"OVIDIUS" UNIVERSITY OF CONSTANTA
DOCTORAL SCHOOL OF MEDICINE
MEDICINE FIELD

DOCTORAL THESIS

Analysis of the epidemiological and clinical aspects of Clostridioides Difficile infection

-SUMMARY-

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Contents

Key words: evolution, Clostridioides Difficile, relapse, enterocolitis, faecal transplant, antibiotics, intestinal microbiome

INTRODUCTION

In 1935, Hall and O'Toole (1) discovered a new microorganism in the stool of newborns. It was considered to be part of the normal bacterial flora and given the name Bacillus difficilis. In 1977, because of the difficulty with which the bacillus was isolated and because of its relatively slow growth compared to most members of the genus Clostridium, it was renamed Clostridium difficile (2). It was later reclassified in 2015 by Lawson and Rainey as Clostridioides difficile. Also in 1977, Larson et al. (3) found that colitis occurring after the administration of antibiotics was caused by C. difficile, and in 1978, Bartlett et al. (4) showed that it was the causative agent of pseudomembranous colitis. Finney made the first anatomopathological studies of colitis with C difficile, and in 1893 he reported postoperative pseudomembranous changes in the intestinal tract of a 22-year-old patient (5). Later, in the 20th century, cases of Clostridioides infection were found after administration of antibiotics such as chloramphenicol and clindamycin (6,7). The incidence of C. difficile (ICD) infection has increased over the past decade, with more virulent strains emerging that affect new groups of patients (8), producing changes in disease manifestations and response to treatment, and influencing disease complications.
C. difficile affects up to 8% of hospitalized patients and is the most common cause of nosocomial diarrhea worldwide. It is the causative agent of 20-30% of antibiotic-associated diarrhea, 50-75% of antibiotic-associated colitis, and 90-100% of antibiotic-associated pseudomembranous colitis. Additionally, 20 to 28% of CDI are estimated to be community acquired, with an incidence of 20–50 cases per 100,000 population in the United States, Sweden, and England (9).

PERSONAL CONTRIBUTION

1. Objectives

In the present research, I set out to obtain new data regarding the spread of infection with Clostridioides Difficile in Constanta County and the factors that predispose to this type of infection. I also set out to observe the clinical pattern and evolution of our patients in order to highlight possible unfavorable prognostic factors regarding the risk of death and relapse.

2. Material and methods

The research includes two studies:

Study 1

It includes the epidemiological and clinical-evolutionary aspects of a group of patients diagnosed with Clostridioides Difficile admitted to the Constanta Clinical Hospital for Infectious Diseases. The study is prospective, for a period of 3 years (January 2014 - December 2016), using inclusion and exclusion criteria and a file for collecting the information necessary for the analysis.

Definitions

To include patients in this study we used the recommendations of the European Society for Microbiology and Infectious Diseases (ESCMID) regarding:

- Case definition for C. Difficile infection
- Case definition regarding the place of infection (nosocomial, community)
- Case definition for severe forms, relapses and mortality rate

Study 2

I monitored the impact on the quality of life from a psychosocial and somatic point of view, based on a specific test (Rotterdam Symptom Checklist).

Since a long period of hospitalization, eating disorders, diarrhea symptoms can cause affective and emotional balance disorders, increased anxiety, decreased self-image, the onset of depression as well as changes in the patients quality of life, I studied how C. Difficile infection can disturb the psychosocial balance of the patient.

I obtained the data through this specific test that I carried out and which led to the establishment of the degree of severity of the state of anxiety and depression, corroborated with the data obtained through the clinical interview and the observation of the patient's behavior.

Inclusion criteria:

- adult patients, 18 years old or over
- diarrhea with watery stools, 5-7 on the Bristol scale, >3/day or with greater frequency
- with discharge diagnosis (secondary or main) of diarrhea with Clostridioides Difficile
- with positive bacteriological tests for CD (toxins, cultures).

Exclusion criteria:

- patients younger than 18 years old

-with discharge diagnosis (secondary or main) of diarrhea of unspecified etiology

In all cases, complete history and detailed clinical examination were performed. The patient observation sheet was used as working tools.

I collected data regarding:

- risk factors: advanced age (>60 years old), previous antibiotic therapy, comorbidities, contact with the health environment, previous surgical interventions, likely site of infection

-clinical and paraclinical data: temperature, leukocyte count, urea, creatinine, TGP, TGO, procalcitonin, inflammatory tests

-microbiological results: PCR or EIA for C. Difficile, cultures, rapid C. Difficile toxin detection tests

- the etiological treatment performed and the other use of systemic antibiotics
- the evolution (healing, relapses, death) but also the duration of hospitalization
- the microbiome transplant

I monitored the patients by following: the clinical and paraclinical evolution, the relapses that occurred

Statistical analysis

The obtained experimental data were processed using the statistical processing program IBM SPSS Statistics v 23 IMB. The procedures used were: Descriptive statistics (for the characterization of discrete and continuous variables defined at the database level), Graphs, Parametric statistical tests (t-test for independent variables), Non-parametric statistical tests - addressed to categorical variables ($\chi 2$ test of the association, of the link between two categorical variables with OR calculation, $\chi 2$ test for comparing two proportions).

Ethical considerations
The research was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was explained in detail to all patients. Informed consent was obtained from all subjects for participation in the study.

3. Results

Study 1

Analysis of epidemiological and clinical evolution aspects
Between January 2014 and December 2016, 221 patients with ICD were diagnosed at the Constanta Clinical Hospital for Infectious Diseases.
I performed a comparative analysis of different characteristics in patients with relapses versus those without relapses in an attempt to find out if there is a pattern of relapses in CD infection at the Constanta Hospital for Infectious Diseases.

