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Analysis of risk factors and outcomes of *Clostridium difficile* infection

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Abstract: Introduction: *Clostridium difficile* (C. difficile) is a Gram-positive, anaerobic rod-shaped bacteria, widely spread in the human environment. In the last decade, the frequency and severity of *Clostridium difficile* infection (CDI) have been increasing, making this particular disease one of the most significant nosocomial infections. The aim of our study was an analysis of CDI risk factors, its course and consequences.
Materials and Methods: Medical documentation of the patients treated for CDI in the University Hospital in Cracow and St Anne's Hospital in Miechów has been analysed. The analysis focused on epidemiological data, blood parameters, comorbidities, recurrence rate, and complication rate (deaths included). As part of risk factors analysis, antibiotic use or hospitalisation in a period of 3 months before the episode of infection was considered relevant. Blood tests have been performed using routinely employed, standard methods.
Results: We evaluated data of 168 people infected with C. difficile, out of which there were 102 women (61%) and 66 men (39%). The median age of the patients was 74 years for the entire population with 76 years for women and 71 years for male patients. One hundred_thirteen people (67%) had been previously hospitalised, and 5 person was a pensioner of a nursing home. 99 people (59%) were treated with antibiotics within 3 months before the first episode of infection. An average length of the hospital

stay because of CDI was 11 days. One hundred thirty persons (77%) experienced only 1 episode whereas 38 people (23%) had more than 1 episode of infection. The person with the largest number of recurrences had 9 of them.
C o n c l u s i o n s: The development of CDI is an increasing problem in a group of hospitalised persons, particularly of an old age. The general use of beta-lactam antibiotics is the cause of a larger number of infections with C. difficile. Vast majority of patients have had at least one typical risk factor of CDI development.

Key words: antibiotic related diarrhoea, *Clostridium difficile*, comorbidities, death, risk factors.

Introduction

Clostridium difficile (C. difficile) is a Gram-positive, anaerobic, spore-forming bacillus. It was first isolated in 1935 by Hall and O'Toole from meconium of a healthy neonate and named *Bacillus difficilis* [1]. The importance of this bacterium in pathogenesis of diseases of the large intestine in humans increased with the introduction of antibiotics. In 1974 Tedesco *et al.* reported diarrhoea in 21% of patients treated with clindamycin and found pseudomembranes in endoscopy in half of those subjects [2]. The syndrome resulting from C. difficile infection and presence of its toxins in large intestine was named *Clostridium difficile* infection (CDI). In recent decade prevalence of CDI has increased, as has the incidence of severe course of the disease. The increasing severity of infections was associated with the emergence of a new, fluoroqinolone-resistant, hypervirulent BI/NAP1/027 strain (North American pulsed-field type 1, polymerase chain reaction ribotype 027). This strain produces multiple-fold amounts of toxin A and toxin B, as well as a binary toxin. BI/NAP1/027 strain has been first isolated in North America, then in many countries in Europe, including Poland, and in the following years on nearly every continent [3–9].

The aim of our study was an analysis of CDI risk factors, its course and consequences.

Materials and Methods

Data analysis

In our retrospective study medical documentation of the patients treated from January 2011 till December 2013 for CDI in the Department of Infectious Diseases of University Hospital in Cracow (n = 108) and treated from January 2013 till December 2013 in St Anne's Hospital in Miechow (n = 60) has been analysed. An episode of CDI was defined according European Society of Clinical Microbiology

and Infectious Diseases (ESCMID) guidelines as a clinical picture compatible with CDI and microbiological evidence of free toxins and the presence of C. difficile in stool without reasonable evidence of another cause of diarrhoea [3]. Recurrence was defined when CDI re-occured within 8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment [3]. Diarrhoea was defined as passage of 3 or more unformed stools in 24 hours. The infection was confirmed by detection of C. difficile toxins in faeces using the TOX A/B Quick Check Complete test kit (WAMPOL, TechLab, USA).

The analysis focused on epidemiological data, blood parameters, comorbidities, recurrence rate, and complication rate (deaths included). As part of risk factors analysis, antibiotic use or hospitalisation in a period of 3 months before the episode of infection was considered relevant. Blood tests have been performed using routinely employed, standard methods.

