Corelations Between the Use of Medical Substances and the Incidence Infections Produced by Clostridium Difficile Species

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Antibiotherapy is the main determinant of Clostridium difficile infection due to the imbalance determined in the intestinal flora. Clostridium difficile infection can be considered a current public health problem, given the increased incidence, both as a nosocomial infection as well as at community level, by excessive, uncontrolled and unjustified use of antibiotics, high contagiousness, negative influence on health systems in the increased number of days of hospitalization and implicitly increased costs, and last but not least, the substantial deterioration of the quality of the patient's life. The retrospective study over a 12-month period over a group of 106 patients revealed the following profile of the patient affected by Clostridium difficile infection: females, aged 66.75 ± 15.66, from the urban area, hospitalized in the medical section, who had diarrhea more than 2 days after admission, but up to 28 days after this event, due to prolonged antibiotic therapy with ceftriaxone, associated with a gastric secretion inhibitor, on a background of multiple associated pathologies. It is very important for all health systems to control this phenomenon and this is only possible by applying effective measures to prevent the onset of CDI, of relapses and contamination, thus identifying the judicious use of antibiotics.

Key words: antibiotherapy, Clostridium difficile, nosocomial infection

Clostridium difficile infection (CDI) can be considered a current public health issue, given the increased incidence, both as a nosocomial infection as well as at community level, by excessive, uncontrolled and unwarranted use of antibiotics, high contagiousness, negative influence on systems in the increased number of days of hospitalization and implicitly increased costs, and last but not least, the substantial deterioration of the quality of the patient's life. This latter aspect has the greatest weight in managing in CDI case, given the duration of the disease and treatment episode, the increased risk of relapse, the extent of contamination prevention measures, a fairly high mortality rate, impairment of the communication relationship with the patient and their family [1], but especially the suffering of the patient, which cannot be quantified.

In Europe, CDI records a rate of 4.1/10,000 patient-days, with a 90-day mortality rate of 22% [2]. In 2011, 453,000 CDI cases were hospitalized in the USA, of which 29,000 resulted in the death of patients [3]. Statistics show that CDI has a 21% higher frequency than MRSA infection [4]. Managing CDI cases increases the cost of hospitalization four times, accounting for USA $ 1.5 billion annually [5.6]. The increased incidence of CDI, augmented by the severity of the disease by gaining antibiotic resistance, is reflected in significant healthcare costs, estimated at between $ 750 million and $ 3.2 billion [7].

Clostridium difficile (*C. difficile*) is a gram-positive anaerobic germ that normally colonizes the digestive tract (colon) in 3% of adults, reaching more than 50% for children under 1 year old [8]. Antibiotic therapy is the key determinant of the imbalance in the normal saprophytic flora of the digestive tract, an imbalance that allows the excessive development of *C. difficile* populations resistant to the respective antibiotic, especially in the presence of risk factors such as advanced age, prolonged hospitalization, various associated co-morbidities (cardiac, respiratory, digestive, neoplastic), immunodeficiency, digestive tract diseases, proton pump inhibitor treatment(PPI) or H2-type antihistamine, chemotherapy [9,10]. Previous meta-analyses have suggested a strong association between cephalosporin use and CDI, and many national programmes on CDI control have focused on reducing cephalosporin usage. Despite reductions in cephalosporin use, however, rates of CDI have continued to rise [11]. Recent studies have evaluated the association between antibiotic use and CDI and was reported that cephalosporins and clindamycin were most strongly associated with hospital-associated CDI, while for community-associated infection, the strongest association was seen with clindamycin, cephalosporins and quinolones [12]. Cephalosporin is a β-lactam antibiotic that inhibits bacterial cell wall synthesis (fig. 1).

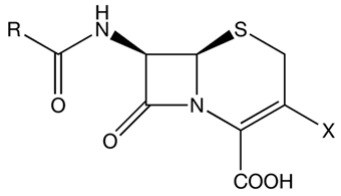

Fig. 1. General structure of cephalosporins

Cephalosporins, like penicillins, have a beta-lactam structure that is the critical determinant of their antibacterial activity. The five-member thiazolidine ring that is characteristic of penicillins is replaced in cephalosporins by a six-member dihydrothiazine ring. This ring is responsible for the agents' ability to resist inactivation by certain bacterial enzymes. Cephalosporins disrupt synthesis of the peptidoglycan layer of bacterial cell walls. Peptidoglycan is a strong structural molecule specific to the cells walls of bacteria. With the cell wall structure

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All authors have equal contribution to the study and the publication

compromised, the bactericidal result is lysis and death of the cell.

