

**"VICTOR BABEȘ" UNIVERSITY OF MEDICINE
AND PHARMACY OF TIMIȘOARA
THE FACULTY OF MEDICINE
DEPARTMENT XIII – INFECTIOUS DISEASES**

MARINESCU ADELINA RALUCA



PhD THESIS

**PARTICULARITIES OF ENTEROCOLITIS WITH
CLOSTRIDIODES DIFFICILE IN WESTERN ROMANIA:
RIBOTYPE / CLINICAL FORM / TREATMENT
CORRELATIONS; CO-INFECTION WITH SARS-CoV-2**

SUMMARY

PhD Supervisor
PROF. LICKER MONICA SORINA, PhD

**Timișoara
2022**

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Keywords: *Clostridioides difficile*, ribotype, clinical form, treatment, co-infection with SARS-CoV-2

INTRODUCTION

Infectious pathology, as well as other clinical manifestations specific to living beings, can not remain unchanged, being subject to various interactions with many environmental factors. We are witnessing an infectious pathology, always changed and current, which must be known by every doctor. In this way, a "pathomorphosis" of infectious diseases is carried out in front of us, which must be followed carefully.

Among the factors that have significantly influenced in recent decades, the evolution of infectious diseases, are primarily socio-economic conditions, hygiene and nutrition much changed, improved, but so varied depending on the socio-economic system of each country.

The spread of antibiotic resistance in the microbial population, especially that transmitted by resistance factor R, between germs of the same species, or of different species, poses particular problems in the future, especially in the therapy of intestinal infections. Antimicrobial resistance can be considered a negative cliché of their use in every hospital, region or clinical ward of a hospital.

The continuous circulation of pathogens around the world and the possible transmission of infectious and parasitic diseases (including quarantine ones) is a problem with considerable expansion in the future, which imposes important obligations of responsibility and international cooperation. These measures are all the more necessary if we take into account the current means of transport, as well as the increase in the number of tourists and / or other passengers, to several hundred million annually. The persistence of quarantine outbreaks in many parts of the world makes it possible at any time for these infections to spread across borders and continents, as has been shown in recent years in the case of various infectious diseases. Today we have the most eloquent example of the infection with the new coronavirus SARS-CoV-2 which has become a pandemic for about 2 years despite all the measures taken, with multiple and serious consequences for all countries.

It is very important for a doctor, regardless of his specialty, to have a complex approach to the medical act, the result being the healing of the patient as quickly as possible and at the lowest possible cost. For example, an immunocompromised patient with neoplasm, treated with cytostatics and subsequently aggravated, may become infected with *Clostridioides difficile* (formerly known as *Clostridium difficile*) during hospitalization, with severe consequences for the patient, prolonged hospitalization, and additional costs. [3].

A justified question is whether some cases of *Clostridioides difficile* (ICD) infection could be avoided. The purpose of this doctoral thesis is to try to decipher the complex mechanisms involved in this infection, which is a major public health problem worldwide; we also try to present some practical, realistic, useful solutions, in addition to the current

protocol, taking in the diagnostic equation of each case all the data and understanding the pathophysiology of this disease with such high mortality.

In the last decade, a number of changes have been reported in the epidemiology, clinical symptoms and evolution of *C. difficile* infection. The number of diagnosed cases has increased, especially in people over the age of 65, there has been an increase in the share of severe forms of the disease, the number of patients with recurrent infections and the lethality caused by this condition. These changes are mainly due to the appearance in circulation of hypervirulent subtypes of *C. difficile*. In addition, the increase in the number of patients at risk, the more frequent orientation towards the diagnosis of *C. difficile* infection and the improvement of the diagnostic capacity of this etiology, have contributed to the increase of the amplitude of these changes.

The pathogenesis of *C. difficile* is mediated primarily by the release of two toxins, toxin A and toxin B. These large toxins (TcdA, 308 kDa, TcdB, 270 kDa) function as glucosyltransferases that inactivate small GTPases such as Rho, Rac and Cdc42 in eukaryotic target cells [5]. Highlighting the ribotype in this pathology, through specific laboratory tests, becomes a key issue both by increasing the success rate in curing the disease, reducing the risk of recurrence, and by targeted and non-empirical administration of the antibiotic.

1. SCIENTIFIC OBJECTIVES

The data presented above led me to evaluate which of the following four factors can be invoked in triggering the endemic diarrhea episode caused by the *C. difficile*:

- some particular field conditions or therapeutic context;
- the degree of efficiency of the diagnostic act by using the test for the cytotoxin *C. difficile*;
- the appearance of "gaps" in the supervision of personal and intra-institutional hygiene;
- concretization of a process of "pathomorphosis" of infectious diseases.