Table I Demographic and epidemiological characteristics in patients with relapses vs. without relapses

Characteristics of pacients with CDI	**Without relapses n=181**	**Relapses n=40**	**Chi square tests $\chi 2$**	**Chi square tests p**
Age groups:				
<20 years	3(1,7%)	1(2,5%)	0,095	0,757
20-39 years	27(14,9%)	4(10%)	0,309	0,578
40-59 years	51(28,2%)	8(20%)	0,745	0,388
60-79 years	83(45,9%)	21(52,5%)	0,338	0,560
>80 years	17(9,4%)	6(15%)	0,583	0,445
Sex:				
Women	112(61,9%)	29(72,5%)	1,168	0,280
Men	69(38,1%)	11(27,5%)	1,168	0,280
Number of comorbidities:				
1	78(43,1%)	16(40%)	0,033	0,855
2	37(20,4%)	12(30%)	1,139	0,265
>3	23(12,7%)	2(5%)	1,245	0,264
Previous surgery:				
No	99(54,7%)	16(40%)	2,268	0,132
Yes	82(43,3%)	24(60%)	2,268	0,132

Previous antibiotics:				
No	46(25,4%)	6(15%)	1,434	0,231
1 AB	52(28,7%)	12(30%)	0,001	0,977
2 AB	30(16,6%)	8(20%)	0,080	0,776
> 3AB	11(6,1%)	0(0%)	1,444	0,229
AB unspecified	42(23,2%)	14(35%)	1,828	0,176
Proton pump inhibitors:				
Yes	51(31,3%)	12(30%)	0,001	0,974
No	112(68,7%)	28(70%)	0,001	0,974
Unspecified	58			

The comparative analysis of the demographic and epidemiological characteristics of the patients in the two categories, who had relapses compared to those without relapses, showed particularities but no statistically significant differences were observed.

Table II Clinical and paraclinical characteristics in patients with relapses vs. without relapses

Characteristics of pacients with CDI	**Without relapses n=181**	**Relapses n=40**	**Chi square tests $\chi 2$**	**Chi square tests p**
Admission Temperature:				
< 37,5	140(77,3%)	32(80%)	0,026	0,871
37,6- 38,5	41(22,7%)	8(20%)	0,026	0,871
>38,6	0	0 (0%)		
Leukocyte count:				
<16.000	151(83,4%)	35(87,5%)	0,163	0,686
16.000-25.000	23(12,7%)	5(12,5%)	0,052	0,819
>25.000	7(3,9%)	0(%)	0,597	0,438
Serum creatinine:				
1,36 mg/dl	151(83,4%)	33(82,5%)	0,009	0,923
1,37-2 mg/dl	13(7,2%)	3(7,5%)	0,073	0,786
> 2 mg/dl	17(9,4%)	4(10%)	0,032	0,856
Serum albumin:				
>3,5 g/dl	8(33,3%)	0	16,675	< 0.001
2,6- 3,5 g/dl	12(50%)	3(50%)	0,030	0.861
<2,5 g/dl	4(16,7)	3(50%)	18,833	< 0.001
Unspecified	97	34		

CDI diagnostic:				
Positive culture	5(2,8%)	2(5%)	0,048	0,826
Negative culture	10(5,5%)	1(2,5%)	0,151	0,697
Unspecified culture	166(91,7%)	37(92,5%)	0,023	0,879
Toxin positive ELFA	112(61,9%)	24(60%)	0,001	0,964
Toxin negative ELFA	42(23,2%)	12(30%)	0,494	0,482
Toxin unspecified	27(14,9%)	4(10%)	0,309	0,578
PCR positive	18(9,9%)	6(15%)	0,434	0,510
PCR negative	4(2,2%)	2(5%)	0,202	0,653
PCR unspecified	159(87,8%)	32(80%)	1,097	0,295
Rybotipe:				
Rybotipe PCR GeneXpert subtipe R027 positive	17(9,4%)	0(%)	2,858	0,090
Rybotipe PCR GeneXpert subtipe R027 negative	1(0,6%)	0(%)	0,575	0,448
Ribotipare PCR GeneXpert subtipe R027 unspecified	163(90,1%)	40	3,083	0,079

The comparative analysis of the clinical and paraclinical characteristics of the patients in the two analyzed categories, those with relapses versus those without relapses, showed that the value of serum albumin not correlates with the occurrence of relapses.
The other variables, even if they present certain particularities, have no statistical significance.

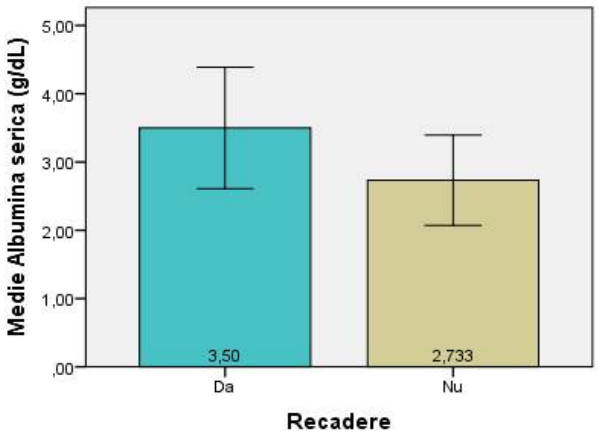

Figure 1 Representation of mean serum albumin values in patients with relapses vs. without relapses

As can be seen from Figure 1, there are significant differences between the mean value of serum albumin in patients who experienced relapses MDA = 3.5 mg/dL and the mean value of serum albumin recorded in patients who did not experience relapses MNU = 2.73 mg/dL: t = 2.376, df = 28, p = 0.025 < α = 0.05, Mdif = 0.766 mg/dL, 95% CI of the Difference = (0.10; 1.42)

Table III Therapeutic characteristics in patients with relapses vs. without relapses

