MINISTRY OF EDUCATION AND TRAINING

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EPIDEMIOLOGICAL, CLINICAL CHARACTERISTICS AND RISK FACTORS OF THE DIARRHEA CAUSED BY *CLOSTRIDIUM DIFFICILE* AMONG THE ADULTS AT BACH MAI HOSPITAL, 2013 - 2017

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LIST OF PUBLISHED SCIENTIFIC ARTICLES RELATED TO THIS THESIS

1. Nguyen Thi Huong Giang, Pham Thi Thanh Thuy, Vu Thi Thu Huong, Tran Nhu Duong (2019), "Some risk factor contributing the diarrhea caused by Clostridium difficile among the adults at Bach Mai hospital, 2013 – 2017", Journal of Clinical Medicine, No. 112 (11 – 2019), pp. 114 – 120.
2. Nguyen Thi Huong Giang, Pham Thi Thanh Thuy, Vu Thi Thu Huong, Tran Nhu Duong (2019), "The epidemiological and clinical characteristics of diarrhea caused by Clostridium difficile among the adults at Bach Mai hospital during 2013 – 2017", Journal of Vietnam Preventive Medicine, vol. 29, No.13 – 2019, pp. 9 – 17.
3. Nguyen Thi Huong Giang, Vu Thi Thu Huong, Pham Thi Thanh Thuy, Tran Nhu Duong (2019), "Genotypic distribution characteristics of *Clostridium difficile* causing diarrhea in adults at Bach Mai Hospital in 2013 – 2017", Journal of Vietnam Preventive Medicine, vol. 29, No. 13 – 2019, pp. 18 – 25.

INTRODUCTION

Infections due to *Clostridium difficile* are an "emerging" disease that was of particular interest in North American and European countries in the early 21st century. *C.difficile* is the leading cause of diarrhea in industrialized countries (Canada, USA, UK,..) with hundreds of thousands cases and tens of thousands deaths each year. Diarrhea caused by *C.difficile* manifests itself as a common diarrhea, pseudomembranous colitis to fulminant colitis, and toxic intestinal aneurysm, especially in the adults. The disease is related to hospital infections, prolongs hospital stay, increase hospital fees. The risk of death is about 2% - 6%, higher in older people. Studies showing the risk of diarrhea caused by *C.difficile* are: elderly, suffering from many chronic diseases that reduce immunity, hospitalized treatment, antibiotic treatment ... There were not many studies on diarrhea due to *C.difficile*, an absolute anaerobic bacterium, diagnosed by toxin detection, in Vietnam. The difficulty in diagnosis has resulted in a limited understanding of disease caused by *C.difficile*. Bach Mai Hospital is a last treatment facility of the northern region that has many patients with diarrhea of unknown etiology. This study was therefore conducted in order to:

1. Describe some epidemiological and clinical characteristics of diarrhea caused by Clostridium difficile in adults at Bach Mai Hospital, in 2013 - 2017.

2. Analyze some risk factors related to diarrhea due to Clostridium difficile in the adults at Bach Mai Hospital, 2013 - 2017.

3. Identify genotypic distribution characteristics of Clostridium difficile causing diarrhea in adults at Bach Mai Hospital, 2013 - 2017.

*** New contributions of the thesis**:

The thesis expressed the results of a five-year research topic (2013 - 2017), including a systematic epidemiological, clinical features and risk factors of diarrhea caused by *C.difficile* in the adults, the distribution of *C.difficile* genotype that caused diarrhea in the country with 8 genotypes belonging to the toxin strains A+B+ and A-B+, the prevalence and role of *C.difficile* in causing diarrhea among elderly people in the provinces of Northern Vietnam. This is the first study to identify the risk factors contributing diarrhea caused by *C.difficile* in Vietnam: age ≥ 65, urban living and cycle dialysis.

The obtained information is the basis for research and monitoring epidemiological changes; comparing the genetic characters of *C.difficile*

with those obtained from other countries in the region and around the world; analyzing the relationship between genotypes and pathogenesis ... The research results also supplement the materials in training, provide scientific evidence about clinical, risk factors of diarrhea caused by *C. difficile*, contributing to raising the knowledge and vigilance of physicians, helping to guide the diagnosis, access to treatment and prevention of diarrhea due to *C.difficile* in Vietnam.

*** The structure of the thesis**:

The thesis includes 137 pages: 02 pages of Introduction; 35 pages of Literature Overview; 21 pages of Subjects and research methods; 38 pages of Results; 37 pages of Discussion and 02 pages of Conclusion, 01 page of Recommendation.

Chapter 1. LITERATURE OVERVIEW

1.1 General points about diarrhea caused by *Clostridium difficile*

1.1.1 C.difficile bacillus

C.*difficile* is a gram-positive, anaerobic gram-positive bacillus, very difficult to grow, exists in two forms: inactive spore form which is antibiotic resistance and active form that can produce toxins, sensitive with antibiotics.

Two external toxins of *C.difficile* include: toxin A (toxin A, *tcd*A) is an intestinal toxin, and toxin B (toxin B, *tcd*B) is cytotoxic. There are 3 strains of *C.difficile*: A+B+, A-B+ and A-B-, but only A+B+ and A-B+ strains cause disease in humans. Some strains can produce double toxins (binary toxin), causing more severe clinical circumstances (eg: BI/NAP1/*027* and *078* strains).

1.1.2. Diarrhea caused by Clostridium difficile

Diarrhea when there is a change in the normal intestinal motility, increase in water amount, volume or frequency of diarrhea. Called "diarrhea" when the stool does not form or liquid, more than 3 times/day.

Diarrhea due to *Clostridium difficile*: presence of diarrhea (liquid stools ≥ 3 times/24 hours), stool test for toxin or gene responsible for toxin of *C.difficile* or colitis/histopathology with colitis pseudomembranous.

1.2. Epidemiological and clinical characteristics of diarrhea caused by *C.difficile*

1.2.1. Epidemiology of diarrhea caused by Clostridium difficile

C.difficile produces toxin causing diarrhea has been identified since the late 1970s. At the beginning of the 21st century, a series of hospital diarrhea outbreaks due to highly toxic pathogenic *C.difficile* that caused

serious illness, many complications, death and high recurrence have been recorded in Canada, the United States, the United Kingdom, Belgium, the Netherlands ... Infections caused by *C.difficile* were hospitalized in the United States from 25,200 cases (in 1998) to over 450,000 (in 2015) with over 35,000 died. Diarrhea due to *C.difficile* accounts for 10% - 20% of diarrhea cases in some Asian countries. In 2016, C.*difficile* caused diarrhea was identified in Ho Chi Minh City - Vietnam, but there were only few cases of diarrhea caused by this bacteria founded in the North.

1.2.2. Clinical manifestation of disease caused by Clostridium difficile

Clinical symptoms:

• Diarrhea: about <10 times/day. Watery stools, may be with mucous, with a specific odor and rarely with blood in the stool.

• *C.difficile* colitis: common

• Pseudomembranous colitis: typical for disease due to *C.difficile*.

• Acute colitis: about 3%, including perforation, intestinal obstruction, aneurysm and death.

