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Review

Clustering Disease of Clostridioides Difficile Infection: Implication for the Management in Internal Medicine

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Abstract: Clostridioides difficile is a bacterium responsible for a healthcare-associated gastrointestinal infection, primarily affecting people who have undergone prolonged antibiotic treatment or who have compromised immune systems. The CD is of particular concern due to its high recurrence rates and the potential for serious outcomes, including life-threatening conditions such as pseudomembranous colitis, septic shock, and all associated conditions. Since this infection is a disease associated with other health conditions, a general vision of the problems is necessary which aims to obtain a general overview of the manifestations that generally correlate with care. Clinical reasoning following the disease-clustering method is able to produce a categorization process by grouping the possible correlations of the various conditions or factors underlying diseases on the basis of certain similarities or common models. The clustering process is performed using data analysis techniques which, by statically correlating each other, give an exact dimension of all the information related to a particular disease. In the case of CD, reasoning based on disease clustering has better clarified the practices, appropriateness in infection control, judicious use of antibiotics, and research into therapeutic and preventive strategies. This review, taking advantage of the clustering strategy, aimed to analyze the contingent conditions of the infection under examination, to reduce the incidence and impact of CD, having as its mission the improvement of the results deriving from the contrast of all those correlated pathological conditions to healthcare for the improvement of public health.

Keywords: clostridioides difficile infection; clustering disease approach; frailty

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1. Introduction

Clostridioides difficile (CD) is a gem discovered in 1935 but was thought not to be linked to any particular manifestation. Only in 1978 was it considered the agent responsible for a form of watery diarrhea related to the use of antibiotics which from an anatomical–pathological point of view manifested itself with pseudomembranous inflammation of the intestinal wall until 1978 [1]. CD originates from spots that are resistant to color and oxygen. They are also alcohol-resistant but are affected by sodium hypochlorite. Its pathogenic power is linked to the ability to produce toxins and various defense mechanisms and evasion of the host's immunological surveillance. This causes cytotoxic damage to the intestinal wall, which can range from mild clinical pictures to creating very serious complications with toxic megacolon, septic shock, and other systemic manifestations with multiorgan failure and disseminated vascular coagulation. The people most at risk of CD are fragile, institutionalized people with the presence of persistent infections who, therefore, require cumulative doses of antibiotics, antimicrobial polytherapy, and long exposure to these agents [1]. In addition to the virulence ensured by the toxins of which it is a skilled synthesizer, another important characteristic is the high rate of recurrence which sometimes makes it quite resistant to the already few classes of antibiotics capable of inhibiting its growth [1]. The need to write this review was to combine the clustering strategy and current knowledge in order to obtain a more delineated and clearer picture of one of the most important infections related to healthcare. A detailed overview of the review is outlined in Figure 1.

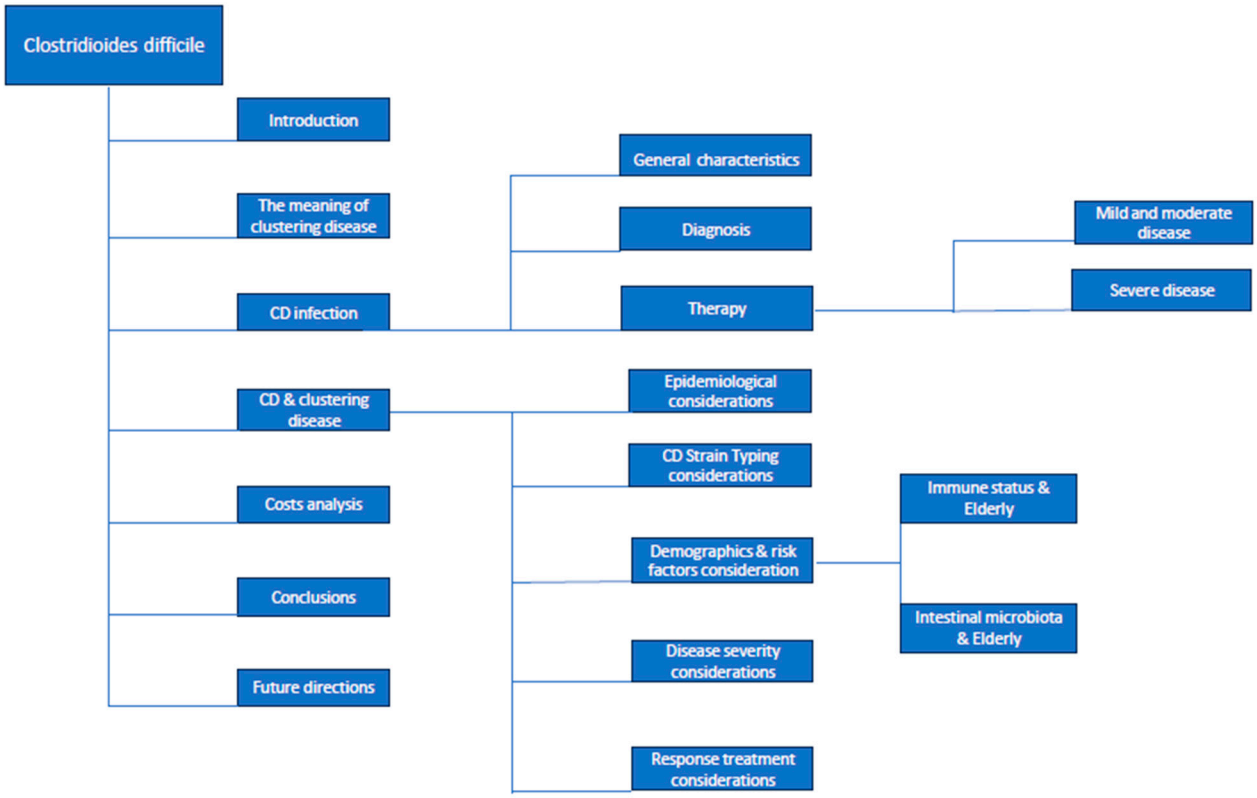

Figure 1. Chart diagram of the overall review.

