

Clostridium difficile – emergent hospital flora

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Abstract: *Clostridium difficile* (*C. difficile*) is a Gram-positive sporogenous bacillus strictly anaerobic, which in the last decade has become the most important anaerobic bacterium in nosocomial human pathology. *Cl.difficile* is the etiological agent of more than 20% of diarrhea postantibiotics, over 95% of pseudomembranous colitis and the first cause of nosocomial infectious diarrhea in adults.

Although this bacterium usually colonizes the intestine of vertebrates (the normal microbiota), the toxinogenic strains (*tcdA* and *tcdB*) are pathogenic in the digestive tract. Given the excessive use of antibiotics and the increased spores resistance, it is possible an environment contamination, with strains which may already be resistant to antibiotics. The main causes of this infection are decreased resistance to antibiotic-induced colonization, contamination with a pathogenic strain of *Cl.difficile*, secretion of A and/or B toxins and deficient immune response.

Due to the increasing worldwide incidence of infections with *C. difficile* on one hand and to the discovery of new ways of transmitting the infection according with some studies regarding the genetic diversity of bacterium strains on the other hand, a new approach is necessary for *C. difficile* related topics..

Keywords: antibiotics, *Clostridium difficile*, epidemiology, nosocomial infection, toxins.

INTRODUCTION

Clostridium difficile (*C. difficile*) is a Gram positive sporogenous bacillus strictly anaerobic, which in the last decade has become the most important anaerobic bacterium in nosocomial human pathology. *Cl.difficile* is the etiological agent of more than 20% of diarrhea postantibiotics, over 95% of pseudo-membranous colitis and the first cause of nosocomial infectious diarrhea in adults.

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excessive use of antibiotics and the increased spores resistance, it is possible an environment contamination, with strains which may already be resistant to antibiotics.

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Microbiological diagnosis is made by several methods and techniques for bacteria or toxins identification.

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Cytotoxicity test reveals the cytopathic effect of fecal filtrate with pg sensitivity. Immunoenzymatic assay enables a rapid diagnosis, first generation with ELISA, the second generation by immunoenzymatic or immuno-chromatography cassette. Molecular biology techniques based on quantitative real-time PCR detect *tcdA* and *tcdB* genes in stool, responsible for toxigenesis with very good sensitivity and specificity. Through cultivation and microscopy *C. difficile* can be revealed in the stool or on contaminated surfaces; spores are resistant in the environment and are found in nosocomial flora. A characteristic enzyme, glutamate dehydrogenase (GDH) can be revealed in stool by immunoenzymatic assay correlated with the outcome of cultivation, or latex agglutination test with antiGDH antibody.

Due to the increasing worldwide incidence of infections with *C. difficile* on one hand and to the discovery of new ways of transmitting the infection according to some studies regarding the genetic diversity of bacterium strains on the other hand, a new approach is necessary for *C. difficile* related topics.

CLINICAL

Clostridium difficile (*C. difficile*) is a Gram positive, spore forming bacteria, spread by the fecal-oral route. It is non-invasive, produces toxins A and B, which cause disease, ranging from asymptomatic carriage, to mild diarrhea, to colitis, or pseudo-membranous colitis. *Clostridium difficile* infection (CDI) is defined as the acute onset of diarrhea with toxigenic *C. difficile* or its toxin and no other cause for diarrhea.

Since 2000 the rate of CDI has been increasing, especially in the elderly with a recent hospitalization or residing in long-term care facility (LTCF).

Carriage of *C. difficile* occurs in 5–15% of healthy adults, up to 57% in residents in LTCF and can reach 84.4% in newborns and healthy infants.

In simple diarrhea cases, the classic symptoms may not occur and the endoscopic examination shows

normal or ulcerated mucous; in 25% of cases ending the antibiotic therapy was followed by clinical recovery in 2-3 days. Further on antibiotherapy is a prolonging factor of diarrhea relapse.

Pseudo-membranous colitis represents up to 9% of CDI and starts with abundant watery diarrhea, over 7 stools a day, with heterogeneous no bleeding aspect. They are accompanied by fever in 75% of cases and abdominal pains in 70% cases. These symptoms are non-specific, leukocytosis up to $\times 80.000$ PMN/ μ l, extracellular dehydrating caused by exudative enteropathy.

