blood in anaerobiosis and absent growth in Colombia blood in aerobiosis. These strains, when analyzed under optical microscopy, showed Gram positive bacilli, with terminal or subterminal endospores. These 51 strains were inserted into the Vitek apparatus for identification. Only 45 strains representing 88.23% of the total strains introduced for identification and 93.75% of the total strains identified presented a positive identification result for Clostridium difficile. PCR testing was performed for 50 strains with immunochromatographic test GDH-positive and at least one toxin A or B. The results were: 48 valid tests (2 errors), of which one test with non-oxygen strain, 3 tests with toxigenic strains other than 027, 44 tests with 027 strains (93.61% of the toxigenic strains). The non-oxygenous strain showed non-specific growth on the chromogenic medium, so it was most likely another germ even if the immunochromatographic test was positive. The most widespread type of CD strain in the US was ribotype 027 (28.4%), in a CDC study in 2009, and in England it had a prevalence of 55% in 2007 and 21% in 2010 [152]. This decrease in prevalence of this strain in England has been associated with significant decreases in reported ICD incidence and associated mortality, and has been attributed to decreased use of fluoroquinolones, increased awareness of ICD, and improved control of ICD.

Some studies in Romania show that the main circulating strain is ribotype 027 [14], [15]. The high percentage of strains 027 identified by the own study - 93.61%, draws attention to the possibility of the evolution of cases towards severe forms with reserved prognosis. High percentages of identification by high sensitivity methods: 85% of the total cultivated strains showed specific growth on chromogenic environment, growth present on blood in anaerobiosis and absent growth on blood in aerobiosis; 93.75% of the total strains identified by Vitek were Clostridium difficile; 97.91% of the strains were toxigenic, ribotype 027 or other at PCR identification; justifies the diagnosis of this infection only by immunochromatographic test with GDH and A and B toxins of first intention. The cases where there are risk factors and clinical arguments and uncertain or negative immunochromatography should also be tested by a CD gene detection PCR test in the stool. The cost of these analyzes is another strong argument for choosing immunocromatography as a first-intention test and the fact that it is a fast technique that gives a result in 30 minutes. The cost of the reagents for: a CerTest immunocromatographic test is 29.09 lei, an anaerobic culture, with Vitek identification is 84.82 lei (including only the plate with CROMID C. difficile, Anabag anaerobic bag, Vitek 2 ANC ID), and a GenExpert PCR test 208.25 lei. The average cost of all the analyzes necessary for the diagnosis and monitoring of ICD, calculated on a batch of 166 episodes of ICD treated in our hospital during the period 1.07.2018-31.12.2018 was 266.97 lei (with a minimum of 0 lei and a maximum of 1365.93 lei and a median of 234.32 lei) of which the etiological investigation is 60 lei, representing the cost of an immunocromatographic test for the detection of toxins A and B and GDH of Clostridium difficile. The Infectious Diseases Clinical Hospital “Sf. Cuv. Parascheva ”Galați is a university clinical hospital, with two specialties, Infectious Diseases and Dermatology, classified by rank 5. The budget settled by the County Insurance House for an ICD case is 1430 lei, compared to the disease complexity index of 0.80, is 1144 lei.

The average of the total expense statement of a patient with ICD, calculated on a batch of 166 episodes of ICD treated in our hospital during the period 1.07.2018-31.12.2018 was 4434.45 lei (with a minimum of 166.58 lei and a maximum of 19085.19 lei and a median of 3909.63 lei). This average of the expense statement in the DCI represents 387.62% of the amount allocated by the County Insurance House for a DCI case.

2.2.4. Conclusions

The main circulating CD strain in the Galați area is ribotype 027 (93.61% of the toxigenic strains). This strain is responsible for the 2010-2011 epidemic in the United States of America and Europe, is associated in clinical trials with higher severity of disease episode and higher death rate.

This ribotype has increased capacity for secretion of toxins and secretes an additional toxin, namely binary toxin, has increased ability to form spores over the historical strain. Caution should be exercised when interpreting immunocromatographic tests with poorly positive results and in case of clinical non-correlation a CD gene detection test was performed in the stool by PCR. The low budget allocated by the County Health Insurance House for a patient with ICD, the high percentages of matching of the results of the immunocromatography with the other diagnostic methods (culture in anaerobiosis with Vitek identification and detection of CD genes in the stool by PCR) and the speed of the result, indicates this method as the first intention in the etiological diagnosis of ICD.

2.3. Study 2 - Demonstration of the stability of vancomycin and metronidazole in gastric and gastrointestinal juices using UV-Vis spectra

2.3.1. Working hypothesis

This study analyzed the two main antibiotics used in the therapy: metronidazole and vancomycin, the evidence of their effectiveness in the literature and the chemical stability of antibiotics in the simulated gastrointestinal fluid.

Chemical, physical and microbiological stability of the vancomycin solution

Considering that in Romania there is no pharmaceutical form of capsules available, but the active substance in both capsules and powder from vials for intravenous use is vancomycin hydrochloride and in the clinic used, powder from intravenousvialsdissolved for oral administration, the stability details of the solutions are important. After reconstitution and dilution, the chemical and physical stability of the product was demonstrated for 48 hours at room temperature (<25 °C) and at temperatures between 2 °C and 8 °C [300]. From a microbiological point of view, the drugshould be used immediately. If not used immediately, the storage period should not exceed 24 hours at temperatures between 2°C and 8°C and reconstitution/dilution should be performed under controlled and validated aseptic conditions [300].

2.3.2. Objective

Demonstration of the chemical stability of vancomycin hydrochloride powder from the vials for intravenous use in the presence of gastric and gastrointestinal juices and thus the efficacy of the oral dose administered in patients with Clostridium difficile infection.

2.3.3. Material and method - Testing the chemical stability of vancomycin and metronidazole in artificial gastric juice and artificial gastrointestinal juice using UV-Vis spectra

The chemical stability of vancomycin and metronidazole under acidic conditions was evaluated using simulated gastric and gastrointestinal fluid. The simulated gastrointestinal fluid was obtained by mixing equal volumes of two solutions.

The first solution was obtained using 5 g/L NaCl and 3g/L pepsin and the pH was adjusted to 2 (artificial gastric juice). The second solution was composed of 5 g/L NaCl, 3 g/L pancreatin and 0.45 g / L bile and the pH was adjusted to 8 (artificial intestinal juice). The pH of the final gastrointestinal fluid, obtained by mixing the two mentioned solutions, was adjusted to pH 2.5 (artificial gastrointestinal juice) [303]. UV-Vis spectra were recorded for drug solutions and drug solutions under simulated gastrointestinal conditions at different time intervals after mixing.

The UV-Vis spectra of vancomycin and metronidazole were recorded in their aqueous solutions in the range 170-800 nm.

2.3.4. Results

All absorption bands of vancomycin and metronidazole, as well as the components of the simulated gastric and gastrointestinal juices, were observed in the UV field at time 0,30 minutes and 60 minutes after the preparation of the solutions, figures 10 ÷ 11.

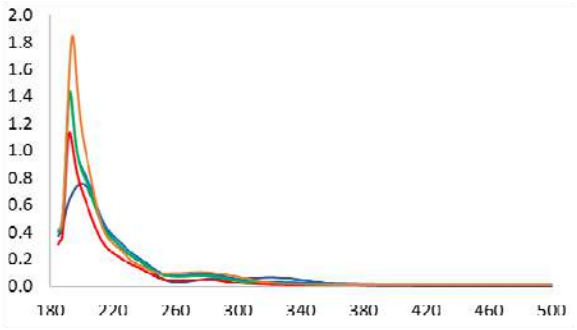

Fig. 10. *UV-Vis spectra of vancomycin solution (blumarine) in solution with pH2.5 (orange) at time 0 (green),30 (blue) and 60 (red) min.*

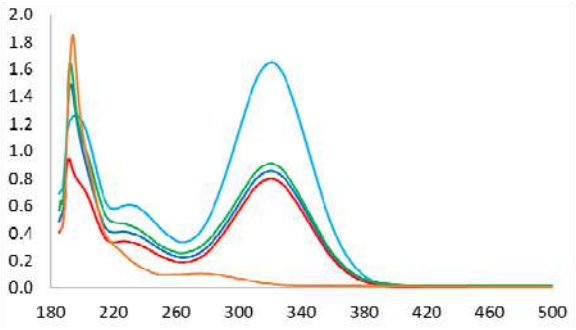

Fig. 11. *UV-Vis spectra of metronidazole solution (light blue) in solution with pH2.5 (orange) at time 0 (green),30 (blue) and 60 (red) min.*

The characteristic intensity of absorption of the initial and mixed solutions are in tables 1 and 2.