2. What Is the Meaning of Medicine Based on Clustering Disease?

The presence of numerous pathologies, sometimes conflicting with each other in the same patient, today constitutes an important challenge for all those who are involved in the world of healthcare complexity in the healthcare sector [2]. Unlike multimorbidity, which implies the simultaneous presence in the same individual of two or more acute or chronic conditions, comorbidity presupposes the coexistence of any pathological event during the clinical course of a certain main disease (index disease), which is complicated by a possible hierarchical order of events between the initial pathology and the contingent conditions [2,3]. The different coexisting conditions in the same individual can influence each other, and it is able to influence the outcome of the treatments of the other coexisting ones, through various modalities: the limitation of life expectancy, the increased intercurrent morbidity, the interactions between pharmacological therapies, and the impossibility of full use of adequate treatments due to contraindications [4]. Grouping the diseases in two or more specific and concomitant conditions in the same patient can contribute to a greater knowledge of the various aspects and of the evolution a main condition may undergo. Therefore, in a study, Nobili et al. [5] have used the term cluster of several diseases to indicate the co-presence of two or more specific conditions in order to be able to establish their prognostic importance based on how they are associated with increasing the risk of mortality in patients with a so-called index disease [6,7]. Clustering diseases refers to the process of categorizing or grouping diseases based on certain similarities or patterns. Clustering is often performed using computational methods and data analysis techniques on large datasets of disease-related information. There are various reasons why clustering diseases can be beneficial:

- Disease Classification: Clustering can help in organizing diseases into meaningful groups based on similarities in symptoms, genetic factors, pathological features, or responses to treatments. This classification can aid in better understanding and management of diseases.

- Identification of Disease Subtypes: Clustering can help identify different subtypes or variants of the disease. This can be especially useful in complex diseases such as cancer, where different subtypes may require tailored treatment approaches.
- Predictive Analytics: Clustering can assist in predicting disease outcomes or patient responses to treatments based on similarities observed among different patients. This can enable personalized medicine and targeted interventions.
- Research and Drug Development: By clustering diseases, researchers can identify common molecular pathways or biological mechanisms underlying diseases within a cluster. This knowledge can guide the development of new drugs or repurposing existing ones.
- Public Health Planning: Clustering can aid in identifying disease hotspots, patterns of disease spread, or common risk factors across clusters. Such information is valuable for public health planning, resource allocation, and preventive measures.

It is important to note that clustering diseases is a complex task and requires comprehensive data, sophisticated algorithms, and domain expertise. Researchers and data scientists employ various techniques such as hierarchical clustering, k-means clustering, or advanced machine learning algorithms to achieve meaningful disease clusters. Additionally, clustering should be seen as a tool to aid in disease understanding and management, but it does not replace the need for rigorous scientific investigation, clinical expertise, and careful interpretation of results. Very few studies have explored the association between multimorbidity and CDI especially regarding the population of elderly patients with prolonged hospital stays and with numerous risk factors other than antibiotic therapy who most frequently develop CDI [7,8]. Therefore, the aim of this narrative review was to highlight the process that drives potential risk factors of CDI to begin a serious pathological condition to explore the main complications that have a greater impact on the mortality of this condition considering the frail patient with the highest care complexity.

3. Clostridioides Difficile Infection

3.1. General Characteristics

Clostridioides difficile (CD) is an obligate anaerobic Gram-positive bacterium capable of producing spores within the colon of even healthy adults and in the large intestine of healthy adults [9,10]. CD infection (CDI) is one of the most important public health problems precisely because it is closely related to hospitalization, frailty, and antibiotic treatment for other infections. The most common manifestation of CDI is the presence of watery diarrhea [11]. CDI is most commonly encountered in hospitalized, community, or nursing home elderly individuals who have received antibiotic therapy or have recovered from multiple courses of broad-spectrum antimicrobial therapy [12]. However, the most important risk factors for CDI are more general and usually include older age ($\geq$65 years), exposure to antibiotics, exposure to healthcare settings, the number of comorbidities, the use of the immune system, or capable of modifying the host's intestinal microbiota and the body's immune barriers (Gastric pH) [11]. The antibiotics most commonly associated with CDI are aminopenicillins, second and third-generation cephalosporins, fluoroquinolones, and clindamycin, which have seen an increase in their use in recent years [9]. The pathogenic power of CD is linked to its ability to produce two enterotoxins, TcdA and TcdB [13], which cause intestinal damage by activating an inflammatory cellular response with mild clinical manifestations ranging from asymptomatic colonization or mild and self-limiting diarrhea to more severe manifestations ranging from pseudomembranous colitis, which can lead to acute intestinal insufficiency with toxic megacolon, intestinal perforation, septic shock, disseminated intravascular coagulation (DIC), and death [14,15].

3.2. Diagnosis

The clinical suspicion of CDI infection is related to the detection of the germ on the stool and the search for the toxins it produces in any case of watery diarrhea related to health care or in case of unexplained diarrhea [16,17]. Although numerous laboratory tests

are available for the diagnosis of CDI, the optimal diagnostic strategy is still debated given the differences in sensitivity and specificity of the various methods. The stool cytotoxicity test (CTA) associated with the detection of toxin B (pathogenically and prognostically thought to be more dangerous) through stool culture is currently considered the gold standard due to its greater accuracy and good sensitivity and specificity [16]. Real-time PCR is the ideal diagnostic test, but performing this test takes too long for routine diagnosis and cannot be used for screening, considering the need to start targeted antibiotic therapy immediately [16]. Another assay currently used is the enzyme immunoassay (EIA) for the detection of glutamate dehydrogenase expressed by all CD strains. This test is distinguished by a good sensitivity but is not able to predict the production or otherwise of toxins in case of positivity [16,18–22]. The lack of sensitivity with respect to CTA precludes its use as a stand-alone test for the diagnosis of CDI. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [23] recommends the use of a two-step algorithm: the algorithm should start with an EIA for the detection of CD GDH and toxins A and B due to its high specificity (over 95%). Other possible combinations can be EIA and CTA, or CTA and toxigenic culture.

3.3. Therapy

Once the diagnosis has been made, the therapeutic options are varied and largely depend on the severity of the manifestations and the risk of severe complications of the infection [23].

