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ORIGINAL ARTICLE

Growing consumption of antibiotics and epidemiology of *Clostridioides difficile* infections in Poland: A need to develop new solutions

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ABSTRACT

Clostridioides (formerly *Clostridium*) *difficile* infections (CDIs) are becoming more common and more serious. *C. difficile* is the etiologic agent of antibiotic-associated diarrhea, pseudomembranous enterocolitis, and toxic megacolon while CDIs recur in 7.9% of patients. About 42.9 CDI cases/10,000 patient-days are diagnosed each day in Europe, whereas in Poland 5.6 CDI cases/10,000 patient-days are reported; however, the median for European countries is 2.9 CDI cases/10,000 patient-days. Epidemiology of CDIs has changed in recent years and risk of developing the disease has doubled in the past decade that is largely determined by use of antibiotics. Studies show that rate of antibiotic consumption in the non-hospital sector in Poland is much higher than the European average (27 vs. 21.8 DDD/1,000 patient-days), and this value has increased in recent years. Antibiotic consumption has also increased in the hospital sector, especially in the intensive care units – 1,520 DDD/1,000 patient-days (ranging from 620 to 3,960 DDD/1,000 patient-days) – and was significantly higher than in Germany 1,305 (ranging from 463 to 2,216 DDD/1,000 patient-days) or in Sweden 1,147 (ranging from 605 to 2,134 DDD/1,000 patient-days). The recent rise in CDI incidence has prompted a search for alternative treatments. Great hope is placed in probiotics, bacteriocins, monoclonal antibodies, bacteriophages, and developing new vaccines.

KEYWORDS

Clostridium difficile infections, bacteriophage, probiotics, epidemiology, antibiotic consumption

INTRODUCTION

Clostridioides (formerly *Clostridium*) *difficile* is a Gram-positive, anaerobic, spore-forming rod causing disease in humans and animals, present in the gut of 14% of healthy people [1]. This pathogen was first described in 1935 and called *Bacillus difficilis* – a rod that is difficult to grow [2].

At present, *C. difficile* infection (CDI) poses an enormous challenge to contemporary medicine. The number of CDIs is constantly growing and their treatment is becoming increasingly more difficult and more expensive. *C. difficile* is the main etiologic agent of antibiotic-associated diarrhea; according to literature data, as many as 16%–35% of them are caused by *C. difficile* [3]. Symptoms of CDI are watery diarrhea, abdominal pain, vomiting, nausea, and fever [4]. Severe forms of infection may lead to pseudomembranous colitis and toxic megacolon (*megacolon toxicum*) [5]. According to literature reports, 25% of patients with CDI do not respond to antibiotic treatment and approximately 7.9% relapse [6, 7]. Severe complications, such as surgeries, *megacolon toxicum*, or even patient death, are determined in 16.7% of all CDI cases [7].

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Hospitalized patients are the most exposed to CDI. Seventy percent of all CDIs are associated with hospital treatment [7]. The most significant risk factor for the development of CDI is taking antibiotics that interfere with the natural intestinal flora [8]. The antibiotics with the highest risk of developing CDI include, first of all, clindamycin that increases the risk of developing CDI approximately 20-fold; while the frequently used fluoroquinolones, cephalosporins, and carbapenems increase this risk fivefold (compared to people not treated with an antibiotic) [9]. Restoring the patient's microbiota to its original state, the one from before antibiotic therapy, takes up to 2 years [10]. There are attempts to look for relationships between development of CDI and the application of drugs that alter the pH of gastric juice, such as H2 blockers and proton pump inhibitors. However, we lack enough evidence to confirm this hypothesis [11]. A meta-analysis made by Furuya-Kanamori et al. demonstrates that exposure to corticosteroids increases the risk of CDI, but more accurate studies of this phenomenon are necessary [12]. Other significant risk factors for CDI are immunosuppression, e.g., in transplant patients, especially heart transplants, as well as cystic fibrosis, vitamin D deficiency, intestinal diseases, age over 65 years, low levels of anti-toxin A IgG, and previous CDI: after the first episode, the risk increases to 45%, and by the third it is 65% [6].