Statistics

Elements of descriptive statistics have been used in the analysis — all data are presented as means or medians and lower (Q_{25}), upper (Q_{75}) quartiles. Normal distribution of variables was checked using the Shapiro-Wilk test. Differences between groups of patients who survived and who died were determined using or the Mann-Whitney U-test if normality was not observed. Calculations were performed using Statistica 13 (StatSoft® Inc. U.S.), and statistical significance was defined as $p \leq 0.05$.

Results

We analysed 168 cases (Kraków = 108, Miechów = 60), including 102 women (61%) and 66 men (39%). In the analysed period in the Infectious Diseases Department of the University Hospital in Kraków there were 3185 hospitalised patients and in the University Hospital as a whole — 221,188. The percentage of people hospitalised due to CDI in the Infectious Diseases Department was 3.4%. In the hospital in Miechów the analysed patients had been treated in various departments, including 40 people from Infectious Diseases, 12 from Pulmonary Diseases, 3 Cardiology, and 3 Internal Diseases, 1 patient from Neurology and 1 — Paediatrics Department. In the analysed period in the hospital in Miechów there were 10,318 hospitalisations, in the Infectious Diseases Department 702, the percentage of patients treated for CDI was 0.6% in the Hospital and 5.7% in the Infectious Diseases Department itself.

The median age of the patients was 74 years for the entire population (range 8–93 years), 76 years in the case of women and 71 years for men. 130 persons (77%) experienced only 1 episode, whereas 38 people (23%) had more than 1 episode of infection. The mean number of CDI episodes was 1.33 for the whole analysed group, whereas for the people experiencing recurrences — 2.5. The patient with the largest number of recurrence episodes had 9 of them altogether. The distribution of age, sex in the analysed group is presented in Tables 1 and 2.

Table 1. The age distribution of CDI in the analysed group.

Age	n	Median (range) years
Total	168	74 (8–93)
Women	102	76 (25–93)
Men	66	71 (8–92)

CDI — *Clostridium difficile* infection.

Table 2. Distribution of the study group based on age and sex.

	Total	Women	Men
<65 y/o	46 (27%)	25 (54%)	21 (46%)
>65 y/o	122 (73%)	77 (63%)	45 (37%)

y/o — years old.

In the study group 113 people (67%) had been hospitalised in the preceding 3 months, in 2 cases we were unable to ascertain the duration. Forty four people declared no previous hospitalisation, 5 had been under nursing home, in 6 cases there is no information on previous hospital stays. The average length of the hospital stay during CDI episode was (median) 11 days (Q_{25}–Q_{75}: 8–17 days), an average length of hospital stay before analysed episode of CDI was very similar (median = 11 days, Q_{25}–Q_{75}: 8–16 days).

Ninety nine people (59%) were treated with antibiotics within 3 months before the episode of infection, 39 (23%) definitely did not have such therapy, whilst in the case of 30 people (18%) this cannot be determined on the basis of the patient documentation. Among the 99 people having received antibiotic treatment — 83 have given the name of the medication, whereas in 16 cases the patients were certain of having taken antibiotics but could not remember the name(s). The type of antibiotics therapy used before the first episode of CDI is presented in Table 3.

The type of CDI treatment and average values of the most significant blood parameters are presented in Tables 4 and 5.

Table 3. Antibiotics used before the episode of CDI.

Antibiotic	n (%)*
amoxicillin/clavulanic acid	29 (35%)
ciprofloxacin	28 (34%)
cefuroxime	20 (24%)
ceftriaxone	14 (17%)
amikacin	4 (4.8%)
amoxicillin	3 (3.6%)
ceftazidime	3 (3.6%)
clindamycin	3 (3.6%)
vancomycin IV	3 (3.6%)
cefalexin	2 (2.4%)
cefotaxime	2 (2.4%)
doxycycline	2 (2.4%)
imipenem/cilastatin	2 (2.4%)
clarithromycin	2 (2.4%)
metronidazole	2 (2.4%)
norfloxacin	2 (2.4%)
sulfamethoxazole/trimethoprim	2 (2.4%)
furazidin	1 (1.2%)
gentamicin	1 (1.2%)
linezolid	1 (1.2%)
meropenem	1 (1.2%)

CDI — *Clostridium difficile* infection

* The percentage was calculated in relation to where the antibiotic used was known (n = 83). Some patients have used more than one antibiotic, therefore the percentage sum does not equal 100%.