3.3.1. Mild and Moderate Disease

Among the most frequently used drugs, oral and non-oral antibiotics sensitive to CD are to be considered first choice (by suspending antibiotic therapy, a possible cause of intestinal activation of CD), immunotherapy [14,24], probiotics, the monoclonal antibodies directed in particular against the neutralization of the toxin, and, finally, the fecal transplant. In the initial forms, the treatment of choice of the symptomatic CDI benefits from the use of oral metronidazole 500 mg three times a day for 10 days or oral vancomycin 125 mg every 6 h for 10 days, considering that the latter is considered superior for the purposes of treating this infection compared to the imidazole derivative [25–29]. However, it was noted that patients treated with vancomycin had a shorter interval of clinical improvement than those treated with metronidazole [30]. In our center located in central Italy, we prefer the association of the two drugs as a first-line treatment for the first manifestations of CDI as suggested by previous studies which concerned in particular frail and immunosuppressed patients with high care complexity [31–33]. Fidaxomycin is an effective bactericidal antibiotic in the treatment of CDI with a specificity restricted to these cases with minimal impact on the intestinal microbiota [34,35]. Some studies have compared fidaxomicin with vancomycin for the treatment of initial CDI, finding a non-inferiority of the first drug but with a much lower recurrence rate than vancomycin [36–39]. Fidaxomicin 200 mg at a dosage of two tablets a day for ten days is currently considered a first-line drug, especially in the treatment of recurrence of CDI or in patients with a high likelihood of recurrence [39,40]. Probiotics seem to be especially helpful in reducing the risk of diarrhea with prophylactic use of antibiotics [41]. However, there is insufficient evidence to recommend probiotics as an adjunct to antibiotics in the treatment of CD diarrhea [42]. Bezlotoxumab is a recombinant human monoclonal antibody IgG1/kappa isotype, effective as adjunctive treatment in patients at high risk of recurrence of CDI [43]. Specifically, bezlotoxumab blocks the action of CD B toxin (tcdB) with a significantly reduced relapse rate in patients with recurrence risk factors (older age, extensive antibiotic use) [44–47]. Currently, bezlotoxumab is recommended to prevent the recurrence of CDI, although it is currently difficult to establish which parameters predict the possibility of recurrent infection or which are the factors that generate short-term recurrence of CDI [48,49].

Fecal Microbiota Transplantation (FMT) consists of the transfer of fecal material from a healthy donor to a recipient infected by CD with the aim of restoring healthy intestinal flora

and re-establishing all the immune and physical components that make up the intestinal barrier so as to prevent colonization by CD [50–52]. Considering that CDI recurrences are often evident in patients with alterations of the microbiota and intestinal permeability, this strategy has been proposed, above all, to prevent the recurrence of the infection [53–55]. In most cases, some mild side effects have been reported, but some serious events (bacteremia, perforations, death) have also been isolated and it is also difficult to predict the long-term effects of this intervention [56,57]. Currently, it is suggested to use FMT in patients insensitive to specific antibiotic therapy after a recurrence of CDI [55].

3.3.2. Severe Disease

Severe forms should be treated preferentially with vancomycin, which appears to be superior to metronidazole in CDI (while fidaxomicin can be administered as a second choice) [55–61]. Patients with unresponsive fulminant CDI who progress to systemic toxicity, peritonitis or toxic colonic dilatation, and intestinal perforation require surgery [62,63]. Early colectomy has been shown to be associated with improved survival [62,63]. There are no universally accepted criteria that stratify severe and fulminant CDI over less severe forms. It can be said that the Severe CDI has hypoalbuminemia (serum albumin < 3 g/dL), WBC count $\geq$ 15,000 cells/mm^3, creatinine $> 1.5 \times$ baseline (or glomerular infiltration rate decreased by 25% from baseline), or temperature > 38.5 °C. Fulminant, defined as CDI, manifests itself with the characteristics of septic shock with severe hypotension and demand for vasopressors, paralytic ileus associated with toxic megacolon, mental status alterations, metabolic acidosis linked in particular to hypokalemia and serum lactate levels > 2.2 mmol/L, organ failure syndrome [64]. In these cases, in addition to the suppository therapy of the intensive type, it is possible to use in case of resistance to commonly used antibiotics, other antibiotics that show activity against CD including teicoplanin, and tigecycline [62]. In any case, the administration of these antibiotics should be strictly controlled as it is paradoxically possible an increase in the severity of diarrhea or persistence of the same and for which this therapy should be interrupted. According to some authors [65–67], such a regime is worth implementing, if the CDI additional treatment does not exceed 7–10 days. Other severe manifestations of CDI include toxic megacolon, colonic perforation, intestinal paralysis, renal failure, systemic inflammatory response syndrome, disseminated intravascular coagulation (DIC) septicemia, and death, and acknowledge intestinal infiltration, and bacteremia as important causes [62–64]. The mortality rate directly due to CDI is estimated at 5%, while the mortality associated with CDI complications reaches 15–25% and up to 34% in intensive care units (ICU) [63,68]. In the fragile immunocompromised patient with high assistance complexity, it has been observed that in the case of CDI, the risk of mortality from all causes increases in the short term [69]. Contrary to the various studies cited so far, severe forms of CDI are burdened by an increased incidence of complications related to coagulation such as deep vein thrombosis (DVT) and disseminated intravascular coagulation (DIC) [70]. Coagulation complications related to CDI depend on inflammasome activation [70]. Inflammasomes are complexes of multiple related proteins present within the cytosol of cells of the innate immune system primarily involved in the first-line activation of inflammatory responses, against pathogens and associated signals that configure tissue damage as occurs in CDI [71]. The inflammasome is activated whenever a pathogen or pathogen-associated signals induce a response of cells of the immune system which results in a structural adaptation of the cytoskeleton and microtubules, neutrophils, and macrophages forming a complex among them [72]. The main function of the inflammasome is to regulate the production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) [73,74]. When the inflammasome senses the presence of specific danger signals (such as microbial components or cellular stress), it becomes activated and triggers a cascade of events leading to the cleavage and secretion of IL-1β and IL-18. These cytokines then initiate an immune response, recruiting immune cells to the site of infection or injury and promoting inflammation [73,74]. The connection between the inflammasome and DIC lies in the fact that the activation of the

inflammasome and the subsequent production of pro-inflammatory cytokines can lead to a state of systemic inflammation. This inflammatory response, if excessive and uncontrolled, can contribute to the development of DIC. Furthermore, the inflammatory process itself can activate the coagulation system, further aggravating DIC [73,74].