Clostridium difficile releases two exotoxins, toxin A (TcdA) and toxin B (TcdB), toxins that cause inflammation and destruction of colon mucosal cells, resulting in diarrhea associated with antibiotherapy and pseudomembranous colitis, shortly after initiation of antibiotic treatment [13]. These toxins belong to the family of clostridial glycosylating toxins and target the family of Rho and Ras GTPases and modify them by glycosylation [14]. Both toxins are large, homologus, single-chain proteins that contain at least four distinct domains: the N-terminus glucosyltransferase domain (GTD), a cysteine protease domain (CPD), a translocation domain (TD), a C-terminus receptor domain (RBD) [15, 16]. Toxins delivery into the host cell cytosol and act on different steps a) toxin binding to the host cell surface receptors; b) toxin internalization through a receptor-mediated endocytosis; c) endosome acidification; d) pore formation; e) GTD release from the endosome to the host cell cytoplasm; f) Rho GTPases inactivation by glycosylation; g) downstream effects within the host cell [17]. The exotoxins are major pathogenicity factors which exert in vivo cytotoxic effect causing reorganization of the cytoskeleton accompanied by morphological changes.

Purpose of this research was to analyze and understand the causal relationship between antibiotics, risk factors and CDI in order to improve prescription of antibiotics at the hospital level, to reduce resistance to them, to increase patient's safety, to ensure proper management of CDI cases and to reduce the number of infections associated with healthcare generated by *C. difficile* and relapses.

Objectives of this study were to identify the incidence of CDI in the population segment analyzed during the defined period; to establish the risk factors that led to the emergence of CDI; to define the demographic and pathological profile of the patient who developed the CDI in order to establish the population at risk; to establish the involvement of proton pump inhibitors in favor of CD development; to emphasize the importance and role of probiotics as adjunctive treatment in the prevention of CDI cases; to establish the involvement of suppression therapy of gastric acid secretion in the development of CDI.

Experiemntal part

Material and Methods

The study is retrospective, for a period of 12 months, in Pitesti County Emergency Hospital. The group consisted of 106 patients who were identified with CDI during that period and reported as healthcare associated infections or community infections (the occurrence of CDI may have been linked to previous admissions or home antibiotic therapies), or relapses, including cases of previous admissions to other healthcare facilities. The data were collected from the FOCG, the epidemiological evidence of nosocomial infection, the CDI declaration forms set up on a case-by-case basis, the Hippocrates computer system, and statistical reports. The establishment of the incidence of CDI in the hospital was achieved by reporting the number of CDI cases to the total number of discharges, except for the discharges from the neonatology department. All patients enrolled in the study were admitted to continuous hospitalization. The CDI incidence on the sections was analyzed by grouping them into two categories: medical and surgical, mentioning for each category those specialties with a higher CDI frequency. In order to define the demographic and pathological profile of the patient with CDI, we applied age, gender, background and associated pathology as criteria. The collection and processing of data was carried out in accordance with ethical norms in research [18, 19].

Results and discussions

In the analyzed period, 0.65% of patients admitted to hospital experienced *Clostridium difficile* infections. Batch distribution by gender revealed a predominance of women (the difference between males and females is statistically significant $=0.000000007709<\alpha=0.01$).