2. RESULTS

2.1 RIBOTYPE/ CLINICAL FORM / TREATMENT CORRELATIONS

2.1.1 PERIOD 2016-2017

Between January 2016 and December 2017, 210 patients were diagnosed with the diagnosis of Acute Enterocolitis with *C. difficile*. All patients tested had a positive A / B toxin *C. difficile*.

In 2016, a number of 95 patients were diagnosed with the diagnosis of *C. difficile* enterocolitis. If we refer to 2017 we can see an increase of 20 hospitalized cases with the same pathology, totaling 115 reported cases.

The incidence of these infections was significantly higher in the age group over 61 years (totaling over half of the case study), followed by adults (40-60 years-over 20% of cases). Although it is the prerogative of the elderly, in the period under investigation there were also cases of young people under 40 (9.47% in 2016, 5.20% in 2017).

The age is considered a primary risk factor for common forms of DCI but also for severe forms.

Because elderly patients are currently the ones who develop the most common ICD, further studies are needed to explore the association between this infection and the areas of comorbidity, fragility, and polypharmacy, which are intrinsic features of hospitalized geriatric patients.

If we correlate the ribotype with the clinical form of the disease by applying the χ^2 test to see the association of the two variables, we obtain $\chi^2 = 5.9$ with $n = 2$ degrees of freedom, and $p\text{-value} = 0.0522 > 0.05$, which is why can draw a conclusion about the association of the two variables without the correction of Cramer's $V = 0.167$, which indicates a weak association. In conclusion, it can be stated that the severity of the disease depends on the shape of the ribotype - *Table 1*.

Table 1. Correlation between disease form and ribotype

Ribotype	Form of the disease			Total
	Light	Medium	Severe	
0.27	16	8	4	28
Untested	130	45	7	182
Total	146	53	11	210

In the present study applying the test χ^2 to see the association of the two variables (ribotype and treatment), we obtain $\chi^2 = 1.55$ with $n = 2$ degrees of freedom, and $p\text{-value} = 0.46 > 0.05$, so the two variables are independent, the conclusion being that there is no association between the two variables - *Table 2*.

Table 2. Correlation between ribotype and treatment

<i>Treatment/ Ribotype</i>	<i>Metronidazole</i>	<i>Vancomycin</i>	<i>Metronidazole + Vancomycin</i>	<i>Total</i>
0.27	15	8	5	28
Netestat	115	34	33	182
Total	130	42	38	210

In order to determine the association between the 3 variables (treatment, clinical form and ribotype) the regression model was used, following which we obtain:

$$\text{logit}(p) = -2.34 - 0.33 \times \text{treatment} + 0.68 \times \text{form}$$

The Hosmer-Lemeshow test shows that the logistic model is a suitable one $P = 0.6859 > 0.05$ and that 86.67% of the cases are correctly predicted - *Table 3*.

Table 3. Correlation of ribotype with clinical form of disease and treatment

Form/ treatment	Untested			Ribotype 027		
	Metronidazole	Vancomycin	Metronidazole + Vancomycin	Metronidazol	Vancomycin	Metronidazole + Vancomycin
Light	95	21	14	13	2	0
Medium	18	10	17	1	5	2
Severe	2	3	2	2	1	2

Applying the test χ^2 to see the association of the two variables (ribotype and hospital of origin of the cases), we obtain $\chi^2 = 36.29$ with $n = 24$ degrees of freedom, and $p\text{-value} = 0.0514 > 0.05$, reason for which is applied the Cramer's V correction = 0.4567, which indicates an average association between the hospital of origin and the ribotype.

Regarding the cost of hospitalization, in 2016 a hospitalized patient on average 10 days totaled a total cost of hospitalization of 2809 lei (laboratory analysis and treatment). Patients with moderate forms of the disease were considered.

In 2017, an identical hospitalization totaled 4471 lei (approximately double the cost in one year). This supports the important use of antibiotic medication against ICD on an outpatient basis. Currently metronidazole and rifaximin are the only antibiotics that can be found and prescribed by specialty pharmacies.