• Systemic symptoms: Pain and bloating. Few cases have nausea, vomiting.

• Severe diarrhea: Fever of >38.30C, blood albumin <25g/l, white blood cells >15 G/l, blood creatinine >133µmol/L (or >1.5 times the baseline value)

Diagnostic tests for diarrhea caused by C.difficile:

• Cytotoxicity test: detect toxins of *C.difficile*.

• EIAs: detect toxins (A, B).

• Stool culture to find *C.difficile*. Testing for detect *C.difficile* toxin (cytotoxicity or EIAs or PCR for genotoxicity) is required.

Treatment of diarrhea caused by C.difficile:

Discontinue unnecessary antibiotic. Use specific antibiotics: metronidazole, vancomycin, fidaxomycin. Combination treatment: intestinal probiotic, stool transplant, surgery (for complication cases).

1.3. Risk factors for diarrhea caused by *C.difficile*

Diarrhea caused by *C.difficile* appeared when having the following factors: firstly, being infected with spores of toxin *C.difficile*; second, there is a change in the existing colonic microorganism population in the colon, allowing *C.difficile* to grow; third, the host's immune system is altered. Disease occurred more frequently and more severe among the elderly and in the group having immune system respond ineffectively.

• High age: 70% - 80% of diarrhea cases caused by *C.difficile* occurred among people aged ≥65 years.
• Chronic illnesses: people with kidney failure, organ transplantation, diabetes... who often have weakened immune system, or use a lot of drugs, easily be infected that need to use antibiotics, frequent hospitalization or frequent exposure to the medical environment ...
• Exposure to *C.difficile* during hospitalization: 94% of cases of diarrhea due to *C.difficile* is related to medical care.
• Use of antibiotics: disorders of intestinal microflora, facilitating the growth of *C.difficile* and cause disease.
• *C.difficile* virulence: sharp increase of diarrhea cases in the early 21st century due to high virulence strain NAP1/*027*/BI in European and American countries, strain *078* caused un epidemic outbreak in Europe, strain *017* caused serious illness in Asia.
•Immunodeficiency: HIV infection, prolonged use of corticosteroids, immunosuppressants, , insufficient antibodies to toxin A of *C.difficile*.
• Use proton pumb inhibitors: reduce the elimination of bacteria in the stomach; decreased neutrophil activity and bacterial disorders in the intestine.
• Using chemicals to treat cancer: changing intestinal microflora, causing inflammation, necrosis of the intestine, creating anaerobic environment suitable for *C.difficile* to cause disease

1.4. Genotypic distribution characteristics of Clostridium difficile

The genome size of *C.difficile* is 4,290,252 bp, the G+C ratio of the whole genome is about 29%. *C.difficile* strains are differentiated into two main groups: PCR ribotype and toxinotype. The first group is the 16S-23S rRNA genotype and the second is to identify the toxin gene. There are about 116 *C.difficile* genotypes based on mutations in the genome coding different toxins.

Epidemiological regions with different time points characterized with circulation of different genotypes of *C.difficile*. In the beginning of the 21st century, the ribotype *027* strain was detected in all provinces of Canada and more than 40 states in the United States. Strains *078* caused serious illness in Europe while strain *244* is the dominant causes of disease in Oceania. Strains *017* were well documented in Asian studies.

Chapter 2. STUDY SUBJECTS AND METHODS

2.1. Study subject

- Patients ≥15 years old, diagnosed with diarrhea due to *C.difficile* (for Objective 1);
- Control case study: Patient group mentioned above, control group is diarrhea cases with stool culture negative for *C.difficile* (for Objective 2);
- *C.difficile* strains isolated from diarrhea patients (for Objective 3).

2.2. Study location

Bach Mai Hospital and National Institute of Hygiene and Epidemiology.

2.3. Study period: In 5 years, from 2013 to 2017.

2.4. Research design

- Descriptive epidemiological design
- Case -control study design

2.5. Sample size and sampling methods

For objective 1: sample size to describe cases of diarrhea caused by *C.difficile* was calculated by following formula:

$$n = Z^2_{1-\alpha/2} \frac{(1-p)}{p.\varepsilon^2}$$

$Z_{1-\alpha/2}$ = 1.96 (reliability α: 95%); p: Prediction rate of diarrhea due to *C.difficile* among hospitalized diarrhea cases (from 10% - 25%). Take p = 0.2. ε: relative error (0.4). n: minimum sample size to achieve is 97.

• Sampling: All 101 patients diagnosed with diarrhea due to *C.difficile* were selected for the study.

For Objective 2: Sample size for patient group in case-control study

$$n = \frac{\left[Z_{1-\alpha/2}\sqrt{2P_2(1-P_2)} + Z_{1-\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$$

p1: rate of individuals exposed to risk factors (a history of hospitalization within 8 weeks before diarrhea) in patient group was 80% (= 0.8). p2: 60% of the individuals exposed to the risk factor in the control group (= 0.6). Reliability coefficient (95% confidence level) $Z_{1-\alpha/2}$ = 1,96. Reliability coefficient (95% confidence level). The strength of the test 1-β = 80%. The calculated sample size: n = 90.

To increase the statistical force of the study and reduce some of the confounding factors, we selected samples according to the disease: control ratio of 1:3. Control group was selected suitable for gender group, treatment department, time of diarrhea in the year.

• Sampling: Among 101 patients with diarrhea due to *C.difficile*, 91 cases having the same criteria as 273 controls were selected.

For Objective 3: All strains of *C.difficile* isolated from patients in goal 1 were included for study.

2.6. Research materials:

- Questionnaire for interviewing diarrhea patients and factors related to diarrhea caused by *C.difficile*
- Stool samples and blood samples taken from diarrhea patients
- Laboratory of anaerobic bacteria in Institute of Hygiene and Epidemiology, laboratory of biochemistry, hematology of Bach Mai hospital in compliance with ISO 15189.
- Positive control samples provided from Microbiologics, Minnesota (USA); Department of Bacteriology II, Tokyo National Institute of Infectious Diseases (Japan); Department of Microbiology – NIHE.

2.7. Laboratory techniques used

- Culture technique to isolate anaerobic bacteria
- PCR technique to detect genotypes of toxins A and B
- Technique for determining the minimum inhibitory concentration MIC
- PCR ribotyping technique determines the ribotype of *C.difficile*

2.8. Research Ethics

The study design was approved by the Ethics Committee for Biomedical Research of the National Institute of Hygiene and Epidemiology (NIHE), No. IRB - VN01057 - 33/2015 and No. IRB - VN01057 - 32/2016; and by The Science and Ethics Council of Bach Mai Hospital, No. 561/QD - BM

Chapter 3: RESULTS

3.1. Some epidemiological clinical characters of diarrhea caused by *C.difficile* among the adults at Bach Mai Hospital, 2013 – 2017

3.1.1. Epidemiological characters of diarrhea due to C.difficile

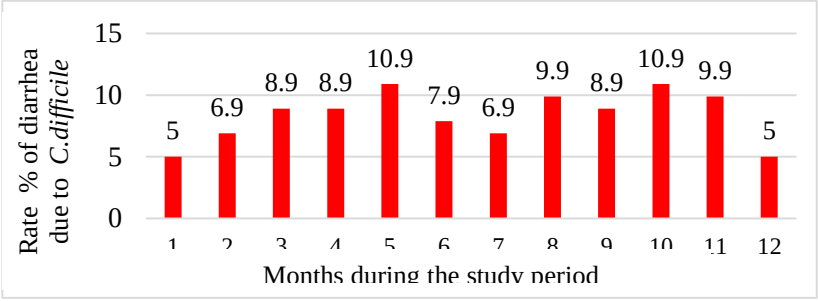

Chart 3.1: Distribution of diarrhea due to *C.difficile* by month (n=101)

Disease was recorded in all months of the year, higher number in May, October (10.9%) and August and November (9.9%). The total number of cases in the months during the 5-year period ranged from 5 to 11 cases (5% - 10.9%).