Table 1. *λ_{max} (nm) / Intensity of absorbance of the initial solutions*

Solution name	***λ_{max} (nm) / Intensity of absorbance***		
Gastric juice - pH2	198.0/2.2377	-	-
Gastrointestinal juice - pH2.5	194.0/1.8532	-	-
Vancomycin	-	200.0/0.7599	320.0/0.0653
Metronidazole	196.0/1.2630	230.0/0.6087	321.0/1.6505

Table 2. *λ_{max} (nm) / Intensity of absorbance of solutions at 0, 30 and 60 minutes*

Solution name	***λ_{max} (nm) / Intensity of absorbance***					
	Time 0'		***Time 30'***		***Time 60'***	
Vancomycin solution mixed with gastric juice pH2	192.0 /1.4308	-	192.0 /1.2242	-	192.0 /1.0318	-
Metronidazole solution mixed with gastric juice pH2	192.0 /1.4321	320.0 /0.8281	192.0 /1.5989	320.0 /1.0841	192.0 /1.0706	320.0 /0.9766
Solution of vancomycin and metronidazole mixed with gastric juice pH2	192.0 /1.6474	320.0 /0.8160	192.0 /1.6345	320 /1.1512	192.0 /1.1879	320.0 /1.0791
Vancomycin solution mixed with gastrointestinal juice pH2.5	192.0 /1.4328	-	192.0 /1.4296	-	192.0 /1.1377	-
Metronidazole solution mixed with gastrointestinal juice pH2.5	192 /1.6400	320 /0.9104	193.0 /1.4903	320.0 /0.8585	192.0 /0.9454	320.0 /0.8007
Solution of vancomycin and metronidazole mixed with gastrointestinal juice pH2.5	193.0 /2.0166	320.0 /0.9896	193.0 /1.5125	320.0 /0.9097	200 /1.0392	320.0 /0.8292

No absorption bands were observed within the visible range.

No significant changes or changes in the frequency of absorption bands of the investigated drugs can be observed after mixing with the simulated gastrointestinal fluid.

A hypsochromic effect [303] and a decrease in the intensity of absorption was observed when the drugs were mixed with simulated gastrointestinal solutions, possibly due to the protonation of the nucleophile groups of the drugs under acidic conditions.

2.3.5. Discussion

The only antibiotics used in the Galaţi Infectious Diseases Clinical Hospital for the treatment of CDI are metronidazole and vancomycin, due to the high cost of treatment with fidaxomycin, not allowed due toa rank 5 hospital budget constraints, although the clinical studies offer evidence of a lower relapse rate of this product.

Oral metronidazole therapy is only reserved for cases of mildor medium severity that have no associated renal or neurological pathology, limited to a single cure of up to 14 days, due to the inferior efficacy evidence of vancomycin as shown in clinical trials and the adverse effects of the antibiotic, potentially cumulative and irreversible.

The first-line therapy, according to the latest therapy guide and the meta-analysis of clinical trials of comparative therapeutic efficacy, is with oral vancomycin which has shown superior efficacy to metronidazole and much less adverse effects being a drug with no reabsorption profile.

In Romania, oral vancomycin capsules are not used as they are not available, instead vancomycin powder from the intravenous vials is used, dissolved and administered by mouth. The active substance in vancomycin capsules and intravenous powder is the same vancomycin hydrochloride. The chemical, physical and microbiological stability of vancomycin hydrochloride in physiological serum, 24 hours, at room temperature, in the original vial, has been demonstrated by the literature studies mentioned above.

The study demonstrates the chemical stability of vancomycin after contact with artificial gastric and gastrointestinal juices, 60 minutes.

This evidence supports the therapeutic efficacy of the oral dose of vancomycin administered to patients with CDI and the possibility of clinicians to use intravenous vancomycin in an indication other than that of the manufacturer, in safe conditionswith the same effective dose and the same adverse effects after dissolution in gastrointestinal juice.

2.3.6. Conclusions

In conclusion, based on UV-Vis absorption spectra, it can be considered that there are no major changes in the chemical structure of the investigated drugs in the presence of gastro-duodenal juices, which could affect their effectiveness in treatment. This evidence is extremely important in the current clinical context in which vancomycin hydrochloride produced for intravenous use, is used orally in clinical practice.

2.4. Study 3 - Epidemiology of Clostridium difficile Infection in South-Eastern Romania

2.4.1. Working hypothesis

Following the outbreak of epidemics in Europe, the United States (USA) and Canada since 2011, infection with Clostridium difficile is considered a public health issue.

The incidence of CDI in Romania has been steadily increasing since 2011. The CDI surveillance system was implemented at national level in March 2014, the target population being represented by all patients admitted to the state or private healthcare units in the country. In Romania, in 2018, 10241 confirmed cases entered the CDI surveillance system, with 2% more cases than in 2017 (10080 cases).

Clostridium difficile infection in Galați County is an important public health problem, the number of cases per 100 discharges being in the first 5 at national level, between 0.44 and 0.66, maximum value at national level, in 2018 [79].

2.4.2. Objectives

- Analysis of the number of cases, the circulating ribotypes, the costs and the share of infections associated with healthcare, at global, European, national and regional level.
- Comparison of the number of cases in the host hospital of the clinical study with those of the other hospitals in the region, which report cases of CDI.
- Identification of hospitals and wards as source of infection of the patients in the study, the percentage of community cases and of unspecified source in order to identify appropriate prophylactic measures and to define the spread of the infection in the community.

2.4.3. Results

Table 3. *CDI cases from the main hospitals in the South-Eastern region of Romania during the period 1.01.2017 ÷ 31.12.2018*

Hospital	***2017 - No. total CDI cases***	***2017 - No. CDI cases associated with healthcare***	***2018 - No. total CDI cases***	***2018 - No. CDI cases associated with healthcare***
Emergency County Clinical Hospital "Sf. Andrei Ap" CT	148	118	104	92
Pneumophysiology Clinical Hospital CT	15	15	25	25
Clinical Hospital for Infectious Diseases CT	85	67	122	104
The County Emergency Hospital "Sf. Pantelimon" Focșani - VN	194	174	165	131
Emergency County Hospital BR	138	85	203	147
Emergency County Hospital BZ	194	72	170	39
Emergency County Hospital TL	81	45	99	70
Emergency County Hospital "Sf. Ap. Andrei" GL	386	370	348	327
Pneumophysiology Hospital GL	64	61	76	74
Emergency Clinical Hospital for Children "Sf. Ioan" GL	0	0	1	1
Clinical Hospital for Infectious Diseases GL	375	299	345	277

Table 4. *Total number of CDI cases / number of beds / percentage of IAAM / prevalence per 1000 inhabitants in the counties in the South-Eastern region of Romania [304]*

County	***Number of CDI cases 2017 and 2018***	***Number of beds***	***Number (%) CDI cases associated with healthcare***	***Number of inhabitants 1.01.2016 / Incidence CDI at 1000 people***
Galați	1595	2838	1109 (69,52%)	631669/1.75
Constanța	509	4418	421 (82,71%)	769768/0.54
Buzău	364	1863	111 (30,49%)	478811/0.23
Vrancea	359	1258	305 (84,95%)	391169/0.77
Brăila	341	1777	232 (68,03%)	356196/0.65
Tulcea	180	790	115 (63,88%)	244249/0.47

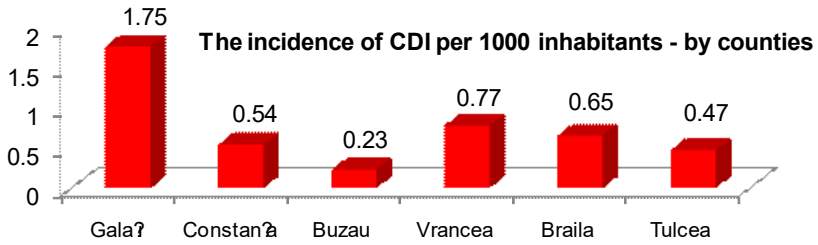

Fig. 12. *Total number of cases CDI / No. IAAM - by counties*

The incidence of CDI in the US epidemic in 2010-2011 was 147.2 cases per 100,000 population, decreasing to 130.28 cases per 100,000 population in 2017.

In the South-Eastern region of Romania in 2017-2018, the incidence of CDI was 116.57 cases per 100,000 inhabitants (calculated from the population reported on 1.01.2016), with a maximum in Galați County, of 175 cases per 100,000 inhabitants and a minimum of 23 cases per 100,000 inhabitants in Buzau County. In 2018, at national level, Galati County was in the top 5 counties with the highest number of CDI cases per 100 discharges.

The percentage of community infections from total CDI in the USA in 2010-2011 was between 6-32%, increasing until 2017, when it was 51.39%.

In the EU, in 2016, the percentage of community infections from total CDI was 25.4%, in Canada, between 2005 and 2006, it was 27%, and in Australia, between 2010 and 2012, it was 26%.

In Romania, in 2018, the percentage of community infections out of the total CDI was 19%.

In the Southeast region, between 2017 and 2018, the percentage of community infections from the total CDI was 31.52% with a maximum in Buzău county of 69.51% and a minimum in Vrancea county of 15.05%. In Galați County, between 2017 and 2018, the percentage of community infections from the total CDI was 30.48%, and at the Clinical Hospital for Infectious Diseases Galați was 20%. The average cost of care for a hospitalized patient with CDI is: 7000-7500 euros in the EU (with data from Italy 2009-2012, Germany 2010, England 2010), 3427-9960 US dollarsin the US (with data from 2010-2011) and 4434.45 lei in the Clinical Hospital of Infectious Diseases Galaţi (with data from 1.07.2018-31.12.2018).