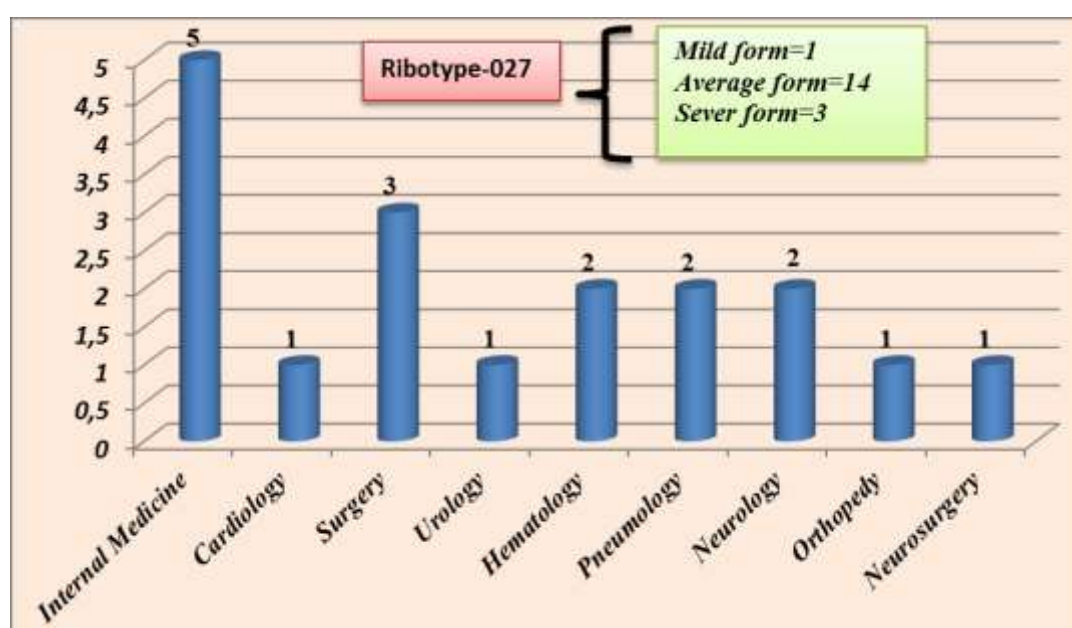
CONCLUSIONS:

- the incidence of ICD increased by 2 percent per patient compared to the period 2013-2014;
- in terms of gender distribution, both in 2016 and in 2017, females predominated;
- from the point of view of the environment of origin, the cases from the urban environment predominated, but with a slight increase of those from the rural environment compared to the period 2013-2014;
- the incidence of ICD was significantly higher in patients over 60 years of age;
- age is a primary risk factor for common forms of ICD, but also for severe ones;
- cardiovascular diseases were the comorbidity most frequently associated with DCI;
- there is an association between ribotype and ICD severity;
- no association between ribotype and treatment could be demonstrated.

2.1.2 PERIOD 2018

Between January 2018 and December 2018, there were 207 patients hospitalized in the Infectious Diseases and Pneumoftiziologie Hospital “Doctor Victor Babeș” Timișoara, with the diagnosis of Acute Enterocolitis with *C. difficile*.

All 207 patients in the study tested positive for *C. difficile* A / B, A, or B toxins. Ribotyping was performed in 18 cases. The patients were transferred from various wards of medical hospitals in the western part of the country. Binary toxin with strain 027 was identified in all cases - Image 1.