Table 4. Values of analysed blood parameters.

Parameter	Study group				CDI patients who died			
	n	median	Q_{25}–Q_{75}	range	n	median	Q_{25}–Q_{75}	range
WBC × (10^3/ul)	165	11	7–16	3.1–46.8	18	18.5	11.4–26.6	6.5–46.8
CRP (mg/l)	160	60	16–108	0.2–333	18	116	54–146	19–333
Creatinine (µmol/l)	166	80	62–122	42–783	18	168	115–274	61–404

CDI — *Clostridium difficile* infection; CRP — C-reactive protein; Q_{25} — lower quartile; Q_{75} — upper quartile; WBC — white blood cells

Table 5. The frequency of the therapy used in the treatment of CDI.

Type of antibiotic therapy	n
metronidazole P.O.	90 (54%)
vancomycin P.O.	45 (27%)
vancomycin P.O. + metronidazole IV	19 (11%)
P.O. metronidazole poorly tolerated or deemed ineffective, subsequently switched to P.O. vancomycin	12 (7%)
no treatment	2 (1%)

CDI — *Clostridium difficile* infection; IV — intravenously; P.O. — orally

There were 18 deaths in the study group (10.7%). Those patients who died were statistically significantly older ($p = 0.049$), exhibited higher values of white blood cells — WBC ($p < 0.001$), CRP ($p = 0.004$), creatinine ($p < 0.001$) in comparison to those who survived.

The frequency of the comorbidities which are known risk factors for CDI development are presented in Table 6. In 53 people (32%), found comorbidities may have been a predisposing factor to CDI development. Among the 15 people with gastrointestinal disorders, in 9 cases it was diverticulosis. Six out of 19 people with malignancies had undergone chemo- and/or radiotherapy. Eight out of 16 people with chronic kidney failure required dialysis.

Table 6. The incidence of comorbidities or therapy other than antibiotics that are risk factors for CDI.

Predisposing factor	n (%)*
Malignancy	19 (11.3%)
Chronic kidney disease	16 (9.5%)
Gastrointestinal disorders	15 (8.9%)
Immunosuppressive therapy #	9 (5.4%)
Autoimmune disease	4 (2.4%)
HIV infection	1 (0.6%)
Cirrhosis	1 (0.6%)

CDI — *Clostridium difficile* infection

* the percentage was calculated in relation to the whole analysed group (n = 168).

In some patients, multiple risk factors were present, therefore the percentage sum does not equal 100%.

\# malignancy treatment not included.

The next question asked was how many patients presented with no known risk factors of CDI. Having excluded those not previously hospitalised, left were 44 (26%) persons who had not been hospitalised during last 3 months, nor had they been in nursing homes. Among them, there were 26 persons who had used antibiotics previously, 9 people had definitely not used them and in case of 9 there was no certain data. Looking for the risk factors in a remaining group of 9 persons with no hospitalization and no certain antibiotic we found that 6 of them were above age of 65 years. Finally, 3 people aged 54–55 were left; one of them suffered from a chronic autoimmune disease (scleroderma), another was additionally diagnosed with salmonellosis. Ultimately, in the group of 168 people, there was only 1 person with no known CDI risk factor found.