4. Clostridioides Difficile Infection from the Perspective of Clustering Disease

CDI is a bacterial infection that primarily affects the colon and causes symptoms ranging from mild diarrhea to severe life-threatening conditions [14,15]. Clustering CDI involves grouping cases of the infection based on various factors to gain insights into its epidemiology, risk factors, and potential treatment approaches. Here are some key aspects of clustering CDI (Figure 2):

- Epidemiological Clustering: Clustering CDI cases based on geographic location and time can help identify outbreaks or localized clusters of the infection. This information can be valuable for implementing targeted infection control measures and investigating potential sources of transmission.
- Strain Typing: Clustering CDI strains based on their genetic characteristics can provide insights into the diversity and relatedness of C. difficile isolates. Techniques such as polymerase chain reaction (PCR) ribotyping, pulsed-field gel electrophoresis (PFGE), and whole-genome sequencing (WGS) can be used to determine strain types and identify clusters of genetically similar strains. Strain typing can help understand the transmission dynamics, track outbreaks, and assess the effectiveness of infection control measures.
- Patient Demographics and Risk Factors: Clustering CDI cases based on patient demographics (age, sex, comorbidities) and risk factors (previous hospitalizations, antibiotic usage, immunosuppression) can help identify high-risk populations or specific factors contributing to CDI occurrence. This information can guide targeted prevention strategies and clinical management.
- Clinical Presentation and Disease Severity: Clustering CDI cases based on clinical presentation (mild, moderate, severe) and disease severity can aid in understanding the spectrum of disease and identifying factors associated with severe outcomes. This knowledge can inform treatment decisions and guide interventions for high-risk patients.
- Treatment Response: Clustering CDI cases based on response to different treatment modalities (e.g., antibiotics, fecal microbiota transplantation) can provide insights into the effectiveness of various treatment approaches. It can help identify factors associated with treatment failure or recurrence and guide the development of improved therapeutic strategies.

By clustering CDI cases based on these factors, researchers and healthcare professionals can gain a better understanding of the infection, develop targeted interventions, and contribute to the overall management and control of CD infection.

4.1. Epidemiological Consideration Approaching CDI with Clustering Disease Method

In the last century, the average life expectancy has increased considerably from decade to decade in all the more developed countries and more than a quarter of the European population has at least reached the threshold of old age [75]. At first impression, this would seem to be excellent results to be attributed to scientific and technological progress, but in reality, the improved life expectancy is only partially a positive phenomenon as the social and health costs associated with the chronicity of diseases and disability have also increased. This constitutes an important economic burden and a threat to the sustainability of the countries' welfare more industrialized. The elderly population is easily targeted by the impairment of the various biological and physiological functions and, therefore, more easily the victim of infections related to the development of bacterial strains that are multi-resistant to the most common antibiotic therapies. From an epidemiological point of view, in the last twenty years, the prevalence of CDI has especially in the more industrialized western

countries and with greater availability in terms of economic resources and treatments; furthermore, it is the same countries in which the incidence of new cases of sepsis caused by multi-resistant pathogenic germs [76–78]. In Italy, the incidence of CDI among patients hospitalized in highly complex hospital wards varies from 0.3 to 22.3 episodes/10,000 days with an estimated incidence rate of 5.3/10,000 patient–days [79,80]. The risk of CDI has been shown to increase in patients with increasing age, especially in individuals residing in nursing homes. In addition to the causes linked to the economic factor, the growing incidence can be correlated to various socio-welfare factors: improvement of diagnostic technologies, adaptation of therapies and health procedures, increase in the number of elderly people in the population, and, above all, a growth of institutionalized patients with a high degree of disability and frailty [79]. Furthermore, emerging data confirm that during the recent pandemic, CDI often complicated the clinical course of patients with COVID-19, mainly among those who had more comorbidities and previous experiences of exposure in healthcare settings [81]. In addition to these causes, an important role is played by the diffusion of new hypervirulent strains and an increase in community-acquired CDI has recently been reported among individuals free of the major risk factors hitherto recognized [82–84]. Once the size of the problem has been analyzed, the adaptation of the health structures, the education of the personnel, and the containment of the infection and, therefore, of the consumption of antibiotics are the measures to be implemented to limit the spread of CD and, therefore, of its infection at both the nosocomial and community level [85,86]. Health education about CDI should include information about virulence factors and germ development, reservoirs and transmission routes, and infection and hygiene rules to be respected.

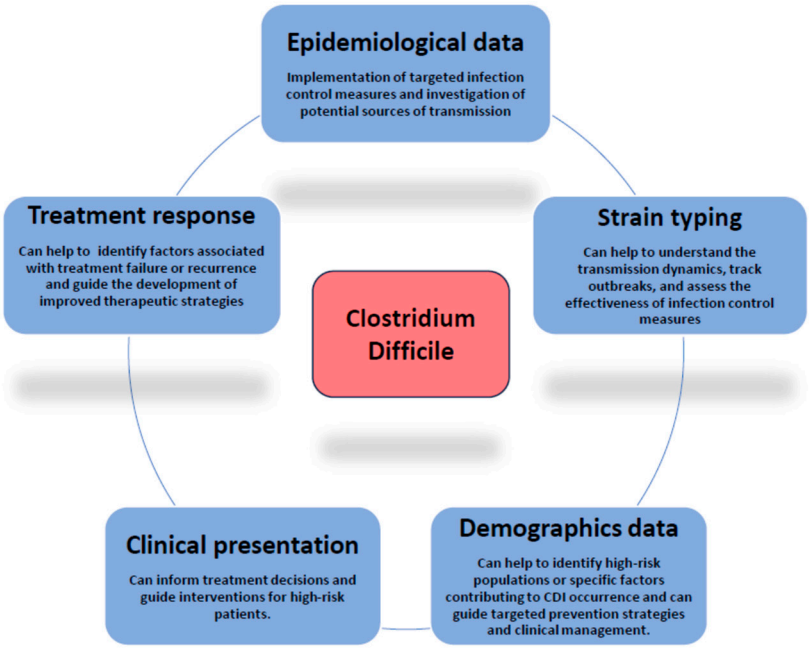

Figure 2. Clustering disease of *Clostridium difficile* infection.