Characteristics of pacients with CDI	**Without relapses n=181**	**Relapses n=40**	**Chi square tests χ^2**	**Chi square tests p**
CDI treatment:				
Yes	181(100%)	35(87,5%)	17,841	< 0.001
No	0	5(12,5%)	17,841	< 0.001
Metronidazol (Mz)	24(13,3%)	9(25,7%)	2,587	0,107
Vancomicin(Va)	14(7,7%)	6(17,1%)	2,074	0,149
Mz+Va	138(76,2%)	20(57,1%)	4,514	0,033
Mz+ Va+Tigeciclin (Tg)	3(1,7%)	0(%)	0	0,998
Va+ Tg	2(1,1%)	0(%)	0	0,731
Extended scaled scheme:				
Yes	28(97%)	1(3%)	5,336	0,020
No	151(81%)	36(19%)	5,336	0,020
Associated antibiotics:				
Without	135(75%)	32(80%)	0,216	0,642
1 AB	39(21,7%)	8(20%)	0	0,980
2 AB	6(3,3%)	0(%)	0,391	0,531
Fecal transplant:				
Yes	10(6,2%)	10(25%)	0,587	0,049
No	161(93,8%)	40(75%)	0,587	0,049
Evolution:				
Death	3	0(%)	0,007	0,934
Discharge	178	40(100%)	0,007	0,934
Scor ATLAS				
1-5	17(70,8%)	5(83,3%)	0,010	0,917
6-10	7(29,2%)	1(16,7%)	0,010	0,917
Unspecified	16	175		

The comparative analysis of the therapeutic characteristics of the patients in the study of the two groups, with relapses vs. without relapses, showed that between the proportion of patients who relapsed at most 8 weeks in the group of those with deescalated treatment with oral vancomycin (1 patient, i.e. 3%) and the proportion of patients who relapsed in the group of those without deescalated treatment with oral vancomycin (36 patients, i.e. 19%) are considered to have statistically significant differences: $p = 0.020 < \alpha = 0.05$; Chi-squared = 5.336; df = 1.

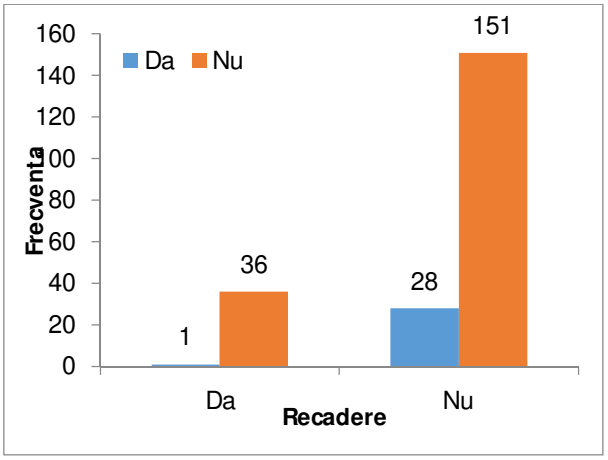

Figure 2 Graphical representation of extended, staggered CD-specific treatment use in relapsed vs non-relapsed patients

Study 2

The following is an analysis containing the results of the assessment of the quality of life in patients with the diagnosis of CD infection. The group consisted of a number of 54 patients who were hospitalized between January 2014 and December 2016 at the Constanta Clinical Hospital for Infectious Diseases and agreed to participate in completing the questionnaire.
I analyzed from a psychosocial and somatic point of view the impact on the quality of life based on a specific test (Rotterdam Symptom Checklist).
The Rotterdam Symptom Checklist was developed primarily as a tool to measure symptoms reported by cancer patients when participating in clinical research. It is also applicable to monitoring the patient's anxiety and depression levels and thus reflects the presence of psychological impairment in the case of other conditions as well.
Because a long period of hospitalization, eating disorders, diarrhea symptoms can cause affective and emotional balance disorders, increased anxiety, decreased self-image, the appearance of depression as well as the change in the patient's quality of life, I want to observe how extent C. Difficile infection can disturb the psychosocial balance of the patient.
I achieved this with this specific test that helped me establish the degree of severity of the state of anxiety and depression, corroborated with the data obtained through the clinical interview and the observation of the patient's behavior.
It is an observational, clinical-sociological study of the quality of life in patients with CD infection, being structured on several stages that include data collection, their analysis and interpretation.
As I mentioned, the study includes 54 patients out of the 221 analyzed in Study 1 of the present paper.
I pursued studying the impact of the disease on the quality of life of these patients.

Quality of life assessment was performed after obtaining informed consent to participate in this study.
The questionnaire we used is improved and expanded, being composed of 30+8+1 = 39 items and 3 main subscales:
1. The scale of physical suffering caused by the disease (with 22 items, for example -I felt tired. I had muscle pain)
2. The scale of mental suffering generated by the disease, (with 8 items, for example - I was very nervous, irritable. I was depressed, sad, angry, melancholic)
3. Daily activities scale (with 8 items, for example - To climb the stairs at home, to go shopping, to take longer walks, far from home)
A general assessment of health status on a scale from 1 (very poor) to 7 (excellent).
Each item can have a score in the range of 1–4 points, except for the daily activities scale, where the values are in the range of 1–7. A global score of the entire questionnaire can also be calculated, which can take values between 39-155 points.
The higher the scores on the first two scales, the more serious or unfavorable the patient's health status. On the scale of daily activities and on the scale of general assessment of the state of health, high scores indicate a better functionality of the patient.
The questionnaire is anonymous and is applied by self-administration, possibly with minimal technical assistance from the medical staff and lasts, on average, 8 minutes.
I noted the age, sex. The patients in the study group were instructed to rate the extent to which each symptom bothered them in the last week.
Then through a software scoring system I interpreted the results, following the impact on the quality of life through the answers given by the patients in the questionnaire. Next, I also analyzed the correlation between the characteristics related to sex, age and psychosomatic impairment.
The quantification and appreciation of the results of these scores was also achieved in a direct, simple way, by which we used a scale of total values of the test between 39 points minimum and 155 points maximum, by following the answers of the type "A lot" and "Very much" .