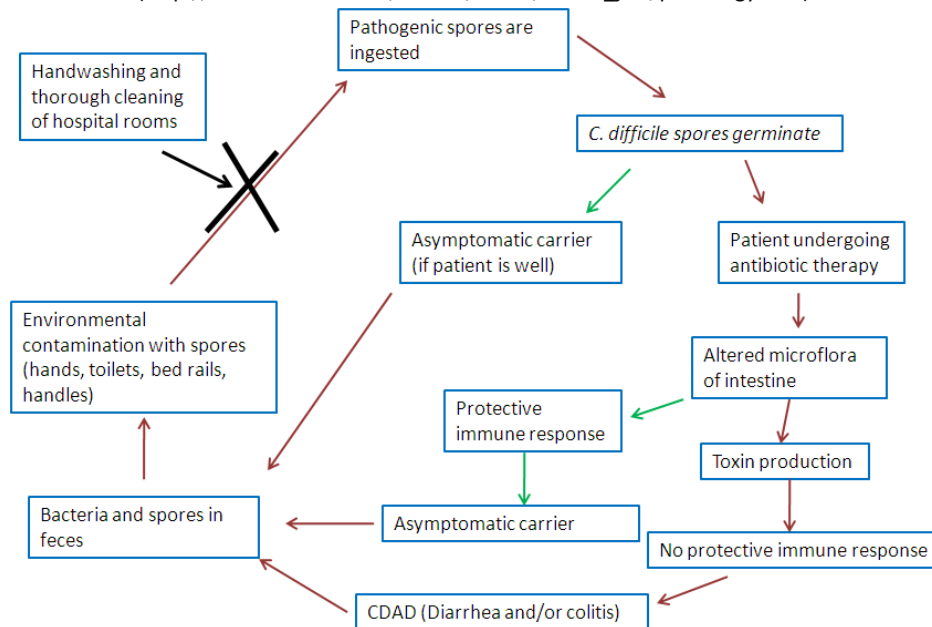
Digestive endoscopy confirms the diagnosis, allowing canker yellowish sores visualization, named pseudo-membrane, on mucous colon membrane. In the first stage they are isolated, afterwards they come together. In CDI forms with severe onset and no obvious etiology of diarrhea an endoscopy is recommended, but this test is difficult to perform on aged and fragile patients. Complications such as septic shock and toxic megacolon may occur, septic shock and toxic megacolon occur and provoke the colon perforation (colectomy required) and even death.

The ratio of severe forms differs (7-18%), depending on the studies we consider. Consecutive mortality with *C. difficile* varies 0,6-3% and when complications occur is 35-50%. Some studies show increased mortality in North America, a double number of cases in EU, heading to 24/million, *C. difficile* being involved in death cases three times more frequent than *Staphylococcus aureus* MRSA. In 20% of cases, relapses appear in the first two months after the initial episode. In over 50% of cases they are connected with the persistence of pathogen strain (spores) inside digestive tract; a new strain could appear and provoke reinfection especially during hospital admission. Multiple strains have been identified during one episode of infection. Approximately 3% of adults are asymptomatic carriers and often with toxin-free strains and sometimes specific toxins may be identified in some asymptomatic patients stool. The asymptomatic transmission of toxinogen strains in neonates is 5-70%, but there is no explanation what

so ever. Although nosocomial infections are the most frequent, some of them could be communal. There are recorded 17.5% postantibiotic diarrhea in EU, from which 66% have one day manifestation. After two weeks of antibiotherapy, the frequency becomes 3.8%, from which 70% are toxic. In North America were identified a lot of cases but no strain high pathogen 027 had been isolated in

communal infectious. Differential diagnosis will be made with other infectious diarrhea: bacterial, viral, fungus and parasitic or non-infectious causes; for example, the outcome of some "cool" drugs is in reality laxative ones (supplements for straitening the immunity, sugar free sweets, food with magnesium and decaf products) with no connection with CDI etiology. [Duker Freuman T., 2014]

Figure 1. Pathogenesis of *Clostridium difficile* –associated disease (http://bioweb.uwlax.edu/bio203/s2009/kumm_jakl/pathology.htm)



MICROBIOLOGICAL DIAGNOSTIC

CDI diagnostic is based on revealing the toxins in stool or isolating a toxinogenic strain of *Cl. difficile*, this being the only pathogenic strain. Diagnostic testing for *C. difficile* has rapidly evolved in the past decade. Previously, toxin A + B EIAs were the most widely used diagnostic tests because of ease of use and objective interpretation.