Discussion

The risk factors for the development of CDI are those which disrupt the normal intestinal flora, mainly therapy with broad-spectrum antibiotics, host-related factors (of which age is the most significant), as well as environmental factors, such as the exposure to C. difficile usually associated with hospital stay with the source being the hospital environment [10–12]. Gastrointestinal infections also lead to destruction or impairment of gut microbiota, possibly facilitating CDI even in a younger population, as demonstrated in our 2018 study [13]. Practically the majority of antibiotics may lead to development of CDI, yet most often the infection is caused by clindamycin, third-generation cephalosporins, fluoroquinoles and broad-spectrum penicillins [12]. The risk in age >65 years old is 5 to 10-fold higher than in people aged <65 years [10, 14, 15]. Hospitalisation is an important risk factor of CDI. According to epidemiological data, the risk of C. difficile colonisation in hospitalised patients is approximately 3–40%, average 10–15%. C. difficile is the most common cause of nosocomial gastrointestinal infection and nosocomial diarrhoea (10–30% of cases). Notably, only 25–30% of people colonised with this pathogen during hospitalisation will develop diarrhoea. The incidence of colonization is related to the duration of hospitalisation [16, 17]. Johnson *et al.* observed the colonisation in approximately 1% of patients hospitalised for up to 1 week, while it increased to approximately 50% in patients hospitalised for over 4 weeks [18]. Sources of the pathogen are the people infected with C. difficile and the hospital environment. The spores remain in the environment for several months and can be found in the vicinity of toilets, but also on hospital furniture, phones and medical equipment like thermometers and stethoscopes. The role of medical personnel in the transmission of the pathogen from the hospital environment to patients should be emphasized. Johnson *et al.* demonstrated that consistent use of latex gloves was associated with reduction of risk of CDI [19].

To sum up the above-named factors' triad, i.e. therapy with antibiotics, old age and hospital stay are the main causes for the development of CDI. An elderly person who is hospitalised for pneumonia is a model example for the risks factors of CDI development. In our analysis, people above 65 years of age were the majority and amounted to 73%. The percentage was higher for women than for men, which probably is due to a longer lifespan of women. Nevertheless, approximately 25% of the infected people were below 65 years of age, whilst the youngest was 8 years old. Most probably an overall increase of the frequency of infections facilitates the spread among younger age groups. In our study we tried to determine the significance of the presence of one of the 3 risk factors, i.e. elderly age, antibiotic therapy and hospital treatment. The most frequently present risk factor was the age >65 (73%), then hospital stay (67%), and treatment with antibiotics (59%). However, the data concerning antibiotics could be underestimated as with as many as 18% persons it was not possible to determine whether there had been any antibiotics used recently. Having analysed the risk factors of CDI, it bears emphasising that only 1 person with no known CDI risk factors was found. This allows to conclude that the risk of development of CDI in a group with no risk factors is minimal. The role of protein pump inhibitors (PPI) remains controversial. Some correlation was initially reported, but subsequent analysis adjusted for other comorbidities did not confirm this hypothesis; especially since it has been demonstrated that C. difficile spores are resistant to the acidic environment of the stomach. With that in mind, the effect of PPI on risk of CDI has not been assessed in our study [20, 21]. The analysis of the antibiotics used before the infection with C. difficile shows that broad-spectrum penicillins and fluoroquinoles are most likely to facilitate the development of infection. In the study group only 3.6% people had been treated with clindamycin, which has a general opinion of the antibiotic responsible for the development of CDI. The explanation of this phenomenon is quite difficult. On one hand, this might be explained with a larger awareness of the risk of CDI after treatment with clindamycin and thus the limitation of its use, on the other — with an exaggerated significance of clindamycin in CDI development.

The clinical picture of CDI is diverse and ranges from asymptomatic carrier status, through various degrees of diarrhoea, to the most severe, life threatening cases. CDI infection may affect every part of the large intestine, but usually it is located in its distal portion [12]. The diarrhoea, which is usually watery and sometimes intense, may be accompanied by abdominal pain, fever, nausea, vomiting, fatigue and anorexia. Occult blood is frequently present in faeces, but overt bleeding is usually absent. In the most severe form of the disease, the signs and symptoms are similar to those of colitis, but they are more intense and may lead to significant dehydration, oedema and shock. The most serious complications are: toxic megacolon, perforation of large intestine, paralytic ileus, renal insufficiency and sepsis. The most significant adverse prognostic factors in CDI are age, high WBC counts, CRP and creatinine levels.

The high WBC count and CRP level reflect the inflammation of the colon, while elevated creatinine levels correlate with dehydration caused by diarrhoea [12, 22, 23]. Our study confirms increased values of WBC, CRP, creatinine. It is worth pointing to significantly larger values of these parameters among those in whose case the disease led to death in comparison to the whole group (CRP 116 vs 60 mg/l, respectively), (creatinine 168 vs 80 μmol/l, respectively) and, in particular, very high leukocyte count (18 vs 11.5 × 10^3/ul, respectively). In clinical practice this is always a factor which worsens the prognoses and in such situations, a combined therapy of intravenous metronidazole and oral vancomycin should always be considered. Our study does not include one parameter important in CDI- blood albumin levels. This particular test was, unfortunately, performed very rarely in the analysed patients.