2.4.4. Conclusions

The incidence of CDI in the Southeast region is lower than that recorded in the 2010-2011 epidemic in the USA, but the incidence in Galati County exceeds this figure. At the global level, the total number of CDI is decreasing, in Romania it is still increasing, at the Clinical Hospital of Infectious Diseases Galaţi it was recordeda decrease in the number of cases for the first time in 2018. The trend of the proportion of community infections is increasing worldwide.

There is a percentage of community infections in Galați County, comparable to the one at regional level, higher than the national and European ones, which draws attention to the spread of the infection in the community and the need to intensify the prophylaxis measures of CDI in primary care medicine.

The cost of treating a hospitalized patient with CDI is high, even though in the Galaţi Infectious Diseases Clinical Hospital, it is lower than in the USA and Western Europe, it is much higher than the budget granted by the County Health Insurance House for this pathology, representing a difficult burden to balance for this hospital.

The main source of CDI remains associated with healthcare, associated with the intensive and / or inadequate use of antibiotics, but the spread of infection is also found in the community.

Increase in the number of community diseases cases can be correlated with easy access to the use of antibiotics and proton pump inhibitors, even without a prescription.

Prolonged hospitalization, antibiotic errors and inadequate hygiene are associated with the onset of CDI associated with healthcare. The promotion of CDI transmission prevention rules and "antibiotic stewardship" strategies is the key to limiting CDI.

2.5. Study 4 - Clinical and epemiological study of Clostridium difficile Infection in a group of 706 patients admitted to the Clinical Hospital of Infectious Diseases "Sf. Cuv. Parascheva "Galați from 1.01.2017 to 31.12.2018

The prospective, observational, actively controlled, non-randomized study was performed on a sample of 706 patients with Clostridium Difficile Infection (CDI) admitted to the Clinical Hospital of Infectious Diseases "Sf. Cuv. Parascheva "Galați, between 1.01.2017 and 31.12.2018 with the diagnosis of Clostridium difficile Infection, representative sample for a population of patients with CDI who are treated in a hospital unit specialized in infectious diseases.

The information was extracted from the study sheets of each patient, the data during the hospitalization period being recorded in the patient clinic notes.

2.5.1. Objectives

- Clinical-biological characterization of the studied groupsand their comparative statistical analysis, identification of demographic, clinical and laboratoryfactors that influence the risk of death, recurrence and duration of treatment in CDI.
- Quantifying the response to the treatment and the prognosis of the studied groups.
- Developinga protocol for the prophylaxis, diagnosis and treatment of CDI for the Galaţi Infectious Diseases Hospital, a course for nurses, scientific seminarsfor doctors and an educational brochure forpatients.

2.5.1.1. The stages of the study

1. Screening period

Patients admitted to the Clinical Hospital of Infectious Diseases „Sf. Cuv. Parascheva "Galaţi between 1.01.2017 ÷ 31.12.2018 with the suspicion of diagnosis of Clostridium difficile Infection received the informed consent form of the study, they were read and explained this consent by the doctor and were allowed 30 minutes to think about whether they want to participate in the study.

2. Treatment period

Patients who decided to participate in the study and signed the consent were evaluated for inclusion and exclusion criteria, and the protocol for investigations, treatment and monitoring was applied. This period includes the hospitalization period, the patient being evaluated daily and the days of treatment received at home decided by the attending physician.

3. Monitoring period

Patients were evaluated by telephone or in hospital for one month, 2 months and 6 months after discharge.

2.5.1.2. Criteria for inclusion in the study

- adults with soft stools, over 3 in the last 24 hours, with a consistency of 6-7 on the Bristol scale and toxins of Clostridium difficile A or B or A and B positive;
- patients who provided written informed consent for participation in the study, personally and fully competent (informed consent model).

2.5.1.3. Exclusion criteria from the study

- unconscious patients or unable to sign informed consent;
- patients who refused to participate in the study;
- patients who, although admitted with suspected CDI, did not meet the clinical and laboratory diagnostic criteria;
- pregnant or breastfeeding women.
- patients under 18 years.
- patients who had extreme values ("outliers") in the usual laboratory tests (leukocytes, hemoglobin, serum albumin, ionogram, serum creatinine).

2.5.1.4. Study sublots

- 706 patients who have experienced one or more episodes of illness in the "Sf. Cuv. Parascheva" Infectious Diseases Clinical Hospital Galați between 1.01.2017 ÷ 31.12.2018;

- Patients were divided into 2 groups: group A (N = 553) consisted of patients with healthcare-associated infection, while group B (N = 153) consisted of patients with infection of indeterminate and community source. It is worth mentioning that not all patients have complete data available.

2.5.1.5. Endpoints of the study

The endpoints of the study were: number of deaths, number of relapses / re-infections, number of days of therapy (regardless of the form of therapy, oral with metronidazole or oral with vancomycin or combined, intravenous metronidazole and oral vancomycin associated or not with vancomycin enemas).

2.5.1.6. Statistical analysis of demographic data

For statistical analysis we used R program, version 3.5.3 (c) R Core Team (2019), R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, URL https: // www. R-project.org/ [310].

Statistical analysis of the demographic data was performed on the group of patients with CDI associated with healthcare (group A) compared to the one with CDI of community and indeterminate origin (group B). The presentation is in Mean ± D.S (standard deviation) for continuous variables and in absolute frequency (relative frequency) for categorical variables, the test used in the inferential analysis being specified for each variable, table 5.

Table 5. *Comparative statistical analysis of demographic data and comorbidities*

Variable		***Lot A (IAAM)***	***Lot B (IC)***	***Value p***
Age - Mean ± D.S		68.02 ± 14.36	59.78 ± 17.15	< 0.0001 [1]
BMI - Mean ± D.S		25.35 ± 5.04	25.92 ± 5.59	0.2895 [1]
Sex	**M – No (%)** **F – No (%)**	261 (47.20) 292 (52.80)	53 (34.64) 100 (65.36)	0.0057 [2] -
Smokers	**Yes – No (%)** **No – No (%)**	71 (13.79) 444 (86.21)	18 (12.41) 127 (87.59)	0.6690 [2] -
Environment	**Urban – No (%)** **Rural – No (%)**	361 (65.28) 192 (34.72)	115 (75.16) 38 (24.84)	0.0210 [2] -
IMA	**Yes – No (%)** **No – No (%)**	29 (5.24) 524 (94.76)	4 (2.61) 149 (97.39)	0.2003 [3] -
ICC	**Yes – No (%)** **No – No (%)**	152 (27.49) 401 (72.51)	25 (16.34) 128 (83.66)	0.0049 [2] -
Basque disease Peripherals	**Yes – No (%)** **No – No (%)**	41 (7.41) 512 (92.59)	3 (1.96) 150 (98.04)	0.0128 [3] -
Stroke	**Yes – No (%)** **No – No (%)**	76 (13.74) 477 (86.26)	7 (4.58) 146 (95.42)	0.0018 [2] -
Dementia	**Yes – No (%)** **No – No (%)**	51 (9.24) 501 (90.76)	6 (3.92) 147 (96.08)	0.0328 [2] -
COPD	**Yes – No (%)** **No – No (%)**	73 (13.20) 480 (86.80)	3 (1.96) 150 (98.04)	< 0.0001 [3] -
Autoimmune Diseases	**Yes – No (%)** **No – No (%)**	8 (1.45) 543 (98.55)	1 (0.65) 152 (99.35)	0.6921 [3]
Ulcers	**Yes – No (%)** **No – No (%)**	31 (5.62) 121 (94.38)	6 (3.92) 147 (96.08)	0.4056 [2] -
Hepatitis	**Yes – No (%)** **No – No (%)**	86 (15.64) 464 (84.36)	21 (13.73) 132 (86.27)	0.5606 [2] -
DZ	**Yes – No (%)** **No – No (%)**	104 (18.84) 448 (81.16)	19 (12.42) 134 (87.58)	0.0640 [2] -

IRC	**Yes – No (%)** **No – No (%)**	87 (15.73) 466 (84.27)	7 (4.58) 146 (95.42)	0.0003 [2] -
Neoplasms	**Yes – No (%)** **No – No (%)**	126 (22.78) 427 (77.22)	14 (9.15) 139 (90.85)	0.0002 [2] -
Hemiplegia	**Yes – No (%)** **No – No (%)**	53 (9.60) 499 (90.40)	6 (3.92) 147 (96.08)	0.0248 [2] -
Charlson - Mean ± D.S		4.32 ± 2.56	2.71 ± 2.36	< 0.0001 [1]
Abdominal Surgery	**Yes – No (%)** **No – No (%)**	60 (10.85) 493 (89.15)	2 (1.31) 151 (98.69)	< 0.0001 [3] -

[1] Welch bidirectional test; [2] χ^2 bidirectional test; [3] Fisher exact two-way test

2.5.1.7. Demographic characteristics and comorbidity profile of the groups

Of the 706 infections with Clostridium difficile treated in the Clinical Hospital of Infectious Diseases "Sf. Cuv. Parascheva"Galați between 1.01.2017-31.12.2018, 553 were infections associated with healthcare. The percentage of IAAM in the studied group was 78.32%, higher than the figure reported at national level, by CNSCBT, in 2018, 76%.