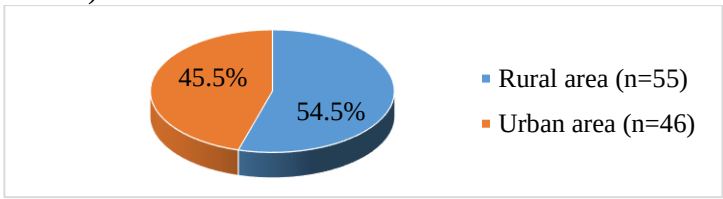

Chart 3.5: Distribution of diarrhea caused due to *C.difficile* by socio-economic zone (n=101)

Chart 3.5 shows that patients were more from rural areas (54.5%) than from urban areas (45.5%).

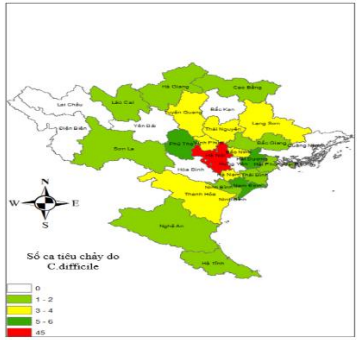

Fig 3.1: Map of distribution of diarrhea due to *C.difficile*

Figure 3.1 shows the distribution of diarrhea caused by *C.difficile* in 21/28 provinces/cities in Northern Vietnam, mostly in Hanoi and neighboring provinces.

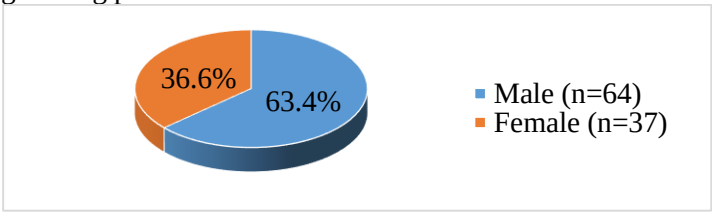

Chart 3.7: Distribution of cases with diarrhea due to *C.difficile* according to their sex (n=101)

Men accounted for a higher proportion (63.4%) than women (36.6%) with the ratio of 1.7: 1.

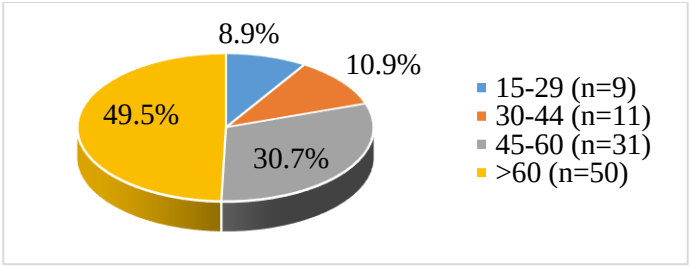

Chart 3.8: Distribution of diarrhea cases caused by *C.difficile* according to their age (n=101)

The number of cases increases with age. The group of patients aged over 60 years old accounted for the most (49.5%) among all age groups.

3.1.2. Clinical characters of diarrhea due to C.difficile

Table 3.1. Symptoms of patients with diarrhea due to *C.difficile*

Symptoms (n=101)	Yes n (%)	No n (%)
Fever	**78 (77,2)**	23 (22,8)
Abdominal pain	**63 (62,4)**	38 (37,6)
Abdominal distention	**79 (78,2)**	22 (21,8)
Nausea, vomiting	15 (14,9)	86 (85,1)
Mucus stools	20 (19,8)	81 (80,2)
Bloody stools	17 (16,8)	84 (83,2)
Hypotension	13 (12,9)	88 (87,1)

Common symptoms in patients suffering from diarrhea due to *C.difficile* were fever (77.2%), abdominal pain (62.4%), distension (78.2%). Nausea – vomiting, mucus stools and bloody stools were recorded with less frequency. There were 12.9% of patients with hypotension.

Table 3.2. The characters of diarrhea due to *C.difficile*

Characters of diarrhea		Frequency (n=101)	Rate%
Maximal number of diarrheas per day	**3-6 times per day**	**66**	**65.3**
	7-10 times/day	25	24.8
	>10 times/day	10	9.9
	X ± SD (min, max)	7 ± 4,9 (3 – 30)	
Number of days with diarrhea	1-3 days	20	19,8
	4-13 days	**50**	**49,5**
	≥14 days	**31**	**30,7**
	Mean (min, max)	8 days (1 – 170 days)	

X ± SD: mean ± SD; min: minimal value; max: maximal value

Diarrhea due to *C.difficile* is usually 3-6 times /day (65.3%). The average number of diarrhea episodes was 7 ± 4.9 times. Diarrhea usually lasts ≥4 days (80.2%), median is 8 days. Especially, 30.7% of diarrhea lasts for more than 2 weeks.

Table 3.3. Results of testing for inflammatory reaction in patients with diarrhea due to *C.difficile*

Test parameters	Normal n (%)	Increased n (%)	Significant increased n (%)
White blood cell count (n=101)	40 (39.6%)	**33 (32.7%)**	**28 (27.7%)**
Pro-calcitonin (n=37)	0	**28 (75.7%)**	**9 (24.3%)**

Table 3.3 shows that the number of white blood cells increased in 60.4% of cases, those with white blood cell count increase of >15 G/L accounted for 27.7%. Among 37 patients tested for pro-calcitonin, most of them increased by 0.05 to 10 ng/mL (75.7%), others have it increased above 10 ng/mL (24.3%).