The analysis of the descriptive statistics of the age variable led to the finding that the series of values is normally distributed (Jarque-Bera $= 4.037 < \chi 2 = 9.21$ and $p=0.0895 \geq \alpha=0.01$), within normal asymmetry and vaulting limits, homogeneous ($C0=0.23$). The average age of patients is 66.75 ± 15.66 years. Most patients are 75 years of age and half of the 106 patients are older than 72 years (table 1). Until the age of 70, the number of males is significantly higher than that of women ($p = 0.0025 <\alpha= 0.01$), and for the following intervals the situation changes, the number of women being significantly higher ($p = 0, 00019 <\alpha = 0.01$)

Batch analysis based on the time from the admission to the disease onset shows that the number of patients who developed CDI between 2 and 28 days is significantly higher than the rest of the intervals ($p = 0$). Thus, compared to the first interval (less than 2 days) it is 2.25 times higher, and compared to the last interval (more than 28 days) it is 36 times higher. For the 2-28 days interval, the number of women is significantly higher than that of men (40 versus 32)-$p=0.00000000771 <\alpha = 0.01$ and the average age of patients in this range is 67.10 ± 16.11, higher than the average age of the lot by 0.35 points (table 2, and fig. 2).

Groups of patients by age	Number of patients	out of which			
		M		W	
		no	%	no	%
20 – 30 years old	3	3	100	0	0
30 – 40 years old	5	3	60.00	2	40.00
40 – 50 years old	9	5	55.56	4	44.44
50 – 60 years old	15	8	53.33	7	46.67
60 – 70 years old	20	11	55.00	9	45.00
70 – 80 years old	37	13	35.14	24	64.86
Over 80 years old	17	6	35.29	11	64.71
Total	106	49	46.23	57	53.77

Table 1
DISTRIBUTION OF PATIENTS BY AGE

Interval from admission to onset (days)	Number of patients	Gender		Average age
		M	W	
≤2	32	15	17	67.53±13.92
2-28	72	32	40	67.10±16.11
> 28	2	2	0	41.50±0.71
total	106	49	57	66.75 ± 15.66

Table 2
ANALYSIS OF BATCH ACCORDING TO THE INTERVAL FROM THE ADMISSION TO THE ONSET OF THE DISEASE

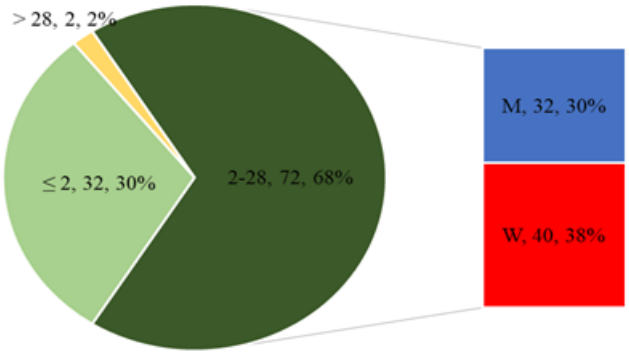

Fig. 2. Incidence of patients according to the interval from the admission to the onset of the disease

An important element for the history of the disease is the pre-CDI admission episodes. Thus, of the 106 patients, 77.36% had had admissions to our hospital, less than 28 days after the onset of the disease, 8 of them received medical care during this period in other hospitals as well, and 18 (16.98%) patients had not been hospitalized before.

A significant cause for CDI triggering is the use of antibiotics at home, often without the doctor's advice, by appealing directly to third-generation or fourth-generation cephalosporins with unreasonable associations or substitutions for extended periods of time. From the history of our patients, 13.21% of them had had antibiotics given at home.

Analyzing the pharmacological therapies used, it was found that 72% of the patients in the batch had received a gastric secretion inhibitor, in most cases the proton pump inhibitor, respectively omeprazole /esomeprazole (fig. 3 and 4).