The average age in the group with infections associated with healthcare (68.02 years) overlaps with the median age reported by CNSCBT, in 2018, namely 68 years. Patients with community infections are younger (mean age = 59.78 years). The worldwide trend is to decrease the number of cases associated with health care and the increase of the community ones and so, in time, we could witness a decrease of the average age in this pathology, now known as pathology of the immunocompromised elderly and with multiple comorbidities.

In both groups the female sex predominated, statistically significantly more women in the group with community infections, which may mean that the female sex will remain more frequent in this pathology, perhaps due to the higher life expectancy in women but perhaps due to particularities of microflora of the colon that will be defined in the future and which could determine their higher susceptibility to CDI. The origin environment in both batches is predominantly urban and this opens up future research perspectives regarding certain eating habits that modify the microbiome and could predispose to CDI.

Both groups had BMI (body mass index) in the overweight area, which could be due to this predominance in the studied population, the Clostridium difficile infection being an acute disease that does not cause significant changes in body weight.

Smoking, too, was not a factor that differentiated the two studied batches, with non-smokers predominating in both batches, and this is probably a general feature of the batch studied, not related to susceptibility to CDI.

Patients with CDI have multiple comorbidities. In our study, chronic heart and kidney failure, chronic obstructive bronchopneumonia, central or peripheral vascular disease, dementia and neoplasm have a statistically significantly higher weight in the group with healthcare associated infections, being chronic debilitating diseases, with potentially immunosuppressive potential. CDI, which for monitoring and treatment requires multiple contacts with the medical system and hence their more frequent association with IAAM type CDI. The accumulation of comorbidities, brought together by the CHARLSON score, is higher in the group with IAAM. This observation is consistent with the data from literature, and worrisome giventhat CDI extends into the community and affects younger patients with fewer comorbidities which clearly opens up research perspectives in the area of susceptibility to infection. Our study shows that the main risk factor for CDI is antibiotic therapy.

The analysis of the comorbidity profile of the studied group revealed:

- statistically significantly higher percentage of patients with ICC who have experienced episodes of CDI, in the group with IAAM and the literature studies emphasize that patients with ICC who develop an episode of CDI have higher mortality than those with only ICC, what draws attention to increased prophylactic measures in the sections in which patients with ICC are treated [314].

- small percentages of patients who associate peripheral vascular disease, CD infection, but statistically significantly more frequent in the group with IAAM, and studies in the literature emphasize that patients who underwent cardiovascular surgery and an episode of CDI required a medium length greater mechanical ventilation, intensive care and hospitalization compared to those without CDI, which explains the need for increased measures of CDI prophylaxis in the vascular surgery sections [315].

- the association of stroke and hemiplegia, statistically significantly higher in the group with IAAM, and clinical studies suggest that stroke is an important risk factor for CDI recurrence [316], which suggests the need for increased measures of CDI prevention in the neurology sections.

- diabetes had similar significance in the group with IAAM and in IC, possibly due to the heavy differentiation of cases with nosocomial contact, taking into account only continuous admissions, but diabetic patients are frequently present in the specialized ambulatory and day hospitalization for the treatment and monitoring of the basic disease, as well as its complications and associated pathologies. Diabetes remains a cause of immunosuppression and thus a risk factor for CDI.

- the psychiatric pathology is not usually accompanied by CDI, but it was noted, however, the associated dementia in the patients from this study group, with a statistically significant percentage higher in the group with AMI. The difficulty with which these patients perform their hygiene and the repeated nosocomial contact, for the treatment of the underlying and associated diseases can explain this association. Clinical studies have observed a higher incidence of CDI in elderly Americans with depression than in those without depression, with the risk of developing CDI being higher in patients using antidepressant medication, in older adults, and in those living alone [317].

- a statistically significant percentage of the association of COPD-CDI in the group with IAAM compared to the group with IC, and the clinical studies emphasize the higher mortality of hospitalized patients with COPD and CDI compared to those without COPD, which draws attention to the hygiene measures in the pneumology sections, but also on the chronic treatment of this condition, which must be monitored as closely as possible in order to prevent acute exacerbations and decompensations and thus avoid nosocomial contact and antibiotic therapy [318].

- a statistically significant percentage of the association of chronic renal failure with CDI in the group with IAAM compared with the group with IC and the clinical studies emphasize the increased risk of death in patients with CKD who develop an episode of CDI compared to those with CKD only, and thus the need for increased prophylactic measures in dialysis and nephrology sections and the beneficial role of prophylaxis with Lactobacillus plantarum 299v [320].

- the presence of neoplasms in the diseases associated with CDI was statistically significantly higher in the group with IAAM, and the studies in the literature observe a mortality during the hospitalization significantly increased in the patients who have associated CDI compared to those only with cancer [321], which attracts the attention on CDI prophylaxis measures to be taken in the wardsthat care for patients with cancers.

- the Charlson score that cumulates the comorbidities and estimates the survival expectancy based on 10 years, was statistically significantly higher in the group with infections associated with healthcare compared to the group with community infections and a study published in 2017 considers the Charlson score as an independent predictor of the evolution CDI and notes the existence of a higher Charlson score in patients who died during hospitalization - 7.37 compared to 4.4 in those who were discharged healed [322].

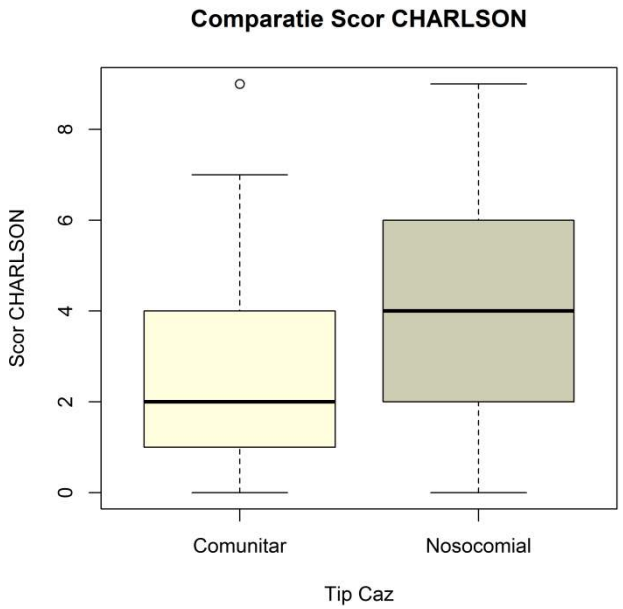

Fig. 13. *Analysis of comorbidities in the Charlson score as a whole in the groupwith community infections compared to the group with infections associated with healthcare*

2.5.1.8. Risk factors incriminated in triggering the episode of infection with Clostridium difficile

Clinical studies in the literature emphasize that the risk factors involved in triggering the episode of CDI are: antibiotic therapy, age over 65, contact with medical care units, cancer chemotherapy, gastrointestinal surgery, nasogastric tube feeding, upper or lower digestive endoscopy, comorbidity of the patient (chronic or disabling diseases with immunodepression) [323], [324], [325], [135].

- Antibiotic therapy is the most important modifiable risk factor for CDI.

The presence of antibiotic therapy in the last 12 weeks before the onset of CDI was identified in 71.42% of patients with IAAM and in 45.75% of patients with IC, as opposed to studies in the literature that initially stated that about 5-10% of patients with CDI have not been exposed to antibiotics [313].

The antibiotic classes considered high risk for CDI are: clindamycin, fluoroquinolones, cephalosporins, aztreonam and carbapenems and those considered moderate risk for CDI are: macrolides, sulfonamides/trimethoprim and penicillins [326]. In this study, there is a higher percentage of high risk antibiotic classes for CDI in the group with IAAM (cephalosporins, fluoroquinolones, carbapenems, clindamycin) than in IC. The biggest difference is in the consumption of cephalosporins, namely 28.21% in the group with IAAM compared to 16.34% in the group with IC. It is noted, however, the use of all these high-risk antibiotic classes and in cases with community source, therefore treated in primary or ambulatory medicine, which may be one of the factors responsible for the spread of infection in the community and which requires the revision of antibiotic guidelines in primary medicine.

- Chemotherapy for cancer is considered a risk factor for CDI, its presence in our study groups being observed in very few patients. In the clinical studies in the literature, CDI was considered the most common bacterial cause of diarrhea associated with healthcare in patients receiving chemotherapy for cancer in 2011 in the USA. The highest rate of CDI was described in patients who received hematopoietic stem cell transplant (allogeneic - 27%, autologous - 9%) and chemotherapy for leukemia (12.4%) and the lowest for patients with solid tumors (1.4%) [319].