However, EIA tests have substantially reduced sensitivities compared with reference standards. Moreover, toxin A immunoassays (without toxin B) lack detecting the small number of pathogenic strains that only produce toxin B. Two major advances in the laboratory diagnosis are the use of GDH detection in stools as a means of screening for CDI and the development of Nucleic acid amplification tests (NAATs) such as PCR to detect toxinogenic strains of

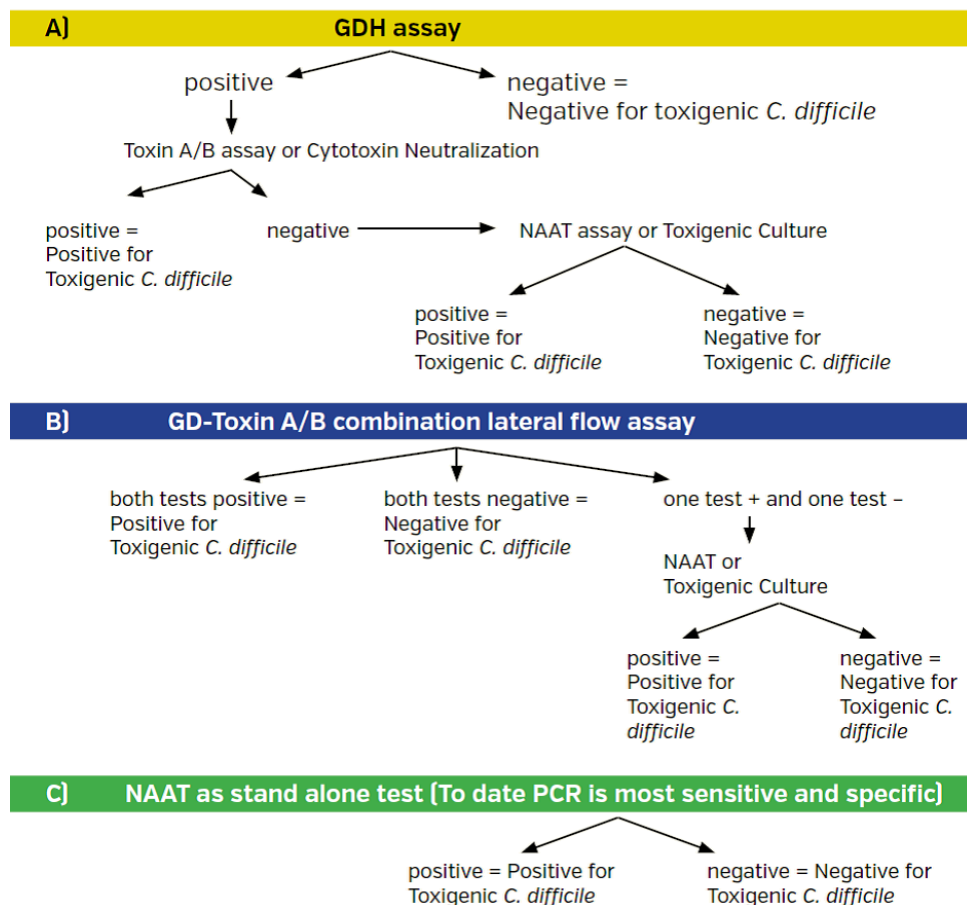
C. difficile. Glutamate dehydrogenase (GDH) screening tests for *C. difficile* can be used in two- or three-step algorithms with subsequent toxin A + BEIA testing, but the sensitivity of such strategies is lower than NAATs [Surawicz et al., 2013] (fig 2).

Testing the toxinogenic *C. difficile* should be limited to patients with > 3 nonformed stool specimens per 24 hr period, unless ileus (obstruction) is suspected. Repeat testing following a positive test (test of cure) is not recommended since patients may carry toxinogenic *C. difficile* for months after clinical cure. Repeated testing following a positive test is appropriate if the patient improves with therapy and relapses after the completion of a treatment regimen (clinical relapse). Testing a second specimen from a negative patient is more likely to be a false positive [American Society for Microbiology, 2010].

The optical microscopy swab is pathognomonic, revealing long gram-positive bacilli with a bulge at terminal ends, with long terminal and isolated spores, visible with Gray coloration. While the presence of *C. difficile* can be suspected, we cannot differentiate the pathogenical strains from the

nonpathogenic ones, therefore the examination should be supplemented with toxigenic and molecular biology tests. In the last years, a very pathogenic and virulent strain, *C. difficile* 027, has been identified, that causes severe epidemic episodes (Fig 3).

