EPIDEMIOLOGICAL CHARACTERIZATION OF CLOSTRIDIUM DIFFICILE INFECTIONS IN A RURAL COMMUNITY SETTING

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Abstract

Clostridium difficile is a microorganism - a Gram-positive anaerobic bacterium that has developed and hyperspecialized the ability to form spores. Outside the host, *Clostridium difficile* germinates spores which are resistant to unfavorable environment and for an extremely long time period. *Clostridium difficile* infection frequently occurs on a background of major disturbances in the balance of the normal intestinal flora caused by use for therapeutic purposes of broad-spectrum antibiotics. Patients infected with *Clostridium difficile* in medical facilities are usually elderly people, immunosuppressed or subjected to an antibiotic medication regimen. Wide-scale community use of antibiotics and proton-pump inhibitors induces favorable conditions for the germination of *Clostridium difficile* spores in the gastrointestinal tract.

Clostridium difficile biocolonizes many domestic and wild animals. *Clostridium difficile* is a complex bacterium, capable of developing resistance forms that parasitize the gastrointestinal tract of humans, and also many animals. An important source of the *Clostridium difficile* infection is represented primarily by antibiotic medication administered in communities without medical recommendation, as well as food.

Keywords: *Clostridium difficile*, biocolonization, resistance forms.

Rezumat

Clostridium difficile este un microorganism, bacterie Gram-pozitivă anaerobă ce are dezvoltată și hiperspecializată capacitatea de a forma spori. *Clostridium difficile* în afara gazdei formează spori rezistenți la mediul nefavorabil și pe o durată extrem de mare de timp. Infecția cu *Clostridium difficile* are loc frecvent pe fondul unor perturbări majore în echilibrul florei intestinale normale, produsă de utilizarea în scop terapeutic a antibioticelor cu spectru larg de acțiune. Pacienții infestați cu *Clostridium difficile* în unitățile medicale sunt, de obicei, persoane vârstnice, imunosupresate sau supuse unei scheme de medicație cu antibiotic.

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Utilizarea comunitară, pe scară largă a antibioticelor și inhibitorilor pompei de protoni induce condiții favorabile germinării sporilor de Clostridium difficile în tractul gastrointestinal.

Clostridium difficile biocolonizează multe animale domestice și sălbatice. Clostridium difficile este o bacterie complexă, capabilă să dezvolte forme de rezistență care parazitează tractul gastrointestinal al omului și nu numai. Sursa importantă în infecția cu Clostridium difficile o reprezintă, în primul rând, medicația cu antibiotice administrată în comunitate și fără aviz medical, precum și alimentația.

Introduction

Clostridium difficile is a microorganism - a Gram-positive anaerobic bacterium that has developed and hyperspecialized the ability to form spores. Outside the host, *Clostridium difficile* germinates spores which are resistant to unfavorable environment and for an extremely long time period ⁽¹⁾. The target anatomy of *Clostridium difficile* is the gastrointestinal tract. *Clostridium difficile* infection frequently occurs on a background of major disturbances in the balance of the normal intestinal flora caused by use for therapeutic purposes of broad-spectrum antibiotics ⁽¹⁾. The sequence of biological and biochemical events that precede the induction of the *Clostridium difficile* infection in the gastrointestinal tract is not known. Thus the *Clostridium difficile* infection occurs after a patient treated with broad-spectrum

antibiotics ingests infectious resistant spores. In the gastrointestinal tract, *Clostridium difficile* spores pass from a latent state into metabolic activity, a process defined and known as the primary germination state ^(2,5). *Clostridium difficile* spores start the germination process in the duodenum. The germination of *Clostridium difficile* spores progresses in two stages, and it depends on: pH in the gastrointestinal tract, bile salt concentration, locally available calcium ions or other bivalent cations, and presence of certain free amino acids ^(2,3,4). The germination process is remarkable and widely studied as it is the first and most important stage of the pathogenesis process. Recent experimental studies have attempted to decipher the molecular signals that lead to metabolic activation of the resistance forms of the pathogen. Initially it was thought that an increase in the gastrointestinal tract leads to a

specific binding to certain enterocyte receptors, intracellular and extracellular^(6,7,8). Unfortunately, subsequent molecular studies did not identify any direct link between enterocyte membrane receptors and the occurrence of *Clostridium difficile* spores^(6, 7, 8). Of amino acids, only serine, glycine and alanine seem to play an important role in the activation of *Clostridium difficile* spores, but the germination mechanism induced by amino acids remains uncertain^(5, 9). Another mechanism of the germination process of *Clostridium difficile* spores is represented by bivalent cations. Of bivalent cations, calcium and magnesium seem to be involved in the germination process of *Clostridium difficile* spores, but their mechanism of involvement in germination remains uncertain⁽¹⁰⁾. Once the germination process is completed, the colonization process and the specific pathogenicity of the microparasite are carried out. *Clostridium difficile* entering metabolic activity will progressively produce enterotoxin A and cytotoxin B that cause diarrhea and pseudomembranous colitis in infected subjects⁽¹¹⁾. Once these toxins have been synthesized, they specifically bind to enterocyte membrane receptors leading to the destruction of the intestinal mucosa, diarrhea and acute colitis.