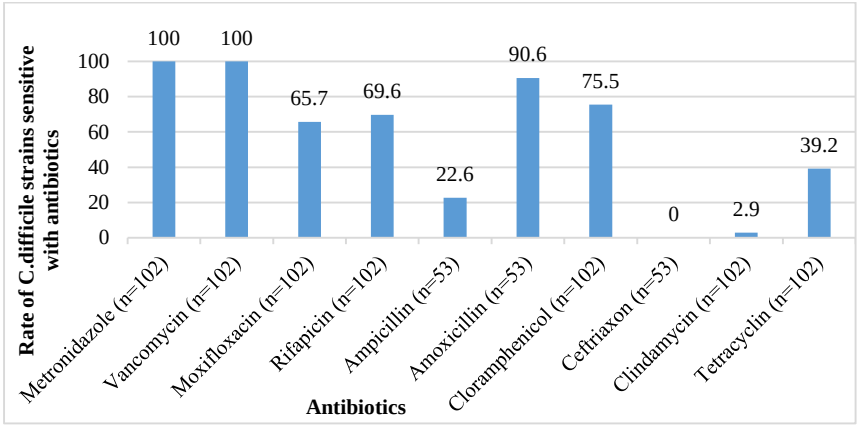

Chart 3.11: Rate of *C.difficile* sensitive with antibiotics

Chart 3.11 shows that all *C.difficile* strains were sensitive to metronidazole and vancomycin. The sensitivity was found reduced more with amoxicillin, chloramphenicol, rifapicin and moxifloxacin. None of the strains was sensitive to ceftriaxon.

Table 3.10. Treatment of diarrhea caused by *C.difficile*

Patients	Cured	Treated & discharged	Referall	Treatment failure	Dead
Patients in ICU (n=35)	4 (11.%)	5 (14.3%)	12 (34.3%)	**9 (25.7%)**	**5 (14.3%)**
Patients in DID (n=53)	23 (43.4%)	13 (24.5%)	11 (20.8%)	5 (9.4%)	1 (1.9%)
Patients of other units (n=13)	4 (30.8%)	3 (23%)	4 (30.8%)	2 (15.4%)	0
Total (n=101)	31 (30.7%)	21 (20.8%)	27 (26.7%)	**16 (15.8%)**	**6 (5.9%)**

Note: ICU – Intensive care Unit; DID- Department of Infectious Diseases

Table 3.10 shows that the proportion of patients with bad progress (death and severe illness) among total studied patients was 21.7%, and death was 5.9%. This rate was higher in patients who treated at the intensive care unit: the adverse event was 40%, the death was 14.3%.

3.2. Risk factors for diarrhea caused by *C.difficile*

3.2.1. Risk factors for diarrhea due to C.difficile by univariate analysis

Table 3.12. Patient's age and diarrhea due to *C.difficile*

Age		Patients (n =91)	Controls (n = 273)	OR (95% CI)	p
Age groups	15-29	7	32	1	
	30-44	9	57	0.72 (0.25-2.12)	0.554
	45-60	29	71	1.87 (0.74-4.71)	0.186
	>60	46	113	1.86 (0.77-4.52)	0.170
Age groups	**< 65**	50	191	1	
	≥ 65	41	82	**1,91 (1,17-3,11)**	**0,009***

Table 3.12 shows that patients ≥65 years old were at risk of C.difficile diarrhea 1.91 times higher than patients aged <65 (95% CI: 1.17 - 3.11).

Table 3.14. Chronic disease/health status and diarrhea due to *C.difficile*

Chronic disease/health status		Patients (n = 91)	Controls (n = 273)	OR	95% CI	p
Diabetes	Yes	21	46	1.48	0.83-2.65	0.186
	No	70	227	1		
Chronic kidney dis.	Yes	11	18	1.95	0.88-4.30	0.99
	No	80	255	1		
Cycle Dialysis	Yes	**5**	**3**	**5.23**	**1.23-22.35**	**0.025***
	No	86	270	1		
Chronic resp. dis.	Yes	**11**	**15**	**2.37**	**1.04-5.36**	**0.039***
	No	80	258	1		

Cycle dialysis possess a risk of *C.difficile*-associated diarrhea in 5.23 times higher (95% CI: 1.23 - 22.35). Having chronic respiratory disease increased the risk of *C.difficile* diarrhea 2.37 times higher (95% CI: 1.04 - 5.36).

Table 3.15. Relation between living place and diarrhea due to *C.difficile*

Living place	Patients (n=91)	Controls (n=273)	OR (95%CI)	p
Rural	47	178	1	
Urbal	44	95	**1.75 (1.08-2.84)**	**0.022***

Patients living in urban might have risk of diarrhea due to *C.difficile* higher 1.75 time in comparison to patients living in rural (95% CI: 1,08 – 2,84).

Table 3.16. History of hospitalization in 8 weeks prior to diarrhea

Hospitalization in last 8 weeks	Patients n = 91	Controls n = 273	OR (95% CI)	p
Yes	67 (73.6)	172 (63)	1.64 (0.97-2.78)	0.066
No	24 (26.4)	101 (37)	1	
Days of hospitalization before diarrhea: mean (min, max)	10 (0-84)	7 (0-90)	1.02 (0.99-1.05)	0.061

The hospitalization in last 8 weeks was a risk for getting diarrhea due to *C.difficile*, OR = 1.64, but the difference was not significant (95% CI: 0.97 - 2.78). Median hospitalization time before diarrhea was 10 days for patient group, 7 days for control group, but the difference was not statistically significant (p>0.05).

Table 3.17. History of antibiotic use within 8 weeks prior to diarrhea

History of antibiotic use within 8 weeks prior to diarrhea	Patient group (n = 91)	Control group (n = 273)	OR (95%CI)	p
Yes	61 (67)	165 (60.4)	1.33 (0.81-2.19)	0.262
No	30 (33)	108 (39.6)	1	
≥ 3 types of antiobiotics	20	52	1.20 (0.67-2.14)	0.544
< 3 types of antiobiotics	71	221	1	

Using antibiotics within 8 weeks before diarrhea possed a higher risk for *C.difficile* (OR = 1.33), but the difference was not statistically significant (p>0.05). The same situation for using various antibiotics in 8 weeks prior to the diarrhea.

Table 3.18. Antiobiotics used in 8 weeks prior the diarrhea

Antibiotics		patients (n = 91)	Control (n = 273)	OR (95% CI)	p
Penicilin	Yes	4	9	1.35 (0.41-4.49)	0.626
	No	87	264	1	
Cephalosporin	Yes	33	74	1.53 (0.92-2.53)	0.098
	No	58	199	1	
Carbapenem	Yes	37	97	1.24 (0.76-2.02)	0.380
	No	54	176	1	
Aminosid	Yes	6	12	1.54 (0.56-4.22)	0.405
	No	85	261	1	
Macrolid	Yes	6	11	1.68 (0.60-4.68)	0.320
	No	85	262	1	
Clindamycin	Yes	1	4	0.75 (0.08-6.77)	0.796
	No	90	269	1	
Quinolon	Yes	19	51	1.15 (0.64-2.07)	0.645
	No	72	222	1	
Cotrimoxazole	Yes	0	2	-	
	No	91	271	1	
Metronidazole	Yes	4	29	0.39 (0.13-1.13)	0.083
	No	87	244	1	
Glycopeptid	Yes	3	37	**0.22 (0.07-0.72)**	**0.013***
	No	88	236	1	

Table 3.18 shows that using glycopeptide antibiotics within 8 weeks before diarrhea to treat other diseases possess a lower risk for *C.difficile*, equal to 0.22 times in the control group, p <0.05 (95% CI : 0.07 - 0.72).

