Diploma Thesis

Colonization by *Clostridium difficile* in long-term care facility residents in Graz, Austria

submitted by

Elisabeth Schreiner

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Section of Infectiology and Tropical Medicine

under supervision of

Assoz. Prof.in Priv.-Doz.in Dr.in med. univ. Ines Zollner-Schwetz

Priv.-Doz.in Mag.a rer.nat. Dr.in scient. med. Eva Leitner-Meyer

Graz, 26.07.2018

Statutory Declaration

I hereby declare that I have authored this thesis independently and without any support from third parties, that I have not used other than the explicitly marked sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 26.07.2018 *Elisabeth Schreiner eh.*

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Abstract

Background: Infection with *C. difficile* is a widespread cause of diarrhoea in acute care hospitals and long-term care facilities (LTCFs). Apart from the infection rates, asymptomatic carriage rates should also be monitored for two reasons: Firstly, carriers may act as a reservoir for the bacteria and thereby contribute to the transmission of the disease. Secondly, they may develop symptomatic *C. difficile* infection themselves. We aimed to evaluate the prevalence of colonization with *C. difficile* in LTCF residents in Graz, as no data is currently available concerning Austria. Secondary objectives include risk factors for colonization.

Methods: The point-prevalence study was conducted in March 2018 in Graz and included residents of four LTCFs who did not show any clinical signs of an infection with *C. difficile.* Stool samples were tested with a glutamate-dehydrogenase-enzyme immunoassay. Positive samples were then tested with a toxin A/B-enzyme immunoassay. The samples were also cultivated on agar plates. Suspicious colonies were confirmed using MALDI-TOF. *C. difficile* isolates were tested for antimicrobial susceptibility. Carriers were retested after one month to see if *C. difficile* can still be detected. Moreover, swabs were taken from their residential units to identify a possible contamination with spores. Demographic and clinical information of the patients were collected, for instance, length of stay in the long-term care facility, level of care, diabetes, mobility, faecal and urinary incontinence or administration of antibiotics in the last 3 months.

Results: Asymptomatic carriage of *C. difficile* was confirmed in 4 (2.78%) of the 144 collected samples. The prevalence ranged from 0 to 7.69% in the different facilities. After one month, two of three available carriers were still colonised. None of the examined strains showed resistance to metronidazole or vancomycin. The rate of diabetes mellitus differed significantly between the carriers (75%) and the non-carriers (20%, p: 0.031). The environmental swabs did not show any contamination with spores.

Conclusion: The asymptomatic carriage rate in Graz is comparable to results from Germany, Switzerland and Italy (2-5.1%). According to our results, diabetes mellitus might be a possible risk factor for asymptomatic carriage of *C. difficile*.

Zusammenfassung

Grundlagen: Eine Infektion mit *C. difficile* ist eine weitverbreitete Ursache für Diarrhö in Akutspitälern oder Langzeitpflegeeinrichtungen. Abgesehen von den Infektionsraten sollte auch die Prävalenz der asymptomatischen TrägerInnen erhoben werden: Einerseits könnten sie als Reservoir für die Bakterien dienen und damit zur Verbreitung der Krankheit beitragen, andererseits könnten sie später selbst eine Infektion mit *C. difficile* entwickeln. Unser Primärziel war daher die Ermittlung der Prävalenz in Langzeit-Pflegereinrichtungen in Graz. Zu diesem Thema gibt es österreichweit momentan noch keine verfügbaren Daten. Die Sekundärziele beinhalteten Risikofaktoren für die Kolonisation.

Methodik: Die Punkt-Prävalenz-Studie wurde im März 2018 in 4 Langzeitpflegeeinrichtungen in Graz durchgeführt. Die TeilnehmerInnen durften keine klinischen Zeichen einer Infektion mit *C. difficile* aufweisen. Die Stuhlproben wurden zuerst mit einem Glutamat-Dehydrogenase-Immunoassay getestet. Alle positiven Proben wurden dann einer Testung mit dem Toxin-A/B-Immunoassay unterzogen. Die Proben wurden auf Agarplatten kultiviert. Verdächtige Kolonien wurden mit MALDI-TOF bestätigt. *C. difficile* Isolate wurden auf antimikrobielle Resistenzen getestet. Die TrägerInnen wurden vier Wochen nach den ursprünglichen Untersuchungen noch einmal getestet. Außerdem wurde Mithilfe von Umgebungsabstrichen untersucht, ob der Wohnbereich der TrägerInnen mit Sporen kontaminiert war. Demographische und klinische Daten der TeilnehmerInnen wurden erhoben, etwa die Aufenthaltsdauer, Pflegestufe, Diabetes mellitus, Mobilität, Stuhl- und Harninkontinenz oder die Antibiotikaeinnahme in den letzten 3 Monaten.

Ergebnisse: Für 4 der 144 gesammelten Proben konnte der asymptomatische Trägerstatus nachgewiesen werden (2,78%). Je nach Einrichtung schwankte die Prävalenz zwischen 0 und 7,69%. Nach einem Monat waren zwei von drei verfügbaren Personen immer noch besiedelt. Keiner der Stämme zeigt eine Resistenz gegen Metronidazol oder Vancomycin. Die Rate an Diabetikern unter den TrägerInnen (75%) unterschied sich signifikant von der Diabetikerrate bei nicht Besiedelten (20%, p: 0,031). Bei den Umgebungsuntersuchungen konnte keine Verunreinigung durch Sporen festgestellt werden.

Schlussfolgerungen: Die Rate von 2,78% an asymptomatischen TrägerInnen in Graz ist vergleichbar mit Ergebnissen aus Deutschland, Italien und der Schweiz. (2-5,1%). Unseren Ergebnissen zufolge könnte Diabetes mellitus ein möglicher Risikofaktor für asymptomatische Besiedelung mit *C. difficile* sein.

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Glossary and abbreviations

AGES	Agentur für Gesundheit und Ernährungssicherheit, Österreich
CCNA	Cell Cytotoxicity Neutralization Assay
CDAD	*Clostridium Difficile*-Associated Disease
CDI	*Clostridium Difficile* Infection
DDD	Defined Daily Doses
DNA	Deoxyribonucleic Acid
ECDC	European Centre for Disease Prevention and Control
EIA	Enzyme Immunoassays
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESGCD	European Study Group on *Clostridium difficile*
EUCAST	European Committee of Antimicrobial Susceptibility Testing
FMT	Faecal Microbiota Transplantation
GDH	Glutamate Dehydrogenase
GGZ	Geriatrische Gesundheitszentren der Stadt Graz
GTP	Guanosine Triphosphate
ICU	Intensive Care Unit
IDSA	Infectious Diseases Society of America
LTCF	Long-Term Care Facility
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NAAT	Nucleic Acid Amplification Tests
NAP1	North American Pulsed Field Type 1
NPV	Negative Predictive Value
PaLoc	Pathogenicity Locus

PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PROHIBIT	Prevention of Hospital Infection by Intervention and Training
PWH AR	Pflegewohnheim Aigner-Rollett am Rosenhain
PWH EH	Pflegewohnheim Erika Horn
PWH PR	Pflegewohnheim Peter Rosegger
REA	Restriction-Endonuclease Analysis
RFLP-PCR	Restriction Fragment Length Polymorphism - Polymerase Chain Reaction
RNA	Ribonucleic Acid
SHEA	Society for Healthcare Epidemiology of America
SRRS	SeniorInnenresidenz Robert Stolz
TC	Toxigenic Culture
UDP	Uridine Diphosphate
VRE	Vancomycin – Resistant Enterococcus
WHO	World Health Organisation

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1. Introduction

1.1. General remarks on biological features and history

Clostridium difficile is a strictly anaerobic, gram-positive staining, spore-forming bacterium. (1) It is rod-shaped, 0.5-1.9 x 3.0-16.9µm big, has polar fimbriae and many strains also have flagella. (1–3) The capsule consists of polysaccharides. (2)

The genus name *Clostridium* suggests its genetic relatedness with other well-known pathogens such as *Clostridium botulinum*, *Clostridium perfringens*, and *Clostridium tetani*. However, it has recently been proposed by Lawson et al. (1) to reclassify *C. difficile* as *Clostridioides difficile*, meaning organism similar to Clostridium, due to phylogenetic differences. All common abbreviations such as *C. difficile* or CDI for *C. difficile* infection could thereby remain unchanged. (1)

The bacillus was first discovered by Hall and O'Toole in 1935 in the stool of healthy new-born children. The name derives from the difficulty they had in cultivating the microbe. (4) Back then, *C. difficile* was thought to be commensal and it was not until 1978 that its connection to pseudomembranous colitis and diarrhoea was established. (3,5)

Different strains of *C. difficile* have been discovered, yet not all of them express virulent potential. (2) They can be categorized using two methods: PCR ribotypes and toxinotypes. During PCR-ribotyping the 16S-23S rRNA gene sequence is typed. (6) Currently, more than 300 ribotypes have been discovered and are available for reference in the Cardiff PCR-ribotyping library. (7) Toxinotyping of *C. difficile* is based on differences in the pathogenicity locus (PaLoc) region coding for toxin A and B, TcdA and TcdB. Strains with the same toxinotype have identical variations in said regions. Currently, 31 different toxinotypes are known. The method is based on RFLP-PCR (Restriction Fragment Length Polymorphism - Polymerase Chain Reaction) and correlates well with ribotyping. Toxinotype 0 corresponds to the reference strain VPI 10463, to which the other strains are compared. (8)

1.2. Pathophysiology

C. difficile spores are ingested, pass the highly acidic stomach, germinate in the terminal ileum and finally multiply in the large intestine. (2) At first, the bacteria need to adhere to the gut cells. A healthy person's commensal microflora is able to inhibit the process even at this early stage: It prevents the adhesion of the bacteria to host cell receptors, inactivates factors that induce bacterial germination and growth, stimulates the host's immune response and even produces molecules that may kill vegetative cells. All these mechanisms combined are known as 'colonization resistance'. If the healthy microbiota is disturbed, the microflora cannot defend itself properly and *C. difficile* might reproduce. (9) Pathogenic strains are able to adhere better than non-pathogenic ones. The more virulent a strain is, the better is its ability to adhere. Toxin A seems to be directly or indirectly involved in the bacterial binding to the gut since the addition of toxin A to non-toxigenic strains improved the adhesion. (2) *C. difficile* is known to have fimbriae and often also flagella, but both have not been correlated with the ability to adhere. The evenly distributed positive charge in the cell wall of *C. difficile* may also help to attach to negatively charged host cells. (2) The most common sites of adhesion are the terminal ileum and caecum. (2) Another factor that is positively correlated with high virulence is chemotaxis: The intestinal mucus acts as a chemoattractant for the bacteria and lures them away from the colonic lumen. Therefore, *C. difficile* has to be mobile, which is accomplished by flagella. (2)

The PaLoc consists of 5 genes that are illustrated in *Figure 1*: tcdA for toxin A, tcdB for toxin B, tcdC, a negative regulator that inhibits toxin production, tcdR, a positive regulator and tcdE for a holin-like pore-forming protein. (4,6)

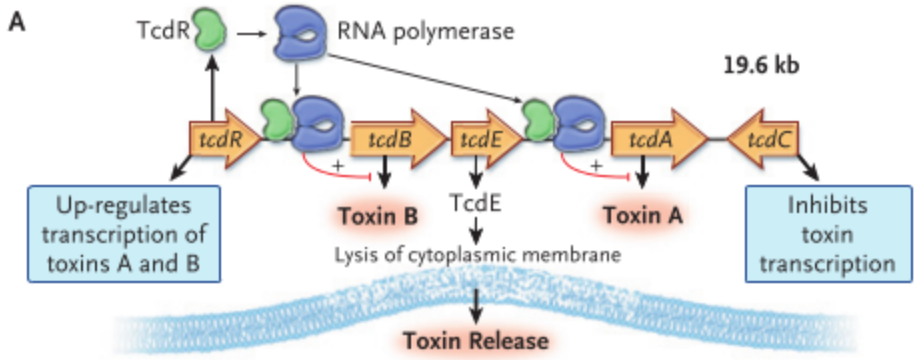


Figure 1: PaLoc region of C. difficile. (4) Reproduced with permission from (scientific reference citation), Copyright Massachusetts Medical Society.

Apart from toxins A and B, a third one has been described: the binary toxin. It is encoded by two genes; cdtA is the enzymatic unit and cdtB the binding unit. (3,10) Both of them are located outside the PaLoc region of *C. difficile*.

C. difficile is not an invasive microorganism which means that the toxins are responsible for the onset of the disease. (3) A large study conducted in the UK (11) that included 12 420 faecal samples supported this hypothesis by showing that only the presence of toxins, but not the presence of toxigenic strains alone, correlated with the clinical outcome. Patients were divided into 3 groups: cytotoxin assay positive (group 1), cytotoxigenic culture positive but cytotoxin assay negative (group 2) and neither cytotoxigenic culture nor cytotoxin assay positive group (group 3). The mortality rate per 1000 inpatient days was significantly higher in group 1 than in groups 2 and 3 (9.03 in group 1, 5.33 in group 2, 6.26 in group 3, p (1 vs. 2) = 0.0195, p (1 vs. 3) = 0.0033). (11)

Presumably, toxin A and B work synergistically, but the exact process has not been sorted out yet. (3) The binding of toxins to colon cells and the following endocytosis is illustrated in *Figure 2*: The tcdB binding domain comes into contact with the cell-surface receptor which leads to receptor-mediated endocytosis. The pH-value in the endosome is acidic and it induces pore formation of the hydrophobic domain, the first conformational change. Now the protease domain is located in the cytosol which triggers a second conformational change. Through autocatalysis of tcdB, the catalytic-DXD glucosyltransferase domain is split from the original unit and released into the cytosol. The glucosyltransferase glucosylates the target Rho GTPases in the cytosol at a threonine residue (Thr) which causes disaggregation of the cytoskeleton and cell death. (4,12) Cell migration is impaired by glucosylated proteins, therefore the integrity of mucosal cells cannot be quickly restored and an inflammation occurs. (3)