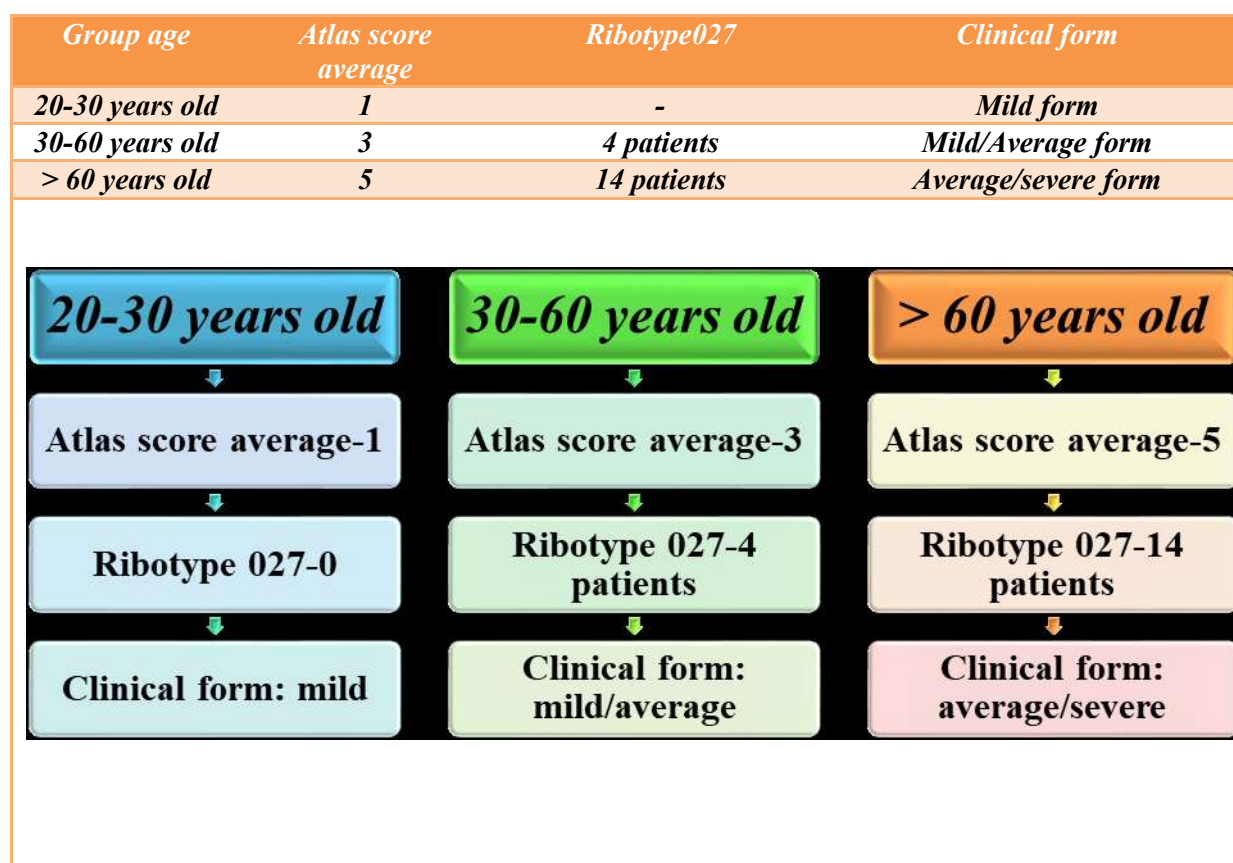


Img. 1 Correlations between the case origin section and the ribotype

The The ATLAS score was developed to predict recovery and recurrence due to ICD. It is used in the patient's bed at the time of diagnosis of ICD and each variable is assigned 0-2 points. We evaluated the usefulness of this scoring system as a predictor of the ICD mortality [115] .

Out of the 207 hospitalized patients, it was possible to observe in the age group over 60 years, 15 cases of ribotype 027 which, corroborated with the Atlas score, showed a moderate / severe form of the disease - Image 2.

The ATLAS score also remains useful for follow-up, respectively, 97% of patients at 60 days post ICD had a favorable evolution.



Img. 2 Correlation between age group, Atlas score and ribotype

Regarding the treatment regimen chosen for hospitalized patients, 58 patients (28%) received metronidazole treatment for 14 days, 93 patients (45%) received vancomycin treatment for 14 days, 34 (16%) metronidazole treatment and vancomycin 14 days and 22 (11%) metronidazole in combination with rifaximin 21 days.

Correlating the ribotype with the chosen regimen, 4 patients initiated on metronidazole treatment, being classified as a mild form, relapsed, subsequently becoming a moderate form of the disease. Ribotyping showed strain 027, which subsequently responded favorably to vancomycin treatment.

Studies in recent years have shown that vancomycin is more effective than metronidazole, and in addition to clinical experience, patients treated with vancomycin from the first episode have a lower risk of recurrence. [110, 111].

Current guidelines recommend initiating treatment with vancomycin from the first episode of ICD, including in mild forms of the disease. [116].

In this study, 3 patients in the group had a severe form of infection, corroborated with ribotype 027 and ATLAS score, which did not respond favorably to specific treatment.

CONCLUSIONS:

- 83% of the ICD cases were of nosocomial origin;
- the departments of Internal Medicine registered the highest incidence of DCI;
- 73% of ICD cases underwent antibiotic treatment prior to hospitalization;
- binary toxin with strain 027 was identified in all ribotyped cases;
- ribotype 027 corroborated with the Atlas score was highly suggestive for a moderate / severe form of ICD;
- strain 027 responded favorably to vancomycin.