Correlation between illness and psychosomatic impairment

Table IV Scores obtained in the study regarding the impact on the quality of life

	Somatic	Emotional	Anxietate	Depresie	Autoingrijire	Scor I (Q1...Q30)	Scor II (Q1b...Q8b)	Scor Total
Minimum	22,00	7,00	4,00	5,00	4,00	39,00	8,00	50,00
Maximum	57,00	23,00	14,00	19,00	16,00	96,00	32,00	134,00
Mean	40,37	14,30	9,65	12,70	9,54	68,65	21,56	95,35
Median	41,00	14,00	10,00	12,50	10,00	70,50	23,50	96,50
Mode	42,00	10,00	11,00	12,00	4,00	60,00	31,00	116,00
Std. Deviation	8,80	4,00	2,53	3,63	4,21	14,79	8,43	22,46
Percentile 25	33,00	11,00	8,00	10,00	5,00	57,00	15,00	80,00
Percentile 75	47,00	17,00	12,00	16,00	14,00	79,00	30,00	114,00

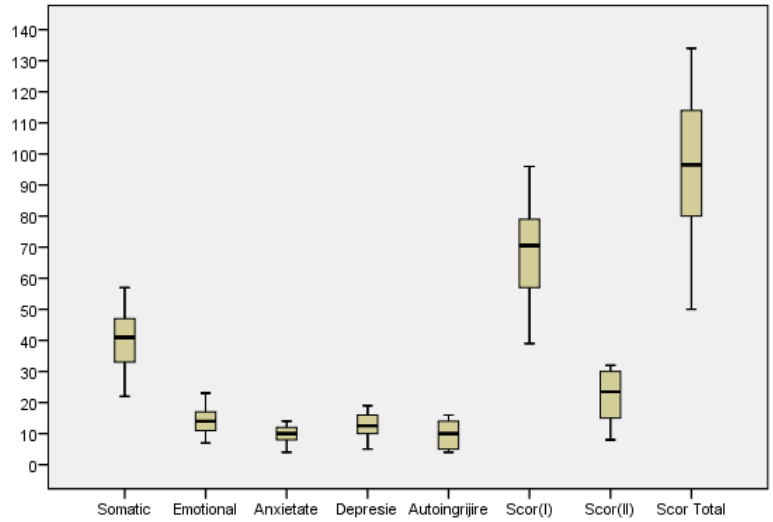

Figure 3 Representation from the scores of the variables under study

In the Somatic Aspects section, the score obtained by the 54 investigated patients was between 22 and 57 points, with an average score of 40.37, a median value of 41 and a most frequently occurring value (module) of 42. Standard deviation calculated is 8.80 and the interquartile range (IQR) between 33 and 47 points. Also, from the total of 54 patients, 24 (44%) patients were assigned to the Much category and 30 (56%) patients were assigned to the Very much category.
In the Emotional stability/instability section, out of a total of 54 patients, 20 (37%) patients were placed in the Much category and 34 (63%) patients were placed in the Very much category.
In the Anxiety section, out of a total of 54 patients, 21 (39%) patients were classified as Much and 33 (61%) patients were classified as Very much.
In the Depression section, out of a total of 54 patients, 16 (29%) patients were assigned to the Much category and 38 (70%) patients were assigned to the Very much category. (Table IV and Figure 3).
This shows an impairment of the quality of life in CDI patients

Table V Correlation between gender and emotional stability/instability

			Stabilitate/Instabilitate Emotionala		Total
			Foarte mult	Mult	
Sex	Masculin	Count	10	12	22
		% within Stabilitate/Instabilitate Emotionala	29,4%	60,0%	40,7%
		% of Total	18,5%	22,2%	40,7%
	Feminin	Count	24	8	32
		% within Stabilitate/Instabilitate Emotionala	70,6%	40,0%	59,3%
		% of Total	44,4%	14,8%	59,3%
Total		Count	34	20	54
		% within Stabilitate/Instabilitate Emotionala	100,0%	100,0%	100,0%
		% of Total	63,0%	37,0%	100,0%

Table V Correlation between gender and emotional stability/instability

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided) **(p)**	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square χ^2_{calc}	4,880[a]	1	,027		
Continuity Correction[b]	3,695	1	,055		
Likelihood Ratio	4,883	1	,027		
Fisher's Exact Test				,044	,027
Linear-by-Linear Association	4,790	1	,029		
N of Valid Cases	54				

In the section related to Emotional Stability/Instability, 12 men (60.0%) and 8 women (40.0%) were placed in the Much category; while 10 men (29.4%) and 24 women (70.6%) were included in the Very Much category.
By applying the CHI-Square Test, it is found that between the two variables under study, Sex and Emotional Stability/Instability, there is a relationship of dependence (association, connection): $\chi 2calc = 4.88$, $df = 1$, $p = 0.027 < \alpha = 0.05$.
Also, the calculated risk/chance ratio is OR = 0.278 and the associated confidence interval is 95% IC for OR = (0.087, 0.886). This value shows that the risk of having male patients for whom the degree of Emotional Stability/Instability was considered Very Much is 3.59 times lower (1/0.278) than the risk of having female patients with the same degree of Stability /Emotional instability.

Table VI Correlation between gender and depression

			Depresie		Total
			Foarte mult	Mult	
Sex	Masculin	Count	12	10	22
		% within Depresie	31,6%	62,5%	40,7%
		% of Total	22,2%	18,5%	40,7%
	Feminin	Count	26	6	32
		% within Depresie	68,4%	37,5%	59,3%
		% of Total	48,1%	11,1%	59,3%
Total		Count	38	16	54
		% within Depresie	100,0%	100,0%	100,0%
		% of Total	70,4%	29,6%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided) **(p)**	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square χ^2_{calc}	4,459[a]	1	,035		
Continuity Correction[b]	3,270	1	,071		
Likelihood Ratio	4,430	1	,035		
Fisher's Exact Test				,067	,036
Linear-by-Linear Association	4,376	1	,036		
N of Valid Cases	54				

In the section related to Depression, 10 men (62.5%) and 6 women (37.5%) were placed in the Much category; while 12 men (31.6%) and 26 women (68.45%) were included in the Very Much category.
By applying the CHI-Square Test, it is found that between the two variables under study, Sex and Depression, there is a relationship of dependence (association, connection): $\chi 2calc = 4.459$, $df = 1$, $p = 0.035 < \alpha = 0.05$.
Also, the calculated risk/chance ratio is OR = 0.277 and the confidence interval associated with it is 95% IC for OR = (0.082, 0.939). This value shows that the risk of having male patients for whom the degree of Depression was considered Very Much is 3.61 times lower (1/0.277) than the risk of having female patients with the same degree of Depression.