- Abdominal surgery in the last 12 weeks is low in both study groups. However, the presence of recent abdominal surgery in the patient history is statistically significantly higher in the group with IAAM.

In the literature studies, the incidence of CDI in patients undergoing gastrointestinal surgery varies from 0.28% to 6.8% [315], [316], [317], [318], [319], [320], [341], [344].

Of all the abdominal surgical procedures, colectomy, small bowel resection and gastric resection have been associated with the highest risk of CDI [317], [320]. The highest percentage of CDI was observed after colorectal cancer surgery [319]. It has been suggested that functional intestinal obstruction predisposes to CD infection by altering the normal bacterial flora of the intestine [315], [319]. On the other hand, the microflora breach and the immune imbalance created by operative trauma may predispose CDI [344].

Surgical patients with CDI have a 3.4-fold increase in mortality compared with patients without CDI [316], [317], [321]. Also, patients with CDI are more likely to have postoperative complications, require ATI care, surgical reintervention, or re-hospitalization within 30 days of initial discharge in larger proportions than those without CDI [316].

- Upper digestive endoscopy or colonoscopy in the last 12 weeks have low significancein both study groups. Regarding the consideration of endoscopy as a risk factor for CDI, a clinical study of287 pairs of case-control patients, with mean age 78 years, 65% women, observed that the absolute risk of developing CDI after endoscopy is very low, 0.48% in the first 90 days after endoscopy [294].

- Age over 65 years was identified as a risk factor in the group with CDI associated with healthcare. Patients with IC were younger, they were 59.78 years old.

- Comorbidities were an important risk factor in the group with IAAM, in which the Charlson score with values over 4 has a higher weight than in the group with IC, the patients with IAAM presenting a greater comorbidity than those with IC.

- Contact with medical units can be considered an important risk factor considering that the percentage of IAAM in the studied group was 78.32%, higher than the nationally reported figure, by CNSCBT, in 2018, 76%.

- In the studied groups there were no patients fed through a nasogastric tube due to the fact that these patients often require intensive care and the study was performed in a rank 5 hospital that does not provide such type of care. Patients who needed this type of care were transferred to the Galati County Emergency Clinical Hospital and left the study due to the lack of adequate monitoring, but they were very rare. A meta-analysis of eight observational clinical studies concludes, in 2018, that there is a statistically significant association between the insertion of a nasogastric tube and the risk of poor outcomes of CDI evolution (the combined risk rate of severe or complicated clinical outcomes of CDI in patients with nasogastric tube insertion was of 1.81 (95% CI 1.17 to 2.81). This finding requires the avoidance of unnecessary use of the nasogastric tube and would improve the results of the therapy of CDI [295].

2.5.1.9. Risk factors for mortality from infection with Clostridium difficile

Studies from the literature identify the following risk factors for mortality from CDI: age, comorbidities, hypoalbuminemia, leukocytosis, acute renal failure, and ribotype 027 infection [329]. In ourown study with impact on the death rate, the following univariate binary logistic regression were identified using the following risk factors for death: age over 80 years, the value of the Charlson score, the cumulative comorbidity of the patient, leukocytes over 16000, albumin, creatinine , serum sodium and potassium, the presence of ascites and the value of the ATLAS score (the severity of the disease episode) and with the help of simple multivariate binary logistic regression the following risk factors for CDI death: serum albumin and the value of the ATLAS score (the severity of the disease episode).

2.5.1.10. Clinical parameters

It was observed that the presence of fever and abdominal pain had similar significancein the two groups, fever being a rare symptom, as opposed to abdominal pain, which is almost present, most commonly in the form of colickypain in the colic or diffuse abdominal area.

The average number of stools in the last 24 hours before the start of therapy and the average number of days from the onset of the disease to the start of therapy are higher in the group with community infections, which draws attention to the neglect of symptoms and the delayed presence at the hospital for investigations and treatment.

The presence of ascites was observed in a small number of patients in the two groups, mainly in the group with IAAM, 12.12% of patients with IAAM and 1.96% of patients with IC. We mention that these figures include both ascites that appeared as a complication of comorbidities, before or during CDI and ascites that appeared in a megacolon context as a complication of CDI.

The mean heart rate in patients with IAAM was 89.93 beats per minute and 87.73 beats per minute in IC patients. Both values are slightly higher than normal values, expressing the dehydration status of these patients at the beginning of therapy.

The mean value of blood pressure was lower in the group with IAAM, 126.66 mmHg compared to that in the IC group where it was 130.62 mmHg, both below the normal value of the average age of the groups.

2.5.1.11. Laboratory parameters

It is observed that all laboratory parameters have higher significance, on the levels of high severity, in the group with IAAM. Thus, it can be stated that patients with community-source infections developed episodes of disease with a lower degree of severity than those with IAAM.

- The highest percentages are observed in all groups of total serum leukocytes below 16000, consistent with the clinical observation that CDI is a bacterial infection that does not accompany large leukocytosis either due to the immunocompromised organisms it appears, or because of its infection specificity, so the occurrence of major leukocytosis in a patient with CDI is a definite criterion for severity of the disease episode.
- Serum albumin has values between 2.5 and 3.5 predominantly in the group with IAAM and in the total group and normal values predominantly in the group with IC. Loss of protein through undigested stools and consequently decreased serum albumin is considered a marker of severity of the clinical episode of CDI or of late presentation to the physician or the association of comorbidities with hypoalbuminemia (mainly liver cirrhosis, nephrotic syndrome and cancers).
- Anemia accompanies episodes of CDI in both groups, with hemoglobin values below 12 being observed in 51.71% of patients with IAAM and in 26.79% of patients with IC.
- Dyselectrolytemias are present in both groups, with the predominance of hypopotassemia in the group with IAAM (30.5%).
- Serum creatinine values above 1.5 mg / dl, considered disease severity factor, were observed in 19.8% of patients with IAAM and 13.28% of patients with IC.

2.5.1.12. ATLAS score

The ATLAS score is a combination of clinical and laboratory parameters, proven in clinical studies to have the highest relevance in assessing the severity of the disease episode.

The mean value of the ATLAS score was higher in the group with IAAM, and in this group, higher percentages of higher severity scores were observed, which allows us to state that the patients in the group with IAAM have developed episodes of illness with greater severity than patients with IC.

2.5.1.13. Prognostic parameters

The evolution of the patients was assessed with the following parameters: the number of days from the beginning of the treatment to the normalization of the stool (1-2 stools/day, consistency 1-4 on the Bristol scale, maintained 48 hours), the number of days of antibiotic treatment (in hospital and home), number of days of hospitalization, number of relapses (new episode of illness occurring in the first 8 weeks from the clinical resolution of the episode of CDI studied), the number of re-infections (new episode of disease occurring in weeks 9-24 from the clinical resolution of the episode of CDI studied), the number of deaths occurring within the first 30 days after discharge (considered to reflect the impact of IDC on death rate) and the number of deaths that occurred in the first 6 months after discharge (considered to reflect the impact of comorbidities and IDC on death rate).

The number of days from the beginning of treatment to the normalization of the stool, the number of days of antibiotic treatment and the number of days of hospitalization were comparable in the two study groups, with a median of 4 days until the normalization of the stool, of 10 days of antibiotic treatment and 7-8 days hospitalization.

The number of days of hospitalization higher than the average of the hospital in which these patients were treated was observed in 69.55% of the patients with CDI, with a higher significance in the group with IAAM compared with the group with IC.

The number of relapses and the number of deaths was higher in the group with IAAM. Both the more severe comorbidities and the higher severity of the episodes of CDI led to a more severe prognosis of the group with IAAM.

In clinical trials, up to 25% of patients experience recurrent IDC within the first 30 days after treatment completion [351]. Less frequently, recurrent CDI may occur up to two months after discontinuation of treatment. Once patients have relapsed, they are at significantly increased risk for subsequent relapses. Risk factors for recurrence include age>65 years, severe underlying medical conditions, the need for continuous treatment with concomitant antibiotics during treatment for CDI, and the lack of an antibody-mediated immune response to toxin B [189], [353], [354], [355], [356]. Symptoms of recurrence may be due to recurrence of the initial infectious strain or reinfection with a new strain [357], [358], [359]. Recurrent CDI is often a relapse rather than a reinfection, regardless of the interval between episodes. Among 134 paired isolates from 102 patients with recurrent C. difficile infections, isolates obtained between 2 and 8 weeks were identical in 88% of cases; isolates obtained between 8 weeks and 11 months were identical in 65% of cases [360].

2.5.1.14. Impact of demographic-clinical factors on the rate of post-infection death with Clostridium difficile

Due to the fact that there were non-homogeneous variables between the 2 groups (there were differences with statistical significance between the 2 groups) these differences were "mitigated", using a propensity score, to analyze the impact on the death rate, the duration of treatment and the recurrence rate. For the calculation of the propensity score, the variables were taken into consideration which in the tests revealed differences with statistical significance, apart from the Charlson score, because this depends on all compared variables and not on all comparisons tests the differences were statistical significant. With the help of the propensity score, it was attained that the statistically significant differences existing before the attenuation are "transformed" in their vast majority into differences without statistical significance.