EPIDEMIOLOGY

Epidemiology of CDI has changed significantly in recent years. It is estimated that the past decade brought a twofold increase in the disease risk, but this change applies especially to the clinical picture as the USA, for instance, experienced a threefold rise in the group of patients requiring hospitalization due to community-acquired CDI (CDI-related hospitalizations) and additionally the disease affects people who have been considered earlier not particularly vulnerable to CDI, among others, healthy persons living in the community and peripartum women [13, 14].

In the USA, the incidence of hospital-acquired CDI (HA-CDI) amounted to 9.3/10,000 patient-days and the community-acquired 4.8/10,000 patient-days [15]. In European countries, the total CDI incidence of HA-CDI was 2.4 cases/10,000 patient-days.

Epidemiology of the disease has changed, which is mainly due to the spread of the highly virulent strain NAP1, rare before 2001 and now responsible for many hospital epidemics [12]. In Europe, the most frequently isolated ribotypes are RT027 (22.9%), RT001 (7.5%), RT014 (6.7%), RT078 (5.1%), RT002 (4.2%), and RT020 (4.2%), which also correlate with the results obtained by Pituch et al. – ribotype RT027 is the most commonly isolated ribotype in Poland [7, 16].

The CDI mortality is high, in the USA, an active population- and laboratory-based study in 2011 identified 5.4% of *C. difficile* fatal case rate, whereas in the EU it was 3.9% [7].

EPIDEMIOLOGY OF CDI IN POLAND

The Polish total HA-CDI incidence was 6.2/10,000 patient-days [7]. Other Polish data, from single-center studies, reported the CDI incidence in intensive care units (ICUs) at 13/10,000 and 10.6/10,000 patient-days [17, 18]. However, such a high number of CDI has been observed in Poland for a short time – the incidence rates were 6.1, 8.6, and 9.6 CDI per 10,000 patient-days in 2011, 2012, and 2013, respectively [19].

In Poland, the first infection caused by 027 ribotype was detected in a hospital in Warsaw in 2005–2006. This isolate had *tcdA*, *tcdB*, and binary toxin genes (*cdtA* and *cdtB*) [20]. Studies conducted in 2011–2013 in 13 Polish hospitals have shown that ribotype 027 is the most frequent (62%) type related to CDI in Poland [21]. The situation is ever worse in Silesia where in 2017 more than 80% of CDIs are connected to ribotype 027 [22]. In a Polish single-center study, the crude mortality rate of HA-CDI was 12.9% in medical wards and 27.7% in the ICU setting [23].

According to Barlam, the risk of developing CDI has, among others, a direct close relationship with the rational use of antibiotics: "Antibiotic Stewardship Program" decreases the risk of CDI in particularly susceptible patients [24]. This is consistent with observations concerning the patients in Poland, where the situation in regard to CDI, and to antibiotic consumption, is serious [25]. A single-center study in a Polish ICU, where the consumption of antibiotics amounted to 1,913 DDD (defined daily dose)/1,000 patient-days, with a simultaneous exceptionally high and disturbing percentage of high level of resistant strains, found a very high CDI incidence: 10.6/10,000 patient-days [18]. Still, the unit examined was not an exception. A multicenter study conducted in Polish ICUs indicated consumption of antibiotics at an average level of 1,520 DDD/1,000 patient-days (ranging from 620 to 3,960 DDD/1,000 patient-days) and was significantly higher than analogous rates recorded in, e.g., Germany, 1,305 (463–2,216), or in Sweden, 1,147 (605–2,134 DDD/1,000 patient-days) [26–28]. What is also worrying is the high percentage of carbapenems (17%) and quinolones (14%) that was found, while it is precisely the proportion of broad-spectrum antibiotics and fluoroquinolones in the total antibiotic consumption that constitutes a parameter evaluating the quality of prescribing antibiotics [28].

Analysis of the consumption of antibiotics in hospitals using a different methodology, considering DDD consumption per 1,000 inhabitants per day (person-days and patient-days) (ECDC program), showed consumption slightly lower than the average for all European countries (1.78 vs. 2.03 DDD/1,000 patient-days), however, with upward trend in the period 2014–2017 (data for previous years are not available for Poland) [29]. In turn, the consumption of antibiotics calculated according to the same methodology for the Polish non-hospital sector in 2017 showed a value 30% higher than the European average (27 vs. 21.8 DDD/1,000 patient-days). The upward trend in the consumption of antibiotics in the non-hospital sector was recorded according to ECDC data even in the longer term (2007–2016, for which published data

are available); additionally, there was an increasing trend of the share of fluoroquinolone consumption [25].