3.2 SARS-CoV-2 CO-INFECTION

According to current reports, the increased emphasis on hand hygiene, environmental cleanliness, isolation of patients and the use of personal protective equipment (PPE) during 2020 could have led to a decrease in the DCI associated with healthcare in 2020 compared to 2019 [123, 126], but given the widespread use of antibiotics during the current pandemic and the overlapping gastrointestinal symptoms of COVID-19, renewed attention to ICD is required.

The characteristics of the ICD, severity, management, as well as 30 days of follow-up of the study group are presented in - *Table 4*.

Table 4. ICD characteristics, severity, management, evolution and 30-day follow-up of the 40 patients with COVID-19 and ICD

	ICD Patients (n = 40)	ICD Patients (Percentage %)
ICD with in-hospital onset	32	80
Community ICD associated with healthcare	8	20
CDI recurrence	2	5
Diarrhea before diagnosis of COVID-19	6	15
Diarrhea after diagnosis of COVID-19	34	85
Severity of ICD at diagnosis		
Light	8	20
Severe	14	35
Complicated	18	45
ICD treatment		
Vancomycin	14	35
Vancomycin and Metronidazole	4	10
Metronidazole	5	12.5
Metronidazole and Rifaximin	4	10
Vancomycin and Rifaximin	13	32.5
Follow up 30 days after discharge		
Death before discharge	9	22.5
Unfollowed follow up after discharge	2	5
Patients followed 30 days after discharge	26	
Recovered at home without rICD later	16	40
Re-hospitalization	1	2.5
Deaths related to ICD	2	5
Death, unrelated to ICD	7	17.5

Caption: rICD: recurrent episode of ICD

Regarding the evolution, 11/40 (27.5%) patients recovered completely and were discharged without complications, 18/40 (45%) developed complications at discharge and 9/40 (22.5%) patients died in hospital. ICD was the leading cause of death in two of these patients, while septic shock was considered the leading cause of death in four patients, followed by respiratory failure in two patients and heart failure in one patient. Of the patients with COVID-19, 80% of those with ICD were discharged at home and 26 patients were followed up to 30 days after discharge from hospital. No data are available for the remaining patients.

Ribotyping was performed by randomization in six patients. Age group, clinical form of DCI and complications are presented in *Table 5*.

Table 5. ICD ribotype, clinical features, treatment and evolution

		ICD group and COVID-19 (n = 6)
Age groups	< 40 years	2
	40–59 years	3
	60–70 years	1
Ribotype	027	6
Clinical form	light	-
	sever	3
	complicated	3
Treatment	vancomycin	2
	vancomycin and rifaximin	4
Evolution	deceased, related to ICD	1
	home recovery without subsequent rICD	5

CONCLUSIONS:

- 62.5% of patients with ICD and COVID-19 were female;
- 95% of patients were diagnosed with a first episode of ICD;
- in 85% of cases the onset of diarrhea and the diagnosis of ICD followed the diagnosis of COVID-19;
- cardiovascular diseases were found to be associated in a proportion of 67.5%;
- 75% of patients with ICD and COVID-19 used antibiotics before hospitalization;
- macrolides were the most common antimicrobials administered;
- logistic regression analysis identified the administration of antibiotics during hospitalization as an independent risk factor;
- 48% of ICD and COVID-19 cases developed complications;
- monitoring the emergence of *C. difficile* infection.

OWN CONTRIBUTIONS

- ICD testing in patients confirmed with SARS-CoV-2 and digestive symptoms;
- Monitoring the effectiveness of current treatment for DCI in the at-risk population;
- Supervision of the use of empirical antibiotic treatment;
- Assessment of risk factors in the occurrence of DCI;
- Identification of changes in blood parameters usually determined in an emergency, which have been shown to be risk factors for the severity of ICD;
- Calculation of costs with ICD hospitalization (2016, 2017, 2021);
- SARS-CoV-2 infection has been shown to cause haematological and immunological changes that may cause more severe forms of DCI, but is not an independent risk factor for the risk of mortality in these patients.

Starting from the evaluation of the four factors mentioned at the beginning of the thesis, invoked in triggering the endemic diarrheal episode caused by *C. difficile*, we consider that the degree of efficiency of the diagnostic act remains useful in ICD but ribotyping proved to be really effective in assessing disease therapeutic. The association of particular field conditions, such as SARS-CoV-2 infection, increases the susceptibility of individuals to the occurrence of ICD and at the same time influences its evolution. Even at a time when protective equipment was urgently needed, ICD was able to create a breach due to the process of continuous pathomorphosis.