DISCUSSIONS

In this study I aimed to find answers to the following questions:
- Are there epidemiological peculiarities in patients infected with CD in Constanta, Romania?
- Is the hypervirulent ribotype 027 also found in the circulation of CD strains?
- What are the factors that favor, predispose to CD infection?
- Do we have clinical-evolutionary peculiarities in patients hospitalized with CD in the Constanta Clinical Hospital for Infectious Diseases?
- Are there laboratory and clinical-epidemiological elements that lead to the prediction of the risk of unfavorable evolution, to the relapse of CD infection?
- Is there an impairment of the quality of life from a psychosocial and somatic point of view in patients infected with CD?

In Study 1, we performed an analysis of the epidemiological and clinical-evolutionary aspects of CD infection, which included a group of 221 patients admitted to the Constanta Clinical Hospital for Infectious Diseases between January 2014 and December 2016.
The primary objectives were to identify risk factors for CD infection and factors associated with unfavorable progression to disease relapse.
The 221 patients included in the group met all inclusion and exclusion criteria.
In terms of the year of hospitalization, 2015 represented the year with the most cases - 94 (42%) out of a total of 221. Then 2014 with 67 (30%) and 2016 with 60 (27%). The proportion in the 3 years remained relatively the same, showing a constant of cases of CD infection.
The distribution by gender was with the predominance of women in the studied group, 141 (63%) and men 80 (36%).
The breakdown by age groups shows that most patients are in the 60-80 age group (47%), followed by those in the 40-60 range (26%), 14% are between 20 and 40 years old, 10% are over 80 years and only 4 (1%) under 20 years. Studies carried out in the early 80s and the most recent ones confirm that the occurrence of CD infection is 10 times higher in the 60-90 age group (10,11).
Regarding the correlation between gender distribution and age groups, in each age range the percentage of women is higher than that of men. No correlation was found between the two analyzed variables.
For 148 patients (66%), the environment of origin was urban compared to 73 patients (33%) where the environment was rural. This is explained by easier access and greater addressability of urban patients and better health education.

The analysis of risk factors associated with CDI showed the same risk factors considered in the specialized literature:

Comorbidities

Most of the 221 patients had one comorbidity 94 (42%), 49 (22%) patients with 2 comorbidities and 25 (11%) patients with at least 3 comorbidities. The statistical analysis showed that the age of the patients correlates with the comorbidities, as the age increases, so does the number of comorbidities.

Surgical intervention in the antecedents

106 (48%) patients infected with C. Difficile had a recent surgical intervention (within the last 3 months).

Previous antibiotic treatment

From the group of 221 patients, 169 (76%) had this risk factor in the antecedents.
64 (29%) patients underwent antibiotic therapy with 1 drug.
38 (17%) patients underwent antibiotic therapy with 2 drugs.
11 (5%) patients underwent antibiotic therapy with 3 or more 3 drugs, the rest being with an unknown antibiotic in the antecedents.
The most frequent classes of antibiotics were cephalosporins (n=52), fluoroquinolones (n=48), other beta-lactams (n=35).

Proton pump inhibitors were administered before the onset of digestive symptoms in 63 (31%) of the patients.

Contact with the health system

Analyzing the epidemiological information, I was able to find out the origin of the infection in 218 (98%) of the patients. The source of the infection proved to be nosocomial in most of the patients - 145 (65%), therefore associated with medical care and 73 (33%) of the patients contacted the infection from the community.
Regarding the **hospital origin**, I found out that in most cases it was the Sfantul Apostol Andrei County Emergency Clinical Hospital from Constanta (73 patients; 50% of cases) followed by a group of several hospitals as a contact with the sanitary environment. Constanta Clinical Hospital for Infectious Diseases (n=7; 5%), Palazu Pneumology Hospital Constanta (n=11; 8%), Agigea Constanta Orthopedic Hospital (n=4; 3%) and other hospitals in Constanta without specifying (n=50; 34%).
The most frequent departments were Internal Medicine (n=10), Pneumology (n=10), Infectious Diseases (n=8) and General Surgery (n=46), Orthopedics (n=33) and Urology (n=13) .
The fact that patients came into contact with many medical departments/units in Constanta and its surroundings shows that we are not facing outbreaks of CD infection in Constanta, but rather in an endemic situation, with the infection spreading in all medical units.
Age equal to or over 60 years was found in 47% of the patients we studied.
Regarding the **analysis of clinical and laboratory data**, we found that the majority of patients did not have a fever at admission, n=172 (over 77%).
Leukocytes for most patients (n=186, 84%) were below 16,000 cel/mmc.
For 28 of them (over 12%) the leukocytes were between 16,000 and 25,000 cel/mmc.
Determination of procalcitonin was done only in 4 patients for financial and logistical reasons.
Procalcitonin had a positive value in 50% of the patients analyzed.
Serum albumin - a number of 8 patients (26%) of the 30 analyzed had a serum albumin level below 2.6 g/dl, and 15 (6%) had values between 2.6 and 3.5 g/dl .

37 patients (17%) had changes in **serum creatinine** values and 39 (17%) had increases in serum urea.
Normal values of oxaloacetic glutamic transaminase (**TGO/AST**) were found in the majority of 200 patients (90%) and pyruvic glutamic transaminase (**TGP/ALT**)- 195 patients (88%).
The method of confirming the diagnosis of ICD was made by immuno-enzymatic analysis.
The production of CD toxins was highlighted in the laboratory by immuno-enzymatic tests in 136 (61%) of the patients, the tests being positive and negative in only 54 (24%) of the cases.
Laboratory access to highlight the R027 ribotype was extremely limited due to difficulties in procuring the necessary kits. 18 samples from the entire group were processed, of which 17 samples came out positive for **ribotype 027**.
It should be noted that they came out positive for ribotype 027 in 99% percent.
This shows that the circulation at the level of the studied lot of ribotype 027 in Constanta is predominant.