The death rate in the first 6 months after the episode of CDI was in the total group of 18.13% (128 of 706 patients), in group A of 20.43% (113 of 553 patients), and in group B of 9.80% (15 of 153 patients).

The impact of demographic-clinical factors on the risk of post-infection death with Clostridium was analyzed with a simple binomial logistic regression (a single predictor included in the model), which suggested that the risk of death in patients with nosocomial type infection is close to 2.5. times higher than in patients with community infection, the effect being statistically significant ($p < 0.01$), but this result is influenced by the non-homogeneities regarding the comorbidities between the 2 categories of patients, the analysis attenuated by the propensity score shows that the type of the infection it does not seem to influence the risk of death ($p > 0.05$), comorbidities seem to play a more important role than the type of infection.

Table 6. *Analysis of the influence of the nosocomial origin of the cases on the risk of death, after subtraction using the propensity score*

Variable	***Coefficient***	***Value p***	***OR [IC95%]***
Community Case (Attenuated)	REFERENCE	-	-
nosocomial	0.34	0.2340	1.40 [0.80 la 2.46]

The role of comorbidities in death risk was analyzed by simple univariate binary logistic regression with independent variable Charlson score and it was observed that a 1-unit increase in Charlson score is associated with a 1.26-fold increase in death risk at 30 days from episode of CDI (effect statistical significant, p <0.01), whereas a 1-unit increase in Charlson score is associated with a 1.29-fold increase in riskdeath at 6 months from the episode of CDI (effect also statistical significant, p <0.001).

Table 7. *Analysis of the influence of the Charlson score on the risk of death (OR = odds ratio univariate analysis)*

Variable	***Value p***	***OR [IC95%]***
CHARLSON score / death 30 days	< 0.0001	1.26 [1.15 la 1.40]
CHARLSON score / death 6 months	< 0.0001	1.29 [1.19 la 1.40]

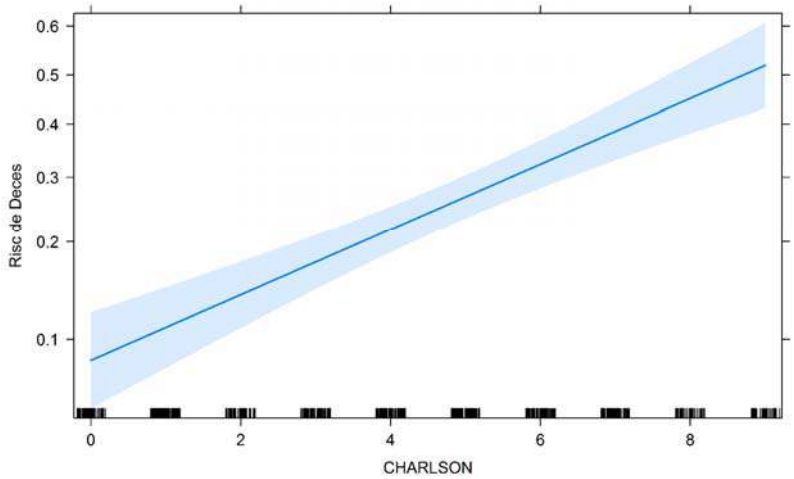

Fig. 14. *Diagnostic graph of the score calculation algorithm of propensity, the transformation of the significant statistical differences*

This analysis highlights the important role of comorbidities in the risk of post-CDI death, which suggests that Clostridium difficile infection is only a potential aggravating or precipitating factor, the primary cause of death being the underlying disease. The onset of Clostridium difficile infection can be considered a predictive factor of death in these patients with multiple comorbidities.

Knowing that in the patients in this study comorbidities play a major role in the risk of death, another analysis considered the Charlson score for each independent variable, the model being that of multiple univariate binary logistic regression (2 independent variables in analysis) and revealed the fact that the following parameters influence the risk of death: the number of serum leukocytes, the albuminemia, anemia, the serum levels of Na, K and Cl, the value of the Atlas score and the presence of ascites at the beginning of the treatment.

Table 8. *Influence of clinical and laboratoryparameters on the risk of death*

Variable	***Coefficient***	***Value p***	***OR [IC95%]***
No. days from onset to starting therapy	0.007	0.4570	1.00 [0.98 la 1.02]
AV	0.006	0.2340	1.00 [0.99 la 1.01]
Leukocyte count (thousands)	0.06	< 0.0001	1.06 [1.03 la 1.09]
Albumin	-1.66	< 0.0001	0.18 [0.12 la 0.28]
Hb	-0.20	< 0.0001	0.81 [0.74 la 0.89]
Na	-0.04	0.0130	0.95 [0.91 la 0.98]

K	-0.32	0.0318	0.72 [0.53 la 0.96]
Cl	0.01	0.9450	1.00 [0.96 la 1.03]
Creatinine	0.28	0.006	1.32 [1.08 la 1.63]
ATLAS	0.40	< 0.0001	1.49 [1.32 la 1.69]
Ascita **Yes**	REFERENCE	-	-
No	-1.44	< 0.0001	0.23 [0.13 la 0.39]
Chemotherapy **Yes**	REFERENCE	-	-
No	-0.91	0.0793	0.40 [0.14 la 1.12]
Toxin A **Negative**	REFERENCE	-	-
Positive	0.01	0.9600	1.01 [0.61 la 1.69]
Toxin B **Negative**	REFERENCE	-	-
Positive	0.29	0.1990	1.33 [0.86 la 2.11]
GDH **Negative**	REFERENCE	-	-
Positive	-0.18	0.3320	0.83 [0.57 la 1.21]

Another analysis in which multiple univariate binary logistic regression was used using, in addition to the Charlson comorbidity score (kept in the permanent model), the independent variables for which the value was not statistically significant ($p<0.05$), the final model being constructed based on a retrograde selection algorithm ("backward selection") and it was observed that serum albumin and the presence of ascites have a statistically significant influence on the death rate.

Table 9. *Influence of serum albumin and creatinine, presence of ascites and the Charlson score on the risk of death*

Variable	***Coefficient***	***Value p***	***OR [IC95%]***
Albumin	-1.50	< 0.0001	0.22 [0.14 la 0.33]
Creatinine	0.21	0.0658	1.24 [0.98 la 1.57]
Ascita **Yes**	REFERENCE	-	-
No	-0.92	0.0080	0.39 [0.19 la 0.78]
Charlson Score	0.12	0.0136	1.12 [1.02 la 1.24]

2.5.1.15. Impact of demographic-clinical factors on the number of days of treatment of Clostridium difficile infection

The analysis of the impact of the demographic-clinical parameters on the number of treatment days was performed after the elimination of the deceased patients, on a number of 538 patients and the attenuation calculated previously in the analysis for the risk of death was used, using a linear regression.

The median duration of therapy for survivors was 10.61 days (10.70 days for AMI patients and 10.34 days for CI patients). In the non-attenuated as well as the attenuated analysis, the type of infection does not influence the duration of the therapy ($p> 0.05$).

Table 10. *Attenuated analysis of the influence of the origin of the case on the duration of treatment*

Independent parameter	***Statistics T***	***Value p***	***Coefficient [IC95%]***
Community Case (Attenuated)	REFERENCE	-	-
Nosocomial	0.73	0.4620	0.25 [-0.42 la 0.94]

The Charlson score did not appear to influence the duration of therapy ($p> 0.05$).

Table 11. *Analysis of the influence of Charlson score on treatment duration*

Independent parameter	***Statistics T***	***Value p***	***Coefficient [IC95%]***
Charlson score	0.49	0.6220	0.03 [-0.10 la 0.17]

Analysis with a simple univariate linear regression model, the Charlson score not being included in the model, because it did not influence the treatment duration, observed that a lower value of Na, a higher ATLAS score and a higher creatinine value are associated with a higher duration of therapy ($p < 0.05$). The values considered for serum sodium and creatinine are those from the hospitalization and reflect the degree of dehydration of the patient at the beginning of antibiotic therapy. The statistical analysis certifies the clinical observation that a patient presenting for investigations and treatment from the first signs of disease without significant dehydration or those with mild episodes of the disease require shorter therapies. The clinical signs of acute enteritis are sometimes ignored and in patients with multiple comorbidities they can cause significant hydroelectrolytic imbalances, and their presence at hospitalization, as in the case of severe or fulminant disease episodes, announces the need for long-term therapy.