Table 3.20. Clinical symptoms of diarrhea due to *C.difficile*

Symptoms		Patient group n = 91	Control group n = 273	OR (95%CI)	p
Fever	>37.5°C	74	222	1 (0.54-1.84)	1
	≤37.5°C	17	51	1	
Abdominal paint	Yes	56	171	0.95 (0.59-1.56)	0.851
	No	35	102	1	
Muscus stools	Yes	17	12	**4.98(2.28-10.89)**	**<0.001***
	No	74	260	1	
Bloody stools	Yes	15	21	**2.36 (1.16-4.80)**	**0.018***
	No	76	251	1	
Hypotension	Yes	12	33	1.10 (0.54-2.24)	0.783
	No	79	240	1	

Mucus stool was found associated with the diagnosis of diarrhea due to *C.difficile* with 4.89 times higher (95% CI: 2.28 - 10.89). Blood stools were also an indicator factor in the diagnosis of diarrhea due to *C.difficile*, with 2.36 times higher than bloodless stools (95% CI: 1.16 - 4.8).

Table 3.21. The frequency of diarrhea per day

Diarrhea frequency	Patient group (n = 91)	Control group (n = 273)	OR (95%CI)	p
≤6 times	60	205	1	
7-10 times	21	37	**1.94 (1.06-3.56)**	**0.033***
>10 times	10	31	1.1 (0.51-2.38)	0.804
Average No. of diarrhea/day	7.0±5.1 (3-30)	6.0±3.7 (3-20)	1.06 (1.0-1.11)	0.049*

Diarrhea 7-10 times a day is a factor related to diarrhea caused by *C.difficile*, which was 1.94 times higher than less frequency diarrhea (95% CI: 1.06 - 3.56).

3.2.2. Risk factors for diarrhea due to C.difficile by multivariable analysis

Table 3.25. The risk of diarrhea caused by *C.difficile* by multivariable analysis

No	Variable	Patients n = 91	Controls n = 273	OR (95%CI)	p
1	≥ 65 y.o.	41	82	**2.01 (1.20-3.40)**	**0.009**
2	Living in urban	44	95	**1.76 (1.05-2.96)**	**0.032**
3	Need dialysis cycle	5	3	**7.32 (1.55-34.6)**	**0.012**
4	Glycopeptide used in 8 weeks before	3	37	**0.18 (0.05-0.67)**	**0.011**
5	Muscus stools	17	12	**5.94 (2.5-14.12)**	**<0.001**
6	Diarrhea 7-10 times/day	21	37	**1.98 (1.04-3.77)**	**0.037**

3 risk factors for diarrhea due to *C.difficile* were found in this study: age of ≥65 years old (OR = 2.01); urban living (OR = 1.76); hemodialysis (OR = 7.32). Two factors were found related to the diagnosis of diarrhea due to *C.difficile*: mucus stools (OR = 5.94); diarrhea 7-10 times/day (OR = 1.98). The protective factor against diarrhea *C.difficile* is the use of glycopeptide antibiotics to treat other diseases for 8 weeks before diarrhea (OR = 0.18).

3.3. Genotypic distribution characteristics of *C.difficile* caused diarrhea among the adults at Bach Mai Hospital, 2013 - 2017

3.3.1. Genes expressed toxin of C.difficile

Table 3.26: Rate of genes expressed toxin of *C.difficile* in patients

Toxin expressed gene	Patient number (n=101)	Rate %
A+B+	50	49.5
A-B+	45	44.6
A+B+ and A-B+	6	5.9

Both toxin genes A+B+ (49.5% of patients) and A-B+ (44.6% of patients) of *C.difficile* causing diarrhea were detected. 6 patients (5.9%) were infected with 2 strains of *C.difficile* which carried toxin gene A+B+ and toxin gene A-B+.

Table 3.30: History of hospitalization for 8 weeks prior to diarrhea by toxin gene

History of hospitalization for 8 weeks prior to diarrhea	A+B+ n (%)	A-B+ n (%)	Having both toxins n (%)	Total n (%)	p
Yes	32 (64.0)	37 (82.2)	6 (100)	75 (74.3)	**0.046**
No	18 (36.0)	8 (17.8)	0	26 (25.7)	
Total	50 (100)	45 (100)	6 (100)	101 (100)	

(Fisher's exact test was applied)

C.difficile diarrhea cases caused by *C.difficile* carrying toxins A+B+, A-B+ or both toxins, showed to have a history of hospitalization within 8 weeks before diarrhea with higher rate. This difference was statistically significant with $p < 0.05$ (Fisher's exact test).

Table 3.32: History of antibiotic use in 8 weeks prior to diarrhea

Use of antibiotics in last 8 weeks	A+B+ n (%)	A-B+ n (%)	Having both toxins n (%)	Total n (%)	p
Yes	27 (54.0)	33 (73.3)	6 (100)	66 (65.4)	**0.024**
No	23 (46.0)	12 (26.7)	0	35 (34.6)	
Total	50 (100)	45 (100)	6 (100)	101 (100)	

(Fisher's exact test was applied)

Diarrhea due to *C.difficile* having toxins A+B+, A-B+ or both toxins, with a history of using antibiotics within 8 weeks before diarrhea was detected higher than the group without a history of antibiotic use in 8 last week. This difference was statistically significant ($p<0.05$) (Fisher's exact test).

3.3.2. Characteristics of the ribotype genotype of C.difficile

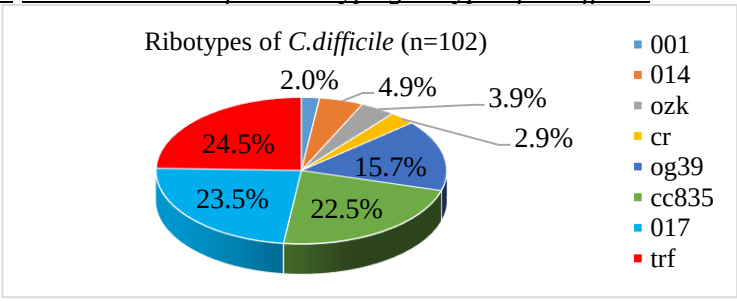

Chart 3.13: Ribotype genotype of *C.difficile* (n=102)

8 ribotype genotypes of *C.difficile* were identified, the most were ribotype genotypes *trf* (24.5%), *017* (23.5%) and *cc835* (22.5%).

Table 3.34: Distribution of ribotype genotype of *C.difficile* and genotoxic genes

Ribotype genotype (n = 102)	A+B+ (n = 53)	A-B+ (n = 49)
001	2 (3,8)	0
014	5 (9,4)	0
ozk	4 (7,5)	0
cr	3 (5,7)	0
og39	**16 (30,2)**	0
cc835	**23 (43,4)**	0
017	0	**24 (49,0)**
trf	0	**25 (51,0)**

C.difficile carries the A+B+ toxin gene with 6 genotypes of ribotype: *001, 014, ozk, cr, og39* and *cc835. 2* genotypes ribotype *017* and *trf* carry the A-B+ toxin gene.