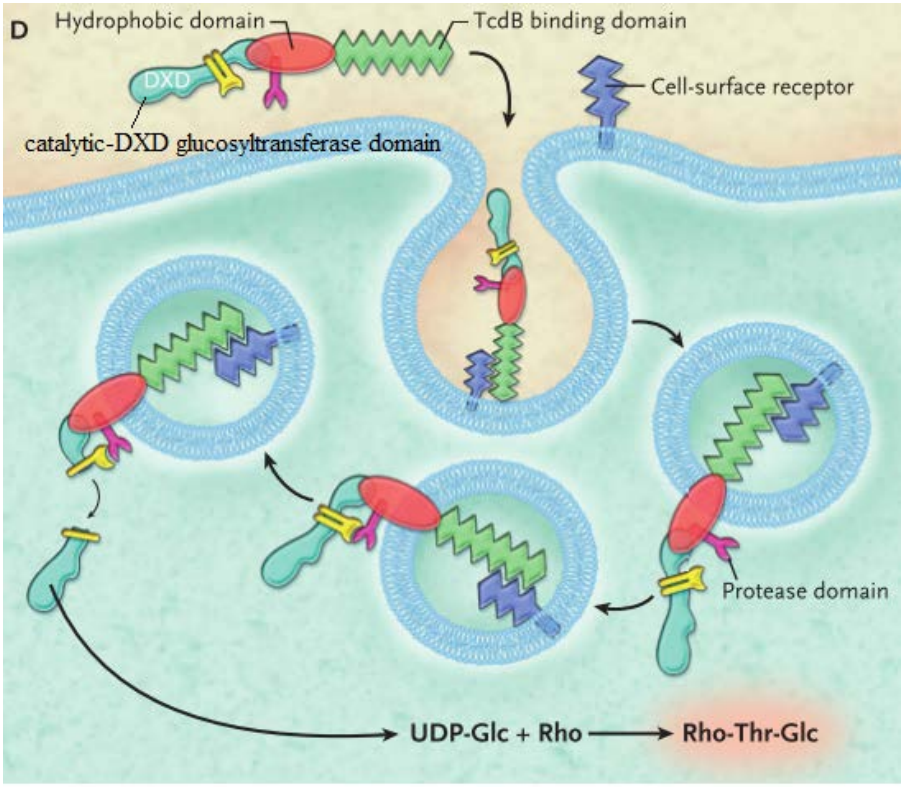


Figure 2: Endocytosis of C. difficile. UDP – uridine diphosphate, Glc – D-glucose. (adapted from (4)). Reproduced with permission from (scientific reference citation), Copyright Massachusetts Medical Society.

The role of the host's immune system in the pathogenesis of CDI is not fully understood yet. After colonization with toxigenic strains of *C. difficile*, some patients show an immediate increase of IgG antibodies against toxin A and remain carriers. Others develop an initial episode of CDI, show an early rise of IgM and eventually also develop IgG antibodies. Patients with low titres of IgG are at a higher risk for recurrent CDI. (4)

1.2.1. Transmission

Although the vegetative form cannot survive more than a few minutes in unsuitable conditions, the spores may survive months to years in almost any environment. (13) They can be found in marine sediment, soil, sand, dung and faeces of different vertebrates. (1) In a hospital environment, they are most frequently present on the floor, the bed frame, the toilet and the wet room. (3,14) The spores are transmitted from one human to another through the faecal-oral route. (5) The structure of those spores has been identified and is schematically depicted in *Figure 3*.

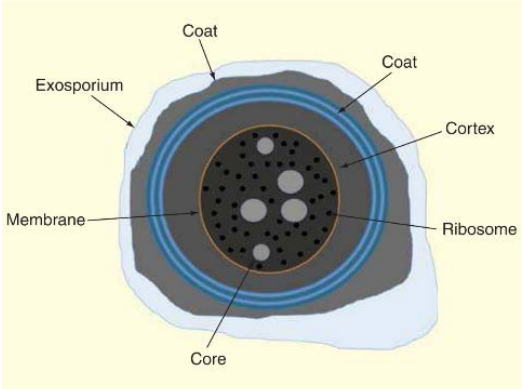


Figure 3: C. difficile spore structure with coat, cortex, membrane, ribosomes and core. (6) Reproduced with permission from Taylor&Francis.

Most of the CDI-cases are health-care-associated; however, 11-28% of new cases may have been acquired outside of hospitals. (15) Community-acquired infection with *C. difficile* is defined as the patient having had no overnight stay in a healthcare facility within 12 weeks before the infection. Nevertheless, this definition does not exclude the possibility that the disease is acquired in a healthcare facility, where the patient did not stay overnight. Patients who have community-acquired CDI are often younger and often have no identified exposure to antibiotics before the onset of CDI. (5) Some studies further differentiate between health-care-associated-cases occurring after 48h of admission or within 4 weeks after discharge and indeterminate cases of infections occurring between 4 and 12 weeks after discharge. (7)

The transmission paths differ in community-acquired and healthcare-associated cases: The most important source of *C. difficile* spores in healthcare-associated scenarios are patients with current CDI. (3,16) The risk of infection is high, as the patient excretes a large

number of germs (10^7-10^9/g faeces) and only a small number of cells are necessary to infect another person. It has been shown in an animal model that the inoculation of only two bacterial cells is sufficient to cause CDI. (3) The transmission can either be direct or indirect. Spores are transmitted indirectly, if the germs are spread by a vehicle from stool to mouth, for instance by the hospital staff, contaminated objects or surfaces. This form of vehicle is missing in direct transmission, where the hands of the person to be infected had direct contact with the contaminated faeces. (3) Fawley and Wilcox (17) conducted a study about the spread of *C. difficile* strains in hospital wards. They DNA-fingerprinted isolates from infected patients and from the inanimate ward environment over a period of 22 months, starting from when the ward opened. Immediately before opening, *C. difficile* could not be found in either ward, but that changed within 1-3 weeks. Spores could be found on approximately a third of the sites in both wards (34 and 36%). Most frequently, spores were detected on toilet or sluice room floors (52%), but also on radiators and air vents (16%). (17)

Concerning community-acquired disease, direct or indirect contact with animals has been suggested as a major cause. Modes of transmission include the contact with retail meat, dog food or animal faeces. Dogs visiting patients in hospitals may also contribute to the transmission of spores. (16) Strains of *C. difficile* affecting humans and animals are often indiscernible. In the Netherlands, PCR ribotype 078 was the third most common strain among CDI cases in hospitals in 2008. The same strain proved to be the most prevalent in swine and cattle. (18) An Austrian study examining animal faeces and retail meat detected six toxigenic isolates. Five of the strains were identical to those causing CDI in humans. (16)