Table 12. *Simple linear regression analysis of the influence of clinical and laboratory parameters over the duration of treatment*

Independent parameter	***Statistics T***	***Value p***	***Coefficient [IC95%]***
Age Disease	0.33	0.7390	0.01 [-0.03 la 0.04]
AV	1.08	0.2800	0.01 [-0.01 la 0.03]
Leukocytes (Thousands)	1.61	0.1080	0.05 [-0.01 la 0.11]
Albumin	-0.49	0.6200	-0.14 [-0.74 la 0.44]
Hb	-1.58	0.1130	-0.14 [-0.33 la 0.03]
Na	-2.38	0.0177	-0.09 [-0.16 la -0.01]
K	-0.40	0.6850	-0.12 [-0.73 la 0.48]
Cl	-1.82	0.0691	-0.07 [-0.15 la 0.01]
Creatinine	2.14	0.0325	0.47 [0.04 la 0.90]
ATLAS	2.01	0.0449	0.23 [0.01 la 0.47]
Ascite Present **Absent**	REFERENCE 0.12	- 0.9040	- 0.08 [-1.35 la 1.53]
Toxina A Negative **Positive**	REFERENCE -1.72	- 0.0852	- -0.81 [-1.73 la 0.11]
Toxina B Negative **Positive**	REFERENCE -0.38	- 0.7040	- -0.14 [-0.91 la 0.62]
GDH Negative **Positive**	REFERENCE 1.05	- 0.2910	- 0.38 [-0.32 la 1.08]

The analysis by simple linear regression was followed by a multiple linear regression, the algorithm being of retrograde selection, the final model containing a single independent parameter, namely the serum Na value considered to be impactful during the therapy.

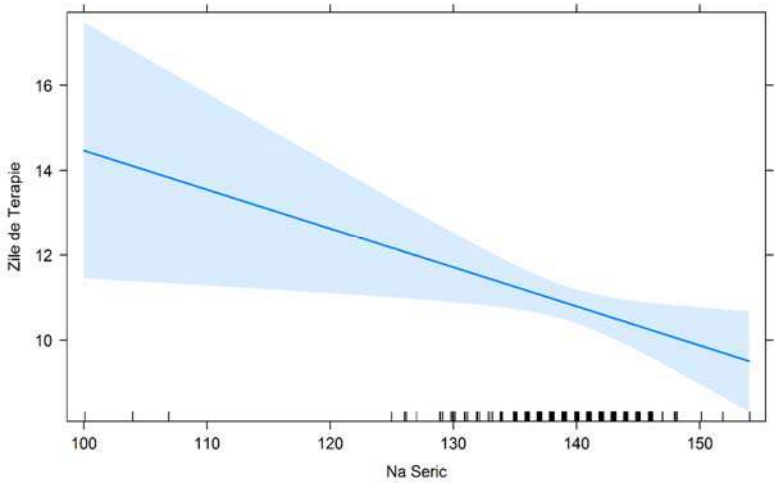

Fig. 15. *The influence of the serum sodium value on the duration of treatment*

Table 13. *Multiple linear regression analysis of the influence of clinical and laboratory parameters over the duration of treatment*

Independent parameter	***Statistics T***	***Value p***	***Coefficient [IC95%]***
Na	-2.38	0.0177	-0.09 [-0.16 la -0.01]

2.5.1.16. Impact of demographic-clinical factors on the number of days of hospitalization for an episode of Clostridium difficile infection

The analysis used a simple univariate linear regression (only for the statistically significant p-value independent variables) and found that in patients with older age, high Charlson score and high ATLAS score, hospitalization is increased, and hospitalization is also increased in patients with hyponatremia and hypoalbuminemia.

Table 14. *Analysis of the influence of the demographic-clinical parameters on the number of days of hospitalization*

Independent parameter	***Statistics T***	***Value p***	***Coefficient [IC95%]***
Age	5.06	< 0.0001	0.04 [0.02 la 0.06]
Score CHARLSON	3.21	0.0010	0.16 [0.06 la 0.26]
Albumin	-5.61	< 0.0001	-1.31 [-1.77 la -0.85]
Na	-3.43	0.0010	-0.09 [-0.15 la -0.04]
ATLAS score	5.44	< 0.0001	0.45 [0.29 la 0.61]

2.5.1.17. Impact of demographic-clinical factors on the risk of recurrence of Clostridium difficile infection

The analysis of the influence of the demographic-clinical parameters on the risk of recurrence was performed identical to the analysis for the risk of death, the dependent variable being represented in this case by the existence/absence of the recurrence. The unannounced analysis reveals that patients with IAAM have a 1.7 times higher probability of recurrence of infection (p <0.05). In the attenuated analysis, the type of infection does not appear to be a risk factor for relapse (p> 0.05).

Table 15. *Attenuated analysis of the influence of the origin of the case on the risk of recurrence*

Variable	***Coefficient***	***Value p***	***OR [IC95%]***
Community Case (Attenuated)	REFERENCE	-	-
Nosocomial	0.29	0.3000	1.34 [0.76 la 2.36]

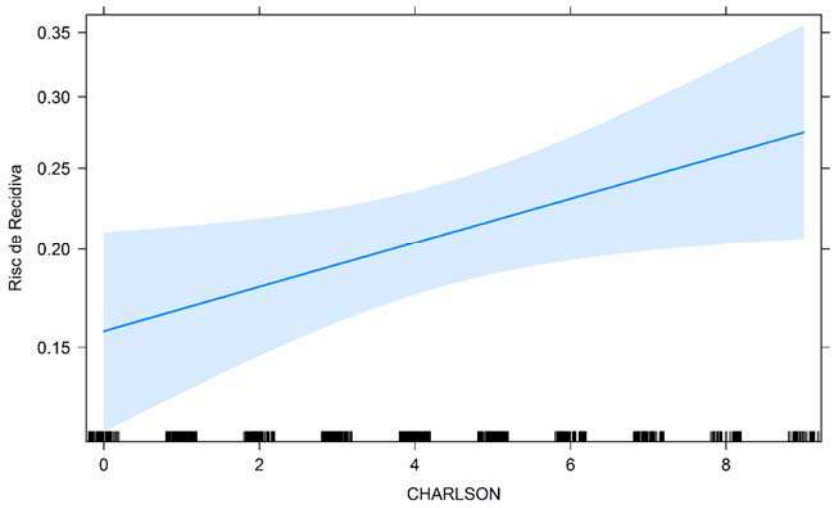

Fig. 16. *The influence of the Charlson score on the risk of recurrence*

The simple univariate binary logistic regression with independent variable, the Charlson score observed that an increase with one unit of the Charlson score, is associated with an 8% increase in the risk of recurrence, an effect with statistical significance ($p < 0.05$).

Table 16. *Analysis of the influence of the Charlson score on the risk of recurrence*

Variable	*Coefficient*	*Value p*	*OR [IC95%]*
Charlson Score	0.07	0.0284	1.08 [1.01 la 1.16]

2.5.1.18. Analysis of the types of treatment

It was noted that in the group with community infections, in which there were younger patients, with less comorbidities and they experienced less severe disease episodes than the patients in the group with IAAM, there were several treatments with metronidazole tablets and there was a smaller percentage of patients requiring human albumin administration.

The number of treatment days is comparable in the two groups, with the lowest average in the case of metronidazole tablets, applied to patients with low comorbidities and clinical forms with low severity and the highest average for combined oral vancomycin combination therapies with vancomycin enema, associated with intravenous metronidazole, applied to patients with clinical severity.

Table 17. *Analysis of the types of therapies is performed in the two study groups*

Variable	*Lot A (IAAM)*	*Lot B (IC)*
No. total patients / lot	553	153
No. patients treated with metronidazole /%	54(9.76)	33(21.56)
No. patients treated with vancomycin /%	402(72.69)	93(60.78)
No. patients treated with metronidazole and vancomycin /%	97(17.54)	27(17.64)
No. patients treated with human albumin /%	24(4.33)	4(2.61)
No. mean days of treatment with metronidazole ± standard deviation	8.55±2.19	8.93±2.43
No. mean days of treatment with vancomycin ± standard deviation	10.08±3.20	10.24±3.11
No. mean days of treatment with metronidazole and vancomycin ± standard deviation	11.42±3.74	12±3.73

2.5.1.19. Cost analysis of the treatment of Clostridium difficile infection

Analysis of the data from the expense statements from 166 observation sheets of outpatients between 1.07.2018-31.12.2018 with the diagnosis of acute gastroenteritis with Clostridium difficile from the Clinical Hospital of Infectious Diseases "Sf. Cuv. Parascheva "Galati pointed out that the biggest share in the total account is spent on accommodation and care and administrative staff.

The cost of drugs, sanitary materials, food, and laboratory blood tests represents 16.22% of total expenses, as follows: 7.61% drugs, 6.02% analyzes, 1.90% food and 0.68% sanitary materials.

The average cost of a patient with CDI treated in the clinic is 4434.45 lei, an amount that represents 387.62% of the amount allocated by the County Insurance House for a CDI case, that is 1144 lei.

Thus, these cases, beyond the complexity of the associated pathology and the evolutionary possibility towards severe or fulminant clinical forms, remain a difficult burden to balance for the management of an Infectious Diseases department of a rank 5 university clinical hospital.

