REVIEW ARTICLE

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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 became the most important anaerobic bacterium in nosocomial human pathology. Cl.dificile 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 Grampositivesporogenous bacillus strictly anaerobic,which in the last decade has became the mostimportant anaerobic bacterium in nosocomialhuman pathology. Cl.dificile is the etiological agentof more than 20% of diarrhea postantibiotics, over95% of pseudo-membranous colitis and the firstcause of nosocomial infectious diarrhea in adults.

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The main causes of this infection are decreasedresistance to antibiotic-induced colonization, contaminationwith a pathogenic strain of Cl.difficile,secretion of A and/or B toxins and deficient immuneresponse.

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

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Cytotoxicity test reveals the cytopathiceffect of fecal filtrate with pg sensitivity. Immunoenzymaticassay enables a rapid diagnosis, firstgeneration with ELISA, the second generation byimmuno-enzymatic or immuno-chromatographycassette. Molecular biology techniques based onquantitative real-time PCR detect tcdA and tcdBgenes in stool, responsible for toxigenesis with verygood sensitivity and specificity. Through cultivationand microscopy Cl. difficile can be revealed inthe stool or on contaminated surfaces; spores areresistant in the environment and are found in nosocomialflora. A characteristic enzyme, glutamate dehydrogenase (GDH) can be revealed in stool byimmuno-enzymatic assay correlated with the outcomeof cultivation, or latex agglutination test withantiGDH antibody.

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the geneticdiversity of bacterium strains on the other hand,a new approach is necessary for C. difficile relatedtopics.

CLINICAL

Clostridium difficile (C. difficile) is a Grampositive,spore forming bacteria, spread by thefecal-oral route. It is non-invasive, producestoxins A and B, which cause disease, ranging fromasymptomatic carriage, to mild diarrhea, to colitis,or pseudo-membranous colitis. Clostridium difficileinfection (CDI) is defined as the acute onset ofdiarrhea with toxigenic C. difficile or its toxin andno other cause for diarrhea.

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

Carriage of C. difficile occurs in 5– 15% of healthyadults, up to 57 % in residents in LTCF and canreach 84.4 % in newborns and healthy infants.

In simple diarrhea cases, the classic symptomsmay not occur and the endoscopic examinationshows normal or ulcerated mucous; in 25% of casesending the antibiotic therapy was followed by clinicalrecovery in 2-3 days. Further on antibiotheraphyis a prolonging factor of diarrhea relapse.

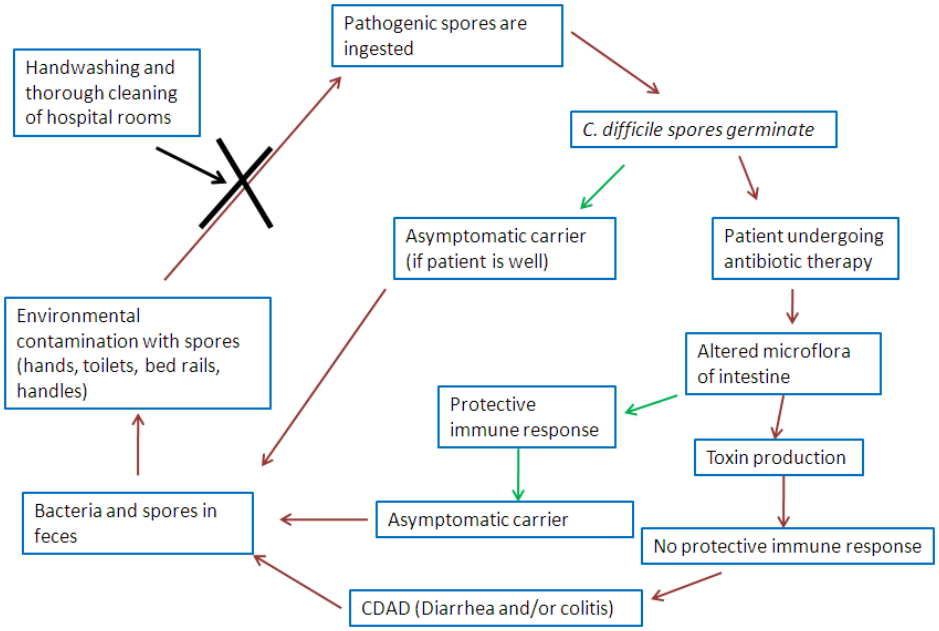
Pseudomembranous colitis represent up to 9%of CDI and starts with abundant watery diarrhea,over 7 stools a day, with heterogenic no bleedingaspect. They are accompanied by fever in 75%of cases and abdominal pains in 70% cases. Thesymptoms are non-specific, leukocytolysis up to ex80.000 PMN/l¼l, extracellular dehydrating caused by exudative enteropathy.