There are five different types of *Clostridium difficile*, each dominant on a particular continent: clade 1 (Europe), clade 2 (North America), clade 3 (Africa), clade 4 (Asia), and clade 5 (Australia)⁽¹¹⁾. *Clostridium difficile*-induced mortality is closely related to the type and synthesis of binary toxins. A percentage of over 50% of the subjects infected with *Clostridium difficile* were recently discharged from the hospital, outpatients or subjects with various immunosuppressive pathologies (inflammatory diseases, various cancers, AIDS, gastrointestinal pathologies, and

metabolic diseases). Most community-acquired *Clostridium difficile* infections are also related to nosocomial infections (onset after hospital discharge or after a visit to a medical facility) or unwarranted use of broad-spectrum antibiotics⁽¹²⁾. Patients infected with *Clostridium difficile* in medical facilities are usually elderly people, immunosuppressed or subjected to an antibiotic medication regimen (Gupta and Khanna, 2014). Compared to the above mentioned patients, community infections often occur in women aged under 50 years, and 20% of these patients have not received any antibiotic treatment during the 180 days prior to diagnosis (De Pestel and Aronoff, 2013). Some epidemiological studies have shown that antibiotic treatment increases approximately 7 times the risk of *Clostridium difficile* infection in certain patient categories⁽¹³⁾. The most common antibiotics previously used by patients with a diagnosis of *Clostridium difficile* infection were: clindamycin, fluoroquinolones, cephalosporins, penicillins, macrolides, and sulfonamides⁽¹⁴⁾. Not only antibiotics are a risk factor in *Clostridium difficile* infections, but proton-pump inhibitors and H2 antagonists may also represent a risk factor in microparasitic infections⁽¹⁵⁾. Wide-scale community use of antibiotics and proton-pump inhibitors creates favorable conditions for the germination of *Clostridium difficile* spores in the gastrointestinal tract.

Material and methods

The data were taken from the Department of Public Health of Constanta, and the reporting and processing of medical information was for the time period between February 8, 2015 - June 4, 2016. Only the medical information of patients aged over 18 years who live in a rural settings was included in the study. The study

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Figure 1. Comparison of *Clostridium difficile* infections by urban and rural settings

	Patient age	Medical history of <i>Clostridium difficile</i>		Patient age	Medical history of <i>Clostridium difficile</i>		Patient age	Medical history of <i>Clostridium difficile</i>	
Male patients	18-30 years old	Yes	No	30-50 years old	Yes	No	>50 years old	Yes	No
	n=3	n=2	n=1	n=1	n=0	n=1	n=8	n=1	n=7
Female patients	n=0	n=0	n=0	n=1	n=1	n=0	n=5	n=5	n=0

Table 2. Reporting of patients in a rural setting by incidence and medical history

protocol was signed and approved by the Institutional Review Board and the Ethics Committee of the Department of Public Health of Constanta. The data was processed in compliance with the legal requirements on the use of personal data. During hospitalization, patients were actively followed to collect data on the evolution of diarrheal episodes, the onset of medical complications, and death. The diagnosis of *Clostridium difficile* infection was confirmed by the determination of toxin A and B and glutamate dehydrogenase via chemiluminescence in faeces.

Of the total number of patients (**n=66**) with a positive result for *Clostridium difficile* infection in the study period, only 18 patients (**n=18**) were from a rural setting.

Results

The table shows the medical history of patients in a rural setting, the incidence by age and gender of *Clostridium difficile* infection, and the source of the infection (community-acquired or nosocomial) can be grossly deduced.

Discussions

Both symptomatic patients and asymptomatic carriers eliminate *Clostridium difficile* spores in extremely high amounts. Recent studies have shown that newborns are the source of infection in over 60% of cases rather than a maternal source. Pseudomembranous colitis may occur infrequently in infants, and could be explained by the cumulative expression of toxin receptors via the two pathways mentioned in the introduction, which are poorly developed. Regarding the information available in literature, women are two times more prone to *Clostridium difficile* infection than men. Infants may be an important source

of active or inactive forms of *Clostridium difficile* microparasite, which may largely explain the incidence of *Clostridium difficile* infection in peripartum women. The major source of the *Clostridium difficile* infection is mainly the antibiotic medication performed in the community without consulting a healthcare professional, but also food. The proven resistance of *Clostridium difficile* spores in thermally processed meat demonstrates the survival ability of resistance forms of the microparasite in extreme environments. Thus, thermal processing of food can be a possible source of infection with *Clostridium difficile*. Farm animals are a good vehicle for the resistance forms of the *Clostridium difficile* bacterium. Therefore, the emergence of extra-hospital infections with *Clostridium difficile* may be related to zoonotic transmission. *Clostridium difficile* biocolonizes many domestic and wild animals, for example, actual carriers of biological forms of the *Clostridium difficile* bacterium can be: dogs, cats, horses, pigs, calves, poultry, goats, and rodents. Another aspect not to be neglected in the transmission and transport of multidrug-resistant forms of pathogenic microorganisms is the use of antibiotics in the zootechnical field. There is sufficient experimental and epidemiological evidence in this respect, but also a series of measures imposed on EU member states. These studies show that the use of antibiotics can indirectly influence spore germination, and hence the susceptibility of rural communities to contact and develop infections with *Clostridium difficile*. Literature analysis and data are a first step in identifying possible transport sources and environmental factors that contribute to the development of *Clostridium difficile* bacteria. The data presented is informative for the medical community and especially important for health education programs.

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Clostridium difficile is a complex bacterium capable of developing resistant forms that parasitize the gastrointestinal tract of humans, and other animals.

Conclusions

Clostridium difficile is a complex bacterium capable of developing resistant forms that parasitize the gastrointestinal tract of humans, and also many animals. An important source of the *Clostridium difficile* infection is represented primarily by antibiotic medication administered in communities without medical recommendation, as well as food.

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