In the case of CDI, these measures are essentially:

- Isolation of affections and contacts;
- Hygiene and attention in the care of affected individuals;
- Environmental disinfection;
- Management of risk factors;
- Antimicrobial management.

Contact isolation is performed in a single room with dedicated toilet facilities for a suitable period of isolation. In the case of CDI, however, it is still not very clear which parameter is decisive for dissolving the isolation, in consideration of the fact that [87] even

after specific therapy for CDI, patients can still have detectable toxins in their feces and continue to excrete the germ as asymptomatic carriers [88].

Hand hygiene and the use of gloves are one of the first aids to reduce nosocomial infections and in the case of CDI to avoid the spread of the germ and its spores [88–90]. Additional precautions can be represented by the use of disposable gowns to prevent contamination of work clothes [91–93].

Environmental sanitation is important to limit the spread of CD spores, considering the latter can persist for long periods on all surfaces due to their intrinsic resistance to heat and disinfectants except hypochlorite-based products or hydrogen peroxide [94–97].

Limiting the excessive use of antibiotics can reduce the incidence of CDI [98,99]. It is very common nowadays for hospitalized patients to receive one or more combinations of antibiotics or consecutive courses of antibiotics [100–102]. Other authors underline the need for a serious vaccination policy aimed at reducing all those bacterial or viral pathologies whose infectious complications can be prevented by effective immunization without the expense of antimicrobial therapy [103–105].

4.2. CD Strain Typing Considerations Approaching with Clustering Disease Method

The CD is a heterogeneous species, which includes both pathogenic strains, i.e., capable of producing toxins, and non-pathogenic strains. For clinical practice purposes, ideal diagnostic tests should ideally detect only toxigenic strains that have the potential to cause intestinal clinical manifestations [106,107]. Infection develops when CD spores lead to toxin production within the intestinal lumen. The two toxins, TcdA (toxin A) and TcdB (toxin B), destroy colonic epithelial cells and stimulate the release of pro-inflammatory cytokines and chemokines, reverberating an intense acute phase response initially of the intestinal type but which soon time can become systemic [106]. New detection techniques such as whole genome sequencing (WGS) and multi-locus variable number tandem-repeat analysis (MLVA) have indicated that CD can be acquired in the community from multiple sources including food [107–109]. The first clone of CD recognized was that BI/NAP1/027 spread worldwide and was associated with significant morbidity and mortality [110]. In addition to toxins A and B, this strain produces a further toxin "the binary toxin" or transferase of C. difficile (CDT), also produced by other strains (ribotype 078, 023) [111–114]. The infection caused by these strains is considered quite severe, although it has not yet been established whether it is the presence of the binary toxin that causes most damage or if there are still other virulence factors not yet known [115,116]. The presence of CD B1/NAP1/027 strains has recently decreased due to the spread of other CD strains but is still very frequent in North America and is increasing in other areas, especially in Eastern Europe [105]. In Australia, 244binary toxin-producing strains known as ribotypes have been isolated, probably as a result of the antimicrobial selection that the various CD strains are undergoing In Asia, toxin A and toxin B-producing strains remain the form more frequently isolated (ribotypes 017, 018, and 014), while cases of CD with the production of binary toxin are not reported [106]. From an epidemiological point of view, it is difficult to establish the frequency of the various strains of CD especially linked to the lack of standardization of the research techniques in each of the various laboratories. Ribotyping is the most commonly used method for CD typing in Europe and Australia and has recently also been popularized in North America [117]. Multi-locus sequence typing (MLST) and ribotyping have similar discriminatory powers but have poor accuracy in distinguishing strains, with the former sometimes being more sensitive and sometimes the latter [106]. Whole Genome Sequencing (WGS), provides high accuracy in distinguishing between strains, further aiding epidemiological investigations [106]. WGS can also be used to assemble phylogenetic trees to investigate the common origins of CD. Taking into account this evidence, it was verified the phenomenon of the migration of the various strains of CD in regions very distant from their phylogenetic origin, and this highlights how the diffusion of emerging infectious organisms is inextricably linked to globalization, and, suggests that there is necessarily a standardized international approach for the typing of CD [106].

4.3. Patient Demographics and Risk Factors Considerations with Clustering Disease Method

As we have already mentioned, one of the predisposing risk factors for CDI is represented by the age of the patient, considering that patients older than 65 are already predisposed to contracting the infection while patients older than 85 are very high risk. In addition to age, exposure to continuous and repeated courses of antibiotics, especially broad spectrum, is closely associated with CDI in older people [114]. Because normal intestinal flora plays a protective role in the prevention of CDI, the use of broad-spectrum antibiotics can cause a large expansion of the CD population [118]. Ampicillin, clindamycin, and cephalosporins are considered to be the major antibiotics predisposing to CDI, while fluoroquinolones may be the major risk factor for ribotype 027 outbreaks. Disruption by broad-spectrum antibiotics impedes cholate metabolism, which, in turn, allows germination and overgrowth of CD [118]. Immunological status is also an important factor, especially in older people: an anergic immune system increases the risk of infection. Furthermore, comorbidities represent predisposing conditions for CDI [114]. Additional factors are represented by some therapies: dialysis, parenteral nutrition, use of laxatives and statins are considered risk factors for CDI. Hospitalization is also one of the major causes of exposure to CD, especially if prolonged over time [114]. According to some meta-analyses, proton pump inhibitors (PPIs) are also related to community-acquired CDI; however, their role in gastric acid suppression facilitates the passage of CD through the stomach to the intestine [119–121].

Why do the elderly population and, therefore, the most fragile frequently suffer from an impairment of various biological functions associated with a predisposition to opportunistic infections?

The answer could be summarized in two main events: the modification of physiological and immune status in the elderly and the changes in the intestinal microbiota composition (Figure 3).