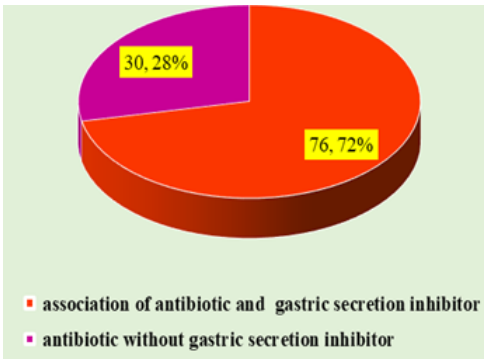

Fig. 3. Incidence of patients receiving gastric secretion inhibitor

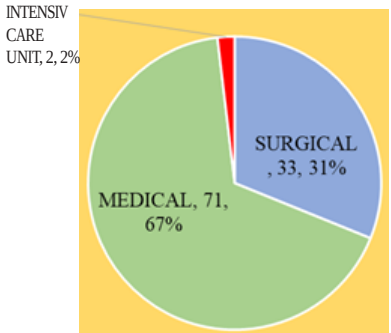

Fig. 5. Incidence of patients according to the type of section

As can be seen, antibiotics represent the common pharmacological class for the patients studied, and the situation found supports the idea that antibiotics are the major cause of CDI development. Most patients are given CEFORT (55 patients, 51.88%), followed by patients receiving SULCEF (33 patients, 31.13%). In 69 cases out of 106 (65%), probiotics are used in therapy (p = 0).

The distribution of patients according to the type of section shows the predominance of the medical sections (67%), while the surgical patients occupy 31% (the difference has statistical significance, p = 0). It should be noted that the main pathology, namely the section where the patient was initially admitted, was considered in the analytical approach. Thus, although multiple cases have been reported by the adult infectious disease department as a result of the transfer from other services, we assigned the patient according to the type of the initial section. The same distribution method determined in our statistics the small number of cases in ATI (fig. 5).

Of the 106 patients, 56% (59 patients) came from rural areas and 44% (47 patients) from urban areas. Statistically, the difference is not significant (p = 0.01046> α = 0.01), which means that the home environment [20] does not influence the prevalence of CDI (fig. 6).

The number of patients with cardiovascular pathology is significantly higher in relation to the rest of the pathologies (p=0.000000000671). It should be noted that most patients had associations of these pathologies, the cardiovascular one being common to most patients. This situation justifies in some ways the complex therapeutic regimens, with CEI as a major option [21] and which determines the increased use of gastric secretion inhibitors.

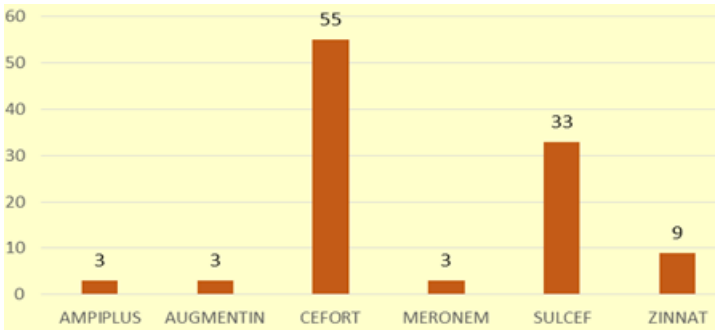

Fig. 4. Distribution of patients according to the type of antibiotic administered

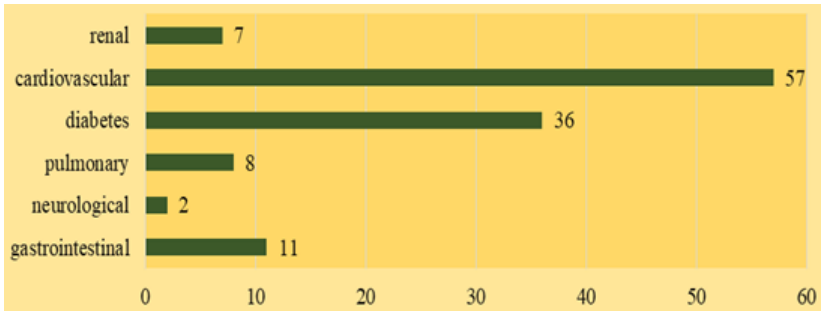

Fig. 6. Batch distribution according to the associated pathology

Regarding the evolution of CDI patients, the analysis revealed that 3.77% of patients had relapses (p = 0) and one patient died.

Epidemiological investigations show that *C. difficile* is the most common etiological nosocomial agent [22]. It is estimated that 20% of patients are infected with *C. difficile* species during their hospitalization, and 30% of them have diarrhea [23]. The number of nosocomial CDIs over time shows an upward path due to the development of *C. difficile* resistance to antibiotics, the occurrence of more virulent and more resistant strains, and the fact that *C. difficile* spores are found throughout the hospital, resulting in increased risk of contamination, and the spore form of existence favors easy fecal-oral dissemination [24]. Nosocomial infections with *C. difficile* may cause deaths, directly or indirectly, in 0.6-1.5% of patients [25].