Table 18. *Analysis of the types of therapy performed in the two study groups*

Variable	*No. hospital days*	*Total discount*	*Cost analysis*	*Food cost*	*Cost of sanitary equipment*	*Cost of medicines*
Arithmetic mean	7.26	4434.45	266.97	84.37	30.24	337.89
Standard deviation	3.76	2665.54	174.88	59.31	31.52	617.87
Minimum value	0	166.58	0	0	0	19.13
Maximum value	27	19085.19	1365.93	500	234.09	6083.13
Median	7	3909.63	234.32	77.5	18.93	189.79

2.5.2. Conclusions

The epidemioclinical study performed in the Clinical Hospital of Infectious Diseases Galați addressed a pathology that represented 7.82% of the total admissions during the period 1.01.2017-31.12.2018 (828 discharges with CDI out of 10578 total discharges), a new pathology for the clinic, the first cases diagnosed and treated here being in the third quarter of 2013, a pathology predominantly associated with healthcare, namely 78.32% of cases, potentially preventable by re-evaluating the hygiene and antibiotic protocols.

This study was conducted in one of the five counties with the highest number of cases to 100 discharges in Romania, in 2018, the county with the highest number of cases in the Southeast region of Romania.

The prospective, observational, actively controlled study analyzed a batch of 706 patients treated in this hospital during the period 1.01.2017-31.12.2018, a batch with statistical significance for this pathology.

The study aimed to identify the differences between the two subgroups of this group, namely between the patients with source the health care units and those with community source and to assess the risk factors for death, recurrence and longer treatment duration.

The study reveals significant statistical differences between the group with IAAM and that with IC in terms of risk factors (age, antibiotic use and PPI, recent abdominal surgery and comorbidities), laboratory parameters (Hb, K), ATLAS score of episode severity disease and prognostic parameters (number of relapses and death rate in the first 30 days after discharge and in the first 6 months after discharge).

The mean age in the group with IAAM was 68.02 years and in the group with IC 59.78 years; above the value considered at risk, 65 years in the group with IAAM.

In both groups, women and urban environment predominated, which was statistically significantly more important in the group with infections of community origin.

The comorbidities of the patients who had a statistically significantly higher weight in the group with IAAM are: heart failure, peripheral vascular disease, history of stroke, hemiplegia, dementia, chronic obstructive bronchopneumonia, chronic renal failure, cancers and Charlson score.

The presence of antibiotic therapy in the last 12 weeks before the onset of CDI was identified in 71.42% of patients with IAAM and in 45.75% of patients with IC, significantly more frequent in the group with IAAM.

The consumption of PPIs in the last 12 weeks was observed in 32.36% of the patients in the group with IAAM, significantly more than the 11.76% of the patients in the group with IC.

Abdominal surgery performed in the last 12 weeks was identified in the anamnesis of 10.85% of the patients of the group with IAAM, significantly more than the 1.31% of the patients of the group with IC.

The clinical parameters did not significantly differentiate between the two groups, but their analysis defines the clinical picture of the disease, namely: abdominal pain is present in most cases (99.34% in AMI and 99.06% in CI), fever is rarely encountered (13.74% in group with IAAM and 17.64% in the IC group), the symptoms are generally ignored for several days, the average number of days from the onset of CDI to the start of treatment is 5.95 days in the group with IAAM and 8.52 days in the IC group, the average number of unformed stools per day were 4.74 in the group with IAAM and 5.35 in the group with IC, patients had mild hypotension and tachycardia at admission (mean values of group IAAM - systolic blood pressure 126.66 mmHg and pulse 89.93 / minute, and in group IC - systolic blood pressure 130.62 mmHg and pulse 87.73 / minute).

The laboratory parameters, analyzed by severity levels, had the following significance:

- the level of leukocytes above 15000 / mm3 in the group with IAAM was observed in 19.52% of patients, and in the group with IC it was observed in 13.72% of patients.
- albuminemia below 2.5 g / dl, was observed in 13.58% of the patients in the group with IAAM and in 8.86% of the patients in the group with IC.
- hemoglobin below 12 g / dL was observed in 51.71% of the patients in the group with IAAM and in 26.79% of the patients in the group with IC.
- serum ionogram: Serum Na below 135mmol/l was observed in 14.64% of the patients in the group with IAAM and in 14.43% of the patients in the group with IC, serum K below 3.5 mmol/l was observed in 30.50% of the patients in the group with IAAM and in 20.19 % of patients in the group with IC, serum Cl below 98 mmol/l was observed in 12.12% of the patients in the group with IAAM and in 9.70% of the patients in the group with IC.
- serum creatinine over 1.5 mg/dL was observed in 19.80% of the patients in the group with IAAM and in 13.28% of the patients in the group with IC.

It is observed that all laboratory parameters have higher influence on the high severity levels in the group with IAAM, which suggests that patients with infections of community source developed episodes of disease with a lower degree of severity than those with IAAM.

The risk of death after CDI was influenced by Charlson score, leukocyte level, albumin, hemoglobin, sodium and potassium in patient's blood, at admission, Atlas score and presence of ascites.

The risk of recurrence was influenced by the level of serum creatinine at admission.

Treatment duration was significantly influenced by the levels of serum sodium at admission and the Atlas score.

Thus, it is considered necessary to calculate the Charlson and ATLAS score when hospitalizing the patient with CDI and, in collaboration with the results of hemoglobin, ionogram and creatinine, to evaluate the optimal treatment scheme for the best prognosis.

The prophylactic measures for CDI should be optimized in such a way as to maintain the downward trend in the number of cases and customized for the top wards as a source for CDI.

CDI prophylaxis measures should be made known in the community (patient brochures, newsletters), personalized CDI prophylaxis and treatment guides should be constantly updated and made known to medical staff through ongoing medical training activities (symposia, scientific papers).

Chapter 3

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The present work contains the first study of the circulating strains of Clostridium difficile in the Galați area, demonstrating the existence of a strain with increased aggressiveness and rapid spread. Anaerobiosis culture allowed the study in electron microscopy of Clostridium difficile bacilli, identifying microbial structural details for the first time at national level, useful for pedagogical practice and understanding of the etiopathogenesis of the disease. Parallel performing a reference technique - culture in anaerobiosis with immunochromatography and PCR allowed comparative analysis of the efficiency of these tests in the diagnosis of circulating strains in the studied area and the conclusion that first-line immunocromatography can be used, and in cases of poor results positives with clinical correlation must be duplicated by PCR.

Testing the chemical stability of vancomycin and metronidazole in artificial gastrointestinal juice using UV-Vis spectra demonstrates the possibility of their effective oral administration, since in practice intravenous vancomycin powder is used for oral administration.

The present study is the first to analyze the incidence of Clostridium difficile infection in the Southeast region of Romania, 116.57 cases per 100,000 inhabitants, compared to the incidence of CDI in the US in the 2010-2011 epidemic, of 147.2 cases per 100,000 inhabitants and 130.28 cases per 100,000 inhabitants in 2017. The maximum of the region was observed in Galați County of 175 cases per 100,000 inhabitants and the minimum of 23 cases per 100,000 inhabitants in Buzau county. Also, the present study is the first to quantify the proportion of community infections at regional level, compared to the national level, with the following results: the percentage of community infections from the total CDI, in Romania, in 2018, was 19%; in the Southeast region, in 2017-2018, was 31.52%, with a maximum in Buzău county of 69.51% and a minimum in Vrancea county of 15.05%; in Galați County, in 2017-2018, it was 30.48%.

The epidemioclinical study was conducted in Galati county, which was in 2018, in the top 5 counties with the highest number of cases of CDI at 100 national discharges.

The epidemioclinical study performed in the Clinical Hospital of Infectious Diseases Galați addressed a pathology that represented 7.82% of the total admissions during the period 1.01.2017-31.12.2018 (828 discharges with CDI out of 10578 total discharges), a new pathology for the clinic, the first cases diagnosed and treated here being in the 3rd quarter of 2013, a pathology predominantly associated with healthcare, namely 78.32% of cases, potentially preventable by re-evaluating the hygiene and antibiotic protocols.

The study compared a group with infections associated with healthcare with a group with community infections and identified the demographic, clinical and laboratory particularities of the two groups and identified the parameters with statistical significance during the treatment, the duration of hospitalization, the risk of death and the risk of recurrence.

Corroborating the parameters with impact on the prognosis of patients with CDI requires the calculation of the Charlson and ATLAS score when hospitalizing the patient with CDI, analyzing the values of hemoglobin, electrolytesand creatinine to form the optimal treatment scheme for the best prognosis.

The paper contains presentations of cases that associate special pathologies or had high severity, or required special therapies, as well as an analysis of a small batch of pediatric cases, extremely useful in clinical and pedagogical practice.

The annexes of the thesis contain an internal diagnosis and treatment protocol for the Clinical Hospital of Infectious Diseases “Sf. Cuv. Parascheva” Galați and a guide for patients with CDI.

Promoting the rules for the prevention of the transmission of CDI and the "antibiotic stewardship" strategies, as well as the personalization of the prevention and treatment guides for each medical specialty can be the key to limiting the CDI

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