Figure 2. Diagnostic algorithm of *Clostridium difficile* (Surawicz et al., 2013)

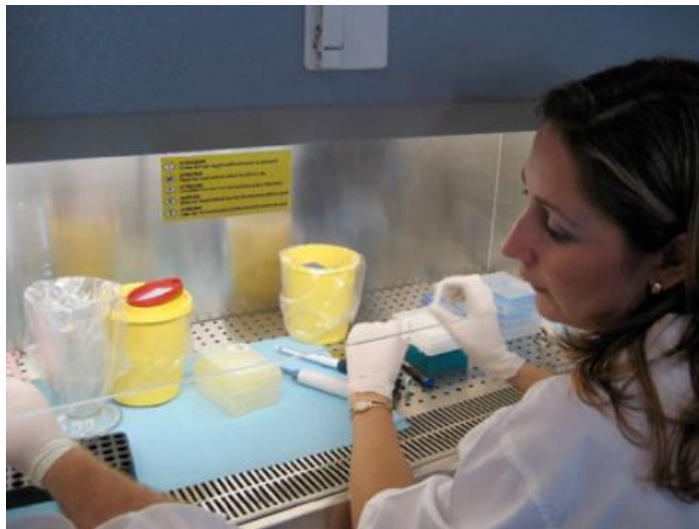


The epidemic strain currently described in North America and EU, has the following features: PCR ribotype 027 in accordance with Anaerobe Reference Laboratory surveillance data [ECDC, 2006], pulsotype NAP 1 on pulsed-field electrophoresis, enzymatic restriction-profile B1, toxinotype III by Rupnik toxinotyping method, positive for binary toxin actinia-specific ADPribozyltransferase, deletion of 18 bp in *tcdC* gene controlling the expression of toxins A and B, hyperproduction of toxins A and B (Ax16 and Bx23) in comparison with strains of other genotypes, resistant to macrolides (erythromycin) and lincosamides (clindamycin).

(moxifloxacin, gatifloxacin and levofloxacin).

Only specialised laboratories are able to perform the techniques for identifying these features and a two weeks period is required for confirmation [INVS, 2006].

In practice, CDI diagnostic is based on toxin B detection in stool or revealing the toxigen strain. A- and B+ strains cannot be detected by current immunoenzymatic assays which detect only A stain.

Figure 3. Analysis of anaerobic bacterial isolates in the microbiology laboratory of CCSMM

The strain isolation through culture is a necessary stage for epidemic clone 027 characterisation; PCR profile identification provides the certainty diagnosis.

This clone presence is clinically suspected if a severe form of the disease is diagnosed, epidemiologically suspected if several cases occur, or microbiologically suspected if the isolated strain is resistant to new fluoroquinolones (moxifloxacin CMI > 4 mg/l) or to erythromycin (CMI > 256 mg/l).

These characteristics are not specific to clone 027, but justify the stool culture in anaerobiosis in order to isolate the responsible stain and to send it to a specialised reference laboratory for further examination.

The genes encoding TcdA and TcdB, *tcdA* and *tcdB*, respectively, have been sequenced and are found in single open reading frames located within a 19.6-kb pathogenicity locus (8, 38).

As expected, both open reading frames are large, with *tcdA* found within an 8,133-nucleotide region and *tcdB* is 7,098 nucleotides in length (fig.4).

Both *tcdA* and *tcdB* are low-G C (28%) genes, which are comparable to the G C content (29%) of the *C. difficile* genome, and the toxins exhibit a high degree of overall similarity (66%).

Given the proximal locations of *tcdA* and *tcdB* and

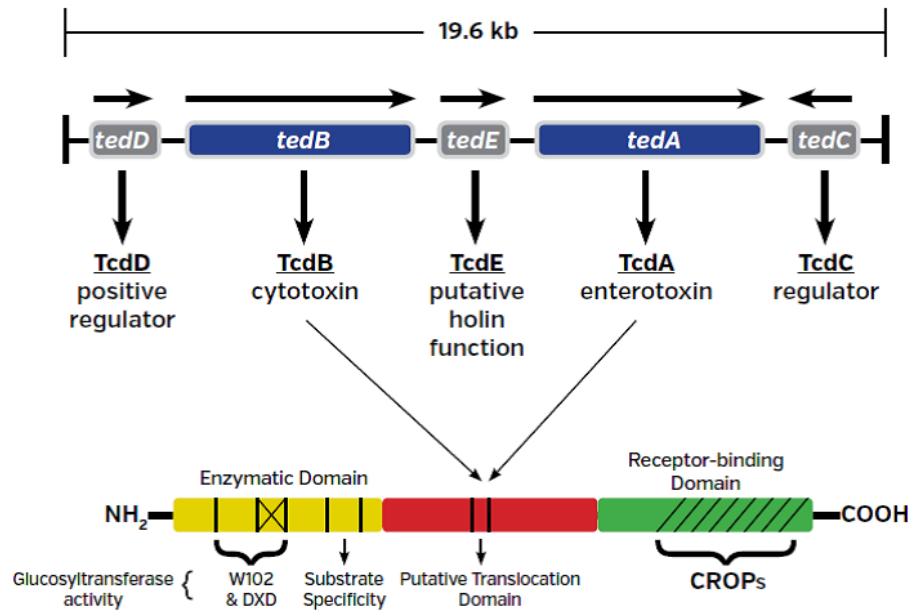
the high sequence and functional homology between the two proteins, it has been proposed that the two genes may have arisen as the result of a gene duplication event.