Digestive endoscopyconfirms the diagnosis, allowing canker yellowishsores visualization, named pseudomembrane, onmucous colon membrane. In the first stage theyare isolated, afterwards they come together. In CDIforms with severe onset and no obvious etiology of diarrhea an endoscopy is recommended, butthis test is difficult to perform on aged and fragilepatients. Complications such as septic shock andtoxic megacolon may occur, septic shock and toxicmegacolon 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. Consecutivemortality with C. difficile varies 0,6-3% and whencomplications occur is 35-50%. Some studies showincreased mortality in North America, a doubleno. of cases in EU, heading to 24/milion, C. difficilebeing involved in death cases three times morefrequent than Staphilococcus aureus MRSA.In 20% of cases, relapses appear in the firsttwo months after the initial episode. In over 50%of cases they are connected with the persistenceof pathogen strain (spores) inside digestive tract;a new stain could appear and provoke reinfectionespecially during hospital admission. Multiple strains have been identified during one episode ofinfection. Approximately 3% of adults are asymptomaticcarriers and often with toxin-free strainsand sometimes specific toxins may be identified insome asymptomatic patients stool. The asymptomatictransmission of toxinogen strains in neonatesis 5-70%, but there is no explanation what

so ever.Although nosocomial infections are the mostfrequent, some of them could be communal. Thereare recorded 17.5% postantibiotics diarrhea inEU, from which 66% have one day manifestation.After two weeks antibiotherapy, the frequency becomes 3.8%, from which 70% are toxic. In NorthAmerica were identified a lot of cases but no strainhigh pathogen 027 had been isolated in communalinfectious. Differential diagnosis will be made withother infectious diarrhea: bacterial, viral, fungusand parasitic or non-infectious causes; for example,the outcome of some "cool" drugs is in realitylaxative ones (supplements for straitening theimmunity, sugar free sweets, food with magnesiumand decaf products) with no connection with CDIetiology. [Duker Freuman T., 2014]

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



MICROBIOLOGICAL DIAGNOSTIC

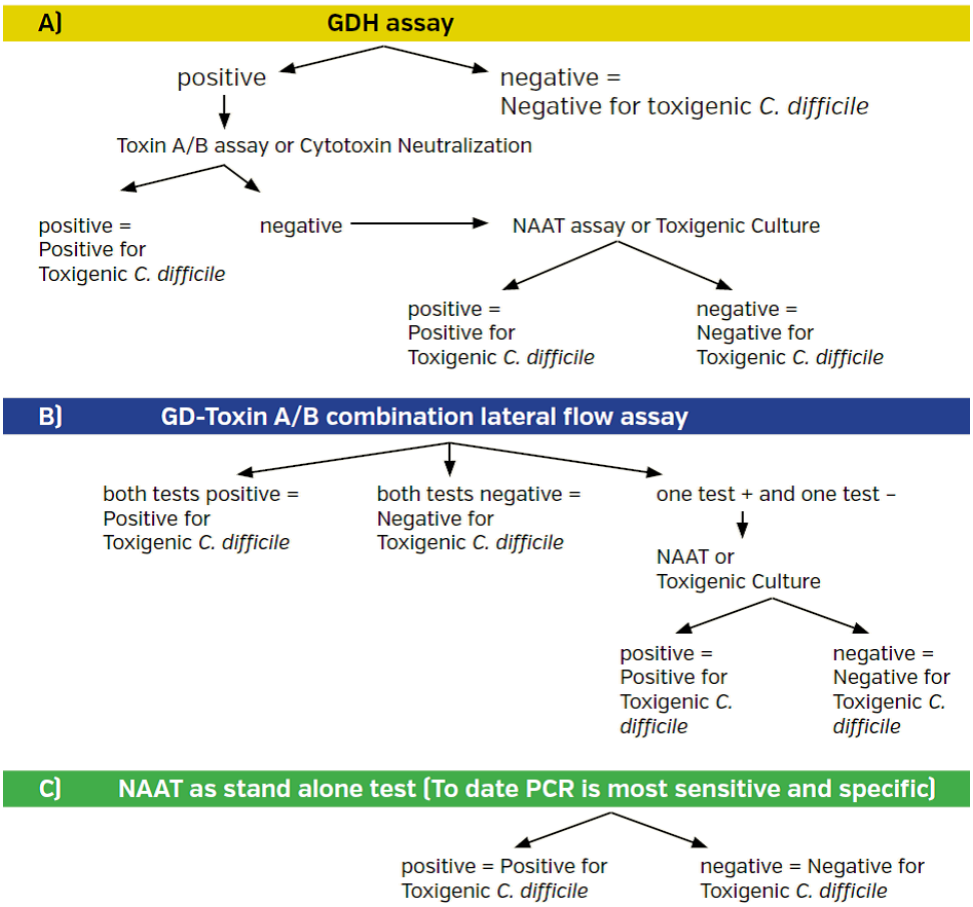
CDI diagnostic is based on revealing thetoxins in stool or isolating a toxinogenic strain ofCl.difficile, this being the only pathogenic strain.Diagnostic testing for C. difficile has rapidlyevolved in the past decade. Previously, toxin A +B EIAs were the most widely used diagnostic testsbecause of ease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