1.2.2. Important strains: 027

One strain has gained special attention in the past years, as it proved to be more dangerous than others: PCR ribotype 027. First isolated in 1988 in France, it was not considered a major problem until higher incidences of CDI were reported from Canada and the USA in the early 2000s. (3) In 2004, Loo et al. (19) conducted a study at 12 hospitals in Quebec to determine the current incidence and mortality rates of CDI as well as the percentage attributable to special strains. In 1997, a mean incidence of 6 per 1000 admissions with an attributable mortality rate of 1.5 percent had been defined for Canada. As opposed to this, the new mean incidence came to 22.5 per 1000 admissions with a 30-day attributable

mortality rate of 6.9 percent in 2004. Both the incidence and the mortality rate markedly increased after the ages of 50 and 60 years, respectively. In 82.2% of the isolates a dominant strain resistance to fluoroquinolones was found, binary toxin genes and partial deletions in tcdC were present in 84.1%. (19) In 2005, McDonald et al. (20) published a study in which they had collected and typed 187 samples from seven acute care hospitals and one long-term care facility in six states that had experienced outbreaks between 2000 and 2003. More than half of the isolates were characterised as restriction-endonuclease analysis (REA) group BI and North American Pulsed Field Type 1 (NAP1), at least 90 percent of the band were identical. In comparison to previous BI isolates, the virulence factors of binary toxin and the deletion in tcdC were identical, but the new isolates were more likely to be resistant to fluoroquinolones. (20) The same strain that accounted for more than half of the isolates in the US-American study was found in 82.2% of the Canadian isolates. It has been typed as toxinotype III and classified as NAP1, PCR ribotype 027. (4) Commonly, it is referred to as PCR ribotype 027 or NAP1/027.

Mainly, there are three bacterial factors contributing to the danger of ribotype 027 (4): Firstly, it produces more toxins A and B, the main pathogenic factors, than other strains. The increased productions result from delete mutations of tcdC, which normally inhibits toxin transcription. Secondly, the strain is resistant to fluoroquinolones, especially moxifloxacin and gatifloxacin. (3,4,19,21) This is a competitive advantage against other strains, especially in facilities with a high use of fluoroquinolones. (3) An assessment of the risk factors after the outbreak in Quebec showed that the use of fluoroquinolones was the most important risk factor for developing CDI. Among the fluoroquinolones with a broader spectrum, ciprofloxacin was shown to be especially associated with CDI. Among the newer respiratory antibiotics, the risk was higher in patients receiving gatifloxacin than in those receiving moxifloxacin. (22)

Thirdly, NAP1/027 produces binary toxin in addition to toxins A and B. It is homologous to the iota toxin of *C. perfringens*, but its role in the pathogenesis of CDI is not clear. Although the sole production of binary toxin seems to be non-pathogenic, the three toxins may act synergistically. All three factors have contributed to the fact that PCR ribotype 027 is considered hypervirulent. (3) Moreover, efficient or even increased sporulation has been observed. (5,15) Ribotype 027 has been linked to a significantly higher morbidity and mortality and a higher rate of complications. (15)

In March 2006, PCR ribotype 027 was isolated in Austria for the first time. The person affected was a 69-year old female tourist from Great Britain, who had previously been treated with antibiotics due to bronchitis. The strain was tested positive for binary toxin and was resistant to fluoroquinolones as well as metronidazole. Treatment with vancomycin was successful. (23) The next cases of ribotype 027 occurred in February and March 2008 in Vienna and Graz, respectively. The two isolates were not clonal; however, both were only resistant to erythromycin and susceptible to clindamycin and fluoroquinolones, which means they were different from the main 027-strains in Europe at the time. (21) Later in the same year, 36 isolates were identified as PCR ribotype 027, all originating from 4 hospitals in Vienna. All of them were tested positive for toxin A, B and binary toxin and showed a characteristic 18bp deletion in the tcdC gene. Resistances varied, although each one showed in vitro resistance against moxifloxacin. (24) This is considered the first outbreak of PCR ribotype 027 in Austrian hospitals.

Since 2010, PCR ribotype 027 has been more common in Austrian hospitals. A study conducted in the Viennese Wilhelminenspital in 2013 showed that a third of the local CDI-cases were caused by ribotype 027. The rate is even higher in recurring CDI-cases (81%). The hospital established an antibiotic stewardship which helped to limit the spread of ribotype 027. (25)

1.3. Epidemiology

Epidemiological studies show that infection with *C. difficile* has become an increasing problem in hospitals and health-care associated facilities, especially in the Western World. The first transnational European study concerning CDI was conducted by Barbut et al. (26) on behalf of the European Study Group on *Clostridium difficile* (ESGCD) in 2005. It covered 38 hospitals in 14 countries; Austria, however, was not part of the study. The mean incidence for all countries was 2.45 per 10 000 patient-days (range: 0.13-7.1), but incidences differed greatly from country to country and from hospital to hospital within the same country. The authors state that the small number of hospitals participating per country makes it impossible to calculate valid incidences for each country, but it was remarkable that countries with recent outbreaks of CDI had higher incidences than others. (26) The second and latest study was carried out by Bauer et al. (7) in November 2008. 34 countries and 106 laboratories participated, including 3 Austrian hospitals in Feldkirch,

Salzburg, and Vienna. The mean incidence was 4.1 per 10 000 patient-days (range: 0.0-36.6) and it also varied across hospitals and countries. For the aforementioned reasons, it is difficult to calculate incidences for each country based on such data. Nevertheless, Austrian physicians proved to have a comparatively medium to high awareness for CDI, measured by the number of patients tested per 10 000 patient-days. The numbers range from only 3 in Bulgaria and Romania to 141 in Finland, Austria has 52 per 10 000 tested. Only 9 participating countries had a higher testing rate. (7)

The Austrian Federal Ministry of Health made it compulsory to notify the authorities about severe or fatal CDI-cases in January 2010. (27,28) Statistics show a total of 338 cases in 2017, 4 thereof came from Styria. (29) These may include non-severe as well as severe cases. Since 2010 there is also a National Reference Centre for *Clostridium Difficile* in Austria, the AGES (Österreichische Agentur für Gesundheit und Ernährungssicherheit) Institute for Medical Microbiology and Hygiene. (30) However, it is not possible to calculate incidences for Austria or individual provinces based on this data from the National Reference Centre. In 2016, for instance, only 57 samples were sent in, but 475 cases were notified in the system (28) Over- and underreporting also renders it difficult to derive a general mortality rate from the passively gathered additional data. Overreporting means, it is more likely to send in a sample, if the patient is critically ill, so mild cases may go unnoticed. Underreporting means, the samples are often sent in before the physician knows about the final outcome of the case and can therefore not report it, even when asked. (28) In 2007, the Austrian Agency for Health and Food Safety collected 142 samples from each of the nine provinces and found a mortality rate of 8.45%. (31)