Furthermore, the similarity in the biochemical activity of TcdA and TcdB, wherein both toxins use a highly conserved N-terminal domain to modify identical substrates, supports the notion of gene duplication. The major regions of homology between TcdA and TcdB fall within the enzymatic and receptor-binding domains of the two toxins. The N-terminal domains of TcdA and TcdB show 74% homology, and this homology provides a basis for the similar substrate specificity of these two toxins.

The C terminus of TcdA and TcdB show a number of short, homologous regions termed combined repetitive oligopeptides (CROPs). TcdA encodes five groups of CROPs, which range in size from 21 to 50 residues and can be repeated throughout the C terminus of the protein. TcdB also encodes five groups of CROPs, four of which show homology to the CROPs of TcdA.

Yet the CROPs found in TcdB are more divergent and less frequent than those found in TcdA. CROPs appear to play a putative role in initial target cell interaction and receptor binding, but the mechanism explaining the necessity for these repeats in cell binding remains unclear [Daniel E. Voth, 2005].

Figure 4. Genetic arrangement of the *C. difficile* pathogenicity locus and proposed protein domain structures of TcdA and TcdB. Both TcdA and TcdB are encoded on the 19.6-kb pathogenicity locus. In addition to the two toxin genes *tcdA* and *tcdB*, three additional regulatory open reading frames are located on this island. *tcdD* is a proposed positive regulator, *tcdE* is a putative holin protein, and *tcdC* is a proposed negative regulator of toxin gene expression. Through deletion mutagenesis, research combined from multiple research groups has revealed a three-domain structure of the large clostridial toxins. The glycosyltransferase activity is located at the N terminus of the protein, and the C terminus is involved in receptor binding. Located in the middle domain of the protein is a putative transmembrane segment that is thought to be involved in membrane translocation. [Daniel E. Voth, 2005]



EPIDEMIOLOGY

C. difficile transmission is made by fecal-oral route, by hands and contaminated objects or environment. The fast transmission in healthcare environments is a result of several factors: strain dissemination in CDI patients, half of samples from patients' rooms being positive; high resistance of spores on inert supports for several months; too many patients crowded in common healthcare settings; numerous healthcare maneuvers creating a high possibility of contamination by the medical personnel hands; inadequate usage of antibiotics which diminishes the resistance to colonization and facilitates *C. difficile* development.

The main individual risk factors are the advanced age and antibiotherapy. There are several studies which correlate the consumption of some classes of antibiotics with CDI incidence: clindamycin, 3rd generation cephalosporins, macrolides, and amoxicillin with clavulanic acid, 1st

generation cephalosporins and fluoroquinolones. It seems that the role of fluoroquinolones in *C. difficile* O27 strains' emergence and spreading is connected to the resistance level towards them [INVS, 2006].

All factors stimulating the digestive ecosystem alteration, like laxatives, antacids, antiseptics, transit retarders, barbiturates, transit, gastrointestinal surgery, etc. may facilitate this infection [Duker-Freuman, 2014].

In March 2014, an epidemic episode with 31 cases of post-antibiotic *C. difficile* infection was recorded in Ploiesti Emergency Hospital (Romania) and the patients were isolated and treated. Most of them were aged people from Neurology, Nephrology and Intensive Care Unit [Libertate newspaper, 2014].

In May 2014 the Ministry of Health of Romania gave the alert for *C. difficile* in Vaslui and Bucharest hospitals. The beginning of the year is worrying, in only 4 months, in Bucharest health facilities were registered 462 infected patients [Pro

TV, 22 Mai 2014].