However, EIA tests have substantially reduced sensitivitiescompared with reference standards. Moreover,toxin A immunoassays (without toxin B) lackdetecting the small number of pathogenic strainsthat only produce toxin B. Two major advances inthe laboratory diagnosis are the use of GDH detection in stools as a means of screening for CDI andthe development of Nucleic acid amplification tests(NAATs) such as PCR to detect toxigenic strainsof C. difficile. Glutamate dehydrogenase (GDH)screening tests for C.difficile can be used in two- orthree-step algorithms with subsequent toxin A + BEIA testing, but the sensitivity of such strategies islower than NAATs [Surawicz et al., 2013] (fig 2).

Testing the toxigenic C. difficile shouldbe limited to patients with > 3 nonformedstool specimens per 24 hr period, unless ileus(obstruction) is suspected. Repeat testing following apositive test (test of cure) is not recommended sincepatients may carry toxigenic C. difficile for monthsafter clinical cure. Repeated testing following apositive test is appropriate if the patient improveswith therapy and relapses after the completion ofa treatment regimen (clinical relapse). Testing asecond specimen from a negative patient is morelikely to be a false positive [American Society forMicrobiology, 2010].

The optical microscopy swab is pathognomonic,revealing long gram-positive bacilli with a bulgeat terminal ends, with long terminal and isolatedspores, visible with Gray coloration. While thepresence of C. difficile can be suspected, we cannotdifferentiate the pathogencal strains from the nonpathogencalones, therefore the examination shouldbe supplemented with toxigenical and molecularbiology tests. In the last years, a very pathogenicaland virulent strain, C. difficile 027, has beenidentified, that causes severe epidemic episodes (Fig 3).

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

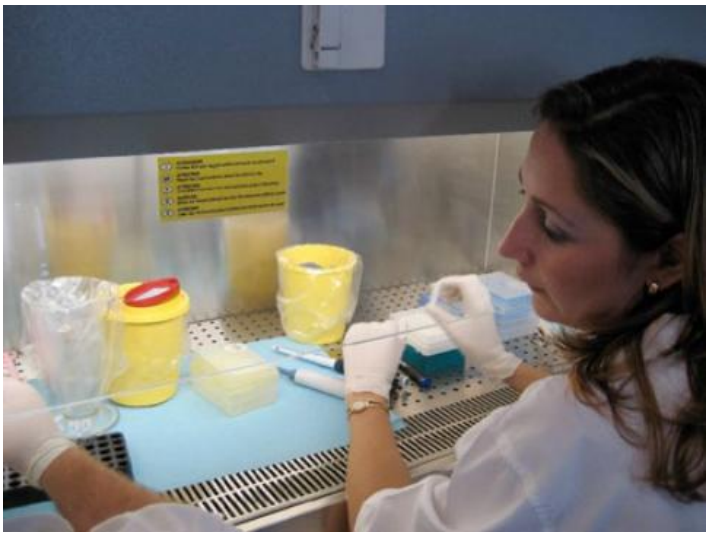


The epidemic strain currently describedin North America and EU, has the followingfeatures: PCR ribotype 027 in accordance withAnaerobe Reference Laboratory surveillance data[ECDC, 2006], pulsotype NAP 1 on pulsed-fieldelectrophoresis, enzymatic restriction-profile BI,toxinotype III by Rupnik toxinotyping method,positive for binary toxin actinia-specific ADPribozyltransferase,deletion of 18 bp in tcdCgene controlling the expression of toxins A andB, hyperproduction of toxins A and B (Ax16and Bx23) in comparison with strains of othergenotypes, resistant to macrolides (erythromycin)and la flororquinolones (moxifloxacin, gatifloxacinand levofloxacin).