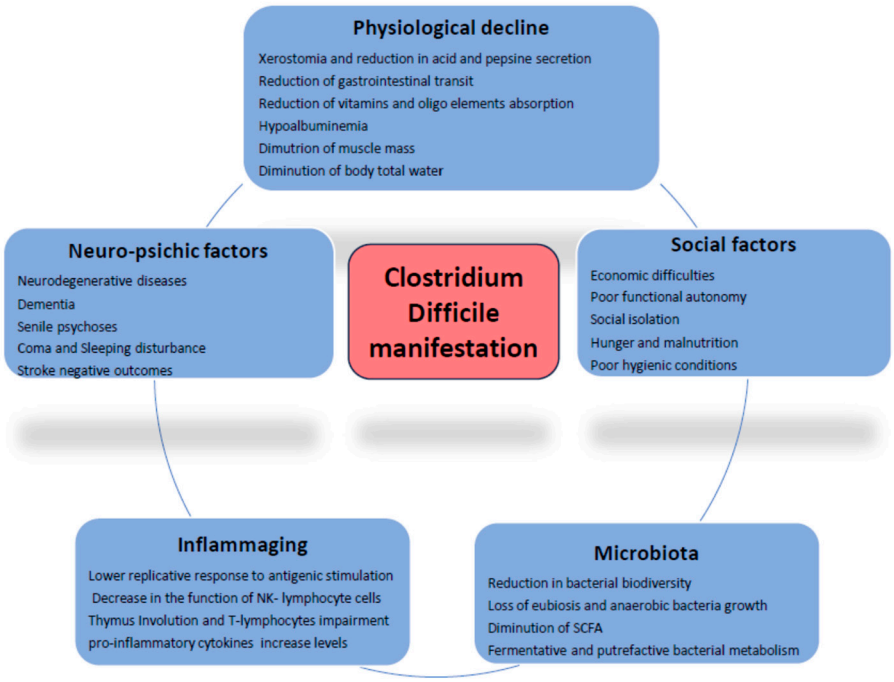

Figure 3. Association of frailty conditions and *Clostridium difficile* pathogenesis.

4.3.1. Changing in Physiological and Immune Status in the Elderly

Aging leads to an involution of the function of the immune system which is essentially linked to a lower replicative response to antigenic stimulation and to a decrease in the function of natural killer lymphocyte cells [122–124]. Furthermore, with age, we witness the physiological involution of the thymus with a consequent numerical and functional

reduction in T-lymphocytes [125,126]. While on the one hand, we find a progressive decline in the immune system, on the other, it is possible to observe an increase in the levels of pro-inflammatory cytokines [127,128]. Furthermore, with advancing, we also have an involution of the functioning of the natural barriers of our organism a decrease in the production of saliva and gastric acid secretion [129,130], and lower absorption of nutrients and essential elements for the correct functioning of the immune system such as iron and vitamin B complex, folic acid, vitamin D [131,132], largely related to the decrease in intestinal transit and the reduced enzymatic digestion and absorption of nutrients [133–135]. Elements not of a physiological nature such as economic difficulties, poor functional autonomy, social isolation, neurodegenerative diseases, and senile psychoses could affect the nutritional status of this segment of the population [136–138]. Constitutional factors affecting the composition of body mass, with an increase in fat mass at the expense of muscle and bone mass the decrease in plasma concentration and total body water content are added elements that can affect the sensitivity to infections, the immune response, and the response to antimicrobial therapy [139–142].

4.3.2. Changing of Intestinal Microbiota in the Elderly

The composition of the intestinal microbiota changes as a function of age and as a function of the physiological changes of the intestine leading to a decline in the normal function of the immune system, which, in turn, increases the risk of opportunistic infections and frailty [143–146]. In the elderly, the intestinal microbiota is characterized by a reduction in bacterial biodiversity with a significant reduction in the various strains with consequent loss of eubiosis (loss of balance) and the affirmation of dominant species, a decline in beneficial microorganisms, an increase in facultative anaerobic bacteria [147].

Gut microbiota changes that occur during old age lead to a decrease in the production of short-chain fatty acids (SCFAs), which are the major bacterial metabolites in the colon [148]. Being that SCFAs are the metabolite substrate of normal bacterial flora intestinal, the lack of this nutrient leads to a change in the metabolism which becomes fermentative and putrefactive [148,149], which is most associated with frailty [150,151]. All of these alterations taken together may explain the increased susceptibility of older people to infections. It is, therefore, easy to think that the microbiota plays an important role in the susceptibility to CD and that, in the event of its alterations, it is the deterrent for the engraftment of intestinal damage linked to its pathogenic power [152]. This deduction underlies the principle according to which intestinal microbiota transplantation could be a treatment option in these cases [153].

4.4. CDI Clinical and Disease Severity Consideration Approaching with Clustering Method

Fulminant colitis occurs in those patients where CDI presents as severe hemorrhagic colitis with bloody diarrhea and can cause systemic manifestations such as severe hypotension, multiple organ failure, and renal insufficiency. Shock, ileus, or megacolon can be complications of fulminant colitis, up to disseminated intravascular coagulation [70]. The reason why the course is indolent in some patients while others are more severe is unclear, but in general, it is possible to state that it depends on the bacterial load, the virulence of CD aggression, and the severity of the inflammatory picture in response to the cytopathic damage. The main characteristic that can determine the severity of the clinical manifestations of CD is the ability to resist most antibiotics [154–156]. In part, the mechanism of resistance of this bacterium can be explained with intestinal microbiota dysfunction [157,158], at least only at the beginning of the pathogenic process, but in most cases, it is poorly understood and multifactorial and ranges from the competitive metabolism of the various intraluminal nutrients, the ability to elicit a greater or lesser immune reaction up to the production of harmful metabolites such as cholate and lithocholate, directly intervening on the metabolism of bile acids [159,160]. Exposure to antibiotics undeniably affects the pathogenesis and severity of CDI [161,162], but in particular, the pathophysiological mechanisms through which CD toxins are able to induce a cytotoxic effect up to intestinal epithelial