C. difficile species secretes an enterotoxin-toxin A and a cytotoxin-toxin B, both of which are high molecular weight proteins with specificity for receptors in the intestinal mucosa, reaching intracellularly by catalyzing a specific modification of Rho proteins (the small binding proteins of glutamyl transpeptidase-GTP which helps polymerization of actin, cytoscheletic architecture and cell movement) and causes inflammation and damage to the intestinal mucosa. The single-chain toxins TcdA and TcdB are the main virulence factors and represent the major cause of antibiotic-associated diarrhea and pseudo-membranous colitis. The *C. difficile* toxins A and B are multifunctional proteins. After binding of the C terminus to the cell wall surface receptors of target cell, the toxins are endocytosed and traffic to early endosomes. The acidic compartment causes conformational changes of the toxin molecule and allows insertion of an internally located hydrophobic region into vesicle membranes, which is accompanied by pore formation and subsequent translocation into the cytosol [26].

Identification of *C. difficile* is by immunocromographic detection of these toxins, but also of an antigen, glutamate dehydrogenase (GDH), a metabolic enzyme produced in significant quantities. This test has a sensitivity of between 72 and 82%, and the specificity ranges from 97 to 98%, compared to the CCCA method (the cell culture cytotoxicity assay) [27].

By affecting the balance of intestinal flora, antibiotic therapy proves to be the major cause of diarrhea and pseudomembranous colitis associated with CDI. The distribution of the batch studied by antibiotic type identified Cefort - ceftriaxone (51.88%) and Sulceph - cefoperazone / sulbactam (31.13%) as the main antibiotics involved in CDI.

Ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) is a broad spectrum cephalosporin of the third generation with proven efficacy in multiple pathologies - respiratory tract infections, otitis, and intraabdominal, urogenital, infections in the skin, especially in cases where antibiotic resistance has developed. Ceftriaxone is not useful in combating *Pseudomonas aeruginosa* sp. and it is inactive for *Enterobacter* sp., although there may be signs of sensitivity, but these are canceled by the increased risk of antibiotic resistance (fig. 7) [28].

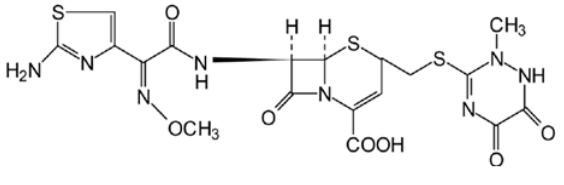

Fig. 7. Chemical structure of ceftriaxone

The effects of antibiotherapy are enhanced by the use of gastric antisecretory, which, by reducing gastric secretion allows development of *C. difficile* species. In our study it was found that omeprazole was most commonly used in therapeutic regimens. This is an imidazole derivative, having low base character, given by the pyridine nucleus, which inhibits the activity of H^+, K+, ATP-ase, thus being a proton pump inhibitor (fig. 8).

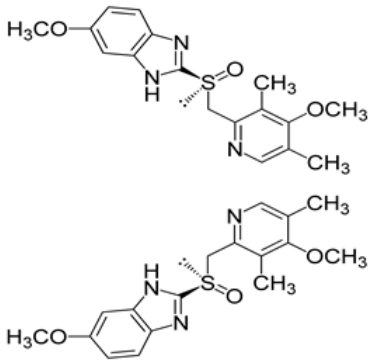

Fig. 8. Chemical structure of omeprazole

Proton pump inhibitors (PPIs) are predominantly used compared to H2 antihistamines, both in the hospital and in the general population. Studies have shown that in the total of drugs released from pharmacies, PPIs have a share of 84.91%, clearly superior to 15.09% of antihistamines H2. Analysis of active substances corresponding to the two pharmacological classes revealed the increased use of Omeprazolum (50.46%) for PPI and Ranitidinum (81.13%) [29].