Only specialised laboratoriesare able to perform the techniques for identifyingthese features and a two weeks period is requiredfor confirmation [INVS, 2006].

In practice, CDI diagnostic is based on toxinB detection in stool or revealing the toxigen strain.A- and B+ strains cannot be detected by currentimunoenzymatic 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 necessarystage for epidemic clone 027 characterisation;PCR profile identification provides the certaintydiagnosis.

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

These characteristics are not specific to clone027, but justify the stool culture in anaerobiosis inorder to isolate the responsible stain and to sendit to a specialised reference laboratory for furtherexamination.

The genes encoding TcdA and TcdB, tcdA andtcdB, respectively, have been sequenced and arefound in single open reading frames located withina 19.6-kb pathogenicity locus (8, 38).

As expected,both open reading frames are large, with tcdAfound 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 exhibita high degree of overall similarity (66%).

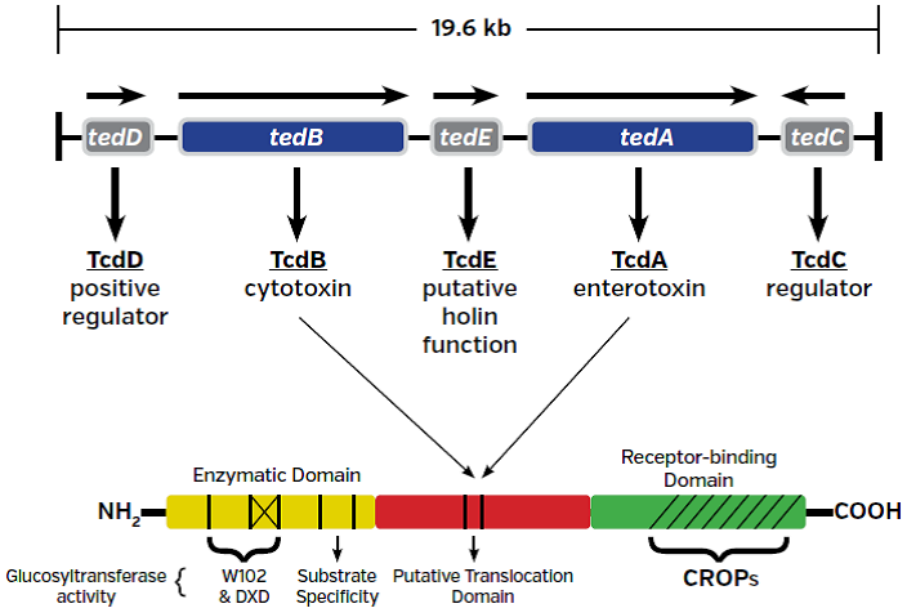
Giventhe proximal locations of tcdA and tcdB and thehigh sequence and functional homology betweenthe two proteins, it has been proposed that thetwo genes may have arisen as the result of a geneduplication event.

Furthermore, the similarity inthe biochemical activity of TcdA and TcdB, whereinboth toxins use a highly conserved N-terminaldomain to modify identical substrates, supportsthe notion of gene duplication. The major regionsof homology between TcdA and TcdB fall withinthe enzymatic and receptor-binding domains of thetwo toxins. The N-terminal domains of TcdA andTcdB show 74% homology, and this homology providesa basis for the similar substrate specificity ofthese two toxins.

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

Yet the CROPs found in TcdBare more divergent and less frequent than thosefound in TcdA. CROPs appear to play a putativerole in initial target cell interaction and receptorbinding, but the mechanism explaining the necessityfor 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 holing 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-oralroute, by hands and contaminated objects orenvironment. The fast transmission in healthcareenvironments is a result of several factors: straindissemination in CDI patients, half of samples frompatients rooms being positive; high resistance ofspores on inert supports for several months; toomany patients crowded in common healthcaresettings; numerous healthcare maneuvers creatinga high possibility of contamination by the medicalpersonnel hands; inadequate usage of antibiotics which diminishes the resistance to colonization andfacilitates C. difficile development.

The main individual risk factors are the advancedage and antibiotherapy. There are severalstudies which correlate the consumption of someclasses of antibiotics with CDI incidence: clindamicyn,3-rd generation cephalosporins, macrolides,and amoxicillin with clavulanic acid, 1-st generationcephalosporins and fluoroquinolones. It seamsthat the role of fluoroquinolones in C. difficile 027strains emergence and spreading is connected tothe resistance level towards them [INVS, 2006].

All factors stimulating the digestive ecosystemalteration, like laxatives, antacids, antisecretors,transit retarders, baritosis transit, gastrointestinalsurgery, etc. may facilitate this infection [DukerFreuman, 2014].