In Poland, *C. difficile* resistance to commonly used antibiotics is high; according to reports of Lachowicz et al., 85.5% studied isolates were resistant to erythromycin, whereas 27.7% had high-level clindamycin resistance, having minimum inhibitory concentrations (MICs) greater than 256 mg/L. All strains were ciprofloxacin-resistant, 83.1% were moxifloxacin-resistant, and 87.9% strains were imipenem-resistant. All strains were sensitive to tigecycline, metronidazole, and vancomycin. All ribotype 027 and 176 *C. difficile* isolates demonstrated high-level resistance to erythromycin (MIC $\geq$ 256 mg/L); multidrug resistance (resistance to at least three classes of antimicrobial agents) was observed in 85.5% of toxigenic *C. difficile* strains [30].

PATHOMECHANISM OF INFECTIONS

CDI is generally contracted endogenously and by fecal–oral route, primarily through the hands of medical staff [1, 31]. *C. difficile* has many virulence factors, including ability to synthesize toxins A, B and binary toxin (CDT), presence of cilia, S-layer, Cwp66 adhesin, GroEL heat shock proteins and fibronectin-binding proteins (Fbp68), as well as the ability to form biofilm and capacity for sporulation [32–37]. Intestinal diseases are caused only by the *C. difficile* strains that produce toxins A and/or B; there is also a correlation between the amount of toxins in the intestines and the disease duration [38]. This has been proven by introducing insertions into the genes *tcd*A and *tcd*B of virulent bacteria, which led to loss of toxin production and absence of disease symptoms [39]. It was an enormous challenge for contemporary medicine when, in the year 2000, the strain *North American Pulsed Field Type 1* (NAP1) appeared; also known as B1/NAP1/027, or in other words ribotype PCR 027, it is characterized by increased virulence, resistance to fluoroquinolones, formation of a greater number of spores, production of larger amounts of toxins A and B and binary toxin [40, 41]. Binary toxin causes disease in people not previously exposed to antibiotic therapy or hospitalized; the incidence of infections with strains producing binary toxin is estimated at 11%–20.5% in children [12]. Toxins produced by *C. difficile* (A, B, and binary) enter enterocytes and then damage their structure; consequently, albumins, electrolytes, and water are lost through the cell membrane [42, 43]. In a healthy individual, the first line of defense against *C. difficile* is the intestinal environment, the mucus present therein, along with antimicrobial substances suspended in it, as well as bactericidal peptides and immunoglobulins, and the natural microbiota.

There is a high percentage of *C. difficile* carriers among children; however, it does not correlate with CDI incidence due to immaturity of mucous membranes and microbiota in young children and the resulting lack or insensitivity of the receptors to *C. difficile* toxin activity. It is only in children over 2 years of age when the microbiota begins to resemble to the one in adults [44].

DIAGNOSIS AND TREATMENT

Due to serious complications, rapid and accurate diagnosis of infection and appropriate treatment play a vital role. The basic tests are the ones that detect glutamate dehydrogenase (GDH), immunochromatic tests, the ones detecting toxins, and the nucleic acid amplification test [12]. GDH is an enzyme produced by toxinogenic and non-toxinogenic strains of *C. difficile*. It is noteworthy that antibodies against GDH can cross-react with other bacterial enzymes of the genus *Clostridium* [45].

Due to the anaerobic growth conditions of *C. difficile*, its drug sensitivity is very rarely determined in routine diagnostics. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), for epidemiological and clinical purposes, sensitivity to eight antibiotics should be determined (moxifloxacin, vancomycin, tigecycline, daptomycin, fusidic acid, metronidazole, rifampicin, and fidaxomicin). There are big problems in terms of interpretation of diagnostic results; therefore, EUCAST does not recommend routine *C. difficile* susceptibility testing (http://www.eucast.org/clinical_breakpoints/, accessed on: December 27, 2018). In 2016, in Europe, 4.6% of *C. difficile* strains isolated were resistant to metronidazole, 69.4% to moxifloxacin, and one isolate was resistant to vancomycin [7].