The analysis of the **therapeutic aspects** showed that most forms of the disease were considered medium forms, being treated among the drugs used with the combination of metronidazole and vancomycin, being the most used in the studied group - 158 (73%).
The number of mild forms allowed 33 patients (15%) to be treated with metronidazole and 20 (9%) of those treated with vancomycin.
Compared to laboratory data that suggested few potentially severe cases, information on how patients in the study group were treated shows an overestimation of case severity. Thus, permanent knowledge and training in medical practice is required to be able to correctly assess the seriousness of the clinical picture based on international and national recommendations. The emergence and selection of CD strains resistant to the specific treatment is thus avoided, but also indirectly by reducing the systemic antibiotics used simultaneously with the treatment for CD.
It was also decided that the treatment schemes should be prescribed according to the national treatment protocol over a long period of time, staggered, with gradually reduced doses to prevent relapses. This occurred in 29 (13%) of the patients.
Concomitant systemic antibiotic treatment was in 47 (21%) patients of the study group who received a single type of systemic antibiotic concurrently with the specific treatment and 6 (2%) patients followed a regimen containing 2 types of systemic antibiotics.
Out of the total of 221 patients in the study group, only 20 (9%) benefited from **faecal transplantation**. I specify that during the study period in Romania this method of treatment was at the beginning level, in Constanta being performed for the first time in the country by only one gastroenterologist.
The analysis of the evolution of the cases showed that in terms of **relapses,** they occurred in 37 (17%) of the 221 patients at most 8 weeks after the end of the etiologically specific treatment.
In-hospital mortality in the group of patients studied was low, 1.3% (3 patients).
To find out the degree of severity, we used the ATLAS score calculation.
Due to material reasons, the limitations in the calculation of the ATLAS score were great. We were able to calculate the score in a number of 30 patients (14%). Most, 22 (10%) had a score between 1 and 5, showing moderate disease involvement and 8 patients (4%) showed severe disease with an ATLAS score between 6 and 10.
The average duration of hospitalization was 9.67 days.

Next, I performed a **comparative analysis** of the different characteristics in patients with relapses versus those without relapses in an attempt to find out if there is a pattern of relapses in CD infection at the Constanta Hospital for Infectious Diseases.
The comparative analysis of the **demographic and epidemiological characteristics** of the patients in the two categories, who had relapses compared to those without relapses, shows peculiarities but which are not statistically significant.
The comparative analysis of the **clinical and paraclinical characteristics** in the patients from the two analyzed categories, **with relapses** compared to those **without relapses**, showed that the serum albumin value does not correlate with the occurrence of relapses. Hypoalbuminemia is present in patients without relapses.
The other constants (temperature at admission, number of leukocytes, procalcitonin, urea, creatinine) did not influence the occurrence of relapses in the current study.
The comparative analysis of the **therapeutic characteristics** showed:
From a total of 187 patients who **did not** receive etiologic treatment with vancomycin for CD in a **prolonged, staggered schedule**, a number of 36 (19%) patients experienced **relapses** at most 8 weeks after the end of treatment. Of the 29 patients who **were treated** with vancomycin in the prolonged, tapering regimen, 1 (3%) patient **relapsed**. Between the proportion of patients who relapsed in the group with deescalated treatment and the proportion of patients who relapsed in the group without deescalated treatment, it is considered that there are statistically significant differences: $p = 0.020 < \alpha = 0.05$.
Thus, it is concluded that prolonged treatment with vancomycin, with gradually reduced doses, prevents CDI relapses.
There are significant differences between **the average length of hospitalization** in patients who experienced relapses (7.95 days) and the average length of hospitalization recorded in patients who did not experience relapses (9.67 days).
This shows the severity of the forms disease and thus the need for a longer period of hospitalization even for the first episodes of CDI.
In the specialized literature, age over 65 years, antibiotic use, gastric acid suppression and infection with a hypervirulent strain are cited as risk factors in CD relapses (12,13,14).
In **Study 2** I performed an assessment of **the quality of life** in patients with a diagnosis of CD infection, considering it useful to assess patients from the point of view of the influence of CDI. I tracked the impact of the disease on the patients quality of life.
Health-related quality of life is a new concept with many definitions. It incorporates negative and positive aspects of the individual's well-being and life. The disease has a significant impact on patients lives. Socio-demographic characteristics are influenced by clinical course, complication rate and mortality. Because functional disability, invalidity, social insertion, psychosomatic development are influenced by the disease.
The group consisted of a number of 54 patients who were hospitalized between January 2014 and December 2016 at the Constanta Clinical Hospital for Infectious Diseases.
I analyzed from a psycho-social and somatic point of view the impact on the quality of life based on a specific test (**Rotterdam Symptom Checklist**). The questionnaire includes the scale of physical suffering produced by the disease, the scale of mental suffering generated by the disease and the scale of daily/everyday activities.
In the **Somatic Aspects** section of the total of 54 patients, 24 (44%) patients were assigned to the "**Much**" response category and 30 (56%) patients were assigned to the "**Very much**" response category.