Chapter 4: DISCUSSION

4.1. Epidemiological, clinical characters of diarrhea caused by *C.difficile* among the adults at Bach Mai Hospital, 2013 – 2017

4.1.1. Epidemiological characters of diarrhea caused by C.difficile

Diarrhea due to *C.difficile* is common in all months of the year, with about an average of 5 to 11 cases per month in 5 years of the study in (5% to 10.9%). *C.difficile* is recognized as a causative agent of diarrhea in hospitals, and is un infectious factors that are not dependent on weather, so the seasonal pathogenicity is not clear.

Patients involved in the study were from 21/28 provinces/cities of Northern Vietnam, the most in Hanoi (45 cases) and neighboring provinces. Bach Mai Hospital is located in Hanoi City, might be related to reason of most patients with diarrhea due to *C.difficile* mainly come from here. Due to convenient transportation and living, patients from provinces/cities closer to Hanoi are also more than other provinces.

The disease is more common in men (63.4%) than in women (36.6%), the ratio of male to female is 1.7: 1. This data is similar to result reported by Vu Thuy Duong et al (2016) in some hospitals in the South and the South Central of Vietnam: among 92 patients with diarrhea due to *C.diffifile* aged >15 years old between 2009 and 2014, women accounted for 39%. Predrag (2016) reports a male to female ratio of 20:17 in Serbia. However, Ngamskulrungroj (2015) reported more women, accounting for 62.3% in Thailand. In the United States, females account for 76% of cases with

diarrhea due to *C.difficile* from the community and 60% of hospitalized. In France, Ogielska (2015) reported a ratio of male to female suffered from *C.difficile* diarrhea in the community of 62: 74.

Diarrhea due to *C.difficile* is more common in older people. The number of patients increased with age. Patients of >60 years old accounted for 49.5% among all diarrhea cases. According to Kurti in Hungary, patients with diarrhea caused by *C.difficile* aged >60 years old accounted for 83.4%. Recent studies have explained that *C.difficile* is the leading cause of diarrhea among elderly people in industrialized countries. Firstly, the prevalence of *C.difficile* in the gastrointestinal tract of older people is higher than that of young people. Secondly, the elderly infected with the *C.difficile* strain that carried the toxin gene with high proportion, only few strains are not producing toxins. Thirdly, elderly people are more susceptible to *C.difficile*, susceptible to disease because of the weakened immune system, lack of antibody antibodies that have protective effects against the disease causative.

4.1.2. Clinical characteristics of diarrhea caused by C.difficile

In our study, the common found clinical symptoms in patients with diarrhea due to *C.difficile* were fever (77.2%), abdominal pain (62.4%) and abdominal distention (78.2%). Less common were nausea, vomiting (14.9%), mucus stools (19.8%) and bloody stools (16.8%). About 12.9% of patients with hypotension need vasopressors. Delays in diagnosis and treatment will increase mortality. Many studies reported about no clinical symptoms specific to diarrhea due to *C.difficile*. According to Bartlett, fever occurs in 28% and abdominal pain occurs in 22% of cases with diarrhea due to *C.difficile*. Similar to ours findings, Oldfield (2014) noted that *C.difficile* diarrhea caused blooding stools in 5% - 10% of cases, and Kim (2011) reported 22.5% of diarrhea patients have mucus stool.

The number of diarrhea caused by *C.difficile* is usually 3-6 times per day (65.3%), those with > 10 times/day accounted for 9.9%, the average is 7 ± 4.9 times. The length of diarrhea caused by *C.difficile* usually lasts more than 4 days (80.2%). Similarly to our findings, in Shanghai, Kim (2011) reported an average duration of 7 ± 6.1 days for diarrhea due to *C.difficile*, and 17.5% of cases had diarrhea lasted for >10 times/day. Our study showed 30.7% of cases had diarrhea in ≥14 days, the longest is 170 days. Humphreys (2014) noted that diarrhea caused by *C.difficile* could last >30 days. Failure to diagnose *C.difficile* etiology and no prompt treatment will increase hospital stay, increase hospital fees, increase complications and death.

Diarrhea due to *C.difficile* is an infectious diarrhea with inflammatory response reaction against bacteria and toxins. In this study, peripheral blood leukocytes increased in 60.2% of cases, of these, 27.7% of cases showed to have an increase above 15 G/L. The pro-calcitonin reaction is valuable in assessing the level of infection, but it is high cost, only applied later in the study. About 37/101 diarrhea cases due to *C.difficile* were tested for pro-calcitonin, all showed to have this increased, of these, 75.7% of cases increased pro-calcitonin moderately (0.5 - 10 ng/ml) and 24.3% increased highly (>10 ng/ml). Bartlett (1980) and Bobo (2011) also noted: leukocytosis increased in 50% of cases, some of them have this reached up to >50 G/L. Little is reported about pro-calcitonin changes in *C.difficile*-associated diarrhea.

All isolated *C.difficile* strains were sensitive to two antibiotics recommended for treatment, metronidazole and vancomycin. *C.difficile* is also sensitive to amoxicillin (90.6%), chloramphenicol (75.5%), rifampicin (69.6%) and moxifloxacin (65.7%). This study is similar to findings of Ngamskulrungroj (2015) in Thailand: *C. difficile* was found sensitive to metronidazole, vancomycin, daptomycin and tygercyclin 98.2% - 100%, only 54.8% of the strains are still sensitive to moxifloxacin.

About treatment results: Patients with serious illness asked for return home, were classified as a bad progression group. Rate of patients with bad progress (severe illness - death) reached 21.7%, (5.9% of death, 15.8% of severe illness). This rate was higher in patients treated in the intensive care unit, including of 40% with a bad progress (death rate of 14.3%, severe recovery of 25.7%). Similar to the comments of Leffler (2015), *C.difficile* is associated with mortality (5%), contributing to 15% - 20% of all mortality causes. At the Intensive Care Unit, the adverse events in diarrhea patients due to *C.difficile* were found related to the initial severe condition of the disease, need therefore the intensive care and continue using antibiotic treatment, facilitating *C.difficile* growth in the gastrointestinal tract.

4.2. Some risk factors for diarrhea caused by *C.difficile* among the adults at Bach Mai Hospital, 2013 - 2017

The study showed that patients aged ≥ 65 years old were 1.91 times more likely to develop diarrhea caused by *C.difficile* than patients under 65 years (95% CI: 1.17 - 3.11). Many studies have also noted the older age is an important risk factor for diarrhea due to *C.difficile*. According to Bauer (2011), the risk of diarrhea due to *C.difficile* in patients over 65 years old was 3.26 times higher than patients of younger age (95% CI: 1.08 - 9.78).

According to Leffler (2015), when diarrhea caused by *C.difficile* occurred in hospitals, the risk of infection in patients over 65 years old years is 10 times higher than in patients of younger age. The hypothesis is that advanced age is related to the likelihood of chronic illnesses, often exposed to medical care and a compromised immune system.