In 2018 new guidelines for CDI treatment were published. In nonsevere cases the first-line treatment is oral vancomycin 125 mg every 6 hours for 10 days or fidaxomicin 200 mg orally twice a day for 10 days. Metronidazole 500 mg orally three times a day for 10 days can be administrated if above agents are unavailable. In severe cases the drug of choice is the oral vancomycin 125 mg every 6 hours or fidaxomicin 200 mg orally twice a day (10 days) and in complicated cases it is recommended to combine oral vancomycin (500 mg every 6 hours) with intravenous metronidazole 500 mg every 8 hours (10–14 days). Moreover, in case of ileus, rectal route of administration of vancomycin can be also used, in dose 500 mg diluted in 100 ml of the 0.9% NaCl every 6 hours [3, 20, 24, 25]. Intravenous use of vancomycin is not recommended because it is associated with insufficient concentrations of the drug in the intestine. Gastrointestinal absorption of vancomycin is minimal and its oral administration allows to achieve therapeutic concentrations within the intestine, while avoiding the side effects observed with parenteral administration. The time of resolution of diarrhoea may be shorter with vancomycin compared to metronidazole [26, 27].

In the study group oral metronidazole was the most frequently used drug (54%). Bad tolerance and/or ineffectiveness of metronidazole was present in a group of 12 people (7%). Vancomycin is also the drug of choice in people with significant liver damage. We analyzed patients with CDI treated before updating of guidelines. Nevertheless, having evaluated the percentage of the P.O. metronidazole + IV vancomycin therapy and the percentage of deaths, it seems that in the analysed group, the use of the strongest therapy was too low. It is important to underline that from 2018 vancomycin and fidaxomicin are the cornerstone of CDI treatment [25].

Mortality in CDI is highest in elderly patients with multiple comorbidities, in whom it may reach 25% [10]. In our study there were 18 deaths in the analysed group (11%). 15 patients died during their first episode of infection, which confirms the observation that among many people with recurrences, the largest intensification

of symptoms occurs during the first episode [28]. Each consecutive recurrence is less dynamic, and, finally no recurrences take place. Asymptomatic carriers of C. difficcile have a high titre of antibodies against A and B toxin [29]. It is believed that some of the recurrences can be attributed to an inefficient creation of protective antibodies, and each episode of the disease increases their number until the level protection against the disease is reached. A recurrence of the symptoms of infection usually takes place about 1 week after the end of the causal treatment. The patients who experienced one recurrence, have a larger risk of further recurrences. CDI recurrence in one half of the cases is caused by the same bacterial strain and in about half of the cases — by a different strain of C. difficile [22, 30]. Also in our group, the average time of recurrence was about one week. With some of our patients, the recurrences took place with very high regularity.

Conclusions

Although any progress in medicine leads to narrowing down the areas of specialisation, it must be stressed that the problem of CDI concerns all hospital wards and specialisations. Infection caused by C. difficile is an increasing problem during hospitalisation, particular among elderly patients, irrespectively of the type of hospital ward. It is paramount to revisit the handling of a CDI patient not only among doctors of different specialties, but the nursing and support staff, extending basic CDI-related care to the patients' families with an emphasis on elderly people. A general use of cephalosporins, and penicillin-type antibiotics is the cause of a larger number of infections with C. difficile. Vast majority of patients have had at least one typical risk factor of CDI development. Based on our observations, blood albumin levels were not tested frequently enough; this test should be performed routinely, as it is important for formulating a prognosis of risk of death, selecting medication and supplementation.

Conflict of interest

None declared.

Abbreviations

C. difficile — *Clostridium difficile*; CDI — *Clostridium difficile* infection; CRP — reactive protein; ESCMID — European Society of Clinical Microbiology and Infectious Diseases; IV — intravenously; P.O. — orally; Q_{25} — lower quartile; Q_{75} — upper quartile; WBC — white blood cells

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