necrosis are of particular importance and are still poorly understood [163]. CD expresses surface layer protein (SLP or S-layer), which is cognate to Toll-like receptor 4 (TLR4), expressed on the surface of the host cell, usually a signal recognition such as dendritic cells [164]. S-layer binding to dendritic cells stimulates nuclear transcription factor kappa β (NF-kB) and interferon regulatory factor 3, resulting in the production of inflammatory cytokines and activation of immune cells including neutrophils [164]. This demonstrates that the activation of the immune system is essential to inhibit the permeability of the intestinal barrier through the damaged mucosa [70]. Additionally, at the level of the CD flagella, there are proteins that act as activators of the immune response (flagellins of b-proteobacteria and g-proteobacteria) which are recognized by TLR5, which is expressed by enterocytes and dendritic cells. The activation of TLR5 decreases the secretion of immunoglobulin A and inhibits the generation of regulatory T cells [164,165]. In addition to Toll-like receptors (TLRs), sensors and other immune mechanisms such as intracellular nucleotide-containing domain 1 (NOD1) and IL-1β/inflammasome domain are also activated during CDI and this is associated at least in animal models, causing a reduction in neutrophil function and, therefore, increased bacterial translocation and increased clinical severity and mortality [164,165]. Toxins are also transported into the cell cytoplasm, where they inactivate the Rho protein which participates in the stabilization of the cell cytoskeleton [166]. The inactivation of Rho allows the non-recognition of the damaged cells by the immune system and lowers the secretion of inflammatory cytokines and chemotactic agents [167,168]. The synchronous action of virulence factors leads to the interaction of the damaged host cell with inflammatory cells causing the formation of the inflammasome. The inflammasome participates in the pathological process by producing inflammatory cytokines and promoting cellular apoptosis, evolving into systemic manifestations based on the virulence and bacterial load of CD. with a time-dependent mechanism [169]. This systemic inflammatory response activates the coagulation cascade and platelet aggregation. These events contribute to a poor prognosis caused by the manifestations of sepsis and by the alterations of the coagulation process leading to a risk of death [170].

4.5. CDI Response Treatment Consideration Approaching with Clustering Method

The clinical response to commonly used antibiotics for CDI depends on the virulence factors of the bacterium and, as we have said, the natural resistance to most antibiotics. This has two important consequences: the first is that the antibiotic treatment options are only somewhat limited and the second is that, thanks to the adaptation of the bacterium to the host's environment, further resistance will be created even to the antimicrobials used so far successfully [170]. Among the virulence factors, the relative contributions of the two main toxins TcdA and TcdB are well known but the pathogenic power of CD does not end with them alone [171]. Closely related to the production of the two toxins, there is also the regulatory activity of TcdC which should repress the production of TcdA and TcdB, but in conjunction with exposure to antibiotics or changes in the intestinal microflora, loses its function on the gene expression of the toxins. As a result, the expression of TcdA and TcdB is upregulated, leading to increased toxin production, which can cause more severe disease symptoms [172,173].

The discovery of the ribotype strain 027 CD, which carried the tcdC mutation and an increased expression of PaLoc responsible for encoding TcdA and TcdB, provided the binary toxin (CDT) [174]. CDT binding to host cells leads to the complete destructuring of their cytoskeleton with loss of barrier function and disruption of tight junctions [175]. The substantial contribution of the CDT is to help increase the virulence and severity of the disease, by activating the inflammatory response, with elevated levels of the cytokine IL-6 and through the Toll-like receptor pathway by suppressing the diffuse immune guest [175,176]. Collectively, these studies suggest that CDT is an important factor in treatment resistance against CD.

Another important pathogenicity factor is biofilms considered significant for the persistence and virulence of CD [177,178]. The formation of a biofilm by CD provides for

the production of a matrix after the adhesion and colonization of extracellular compounds composed of proteins, polysaccharides, and other molecules. This matrix cements the biofilm, providing stability and protection to the bacterial community [177,178]. Over time, the biofilm undergoes maturation by becoming more resistant to external stresses, including antibiotics and the host immune response. Here, the presence is considered an essential element for the response to antimicrobials making complete eradication of the infection difficult and presumably, promoting the persistence of CD in the gut [179]. The collagen-binding proteins allow for greater interaction with the host and allow for immune evasion. Among these, CD2831 is a collagen-binding protein, which interacts with the biofilm making it more stable and evading immune control [180]. The CD also synthesizes collagen-binding surface protein (CbpA) able to improve collagen interaction and adhesion to the extracellular matrix, enhancing contact with the host [181]. CD possesses the S-layer protein, composed of proteins with a lattice structure arranged over the entire bacterial surface to confer physical protection and contribute to bacterial adhesion and colonization, evading the host immune response [182]. The CD also resists the hydrolytic activity of lysozyme and this is reported in animal models as a virulence factor [183].

5. Clostridioides Difficile Costs Analysis

CDI has a high direct and indirect economic cost. In the United States, it is the most common healthcare-associated infection with an annual healthcare expenditure of $4.8 billion [1]. The excessive consumption of economic resources is linked to the fact that CDI is very frequent in older and frail people with a burden of greater pathologies with a high risk of resorting to antimicrobial therapies [184].

Considering the fact that the patient with these pathologies requires hospitalization, the high hospitalization costs are partly linked to hotel care and partly linked to the cost of the drugs used. It is, therefore, of crucial importance to prevent this condition and prevent relapses in order to obtain a reduction in direct medical costs by avoiding readmissions to the hospital [184]. In fact, a parsimonious use of proton pump inhibitors and more careful management of antimicrobial therapy have led to a reduction in the costs linked to the consumption of these drugs and to a reduction in the cases and management costs of CDI [185–187].

The need to prevent new cases and recurrence of CDI are ongoing studies for the development of an effective vaccine which would certainly lead to a decrease in the management costs of this pathology at least related to care [188]. Regarding Europe, the direct costs of treatment and indirect costs due to productivity losses of patients presenting with CDI were significantly higher in infected patients compared to controls, but for patients with relapse, the costs were even higher compared to those who had a single episode of CDI [189,190]. As regards the costs for therapeutic treatment alone, it must be said that for each individual drug, the cost-effectiveness ratio should be evaluated in terms of eradication and prevention of recurrence of CDI cases. In fact, the price of a 10-day treatment scheme with metronidazole is minimal compared to 10 days of treatment with vancomycin, while the use of fidaxomicin is over EUR 1000 if we consider the same duration of treatment. However, it is important to know that the latter drug is more effective both in treating an initial episode of CDI and significantly reducing the rate of relapses [189–191]. A German study [192] also reached the same conclusions, demonstrating that among conventional first-line treatments, fidaxomicin was found to be cost-effective compared to other drugs and this result can be attributed to the lower rate of CDI relapses. Even in the treatment of severe or severe forms of CDI and in the first recurrence of the infection, the cost-effectiveness ratio of fidaxomicin is favorable in favor of this drug [193]. Only in one Canadian study did fidaxomicin effectively reduce relapse rates by improving the cost-effectiveness ratio, although the authors specified that the majority of their patients were affected by severe CDI and a highly virulent strain [194]. It should be noted that the increase in management costs of CDI is linked to relapses and, therefore, further reducing the recurrence of the infection could lead to a saving of resources. Subsequent cost-