Patients on cycle dialysis have a risk for diarrhea due to *C.difficile* higher 5.23 times than patients without dialysis (95% CI: 1.23 - 22.35); patients with chronic respiratory disease have a risk of 2.37 times higher than patients without chronic respiratory disease (95% CI: 1.04 - 5.36). Dubberke (2007) also noted that people with chronic respiratory disease was 1.5 times more likely to develop diarrhea due to *C.difficile* (95% CI: 1.2 - 2.0) and those on dialysis. the risk of diarrhea due to *C.difficile* is 3.5 times higher than that of patients without dialysis (95% CI: 2.5 - 4.8). Patients on cycle dialysis often have to go to health facilities, the risk of exposure to *C.difficile* therefore is higher. According to Dudzicz (2017), dialysis possesses a 3.34 times higher risk for being infected with *C.difficile* than those do not need dialysis.

In urban areas, the risk of diarrhea due to *C.difficile* is higher than in rural areas [OR = 1.75 (95% CI: 1.08 - 2.84)]. There have not been many studies on the risk of diarrhea caused by *C.difficile* in rural and urban areas. Broad-spectrum antibiotics in urban areas may be used more widely, increasing the risk of intestinal bacterial disorders. In urban areas, it is easier to access health facilities, so might increase the chance to exposure to *C.difficile* infection. Further research is needed on the prevalence of *C.difficile* among rural and urban populations in Vietnam.

Hospitalization during 8 weeks prior to diarrhea possess a higher risk of *C.difficile* infection (OR = 1.64) but the difference was not significant (95% CI: 0.97 - 2.78). Our study observed 73.6% of cases with *C.difficile* diarrhea having history of hospitalization in previous 8 weeks. Many other authors noted hospitalization as a risk factor for *C.difficile* diarrhea. Ogielska (2015) reported 80% of diarrhea case due to *C.difficile* had a history of hospitalization in the previous 8 weeks. Data from CDC's Emerging Infectious Diseases Program (2010) showed 94% of cases of diarrhea due to *C.diffcile* related to hospitalization and medical care. According to Kurti (2015), the risk of *C.difficile* is 2.39 times higher in patients with a previous history of hospitalization (95% CI = 1.61-3.51). Wilcox reported a history of hospitalization in the last 6 months as a risk of *C.difficile* diarrhea.

Using antibiotics during 8 weeks before diarrhea or using multiple antibiotics was found to posse higher risk of diarrhea due to *C.difficile*, but

the difference is not statistically significant (p>0.05). Many authors confirmed that antibiotic use is an important risk factor for diarrhea due to *C.difficile*. Antibiotics increase the risk of *C.difficile* diarrhea during treatment, up to 3 months after stopping. The explanation is that antibiotics disrupt the intestinal microflora, enhancing *C.difficile* growth, its toxins and cause disease. In Thailand, Ngamskulrungroj (2015) reported 57.6% of diarrhea cases due to *C.difficile* have used antibiotics in previous 8 weeks. Similar to ours results, Ingle reported mentioned about higher antibiotics use rate prior to diarrhea among patient group than controls, but the difference was not statistically significant. According to a systematic review of studies, antibiotic use is associated with *C.difficile* diarrhea with an odds ratio of 2.86 - 6.92. Our research was conducted at an end-line general hospital, mainly on patients of intensive care unit and of infectious disease unit, many patients might use antibiotics provided by front line health facilities or used themselves before coming to the hospital, explaining the not significant difference in antibiotic use history among groups of patients with diarrhea.

Patients taking glycopeptide antibiotics have a lower risk of *C.difficile* diarrhea [OR = 0.22 (95% CI: 0.07 - 0.72)**]**. There is no difference between other antibiotic groups and the risk of diarrhea caused by *C.difficile*. Unlike us, Predrag (2016) reported that among patients treated in Serbian, the use of all antibiotic groups possesses the risk for *C. difficile* diarrhea. Similar to us, Lv and Peng (2014) in China noted that glycopeptide has a protective effect against diarrhea caused by *C.difficile* [OR = 0.069 (95% CI: 0.008 - 0.563)] and explained that glycopeptide inhibits the germination of *C.difficile* from spore into active form.

Clinical symptoms such as fever, abdominal pain, abdominal distension, nausea - vomiting, and drop in blood pressure did not differ between the patient and control groups (p>0.05). These symptoms are common and nonspecific in patients with diarrhea of various etiologies. Lee (2019) also found no difference in symptoms of fever, vomiting, nausea, abdominal pain between the patient group and the control group. Presence of mucus stools is a risk of diarrhea due to *C.difficile* with 4.98 times higher (95% CI: 2.28 - 10.89) while the bloody stool is a risk of *C.difficile* diarrhea with 2.36 times higher (95% CI: 1.16 - 4.8) compared with group of diarrhea due to other causative, the difference was statistically significant (p <0.05). Skyum (2019) also noted that the factor associated with diarrhea due to *C.difficile* is stool with nasal mucus [OR = 3.5 (95% CI: 1.02–12.1)].

Diarrhea from 7-10 times is more common in patient group of diarrhea caused by *C.difficile* [OR = 1.94 (95% CI: 1.06 - 3.56)]. There are few studies to compare the difference in diarrhea frequency between groups with or not with diarrhea caused by *C.difficile*.

Result of analyzing multivariate logistic regression showed 3 independent risk factors related to *C.difficile* diarrhea include: age ≥65 (OR = 2.01), urban living (OR = 1.76), hemodialysis (OR = 7.32). Two factors related to the diagnosis of diarrhea due to *C.difficile* are mucus stool (OR = 5.94) and diarrhea 7-10 times/day (OR = 1.98). Glycopeptide use during 8 weeks prior to diarrhea is a protective factor against diarrhea due to *C.difficile* (OR = 0.18). Similarly, many studies reported cycle dialysis as a risk by multivariate analysis. Oldfield showed independent risk factors for *C.difficile* diarrhea: hospitalization in the previous 3 months [OR = 2.45 (95% CI: 1.02-5.84)], dialysis [OR = 8.12 (95% CI: 1.80-36.65)], and use corticoid [OR = 3.09, (95% CI: 1.24-7.73)]. In Canada, Demir (2018) presented the independent factors associated with *C.difficile* diarrhea, including cycle dialysis (OR = 13.5) and previous antibiotic use (OR = 4.23).

4.3. Genotypic distribution characteristics of *C.difficile* causing diarrhea in adults at Bach Mai Hospital, 2013 - 2017

4.3.1. Characteristics of the toxin gene of C.difficile

Patients with diarrhea due to toxin *C.difficile* strain A+B+ accounted for 49.5%; strain A-B+ accounted for 44.6% and only 5.9% of strains carries both toxins A+B+ and A-B+. The strain that carries only toxin A without toxin B (A+B-) has not been detected in humans. Before, the pathogenic role of *C.difficile* strain producing toxin A+B+ was recognized and the toxin A has been accepted as the main toxin agent. But recently, the importance of toxin B has been confirmed. Many studies in the world reported about presence of *C.difficile* infections with only toxin B but not toxin A (carrying genotype A-B+) accompanied with serious clinical situation. The isolation of more than one pathogenic *C.difficile* strain from individual diarrhea patient obtained from our study has also been reported, mainly in Asian countries. Putsathit (2017) conducted a study in Thailand, also noted that 4 out of 100 patients were isolated with more than 1 *C.difficile* strain. Cheong (2017) also found that Laos patients had both *C.difficile* and A-B+ and A+B+ toxin genes. Wang (2018) reported an 83-year-old clinical case of diarrhea in China infected with two strains of *C.difficile* carrying toxin genes A+B+ and A-B+ simultaneously.