The following *Figures 4 and 5* are based on the analysis of diagnoses in Austrian hospitals between 1997 and 2015 and between 2001 and 2006, respectively. Both figures show a significant rise in *C. difficile* associated cases in general and in particular age groups. (3,28) In 2002, 813 cases of CDI were reported, while in 2006, the number rose to 2192, constituting a 3.7-fold increase within 4 years. (21)

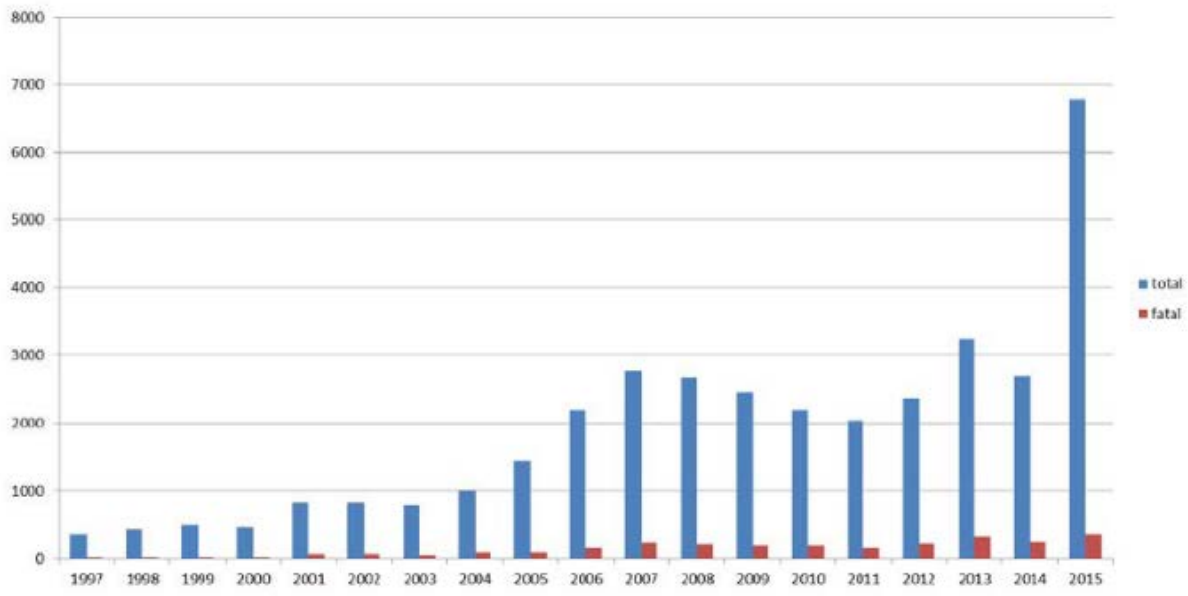
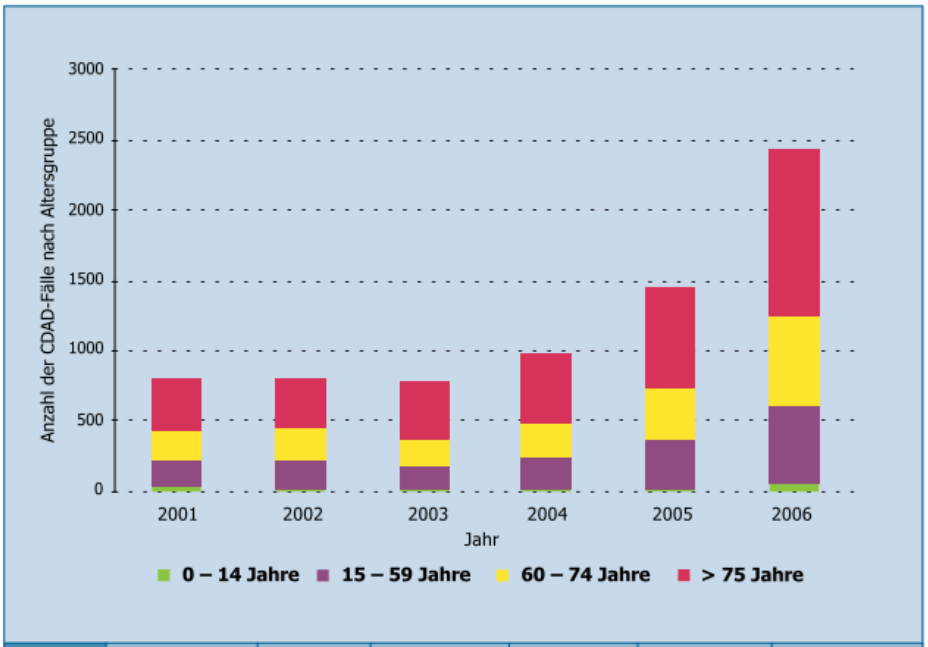


Figure 4: In-patient diagnoses of CDI from 1997-2015 in Austria, main and additional diagnoses. (28)



Letalität	60/807	55/813	54/777	80/997	88/1453	150/2450
	(7,43)	(6,77)	(6,95)	(8,02)	(6,06)	(6,12)

Figure 5: Epidemiology and mortality of CDI in Austria, main and additional diagnoses. (3)

One theory about the recent increase in CDI cases states the rising use of antibiotics as a major cause, especially the use of fluoroquinolones against community-acquired pneumonia which puts a selective pressure on *C. difficile* strains. (16) However, another theory takes account of the increasing numbers of community-acquired CDI and states more and more *C. difficile* spores may originate from animal reservoirs. (16) After toxigenic isolates were found in 11 of 60 retail meat samples in Canada (32), a similar study has been conducted in Austria (16) in 2008. 187 faecal samples of cows, pigs and broiler chicken were tested together with 84 samples of retail meat. Whereas none of the meat samples were contaminated, eight of the faecal samples contained *C. difficile*, six of them were toxigenic. Only one of the strains has never been found in humans in Austria before. This data shows on the one hand that in comparison to Canada, the contamination of retail meat with *C. difficile* is lower. On the other hand, animals have to be considered a reservoir for spores and may contribute to the rising incidences of CDI in Austria and around the world. (16)

1.4. *C. difficile* Infection: The clinical picture

When doing research on *C. difficile*, different abbreviations occur in the literature: CDI which stands for *C. difficile* infection and CDAD which stands for *C. difficile*-associated disease. Both versions are more or less used synonymously, even though recent papers prefer CDI because CDAD can be misleading: It can also refer to *C. difficile*-associated diarrhoea, which does not contain the whole spectrum of the infection. (33)