The negative effects of excessive and/or unjustified use of antibiotics, in fact diarrhea associated with CDI, can be prevented and counteracted by the administration of probiotic species (*Saccharomyces boulardii*, *Lactobacillus rhamnosus GG*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*), which by secretion of bactericidal acids and peptides generates *competition* in intestinal microbial flora, preventing *C. difficile* development [30-32]. Research has shown that probiotics inhibit *C. difficile* species growth in hamsters [33,34]. The bacteriocins have an important practical applications in food preservation as well as in prevention of bacterial infections. They have a limited spectrum of inhibition acting especially on Gram-positive bacteria, but many bacteriocins produced by lactic acid bacteria species are active against food pathogens such as *B. cereus, Clostridium (C.) botulinum, C. perfringens, L.monocytogenes, S. aureus*, etc [35].

CDI treatment recognizes two important levels: eliminating the feasible factors (cessation of antibiotics and gastric secretion inhibitors, measures to reduce the spread) and initiation of etiology therapy - metronidazole for low or moderate severity and vancomycin for severe cases, supported by a supportive treatment-hydro-electrolytic rebalancing, correction of hypoproteinemia and correction of organ dysfunctions. Prevention of recurrences by probiotics or transplantation of enteral flora from a healthy donor is also envisaged in order to restore the saprophytic colon flora [36].

The high percentage of beta-lactam resistant bacteria strains requires careful surveillance of the dynamics of susceptibility profiles for limiting the emergence of these strains in community [36, 37]. The main factors involved in pathogenicity of infections determined by *Clostridium difficile* species are: origin from the natural body flora, deficiencies in the defense mechanisms through antibodies, complement system, leukocytes, polymorpho-nuclear, immediate cell immune response, synergy with other micro-organisms, as well as elements which lead to

the increase of the micro-organism virulence (adherence, invasion, toxins, enzymes and spores). In order to avoid enterocolitis caused by *Clostridium difficile* species, it is recommended to limit the long-term administration of antibiotics which determine an unbalance of the normal intestinal flora as well as prompt renouncement to that medication once the first digestive symptoms occur [8].

Conclusions

It is very important for all health systems to control this phenomenon and this is only possible by applying effective measures to prevent the onset of CDI, relapses and contamination, thus identifying the judicious use of antibiotics.

References

1.CONSTANTIN, DA., CIORICEANU, IH., TANTU, MM., POPA, D., BADAU, D., BURTEA, V., NEMET, GC., ROGOZEA, LM., Rom. J. Morphol. Embryol., 58, nr. 3, 2017, p.1121

2.BAUER, MP., NOTERMANS, DW. ET AL., Lancet, 377, nr. 9759, 2011, p. 63

3.LEFFLER, D., THOMAS LAMONT, J., N. Engl. J. Med., 372, nr. 16, 2015, p.:1539

4.CARLING, PC., BARTLEZ, JM., Am. J. Infect. Control., 38, 2010, p. S41

5.ZIMLICHMAN, E., HENDERSON, D., TAMIR, O., ET AL., JAMA Intern. Med., 173, nr. 22, 2013, p. 2039

6.LOFGREN, ET., COLE, SR., WEBER, DJ, ANDERSON, DJ., MOEHRING, RW., Epidemiology, 25, nr. 4, 2014, p.570

7.DUBBERKE, ER., WERTHEIMER, AI., Infect. Control. Hosp. Epidemiol., 30, nr. 1, 2009, p.57

8.EPURAN, S., RUSU, E., SBARCEA, A., MU[a]AT, S., Rom. J. Infect. Dis. XVII, nr. 1, 2014, p. 28

9.FATEN N ABERRA, Clostridium Difficile Colitis, www.emedicine.medscape.com, accessed in July, 2015

10.BARTLETT, JG., Clin. Infect. Dis., 46, nr. Suppl 1, 2008, p.S4.

11.WILKOX, MH., CHALMERS, JD., NORD, CE., FREEMAN, J., BOUZA, E., J. Antimicrob. Chemother., 72, nr. 1, 2017, pg. 1

12.SLIMINGS, C., RILEY, TV., J. Antimicrob. Chemother., 69, nr. 4, 2014, p. 881

13.PEREZ-COBAS, AE., ARTACHO, A., KNECHT, H., ET AL. PLoS One., 8, nr. 11, 2003, p.e80201.

