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Isolation of Non-Toxigenic Strains of *Clostridium difficile* from Cases of Diarrhea Among Patients Hospitalized in Hematology/Oncology Ward

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A b s t r a c t

Clostridium difficile has become the most common cause of hospital acquired diarrhea after antibiotic treatment. The aim of this study was to determine the frequency of *C. difficile* associated diarrhea among hematology/oncology ward patients and to characterize isolated strains. Twenty three toxigenic and thirteen non-toxigenic strains were detected among fecal isolates. Antibiotic susceptibility testing to erythromycin and clindamycin demonstrated a high degree of resistance (MIC > 256 ug/ml) to both antibiotics in 9 out of 13 nontoxigenic *C. difficle* strains. Out of 7 patients with maximal frequency of diarrhea (10 empties/day) in 4 cases non-toxigenic strains of *C. difficile* were isolated. In these cases duration of diarrhea was longer in time than in cases of diarrhea caused by toxigenic strains. Further investigation with a larger patient population is necessary to better understand the role that non-toxigenic *C. difficile* strains play in disease development.

K e y w o r d s: *Clostridium difficile*, diarrhea, non-toxigenic strains

Clostridium difficile is an anaerobic, spore-forming gram-positive microorganism that was first identified in 1935. Toxigenic *C. difficile* is the etiologic agent of the most common cause of infectious, hospital-acquired diarrhea after long-term antibiotic treatment. Vancomycin and metronidazole are very effective against *C. difficile* strains, however there were reports describing pseudomembranous colitis (PMC) secondary to intravenous vanocomycin treatment (Szczęsny *et al*., 2002). *C. difficile*-associated diarrhea and colitis occur primarily in hospitalized patients in intensive care units, surgical wards, hematology/oncology units and they are more prevalent among older patients (Simon *et al*., 1993). Although, the most important risk factor is the use of antibiotics, other factors such as long-term hospitalization, surgery, immunosuppression, chemotherapy and radiation therapy also are described as a predisposing factors to *C. difficile*-associated diseases (Berild *et al*., 2003; Cohen *et al*., 1997). Disruption of the normal intestinal microflora, reduction or elimination of enteral feeding, immunosuppression from severe illnesses, and sustained ileus are common clinical events that can contribute to potential *C. difficile*-associated diaseases (Anand *et al*., 1993; Szczęsny *et al*., 2002). Toxin production by toxigenic strains of *C.difficile* initiates the pathologic process. Both: toxin A (enterotoxin) and toxin B (cytotoxin) appear to have synergistic effects upon the

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Table I
Occurrence of *C.difficile*-associated diarrhea in hematology/oncology ward after treatment with various medications

Medications	Number (%) of patients with diarrhea
Lincosamides	1 (2.8)
Carbapenems	2 (5.5)
Aminoglycosides	1 (2.8)
Cephalosporins	2 (5.5)
Penicillins with β-lactamase inhibitors	9 (25.0)
Antibiotics and cytostatics	3 (8.4)
Cytostatics	13 (36.1)
Others	5 (13.9)
TOTAL	36 (100)

destruction of gut mucosal cells. Lack of production of these two toxins is seen in nonpathogenic strains of *C. difficile*. In addition to toxins A/B *C. difficile* possesses other potential virulence factors that may contribute to colonization and clinical disease. Additional toxins (binary toxin), proteins, polysaccharide-capsule and enzyme (hyaluronidase and collagenase) production by certain strains of *C. difficile* have also been described (Poxton *et al*., 2001).