An episode of CDI is defined as one of two things by the current guidelines of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID):
Either as a combination of clinical signs of CDI and microbiological detection of free toxins and findings of *C. difficile* in faeces without reasonable evidence of another cause of diarrhoea or as a diagnosis of pseudomembranous colitis. Pseudomembranous colitis can either be found while performing an endoscopy or in colon tissue after a colectomy or during an autopsy. (34)
There are three main symptoms compatible with CDI: diarrhoea, ileus and toxic megacolon. According to the WHO, diarrhoea is defined as loose stools (Bristol chart types 5-7) combined with a frequency of three stools in 24h or fewer consecutive hours or more frequently than it is normal for the individual. (35) Concerning CDI, an abrupt onset

of explosive, watery, foul-smelling diarrhoea often constitutes the beginning of the symptoms. (2) CDI covers a wide spectrum of clinical courses, from self-limiting diarrhoea without colitis to unspecific colitis without pseudomembranes, pseudomembranous colitis, recurrent colitis, septicaemia and perforation of the intestines. (3,5,34,36) Other symptoms include fever, loss of appetite, nausea, dehydration and discomfort. Vomiting is uncommon, as well as great admixtures of blood with stools, although occult blood in the stool can be found regularly. (3,5) Nearly 90-95% of patients with CDI have watery diarrhoea, bloody diarrhoea occurs in only 5-10% of cases. (36)

A patient with ileus shows clinical signs of disturbed bowel functions, for example, vomiting and absence of stool. Furthermore, radiological signs of bowel distension can be detected. If not only a distension of the colon exceeding 6cm in transverse width but also signs of a severe systemic inflammatory reaction are found, the patient has a toxic megacolon. (34)

Severe episodes of CDI are defined differently in US-American and European guidelines. The criteria are compared with each other in *Table 1*. Similar criteria are positioned in the same row. The US-American definition requires two factors: Serum albumin levels lower than 3 g/dl (= 30g/l) plus either a leucocyte count of ≥ 15,000 cells per mm^3 or abdominal tenderness. For the diagnosis of a severe and complicated disease, other factors attributable to CDI are necessary. (37) Five of them overlap with criteria of severe disease suggested by ESCMID, as can be seen in *Table 1*. The ESCMID defines a case of CDI as severe if one or more of the clinical signs listed are present. (34)

Severe CDI can lead to an ICU-admission, colectomy or even death. Even without signs of severe colitis, some patients are considered to be more prone to develop a complicated course of disease: Patients aged 65 or older, patients with serious comorbidities, patients who are already admitted to an ICU when the disease first occurs or immunosuppressed patients. (34)

USA (37)	Europe (34)
Serum albumin < 3g/dl (= 30g/l)	*Decreased blood albumin level < 30g/l*
Leucocyte count ≥ 15,000 cells/mm³	*Leucocyte count over $15x10^9$/l*
Abdominal tenderness	*Signs and symptoms of peritonitis*
Fever ≥ 38.5°C	*Fever (core body temperature > 38.5°C).*
Hypotension with or without required use of vasopressors	*Haemodynamic instability (signs of distributive shock)*
End-organ failure (mechanical ventilation, renal failure, etc.)	*Respiratory failure requiring mechanical ventilation* *Rise in serum creatinine level (≥ 133µM or ≥1.5 times the premorbid level)*
Ileus or significant abdominal distension	*Signs and symptoms of colonic ileus*
Serum lactate levels > 2.2 mmol/l	*Elevated serum lactate (≥ 5mM)*
	Rigours
	Marked left shift (band neutrophils > 20% of leucocytes)
	Pseudomembranous colitis in endoscopy
	Distension of large intestine (> 6cm)
	Colonic wall thickening
	Pericolonic fat stranding
	Ascites not explained by other causes
Leucocyte count ≥ 35,000 cells/mm³ or < 2000 cells/mm³	
Admission to ICU for CDI	
Mental status changes	
printed in **bold: required for severe disease**, printed in *italics: at least one criterium required for severe disease,* normally printed: at least one criterium attributable to CDI required for severe and complicated disease	

Table 1: Comparison of the definition of severe CDI in US-American and European guidelines. (34,37)

1.4.1. Risk factors and recurrences

A previous administration of antibiotics remains the most widely accepted and alterable risk factor for CDI. However, a few patient groups that had been known to have a low risk for CDI are currently showing a rising incidence of cases. These groups include patients without former treatment with antibiotics, peripartum women, patients with inflammatory bowel disease and children. (15) Established and emerging risk factors are listed in *Table 2*.

Adults	**Children**
Factors related to the patient	
Male sex	Male sex
Age > 65 years	Younger age, premature children
Prolonged duration of hospital stay	Prolonged duration of hospital stay, lack of prior hospitalization
Caucasian ethnicity	Caucasian ethnicity
Previous gastrointestinal surgery	Previous gastrointestinal surgery
Comorbidity and underlying conditions	
Inflammatory bowel diseases	Inflammatory bowel diseases
Immunodeficiency and HIV	Immunodeficiency and HIV
Malnutrition	Malnutrition
Low serum albumin levels (< 2.5g/dl)	Hematologic disorders
Neoplastic diseases	Neoplastic diseases
Cystic fibrosis (risk of severe or fulminant disease, mainly post lung transplant)	Cystic fibrosis (risk of severe or fulminant disease, mainly post lung transplant)
Diabetes	Liver and renal diseases
	Systemic lupus erythematosus
	Pancreatitis
Pharmacological therapies	
Prolonged use of antibiotics (mainly clindamycin, cephalosporins, fluoroquinolones, ampicillin/amoxicillin, macrolides, cotrimoxazole, tetracyclines)	Prolonged use of antibiotics (mainly clindamycin, cephalosporins, fluoroquinolones, ampicillin/amoxicillin, macrolides, cotrimoxazole, tetracyclines)
Antineoplastic chemotherapies	Antineoplastic chemotherapies
Use of proton pump inhibitors	
Solid organ transplantation	Solid organ transplantation
Haematopoietic stem cell transplantation	Haematopoietic stem cell transplantation
Narcotic and antidiarrheal medication	Presence of gastrostomy or jejunostomy tube

Table 2: Risk factors for CDI. (15)

A recurrence is defined as a reappearance of CDI within 8 weeks after the beginning of the previous episode. A symptom-free period in between is required. (34) Due to the disruption of the commensal colonic microbiome that is not only the cause but also the consequence of CDI, recurrences are not uncommon and may occur in up to 20-50% of the cases. (4,13,38) The more recurrences a patient already had, the more likely he or she is to have another one (20% after the initial episode, 40 - 60% after two or more recurrences). (4,9) It is important to distinguish between a recurrence of CDI and other conditions with similar symptoms such as postinfectious irritable bowel syndrome, microscopic colitis and inflammatory bowel disease. (4) That is why it is recommended to perform diagnostic testing of *C. difficile* if diarrhoea occurs again. (13)

Risk factors for recurrences include not only the abovementioned disruption of the intestinal microbiota (4,9,37,38) but also the persistence of *C. difficile* spores (38,39), repeated applications of antimicrobial substances (9,38) or a poor antibody response to *C. difficile* toxins. (4,9,39)