14.CHEN, S., SUN, C., WANG, H., WANG, J., Toxins (Basel), 7, nr. 12, 2015, p. 5254

15.JANK, T., AKTORIES, K. Trends Microbiol., 16, 2008, p.222

16.PRUITT, RN., CHAMNERS, MG., NG, KK., OHI, MD., LACY, DB., Proc. Natl. Acad. Sci. USA, 107, 2010, p. 13467

17.DI BELLA , S., ASCENZI, P., SIARAKAS, S., PETROSILLO, N., DI MASI, A., Toxins (Basel), 8, nr. 5, 2016, p. 134

18.ROGOZEA, L., PURCARU, D., LEASU, F., NEME, C., Rom. J. Morphol. Embryol., 55, nr. 2 Suppl, 2014, p.719

19.POPESCU, IG., SECHEL, G., LEASU, FG., TANTU, MM., COTOI, BV., ROGOZEA. LM., Rom. J. Morphol. Embryol., 59, nr. 3, 2018, In Press

20.POSTOLACHE, P., NEMES, RM., PETRESCU, O., MERISANU, IO., Medical-Surgical Journal, 119, nr. 1, 2015, p. 77

21.TANTU, M., BELU, E., BOBESCU, E., ARMEAN, SM., ARMEAN, P., CONSTANTIN, MM., DOMNARIU, CD., Farmacia, 62, nr. 3, 2014, p.443

22.RUSU, D., MIHALACHE, M., CRISTEA, S., BULICREA, R., AMT, II, nr. 4, 2014, p. 25

23.CARROLL, KC., BARTLETT, JG., Annu. Rev. Microbiol., 65, 2011, p.501

24.NITESCU, M., PITIGOI, D., STREINU-CERCEL, A., The importance of decontamination and disinfection in the prevention of Clostridium Difficile infection, 6th National Conference on Microbiology and Epidemiology, 14-16 November 2013, Bucharest

25.MILLER, MA., HYLAND, M., OFNER-AGOSTINI, M., GOURDEAU, M., ISHAK, M., Infect. Control. Hosp. Epidemiol., 23, nr. 3, 2002, p. 137

26.EGERER, M., GIESEMANN, T., HERRMANN, C., AKTORIES, K. J. Biol. Chem., 284, nr. 6, p. 3389

27.SCHUTZE, G., WILLOUGHBY, R., Pediatrics, 131, nr. 1, 2013, p. 196

28.*** https://pubchem.ncbi.nlm.nih.gov, accessed in July, 2018

29.BOBOIA, A., FEHER, LA., CUC, S., MOLDOVAN, M., Farmacia, 65, nr. 4, 2017, p. 635

30.ORRHAGE, K., SJOSTEDT, S., NORD, CE., J. Antimicrob. Chemother., 46, 2000, p.603.

31.HOPKINS, MJ., MACFARLANE, GT., Appl. Environ. Microbiol., 2003; 69, nr. 4, 2003, p.1920

32.WULLT, M., JOHANSSON HAGSLATT, ML., ODENHOLT, I., BERGGREN, A., Dig. Dis. Sci., 52, nr. 9, 2007, p.2082

33.NAABER, P., MIKELSAAR, M., Adv. Appl. Microbiol., 54, 2004, p. 231

34.ELMER, GW., MCFARLAND, LV., Antimicrob. Agents Chemother ., 31, 1987, p. 129

35.RUSU, E., SARBU, I., CRISTESCU, C., COCULESCU, B.I., MOLDOVAN, H., et. al. Rev. Chim. (Bucharest), **67**, no. 12, 2016, p. 2417

36.COHEN, SH., GERDING, DN., JOHNSON, S., ET AL. Infect. Control. Hosp. Epidemiol., 31, 2010, p. 431

37.MITACHE, M.M., CURUTIU, C., RUSU, E., BAHNA, R., DITU, M., MOLDOVAN, H., ET.AL. Rev. Chim. (Bucharest), **68**, no. 3, 2017, p. 566

38.MITACHE, M.M., CURUTIU, C., GHEORGHE, I., LAZAR, V., BUCUR, M., BLEOTU, C., COCHIOR, D., Rev. Chim. (Bucharest), **69**, no. 1, 2018, p. 116

Manuscript received: 26.03.2018