In the **Emotional stability/instability section**, out of a total of 54 patients, 20 (37%) patients were placed in the "**Much**" category and 34 (63%) patients were placed in the "**Very much**" category.
In the **Anxiety section**, out of a total of 54 patients, 21(39%) patients were assigned to the "**Much**" category and 33(61%) patients were assigned to the "**Very Much**" category.
In the **Depression section**, out of a total of 54 patients, 16 (29%) patients were assigned to the **"Much**" category and 38 (70%) patients were assigned to the "**Very much**" category.
This shows a profound impairment of the quality of life in CDI patients. Thus, the conducted study highlights the fact that CDI seriously alters the quality of life, with most patients stating physical and mental difficulties and discomfort.
When analyzing the **characteristics related to sex and the psycho-somatic impairment** for all the analyzed sections, no significant differences were indicated between the answers given by men and the answers given by women.
Thus, in the **Somatic Aspects** section, the score obtained by the 54 investigated patients was between 22 and 57 points, with an average score of 40.
The scores obtained by 22 investigated male patients ranged between 25 and 57 points, with an average score of 38.
The scores obtained by the 32 investigated female patients were between 22 and 57 points, with an average score of 41. There is no relationship of dependence between the two variables under study, Sex and Somatic Aspects.
In the **Stability/Emotional Instability section**, the score obtained by the 22 investigated male patients was between 9 and 21 points, with an average score of 13. Of the 32 investigated female patients, the obtained score was between 7 and 23 points, with an average score of 14. When **analyzing** the correlation between the two variables under study, **Sex and Emotional Stability/Instability**, there is a dependency relationship. Thus, the risk of having male patients for whom the degree of Emotional Stability/Instability was considered "Very much" is **3.59 times lower** than the risk of having female patients with the same degree of Emotional Stability/Instability. It is thus deduced that women are affected more emotionally than men in the context of CDI.
In the **Anxiety section**, the score obtained by the 22 investigated male patients was between 5 and 14 points, with an average score of 9. From the 32 investigated female patients, the obtained score was between 4 and 14 points, with an average score of 10. It is found that there is no dependency relationship between the two variables under study, Sex and Anxiety.
In the **Depression section**, the score obtained by the 22 investigated male patients was between 6 and 18 points, with an average score of 12. From the 32 investigated female patients, the obtained score was between 5 and 19 points, with an average score of 13. When analyzing the correlation between sex and Depression, it is found that there is a dependency relationship between the two variables under study, Sex and Depression. The risk of having male patients for whom the degree of depression was considered "Very much" is **3.61 times lower** than the risk of having female patients with the same degree of depression. Thus, women with CDI in the studied group may develop depression to a greater extent than male patients.
In the **Self-Care/Autonomy section**, the score obtained by the 22 investigated male patients was between 4 and 16 points, with an average score of 9. Of the 32 investigated female patients, the obtained score was between 4 and 15 points, with an average score of 10.

The score obtained for all 30 questions in the first section of the test by the 22 investigated male patients was between 42 and 92 points, with an average score of 65. From the 32 investigated female patients the score obtained was between 39 and 96 points, with an average score of 70.
The score obtained for all 8 questions in the second section of the test by the 22 investigated male patients was between 8 and 32 points, with an average score of 20. From the 32 investigated female patients the score obtained was between 8 and 31 points, with an average score of 22.
The TOTAL score obtained on the test by the 22 investigated male patients was between 60 and 129 points, with an average score of 90. Of the 32 investigated female patients, the obtained score was between 50 and 134 points, with an average score of 98.
When analyzing the **correlation between age and psychosomatic impairment**: the two variables are correlated, the correlation being considered positive, significant ($p < \alpha = 0.05$), classified as weak to moderate. Age affects quality of life.
When analyzing the **correlation between age and depression**, the two variables are correlated, the correlation being considered positive, significant ($p < \alpha = 0.05$), classified as weak to moderate. The risk of depression increases with age.
When analyzing the **correlation between age and anxiety**, the two variables are correlated, the correlation being considered positive, significant ($p < \alpha = 0.05$), classified as very weak.
The correlation between age and emotional stability/instability. The two variables are not correlated.
When analyzing the **correlation between age and somatic**, the two variables are correlated, the correlation being considered positive, significant ($p < \alpha = 0.05$), classified as weak to moderate. With increasing age, somatic damage appears in the context of CDI.
Correlation between age and score on all questions
The two variables are correlated, the correlation being considered positive, significant ($p=0.0004$. $p < \alpha = 0.05$), classified as weak to moderate. Age affects quality of life.
The results obtained according to the quality of life evaluation questionnaire show a significant impact on all aspects (physical and mental) pursued in the context of CDI.

As a **synthesis** of the listed discussions, I consider that the research achieved its initially proposed objectives by carrying out **study 1** in which, by analyzing the epidemiological and clinical-biological data, the factors predisposing to the appearance of this condition and its unfavorable evolution were identified, towards relapse.
Clinical-evolutionary characteristics were analyzed on an extensive number of 221 patients selected for inclusion in the study. The results obtained represent a mirror of the main problems faced by specialists in the field in their daily practice.
Analyzing the epidemiological information, I was able to establish the origin of the infection, which proved to be associated with medical care (nosocomial) in most of the patients. The fact that the patients admitted from other health units came from various sections/departments/units shows that from an epidemiological point of view, we are not facing outbreaks of CD infection, but an endemic situation, with the spread of the infection in all medical facilities and with growing potential for even greater spread in the community.
Study 2 provides the main element of originality of the thesis, through the methodology used, the results obtained and the practical implications, being one of the first carried out at the national level and the first in the Dobrogea region.

Also, from a microbiological point of view, the ribotyping of strains isolated in the Laboratory of the Constanta Clinical Hospital for Infectious Diseases, represents an achievement for Dobrogea. The identification of the presence of the hypervirulent ribotype 027 in 99% of the samples analyzed in the studied group confirms the recent clinical-epidemiological evolution, with the constant increase in the number of cases.