effectiveness analysis studies have included in addition to fidaxomicin, endoscopic fecal microbiota transplantation (FMT) for the treatment of recurrent CDI. Various simulations were used to compare FMT with tera, showing that the latter was less expensive but certainly more effective than vancomycin [195–197] and proved to be more expensive but, at the same time, more effective than metronidazole [195]. Another analysis compared FMT in first-line treatment for recurrent CDI also considering fidaxomicin, showing that the latter proved to be more economically advantageous [198]. A Canadian study [199], in addition to including the three oral antimicrobials also compared FMT, administered during colonoscopy or via a nasogastric tube, highlighting that the endoscopic approach has a greater cost-effectiveness ratio compared to other treatment options. A French study [200] compared vancomycin, and fidaxomicin with FMT performed via enema, duodenal route, and colonoscopy, demonstrating that reduced vancomycin was less expensive than FMT, but also less effective, highlighting how the rectal route of administration the best cost-effectiveness. Based on this evidence and considering the limitations of each study, it is possible to state that FMT does not have a clear indication as a first-line treatment but is to be used only in case of multiple relapses. The aforementioned limitations of the various studies concern the fact that analytical models were used, without having real expenditure data regarding the various treatments available. Furthermore, some studies included the costs of hospital stay while others only considered the expense associated with therapy. Antibiotic treatment with fidaxomicin appears to be the most convenient treatment strategy for CDI at onset and in case of recurrence, while endoscopic FMT acts as an additional treatment also allowing for cost savings linked to the reduction in hospitalization times.

6. Conclusions

The CD is a bacterium that has captured the attention of researchers, healthcare professionals, and the public due to its impact on human health, particularly in healthcare settings. As I reflect on CD, several key aspects come to mind:

- Antibiotic-Associated Infections: CDI is most commonly associated with antibiotic use. Broad-spectrum antibiotics can disrupt the balance of the gut microbiota, allowing the CD to overgrow and cause infection. This highlights the delicate relationship between our gut microbiome and overall health.
- Hospital-Acquired Infections: CDI is a significant concern in healthcare facilities, where vulnerable patients are at increased risk. The spores of CD can persist on surfaces, leading to transmission and outbreaks within hospital settings. Preventing and managing CDI is crucial for patient safety.
- Toxin-Mediated Pathogenesis: The toxins produced by C. difficile, TcdA, and TcdB play a central role in the pathogenesis of CDI. These toxins cause damage to the intestinal lining, leading to inflammation and diarrhea. Understanding the mechanisms of toxin action has paved the way for targeted treatments and research into new therapies.
- Recurrence and Challenges in Treatment: CDI can be challenging to treat, with a significant percentage of patients experiencing recurrence after initial therapy. This is partly due to the formation of spores by CD, which are highly resistant to environmental stressors and standard antimicrobial agents.
- Emergence of Hypervirulent Strains: Certain strains of CD, such as the BI/NAP1/027 strain, have been associated with more severe and recurrent infections. The emergence of hypervirulent strains adds complexity to CDI management and highlights the importance of surveillance and infection control measures.
- Biofilm Formation: CD's ability to form biofilms and surface proteins contributes to its persistence and resilience, making it challenging to eradicate from both medical equipment and the gut mucosa. Biofilms represent an area of ongoing research with potential implications for treatment strategies.
- Prevention and Infection Control: Preventing CDI is of utmost importance. Proper hand hygiene, judicious use of antibiotics, and infection control measures in healthcare settings can reduce the incidence of CDI.

- Vaccines and Therapeutics: Research efforts have focused on developing vaccines and novel therapeutics targeting CD toxins or other virulence factors. These advancements offer hope for better prevention and treatment of CDI in the future.
- CD remains a significant public health concern, but ongoing research and efforts in infection control and therapeutics are making strides in managing this bacterium and its associated infections. Understanding the complexities of CD and its interactions with the host is essential for developing effective strategies to combat CDI and improve patient outcomes.

7. Future Direction

Future directions in the treatment of CD infections may involve the following areas of research and development:

- Novel Antibiotics and Therapies: Researchers continue to explore and develop new antibiotics and antimicrobial therapies that specifically target CD. The goal is to find agents that effectively eliminate the bacterium while preserving the balance of the gut microbiota to reduce the risk of recurrence.
- Bacteriophage Therapy: Bacteriophage therapy involves the use of viruses (bacteriophages) that infect and destroy specific bacterial pathogens, including CD. As antibiotic resistance becomes a growing concern, bacteriophage therapy offers a potential alternative or adjunct treatment for CDI.
- Microbiota-Based Therapies: Fecal microbiota transplantation has shown promising results in treating recurrent CDI. In the future, more refined and targeted approaches may be developed, such as using defined microbial consortia or microbial-derived metabolites to restore a healthy gut microbiome and suppress CD overgrowth.
- Toxin-Targeted Therapies: Since CD toxins (TcdA and TcdB) are major virulence factors, therapeutics that neutralize or inhibit the toxins are under investigation. Monoclonal antibodies targeting toxins have shown potential in clinical trials and could become part of the treatment arsenal.
- Vaccines: The development of vaccines against CD toxins is ongoing. Vaccines can potentially prevent CDI or reduce disease severity by inducing an immune response against the toxins, thereby preventing toxin-mediated damage.
- Biofilm Disruption: As mentioned earlier, CD biofilms contribute to its persistence and resistance to treatment. Research focused on disrupting or preventing biofilm formation may enhance the efficacy of existing therapies.
- Antibiotic Stewardship: Improving antibiotic prescribing practices and promoting antibiotic stewardship is vital in preventing CDI. Reducing unnecessary antibiotic use can minimize the disruption of the gut microbiota and subsequently decrease the risk of CD colonization.
- Combination Therapies: The use of combination therapies, involving a combination of antibiotics, antimicrobials, or other treatment modalities, may prove effective in tackling CDI from multiple angles and minimizing the risk of resistance.

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