Standard CDI therapy involves administration of vancomycin. Treatment with metronidazole is only used in mild cases of infection if the aforementioned antibiotics are unavailable [12]. With mild forms of CDI, the effectiveness of both antibiotics is comparable; however, with severe infections, it was shown that vancomycin proved more effective [46, 47].

IN SEARCH OF NEW SOLUTIONS

A new drug against *C. difficile* in test trials is cadazolid, which inhibits the synthesis of bacterial proteins but is not absorbed from the gastrointestinal tract. The antibiotic demonstrated good activity against *C. difficile*, also against the hypervirulent strain [48]. Another antibacterial agent studied is SMT19969 [2,2′-bis(4-pyridyl)3H,3′H 5,5-bibenzimidazole], which exhibits activity similar to vancomycin in studies [49]. A randomized research with β-lactamase ribaxamase (SYN-004) demonstrated that patients treated intravenously with ceftriaxone along with ribaxamase in lower respiratory tract infections and oral therapy reduced the incidence of *C. difficile* compared to placebo (risk reduction 2.4%). The ribaxamase may prevent CDI in patients treated with intravenous β-lactam antibiotics [50]. The literature lists alternative methods for CDI treatment, such as the use of probiotics, vaccines containing toxins A and B, fecal transplant, and bacteriophage therapy. To investigate treatment options, as well as infection prevention, CDI animal models were created (examples of model animals are Syrian hamsters, mice, pigs, and rabbits), which demonstrate a similar course of the disease as the one in humans [51–53].

Another treatment method for recurrent infections that demonstrates the best results (effectiveness of about 90%) is fecal microbiota transplantation (FMT) carried out in patients with intestinal diseases, irritable bowel syndrome, or nervous system diseases. Usually, fecal samples are collected from a healthy family member as the genetic similarity is reflected in the composition of the microbiota [54]. The doses are usually administered through a lower gastrointestinal series or by means of a colonoscope [55]. The advantages of FMT include low costs of treatment and its effectiveness, whereas the disadvantages are variability of the transplanted microbiota and patients' mental resistance. FMT can take various forms, from a suspension in sodium chloride, through lyophilized powder, to an encapsulated formulation [55].

It is also recommended to use probiotics as adjunctive therapy for CDI treatment. A good probiotic must be resistant to bactericidal substances secreted by other microorganisms, capable of survival in the gastrointestinal tract, and should modulate the host immune response [56]. The most investigated probiotic strain, applicable for *C. difficile* treatment, is the yeast *Saccharomyces boulardii* [57]. Literature data indicate its positive influence on inflammatory bowel disease, a good capacity to colonize the intestine, and the ability to disappear from the body after 5 days upon stopping the supplementation [58]. Through suppression of NFKβ activity, it inhibits IL-8 production, which is one of the mediators of the inflammatory response arising due to CDI, and limits the binding of toxin A to receptors, which was demonstrated during studies in a CDI rat model [58, 59]. According to the studies by Plummer et al. [60], people administered with probiotics showed colonization with the pathogen but the toxin was not detected in their feces, which may point to the fact that probiotics can neutralize the toxin. Literature reports reveal higher proportions of patients with CDI in the placebo groups than in the groups taking probiotics [60, 61]. Apart from *S. boulardii*, other probiotic bacteria listed as adjunctive to CDI treatment are *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus plantarum* [62]. *L. acidophilus* LA-5 alleviates the symptoms of CDI, reduces the concentration of toxins, and improves the histopathological picture of the intestines, whereas *Lactococcus lactis* SL3 reduces the viability of *C. difficile* strains [63, 64]. It should be noted that some strains of the genus *Lactobacillus* are naturally resistant to vancomycin – the antibiotic used for therapy of CDI [65]. Hell et al. [66] gave a multistrain preparation containing bacteria of the genus *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* to 10 CDI-positive people and obtained *C. difficile* eradication in 9 of them, while 1 patient died of pneumonia (not associated with CDI). It should be emphasized that the effect of probiotic strains on *C. difficile* is strain-dependent. A vital role in *C. difficile* eradication is played by H_2O_2 produced by probiotic strains [8]. Due to a limited number of studies and small patient groups, there are no official recommendations for the use of probiotics in the treatment and adjunctive therapy for CDI. At present, there are ongoing clinical studies on the use of probiotics (e.g., *Lactobacillus* spp. and *Bifidobacterium* spp.) in CDI (www.clinicaltrials.gov: NCT03368105 and NCT03647995). It is worth noting that the strains of the *Lactobacillus* sp. produce other antibacterial substances in microaerobic and anaerobic environment, hence the conflicting results of probiotic antagonism against *C. difficile*. In *in vivo* studies, besides a different environment, the eradication of *C. difficile* is also influenced by the immune system and the local microbiota [67]. It should also be pointed out that there were cases of mycoses and fungemia caused by *S. boulardii* [68].