1.4.2. Patients and carriers

Not everybody who carries *C. difficile* in his/her intestines develops CDI, some remain asymptomatic carriers. As opposed to patients with CDI, carriers develop an early increase in serum IgG antibodies to toxin A. (4) New-borns and infants are often colonized with *C. difficile*, but due to a lack of toxin-binding receptors, they remain asymptomatic. (5) The prevalence of colonization by *C. difficile* in adults, especially in high-risk groups such as elderly patients, varies among different studies. Among hospitalised patients, the carriage rate might be up to 25%, whereas it is <5% in otherwise healthy adults. (13) Studies in long-term care facilities from Germany, Switzerland and Italy show a carriage rate ranging from 2 to 5.1%. (40–42) In 2016, a cohort study conducted at a geriatric unit in Hamburg, Germany, tested 262 patients without diarrhoea who were admitted consecutively using PCR. 43 or 16.4% of them presented a positive test result, 16.3% of the asymptomatic carriers developed symptomatic CDI during their hospital stay. (43) In a small US-American prospective study, a carriage rate of 51% was determined during an outbreak. However, the cleaning staff of the examined facility did not use sporicidal disinfectant which might have led to such a high rate. (44)

The most important risk factor for asymptomatic carriage according to Nissle et al. (43) is a previous episode of CDI. Other significant risk factors include previous treatment with

antibiotics and the primary diagnosis ‘post-surgery’. The more hospital stays a patient had experienced in the past 6 months, the more likely he or she was to be a carrier of CDI. (43)

Planche et al. (11) examined 12 420 faecal samples and divided them into 3 groups: CDI positive (cytotoxin assay positive, group 1), *C. difficile* excretors (toxigenic culture (TC) positive, cytotoxin assay negative, group 2) and CDI negative (both methods negative, group 3). Mortality rates did not significantly differ between group 2 and group 3. (11) That means that carrying *C. difficile* without having CDI does not affect the individual’s clinical outcome.

However, an asymptomatic carriage is a risk factor for the development of symptomatic CDI. Nissle et al. (43) suggest that screening for carriage at admission would provide financial and health benefits: Isolation procedures and other hygiene interventions might be undertaken and the spread of spores, as well as the number of secondary infections, might be reduced. (43) Riggs et al. (44) propose that asymptomatic carriers contribute significantly to the transmission of CDI in long-term care facilities. An early recognition of asymptomatic carriers might, therefore, reduce the overall incidence in LTCFs.

1.5. Diagnostic algorithm

In 2016, the ESCMID published an update of the original diagnostic guideline of CDI. All commercially available laboratory tests were considered index tests and were compared to reference tests. A reference test is the best available test for diagnosing a disease. In case of CDI, there are two reference tests: cell cytotoxicity neutralization assay (CCNA) and TC. In short, CCNA positivity demonstrates the presence of free toxin, while TC positivity demonstrates toxin-producing capacity. In a large recent study, CCNA positivity alone correlated with clinical outcome, a positive test result means that CDI is responsible for the patient’s diarrhoea. (11) It is difficult to interpret test results that show a positive TC but negative CCNA. They could be either from a *C. difficile* carrier or a patient with CDI and toxin levels below a certain threshold. (45)

The index tests include enzyme immunoassays (EIA) that detect glutamate dehydrogenase (GDH) and/or toxin A and B and nucleic acid amplification tests (NAAT). GDH is produced by all strains of *C. difficile*, the toxigenic ones and the non-toxigenic ones. All index tests were evaluated concerning their truly and falsely positive and negative test

results. The sensitivity of a test is defined as the probability that a person with the disease will be tested positively. The specificity of a test is defined as the probability that a person without the disease will be tested negatively. The positive predictive value (PPV) of a test is the probability that a person with a positive test result really has the disease. The negative predictive value (NPV) is the probability that a person with a negative test result is free of disease. Both the PPV and the NPV depend on the prevalence in the population. (45)

The GDH EIAs, as well as NAATs, were shown to be more sensitive tests, while Toxin A and B EIAs are more specific. At an assumed prevalence of CDI around 5%, NPVs would be high for all index tests, but PPV would be only 69-81% even for the specific tests. If the prevalence rose, the PPV would also rise, but the NPV would drop in not-sensitive tests. A low PPV means that many patients would get false-positive test results. To avoid that, an algorithm of two tests is recommended: At first, a highly sensitive test is performed to classify negative test results as non-CDI-cases. The first test could be a GDH EIA or NAAT. The second test should provide a high PPV and therefore a high specificity to classify all positive test results as true CDI-cases. A suitable second test is a Toxin A and B EIA. Both combinations (GDH EIA + Toxin A/B EIA or NAAT + Toxin A/B EIA) performed almost identically. (45) A large recent study in the UK found that the optimum algorithm was GDH EIA + NAAT, but GDH EIA and Toxin A/B got almost identical results. The latter permits the categorisation of patients into 3 groups: CDI positive, CDI negative and potential CDI excretor. (11) Since the detection of the last group is crucial for our study design, we chose GDH EIA + Toxin A/B as an algorithm. It is depicted in *Figure 6*.

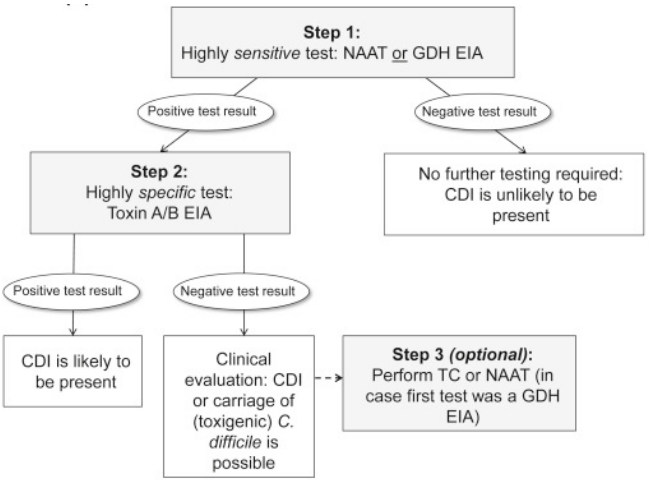


Figure 6: The diagnostic algorithm that will be used in this study. (45) Reproduced with permission of Elsevier (Creative Commons Attribution – Non Commercial - No Derivatives License)

It is also recommended for every laboratory to be able to isolate *C. difficile*, ideally via TC, to perform molecular typing or susceptibility testing. Moreover, TC makes it possible to further examine cases with a positive first and negative second result. They can either be tested falsely positive by GDH EIA/NAAT or contain only a small portion of free toxins that cannot be detected via EIA. This third round of testing can be performed either by TC or NAAT or GDH EIA (if it has not been performed before). (45)

If *C. difficile* is detected, but the Toxin A/B EIA is negative, three options remain: Either the toxin levels are below the threshold of detection, or the toxin results were false-negative or the patient is only a carrier. (45)