In accordance with Annual epidemiological report: Reporting on 2011 surveillance data and 2012 epidemic intelligence data, 2013, uttered by European Centre for Disease Prevention and Control (ECDC), 48% cases of HAI (Healthcare-Associated Infections) associated with gastro-intestinal infections were connected with *C. difficile*, and from all HAI (15.000 cases) in 3 only 5,4% of cases the *Clostridium difficile* has been isolated. Taking into consideration that in Romania over 92.3% of patients were the beneficiary of an antimicrobial prophylaxis during more than a day surgeries, the HAI risk associated with *C. difficile* is very high [ECDC, 2013]

TREATMENT

There is worldwide observed natural resistance and/or acquired to the medicines of the quinolone group.

A mild CDI can usually be controlled by withdrawing treatment with the antibiotics causing the infection (25% of patients could recover in 2-3 days). More severe cases can be treated using an oral specific treatment with metronidazole (1g/day) or vancomycin (1-2g/day) for 10 days. The metronidazole is a better choice, being a less expensive treatment with no risk of selecting glycopeptides resistant germs like golden enterococcus and staphylococcus.

Failure to respond to metronidazole therapy within 5 – 7 days should prompt consideration of a change in therapy to vancomycin at standard dosing. For mild-to-moderate CDI in patients who are intolerant/allergic to metronidazole and for pregnant/ breastfeeding women, vancomycin should be used at standard dosing. In patients in whom oral antibiotics cannot reach a segment of the colon, such as with Hartman's pouch, ileostomy, or colon diversion, vancomycin therapy delivered via enema should be added to treatments (500 mg in 100 – 500 ml of normal saline every 6 h) until the patient improves.

However, relapse is common and requires further treatment with repeated series of

metronidazole or vancomycin, in high doses first and smaller doses associated with probiotics (i.e. *Saccharomyces boulardii*) after improvement. Severe cases may need intensive care for maintaining the vital functions and even surgical treatment for colectomy (in case of toxic megacolon or colon perforation).

CT scanning is an important technique for perforation diagnosis in comparison with colonoscopy technique which presents a perforation risk due to gas inflation. The antibiotic treatment for healthy individuals colonized with *C. difficile* is not recommended, being inefficient for eradicating this bacteria in digestive tract. [Ordeanu, 2010; Ordeanu 2012]

Considering the antibiotherapy limitations, there has been designed the fecal bacteriotherapy, known as "stool transplant"/fecal microbiota transplant (FMT) of bacterial flora acquired from the feces of a healthy donor to reverse the bacterial imbalance responsible for the recurring nature of the infection, with good results [ASGE, 2013].

This "synthetic stool" is a super-biotic obtained using several cultures of saprophyte intestinal culture [Allen-Vercoe, 2013]. Studies show that patients with recurrent CDI (RCDI) have abnormally proportioned colon microbiota, and that reintroduction of normal bacteria via donor feces corrects this imbalance, restoring phylogenetic richness and colonization resistance.

There is no international consensus for defining and surveillance CDI, but we have to consider local (regional and national) epidemiology conditions and possibilities. ECDC created a working group for early detection and monitoring the CDI. They have suggested recorded signals criteria for severe and grouping cases of CDI.

C. difficile infectious can usually be prevented by practicing good hygiene in healthcare environments, such as: individual bed space, washing hands regularly (mechanical action of washing after gloves removal), using gloves, protection mask, glasses and gown in bed space area and in contact with patients, using medical supplies

for one use only, cleaning surfaces using bleach wipes of sodium hypochlorite containing 0.5 % active chlorine, and patient removal limitation. [CCLIN, 2013]

COMMENT

Due to the increasing worldwide incidence of infections with *C. difficile* on one hand, and to the discovery of new ways of transmitting the infection according with some studies regarding the genetic diversity of *C. difficile* strains on the other hand, (<http://www.pharmacypracticenews.com>) a new approach is necessary for *C. difficile* related topics it is important to adopt NAAT testing alone or a 2 or 3 step algorithm for CDI diagnosis.

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If the *C. difficile* is confirmed and classified as a severe form or in an epidemic context it should be reported to Public Health Territorial Authorities and to The Anaerobe Reference Laboratory from INCDMIC Cantuzino, for a clear diagnosis and adequate measures.

CONCLUSION

Due to the increasing worldwide incidence of infections with *C. difficile* on one hand and to the discovery of new ways of transmitting the infection according with some studies regarding the genetic diversity of bacterium strains on the other hand, a new approach is necessary for *C. difficile* related topics.

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