Bacteriocins, produced by certain bacteria, can also be applied in the fight against *C. difficile*. Lacticin 3147 and *L. lactis* niacin demonstrate a bactericidal effect against *C. difficile* but also against other bacteria, including those non-pathogenic, in a way similar to actagardine A and LFF 571. Thuricin CD, produced by *Bacillus thuringiensis*, a bacterium colonizing the intestine, has an inhibitory effect on *C. difficile* that is comparable to metronidazole or vancomycin; however, it shows no toxic effects on other bacteria naturally present in the intestine [69].

Intensive studies are being conducted into monoclonal antibodies against toxins A and B as well as vaccines against *C. difficile* antigens FliD, FliC, and Cwp 84 [70–73]. Research carried out by Wilcox et al. on the influence of bezlotoxumab and actoxumab monoclonal antibodies confirm the safety of their use. The scientists have recorded a smaller number of relapses (by around 10%) in the group taking antibodies than in the placebo group [74]. In addition, there is work underway on vaccines against *C. difficile* and the literature data available suggest that they are safe [59]. Vaccines containing toxoids showed no serious post-vaccination effects. Complications typical of vaccines with aluminum adjuvant have been observed: 46% of people who received the vaccine and 15% of people in the placebo group had a reaction [75]. People vaccinated against *C. difficile* toxins A and B and aged 18–55 years exhibited a greater immune response against toxin A than B, contrary to the situation in the age group of over 65 years. Administering another dose of the vaccine resulted in increased seroconversion of antibodies against toxin A in elderly patients [76]. In the next phase of clinical testing of the vaccine, de Bruyn et al. [77] obtained an immune response against toxin A in 97% and against toxin B in 92% of people. The vaccines deserve special attention, the research into them is in advanced clinical stages and their routine application would allow to prevent the occurrence of infections caused by *C. difficile*.

Another alternative method for CDI elimination is phagotherapy. However, to date, the bacteriophages isolated have displayed a lysogenic life cycle. There are also literature reports on the role of prophages in the expression of genes for *C. difficile* toxins A and B [78]. According to Rea et al., prophages affect the host phenotype and toxin expression, which may result in increased virulence of the pathogen. The bacteriophages obtained to date have been isolated after treating the host strain with mitomycin C [79]. Difficulties to isolate the ones displaying a lytic cycle are explained by the

fact that the prophage stage is conducive to the survival of the phage, which is associated with *C. difficile* spore production. The second argument is the fact that a large number of prophages in the bacterial genome reduce its susceptibility to new bacteriophage infections (mechanism of superinfection) [79]. In their latest studies, Nale et al. demonstrated bactericidal activity against *C. difficile* bacteria of a phage cocktail containing the family *Myoviridae*: CDHM 1, 2, 5, 6 *in vitro* inhibition of biofilm formation by *C. difficile* and *in vivo* reduction in colonization of the hamster gut [80, 81]. Phagotherapy has therapeutic potential in terms of treatment of CDI; however, to date, there are still many unknowns regarding this issue.

PREVENTION

According to European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *C. difficile* (ESGCD), the cornerstones of CDI prevention and control remain appropriate microbiological testing practices based on a two-stage test, CDI surveillance with regular feedback, standard and contact precautions with special emphasis on hand hygiene, use of personal protective equipment and environmental disinfection, antimicrobial stewardship and education of healthcare workers as well as CDI cases and hospital visitors regarding CDI prevention [82].

SUMMARY

Both Europe and Poland are currently facing the enormous problem of high CDI incidence involving the highly pathogenic ribotype 027. Treatment of CDI is lengthy, costly, and sometimes, unfortunately, ineffective. Therefore, it is so important to perform intensive actions to prevent CDI and introduce modern effective methods for their treatment simultaneously.

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