In this communication we present the occurrence of *C. difficile*-associated diarrhea among patients hospitalized in a 38 bed hematology/oncology ward of a tertiary care academic hospital during a 11 months-period. Altogether 369 samples were cultured on TCCA plates for *C. difficile* (294 stools taken from diarrheal patient and 75 environmental samples). Identification of *C. difficile* colonies was done according to routine laboratory-methods (Martirosian *et al*., 1995). Tox A/B ELISA test (TechLab Co., Blacksburg, VA, USA) and PCR for toxins A and B genes (YT 28/YT 29 primers for toxin A and YT 17/YT 19 primers for toxin B) were used to determine toxigenicity of isolates (Kuhl *et al.*, 1993; Martirosian *et al*., 2000). Susceptibility to erythromycin and clindamycin was determined by E-test method (AB biodisk, Solna, Sweden). Thirty six strains of *C. difficile* were isolated from the stools of patients (12.2%). No other intestinal pathogens were detected in stool samples of *C. difficile*-positive patients. Among 36 *C. difficile*-positive patients 15 were males (41.6%) and 21 were females (58.4%). The age range of those patients was between 23 and 84 years (average 53.7). Patients were treated usually with 2–3 medications (Table I) at the same time. The majority of cases of *C. difficile*-associated diarrhea were observed after using of cytostatics (13) and penicillins with beta-lactamase inhibitors (9). Twenty three out of 36 of *C. difficile*-positive patients had undergone previous surgery. The most common type of cancer in this patient population was lymphoma/leukemia in younger patients (younger than 40 years old) while other type of cancers were observed in older patients (age >60 years old) ($p<0,05$). Twenty three of the 36 *C. difficile* isolates were toxin-positive by ToxA/B ELISA and PCR, while remaining 13 isolates were toxin-negative. Antibiotic susceptibility testing to erythromycin and clindamycin demonstrated a high degree of resistance (MIC >256 ug/ml) to both antibiotics in 9 out of 13 non-toxigenic *C. difficile* strains isolated from stool samples (Table II). The clinical analyses of *C. difficile*-positive patients (frequency of diarrhea per day, duration of diarrhea, leukocytosis, max. temperature/days of duration) showed that maximal frequency of diarrhea per day was 10 with duration time of diarrhea from 3 to 8 days. Out of 7 patients with maximal frequency of diarrhea (10 empties/day) in 4 cases non-toxigenic strains of *C. difficile* were isolated. In these cases duration of diarrhea was longer than in cases of diarrhea caused by toxigenic strains. The isolation of non-toxigenic *C. difficile* from cases of diarrhea in patients receiving anti-cancer therapy suggests that colonization with non-toxigenic *C. difficile* may be more prevalent in this patient population. The fact that the majority of these non-toxigenic strains (9) were resistant to erythromycin and clindamycin (MIC > 256 ug/ml) may indicate the potential of these strains to be involved in pathogenesis. Non-toxigenic strains can possess additional virulence factors (*e.g.* binary toxin, enzymes, capsules, flagella, resistance to antibiotics and others) which may play a role in colonization/infection of patient, especially at the hematology/oncology ward (it was described before that patients receiving antineoplastic therapy, particularly leukemic patients and those after undergone surgeries are the main risk group of *C. difficile* infection) (Rupnik, 2001). Further investigation with a larger patient population is necessary to better understanding the role that these strains play in disease development.

Table II
Microbiological characteristics of *C. difficile* strains isolated from of patients' stool samples in hematology/oncology ward

No of strains	*C. difficile* toxins			M I C (μg/ml)		Duration of diarrhea/number of empties	Max. leucocytosis	Max. temperature/ duration	Surgery
	ELISA	PCR							
	TechLab A/B	A	B	Erythromycin	Clindamycin				
1	+	+	+	0.38	1.0	3/8	23.200	38.2/5	–
2	+	+	+	0.5	0.38	3/7	8.000	Subfebrile/7	+
3	+	+	+	0.125	>256	4/10	21.900	39.8/6	–
4	+	+	+	>256	>256	6/8	11.000	Subfebrile/6	–
5	+	+	+	0.19	0.19	5/7	9.800	Subfenrile/4	+
6	+	+	+	0.5	0.38	4/6	7.400	Subfebrile/6	–
7	+	+	+	1.5	>256	6/8	26.200	38.7/4	+
8	+	+	+	>256	>256	7/6	7.100	Subfebrile/5	–
9	+	+	+	0.38	0.75	6/8	8.060	Subfebrile/8	+
10	+	+	+	>256	>256	5/9	7.130	Subfebrile/6	–
11	+	+	+	0.75	2.0	8/10	27.250	39.7/10	+
12	+	+	+	>256	>256	4/8	6.800	Subfebrile/7	+
13	–	–	–	0.38	0.5	8/6	20200	39.6/9	–
14	+	+	+	0.75	1.0	5/7	6.400	Subfebrile/7	+
15	+	+	+	0.75	0.5	7/6	9.010	Subfebrile/6	+
16	+	+	+	0.5	1.0	4/7	11.000	Subfebrile/5	+
17	+	+	+	0.5	0.5	5/8	23.300	39.7/4	+
18	+	+	+	>256	0.5	7/10	10.400	Subfebrile/5	–
19	+	+	+	>256	>256	6/7	20.800	38.9/5	+
20	–	–	–	>256	>256	6/7	9.100	Subfebrile/7	+
21	–	–	–	>256	>256	8/10	7.400	Subfebrile/8	–
22	+	+	+	>256	>256	5/7	6.200	Subfebrile/8	+
23	–	–	–	>256	>256	7/8	11.000	Subfebrile/6	–
24	+	+	+	>256	>256	7/9	9.000	Subfebrile/6	+
25	–	–	–	>256	>256	7/7	22.600	38.9/6	+
26	–	–	–	>256	>256	8/8	7.800	Subfebrile/7	+
27	–	–	–	0.6	0.45	7/10	12.000	Subfebrile/7	+
28	–	–	–	0.7	1.0	6/8	26.450	39.4/5	–
29	–	–	–	>256	0.5	7/7	9.100	Subfebrile/6	+
30	–	–	–	>256	>256	6/10	8.700	Subfebrile/6	+
31	+	+	+	1.0	>256	6/9	21.500	38.7/4	+
32	–	–	–	>256	>256	6/8	6.000	Subfebrile/7	–
33	–	–	–	>256	>256	7/10	21.450	39.2/6	–
34	–	–	–	>256	>256	8/7	7.000	Subfebrile/7	+
35	+	+	+	0.38	0.5	5/7	12.000	Subfebrile/6	+
36	+	+	+	0.38	0.75	6/7	10.600	Subfebrile/8	+

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