All patients with diarrhea due to *C.difficile* strain carrying the toxin genes had a history of hospitalization in the 8 weeks prior to diarrhea, in particular the patients isolated with *C.difficile* strain carrying A+B+ gene having a history of hospitalization during past 8 weeks accounted for 64%, those having strains carrying A-B+ and hospitalized in past 8 weeks accounted for 82.2% and all patients that isolated with strains carrying both types of toxin genes had a history of hospitalization (100%). The difference was statistically significant with $p < 0.05$ (Fisher's exact test) (Table 3.30). Similar to our study, Ogielska (2015) recorded 80% of patients with *C.difficile* diarrhea had a history of hospitalization within the previous 3 months.

Patients with diarrhea caused by C.*difficile* strains carrying toxin genes showed to have history of antibiotic use within the 8 weeks prior to diarrhea with higher rate than those without history of antibiotic use. Patients isolated with strains carrying the A+B+ gene that have used antibiotic during last 8 weeks accounted for 54%, those with strains carrying A-B+ was 73.3% and 100% patients isolated with strains carried both toxin genes had history of antibiotic use. The difference was statistically significant with $p < 0.05$ (Fisher's exact test) (Table 3.32). Luo et al. (2019), based on a multicenter retrospective study also noted that in some cities of Shanghai, Japan and Australia, the rate of *C.difficile*-infected patients that have been used antibiotic during 8 weeks prior to diarrhea was higher (60% - 84.3%), but in some other cities this rate was not high.

4.3.2. Characteristics of the ribotype genotype of C.difficile

We identified 8 ribotype genotypes of *C.difficile* strains isolated from diarrhea patients, the most common were ribotypes *trf*, *017* and *cc*835 (accounting for 24.5%; 23.5% and 22.6%, respectively). Other ribotypes were *og*39, *014*, *ozk*, *cr* and *001* with lower rate. In different regions, at different times, circulate different genotypes. In Japan, Collin (2013) noted that the main ribotype genotype is *smz*/*018*, *014*, *002*, and *001*, and detected an epidemic strain *027*. In Korea in 2008-2010, the 018 genotype was found dominated. In North America, Tenover 2008 - 2009, recorded 5 common genotypes: 027, 002, 106, 017 and 078. In the UK, 2008 - 2009, common strains were 027 (36%), 106 (13%), 001 (7%).

Among the *C.difficile* strains carrying toxin gene A-B+, there are 2 types of ribotype genes: *trf* and 017. The strains of A+ B+ toxin have 6 ribotype genotypes: *cc*835, *og*39, 014, *ozk*, *cr* and 001. Among 116 ribotype

genotypes, Stubbs et al. identified 34 ribotypes carrying the toxin gene AB-, 78 ribotypes with A+B+ toxin and only 4 ribotypes with A-B+ toxin. Among the strains of *C.difficile* circulating in Asia that have toxin A+B+, according to Collins (2019), the most common genotype found in Japan and South Korea was 018 (called *smz*), followed by 012, (also called *cc*835) and 046 (*og*39) was common in China, 014 and 002 were found common in Taiwan and Hong Kong. Among A-B+ toxin strains, the genotype *017* found common in our study (23.5%) is also common genotype in many Asian countries like China, Taiwan, Korea, Japan and Southeast Asian countries such as Thailand, Laos, Malaysia ...

CONCLUSION

1. Epidemiological, clinical characters of diarrhea caused by *C.difficile* among the adults at Bach Mai Hospital, 2013 – 2017

A significant number of adult patients with diarrhea due to *C.diffcile* have been detected at Bach Mai Hospital from 2013 to 2017 (101 cases) with higher rate of men in comparison to women (the ratio of 1.7: 1). Patients come from most provinces/cities of Northern Vietnam (21/28 provinces/cities), from both rural and urban. The prevalence of the disease gradually increases with age, the highest was among people over 60 years old (49.5%), in all months of the year.

Clinical symptoms of diarrhea due to *C.difficile* were found not specific, commonly are fever, abdominal pain, and abdominal distention. Most of cases have moderate diarrhea with 3-6 times/day (65.3%), lasts more than 4 days (80.2%), over 2 weeks accounts for 30.7%. Inflammatory reaction increased in accordance with the increase of leukocytes and blood procalcitonin level was observed in 60.4% and 100% patients, respectively. Rate of patients having elevated leukocytes (>15G/L) and high procalcitonin (>10ng/mL) were 27.7% and 24.3%, respectively. All isolates of *C.difficile* were tested sensitive to the two antibiotics recommended for treatment, metronidazole and vancomycin. The rate of patients badly suffering or died from diarrhea due to *C.difficile* in hospital was high (21.7% and 5.9%, respectively), especially in severe patients requiring intensive care treatment (40% and 14.3%, respectively).

2. Risk factors for diarrhea due to *C.difficile* in adults at Bach Mai Hospital, 2013 - 2017

6 independent factors were found related to diarrhea due to *C.difficile* including 3 risk factors for increasing the disease: age ≥65 (OR = 2.01), urban living (OR = 1.76) and cycle dialysis (OR = 7.32); two related

factors in the diagnosis were mucus stools (OR = 5.94) and average diarrhea of 7-10 times/day (OR = 1.98). A determined protective factor was the use of glycopeptide antibiotics to treat other diseases within 8 weeks prior to diarrhea (OR = 0.18).

3. Genetic distribution characteristics of *C.difficile* causing diarrhea in adults at Bach Mai Hospital, 2013 - 2017

Detected 2 toxin genes, including A+B+ (49.5%) and A-B+ toxin gene (44, 6%) in *C.difficile* strains isolated from adults suffered with diarrhea at Bach Mai Hospital. Patients that isolated with strain carrying both toxin genes A+B+ and A-B+ was accounted for 5.9%. 8 ribotype genotypes were identified, including 2 ribotype genotypes *trf* and *017* coding A-B + toxin gene and 6 ribotype genotypes (*cc835, og39, 014, ozk, cr and 001*) coding A+B+ toxin gene. The most common *C.difficile* ribotype genotypes were *trf* (24.5%), *017* (23.5%) and *cc835* (22.5%).

RECOMMENDATION

1. **Ministry of Health**

Promulgate regimens for diagnosis and treatment of diarrhea caused by *C.difficile*, provide training and disseminating disease related information for health staff of all levels in order not to miss the diagnosis and improve the effectiveness of treatment for patients.

2. **Bach Mai Hospital**

Deploying tests to detect *C.difficile* for early diagnosis and treatment of patients, screen for *C.difficile* among elderly diarrhea patients.

Provide training on diarrhea caused by *C.difficile* to improve the knowledge and vigilance of clinicians about the disease.

Use antibiotics appropriately and enhance infection control to prevent *C.difficile* infection in health facilities.

Continued research on larger scale to comprehensive assessment of risk factors and diarrhea caused by *C.difficile*.