In March 2014, an epidemic episode with 31cases of postantibiotic C. difficile infection wasrecorded in Ploiesti Emergency Hospital (Romania)and the patients were isolated and treated. Mostof them were aged people from Neurology,Nephrology and Intensive Care Unit [Libertateanewspaper, 2014].

In May 2014 the Ministry of Health of Romaniagave the alert for C.difficile in Vaslui and Bucharesthospitals. The beginning of the year is worrying,in only 4 months, in Bucharest health facilitieswere registered 462 infected patients [Pro

TV, 22Mai 2014].

In accordance with Annual epidemiologicalreport: Reporting on 2011 surveillance data and2012 epidemic intelligence data, 2013, uttered byEuropean Centre for Disease Prevention and Control(ECDC), 48% cases of HAI (Healthcare-AssociatedInfections) associated with gastro-intestinalinfections were connected with C.difficile, andfrom all HAI (15.000 cases) in 3 only 5,4% of casesthe Clostridium difficile has been isolated. Takinginto consideration that in Romania over 92.3% ofpatients were the beneficiary of an antimicrobialprophylaxis during more than a day surgeries, theHAI risk associated with C. difficile is very high[ECDC, 2013]

TREATMENT

There is worldwide observed natural resistanceand/or acquired to the medicines of the quinolonegroup.

A mild CDI can usually be controlled bywithdrawing treatment with the antibiotics causingthe infection (25% of patients could recover in2-3 days). More severe cases can be treated usingan oral specific treatment with metronidazole (1g/day) or vancomycin (1-2g/day) for 10 days. Themetronizadole is a better choice, being a less expensivetreatment with no risk of selecting glycopeptidesresisting germs like golden enterococcus andstaphylococcus.

Failure to respond to metronidazole therapywithin 5 – 7 days should prompt consideration of achange 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 usedat standard dosing. In patients in whom oral antibioticscannot reach a segment of the colon, such aswith Hartman's pouch, ileostomy, or colon diversion,vancomycin therapy delivered via enema should beadded to treatments (500 mg in 100 – 500 ml of normalsaline every 6 h) until the patient improves.

However, relapse is common and requires furthertreatment with repeated series of metronidazoleor vancomycin, in high doses first and smallerdoses associated with probiotics (i.e. Saccharomycesboulardii) after improvement. Severe cases mayneed intensive care for maintaining the vital functionsand even surgical treatment for colectomy(in case of toxic megacolon or colon perforation).

CT scanning is an important technique for perforationdiagnosis in comparison with colonoscopytechnique which presents a perforation risk due togas inflation. The antibiotic treatment for healthyindividuals colonized with C.difficile is not recommended,being inefficient for eradicating for goodthis bacteria in digestive tract.[Ordeanu, 2010; Ordeanu 2012]

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

This "synthetic stool" is a super-biotic obtained usingseveral cultures of saprophyte intestinal culture[Allen-Vercoe, 2013]. Studies show that patientswith recurrent CDI (RCDI) have abnormally proportioned colon microbiota, and that reintroductionof normal bacteria via donor feces correctsthis imbalance, restoring phylogenetic richness andcolonization resistance.

There is no international consensus for definingand surveillance CDI, but we have to consider local(regional and national) epidemiology conditionsand possibilities. ECDC created a working groupfor early detection and monitoring the CDI. Theyhave suggested recorded signals criteria for severeand grouping cases of CDI.

C. difficile infectious can usually be preventedby practicing good hygiene in healthcare environments,such as: individual bed space, washinghands regularly (mechanical action of washing aftergloves removal), using gloves, protection mask,glasses and gown in bed space area and in contactwith patients, using medical supplies

for one usageonly, cleaning surfaces using bleach wipes of sodiumhypochlorite containing 0.5 % active chlorine,and patient removal limitation. [CCLIN, 2013]

COMMENT

Due to the increasing worldwide incidence ofinfections with C. difficile on one hand, and to thediscovery of new ways of transmitting the infectionaccording with some studies regarding the genetic diversityof C. difficile strains on the other hand, (http://www.pharmacypracticenews.com) a new approach isnecessary for C. difficile related topicsIt is important to adopted NAAT testing aloneor a 2 or 3 step algorithm for CDI diagnosis.

If the C. difficile is confirmed and classified asa severe form or in an epidemic context it should bereported to Public Health Territorial Authorities andto The Anaerobe Reference Laboratory from INCDMICantacuzino, for a clear diagnosis and adequatemeasures.

CONCLUSION

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

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