CONCLUSIONS

1. During the 3 years of the study, the number of patients admitted, diagnosed and treated with CD infection was relatively constant
2. The prospective research of the epidemiological and clinical-evolutionary aspects in the patients in the group showed:
- 47% of patients were older than 60 years old
- Women were more numerous (63% vs 36% men)
- Most patients came from the urban environment (66% vs 33% rural)
3. For CD infection, the identified risk factors were: contact with the health system, age over 60 years old, present comorbidities, previous antibiotic treatments, previously administered proton pump inhibitors, previous surgical interventions. Their weight was significant showing how high the risk of developing ICD is:
- 65% of the cases with a known source were infections associated with the heathcare, coming from the medical units of the county. This proves that we are facing an endemic evolution of this infection, with worrying extension in the community, 33% of patients coming from home, without recent contacts with the healthcare system
- 75% presented comorbidities: cardiovascular diseases, nutritional diseases, neoplasias
- 76% of patients received antibiotic treatments before the onset of digestive symptoms; the most common antibiotic classes were beta-lactams (especially cephalosporins 3th gen) and fluoroquinolones. Antibiotic abuse is a real national problem. It is extremely important to reduce the number of unnecessary antibiotic prescriptions, as this is the main way to reduce the incidence of CDI
- Proton pump inhibitors were administered before the onset of digestive symptoms in 31% of patients
- 48% had a surgical intervention in the last 3 months
4. The clinical and paraclinical changes in the patients in the group showed, along with diarrhea, the presence of fever (23%), leukocytosis over 16,000 cel/mmc (15%). In patients in whom procalcitonin was analyzed, it was positive in 50% of cases, hypoalbuminemia (32%), increased serum urea and creatinine (17%)
5. The main ways of diagnosing CD infection were:
- Immuno-enzymatic tests (86%)
- Culture in anaerobiosis (9%)
- Confirmation of toxigenesis (61%)
- In the circulating strains that could be analyzed, almost all were represented by ribotype 027 (99%)
6. Drugs used:
- the combination of metronidazole and vancomycin was the most used in the studied group (73%)

- 15% were treated with metronidazole and vancomycin was used in 9% of patients.
- In 13% of cases oral vancomycin treatment was prescribed for a long period of time, staggered, to prevent relapses

7. A percentage of 23% of patients received treatment with systemic antibiotics, other then the etiological one (21% a single type of antibiotic, 2% 2 types of systemic antibiotics).
8. Fecal transplantation was performed in 9% of the studied patients
9. The analysis of the evolution of the cases showed:
- 17% (n=37) had relapses after no more than 8 weeks after the end of the specific treatment
- In-hospital mortality was low, 1% (n=3)

10. ATLAS score calculated in 30 patients:
- 10% had a score between 1 and 5
- 4% had a score between 6 and 10

11. The average duration of hospitalization was 9.67 days
12. The comparative analysis of the epidemiological and demographic characteristics of patients with relapses vs. those without relapses revealed some differences, but without statistical significance
13. The comparative analysis of clinical and paraclinical characteristics of patients with relapses vs those without relapses showed:
- Between the average value of serum albumin in patients who had relapses (3.5 mg/dL) and the average value of serum albumin recorded in patients who did not experience relapses (2.7 mg/dL) there are statistically significant differences ($p = 0.025 < \alpha = 0.05$). Hypoalbuminemia is not correlated with relapses. Malabsorption due to changes in the intestinal mucosa requires in the future greater attention in correcting protein deficiency in patients in current practice.

14. The comparative analysis of the therapeutic characteristics of patients with relapses vs. those without relapses in the studied group showed:
- the rate of CDI relapse up to 8 weeks after the end of treatment was significantly lower in patients who were treated with oral vancomycin, extended regimen, with tapering doses compared to those who were not treated with oral vancomycin, extended regimen - 3% (1 of 29) versus 19% (36 of 187). Thus, it can be deduced that in the studied group the prevention of CDI relapses was done with oral vancomycin in a prolonged, staggered scheme. This conclusion can be extended in current practice by using the deescalated regimen of oral vancomycin in all patients with high risk of CD relapse from the first episode and possibly adding this option to the national treatment guideline.

15. There are statistically significant differences between the average length of hospitalization for patients who experienced relapses (7.95 days) and the average length of hospitalization recorded for patients who did not experience relapses (9.67 days).
16. In the study that analyzes the impact on the quality of life from a psychosocial and somatic point of view in patients infected with CD, it is highlighted that the quality of life is seriously altered, almost all patients affirming difficulties, physical and mental discomfort on the 3 monitored scales (physical suffering scale produced by the disease, the scale of psychological distress generated by the disease and the scale of daily/everyday activities). From the total of 54 patients in the sections:
- Somatic aspects 44% of patients were classified in the "Much" response category and 56% of patients were classified in the "Very much" response category.
- Emotional stability/instability in the "Much" category were assigned 37% patients and in the "Very much" category were assigned 63% patients.

- Anxiety in the "Much" category were assigned 39% patients and in the "Very much" category were assigned 61% patients.
- Depression in the category "Much" 29% patients were classified and in the category "Very much" 70% patients were classified.

17. When analyzing the correlation between the two variables under study, Gender and Emotional Stability/Instability, the results show that the risk of having male patients for whom the degree of Emotional Stability/Instability was considered "Very Much" is 3.59 times lower than the risk of having female patients

18. When analyzing the correlation between the two variables in the study Sex and Depression, the obtained results showed that the risk of having male patients for whom the degree of depression was considered "Very much" is 3.61 times lower than the risk of had female patients

19. When analyzing the correlation between Age and Somatic, Age and Anxiety, Age and Depression, the two variables are correlated, the correlation being considered positive, significant ($p = 0.0009$. $p < \alpha = 0.05$), ($p = 0.0451$. $p < \alpha = 0.05$), ($p = 0.0052$. $p < \alpha = 0.05$).

20. The correlation between Age and the score on all questions of the questionnaire showed that the two variables are correlated, the correlation being considered positive, significant ($p=0.0004$. $p < \alpha = 0.05$). On the scale of physical suffering produced by the disease, the scale of mental suffering generated by the disease and the scale of daily/everyday activities, the impairment of the quality of life in CDI patients is influenced by age

21. The obtained data draw attention to the need for a good collaboration between doctors and hospital psychologists for the supervision, psychological counseling and support given to patients with CDI whose condition greatly affects the quality of life. Functional disability, invalidity, psychosomatic development but also social insertion are influenced by the disease and only a good management of psychological counseling restores the psychological balance with restitutio ad integrum.

22. CDI remains an important public health problem, which requires the intensification of prevention and control measures, especially since the complexity of the disease and its implications in affecting the quality of life of patients can create a long-term challenge on healthcare systems.

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