1.6. Treatment options

In 2014, the ESCMID published an updated treatment guidance document that distinguishes between different patient groups. (34) Regardless of the severity of the case, four measures should always be taken into consideration: Firstly, discontinue unnecessary antimicrobial therapy (34) as antibiotics are the most widely acknowledged risk factor for developing CDI. (15) Secondly, adequately replace fluid and electrolytes (34) since one typical symptom of CDI is watery diarrhoea (2,36) and it is important to maintain the fluid balance. Thirdly, avoid anti-motility medications (34) because they might falsify the outcomes from monitoring of diarrhoea, an important measure of disease response. (37,46) Moreover, they might lead to a more complicated course of disease, for instance by inducing an ileus. (37,46) The American guideline only recommends their use, if the CDI is also treated with antibiotics. (37) Fourthly, review the proton-pump inhibitor use (34), because the suppression of gastric acid might lead to a rise of gastrointestinal pathogens. (47)

The success of the treatment is determined with the help of the markers of severity as well as with the successful reduction of diarrhoea. If firstly, the parameters of disease severity improve and they have not developed new severe signs develop and secondly, the stool frequency decreases or the stool consistency improves, the treatment is deemed successful. The patient's response to the treatment should be observed daily and evaluated after at least 3 days. It may take 3-5 days until a clinical improvement becomes measurable, especially with metronidazole. Even after a clinical response, it might take weeks for the

stool consistency and frequency to normalize.(34) It is not recommended to test for cure, as the patient's stools may contain spores and toxins for some time after the resolution of diarrhoea. (13,45)

Three antimicrobial substances are mainly recommended to treat CDI*:* metronidazole, vancomycin and fidaxomicin. Metronidazole belongs to the nitroimidazole antibiotics. Its production is cheap (48), that is why it is most commonly used for non-severe CDI cases. If oral admission is not possible, the drug can be administrated intravenously. (34) Metronidazole is well absorbed from the gastrointestinal tract and is therefore not able to reach high gut concentrations. (34) The high absorption rate might lead to adverse effects like nausea, headache, taste perversion and peripheral neuropathy. (49) Furthermore, metronidazole is contraindicated for pregnant women. (50)

Vancomycin is a glycopeptide antibiotic. It is more expensive than metronidazole and is mainly used for severe CDI cases. A few studies suggest its superiority to metronidazole in two aspects: Firstly, vancomycin is considered superior with regard to clinical success, defined as the resolution of diarrhoea and abdominal pain. The results under a treatment with vancomycin (81.1%) were significantly better than those under metronidazole (72.7%, p: 0.0134). (51) Secondly, vancomycin showed significantly better results concerning the response time (3.0 days with vancomycin vs. 4.65 days with metronidazole, $p < 0.01$). (52) Special ribotypes like PCR-ribotype 027 are reported to have a higher resistance against metronidazole than against vancomycin. It is discussed if an increasing use of vancomycin facilitates the spread of vancomycin-resistant enterococci (VRE), although a large retrospective analysis did not prove this to be true during a 2-year follow-up period. (34) When treating CDI cases, oral administration of vancomycin is necessary (34), since the concentrations in the gut are not high enough to be effective when administered intravenously. Vancomycin is the first choice for treating CDI in pregnant women for whom the use of metronidazole is contraindicated. (50)

Both of the recommended agents cause a disturbance of the commensal colonic microbiome, which might lead to recurrent CDI or colonization with other nosocomial pathogens. As to that, fidaxomicin (200mg, twice daily for 10 days) can be used as an alternative. (34) It has a limited activity against gram-negative bacteria and thereby better preserves the commensal microflora. (15) Its poor absorption in the gastrointestinal tract leads to few systemic side effects. Fidaxomicin is also less likely to increase colonization

with VRE. (34,49) It is bactericidal and has a longer postantibiotic effect than vancomycin. (49) Hypersensitivity reactions to fidaxomicin have been reported and included in the labelling in the post-marketing phase. (34) Due to lack of sufficient data, the efficacy of fidaxomicin in life-threatening disease is not clear. (34) Fidaxomicin also leads to high expenses (38,48), although it leads to significantly fewer recurrences than vancomycin (15.4% vs. 25.3% in the intention-to-treat analysis, p: 0.005) which would compensate some of the costs. (49)

1.6.1. Initial non-severe disease

For non-severe disease, metronidazole is usually preferred to vancomycin, fidaxomicin or teicoplanin. (34) Two different scenarios are summarised in *Table 3*:

Criteria	*Therapy*
Symptoms of initial CDI such as diarrhoea, non-epidemic setting, infection attributed to concomitant use of antibiotics	**Stop antibiotic treatment** and **observe** patient for 48h. Monitor patient very carefully. In case of deterioration, start treatment immediately.
Deterioration of a non-severe case after observation, no signs of severe CDI	Antibiotics: first choice: **metronidazole** p.o. 3x500mg for 10 days. Second choice: vancomycin p.o. 4x125mg for 10 days.
Oral administration not possible	Antibiotics: intravenous **metronidazole** 3x500mg for 10 days.

Table 3: Therapeutic options for initial non-severe disease. (34)

However, in the latest update of the clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) the use of vancomycin or fidaxomicin is preferred to metronidazole for treating an initial episode of CDI. Only if the other two substances are not available, metronidazole is recommended. (53)

1.6.2. Severe Disease

Vancomycin is recommended in case of severe disease. Four different settings are described in *Table 4*:

Criteria	*Therapy*
Criteria for severe disease as listed in Table 1	Antibiotics: first choice: **vancomycin** 4x125mg for 10 days (raise up to 500mg possible, lack of sufficient evidence for raise in the absence of ileus). Second choice: fidaxomicin p.o. 2x200mg for 10 days
Oral administration not possible	Addition of alternative applications of **vancomycin**: vancomycin retention enema (500mg in 100ml saline, 4x daily) or administration by an oral or nasogastric tube (500mg 4x daily for 10 days) plus metronidazole i.v.
Life-threatening disease	Antibiotics: first choice: **vancomycin** 4x125mg for 10 days (raise up to 500mg possible). No sufficient data for use of fidaxomicin. Do not use metronidazole alone in case of life-threatening disease!
Progression of disease under treatment with antibiotics	Surgical intervention: Subtotal colectomy with end-ileostomy or diverting loop ileostomy and colonic lavage.
Perforation of the colon or systemic inflammation and clinical deterioration under maximal antibiotic treatment	Surgical intervention: Total abdominal colectomy.

Table 4: Therapeutic options for severe disease. (34)

Due to a rising incidence of refractory CDI cases, alternatives to the standard therapy are currently being developed. (34,54) One of them is teicoplanin, another glycopeptide with similar cure rates. It is not included in the treatment recommendations because it is not

available in the USA, but it has a licensed indication for CDI. (34) Another one is tigecycline, a broad-spectrum glycylcycline antibiotic used to treat complicated infections in the abdomen. In a retrospective cohort study with 90 patients, its efficacy in curing patients with severe CDI was compared to that of the standard therapy of oral vancomycin together with intravenous metronidazole. Clinical cure was defined as patient survival and resolution of four important symptoms of CDI: diarrhoea, abdominal pain, fever and leucocytosis. The tigecycline-group had significantly better rates of clinical cure (75.6% vs. 53.3%, p: 0.02), less complicated cases (28.9% vs. 53.3%, p: 0.02) and less cases of CDI sepsis (15.6% vs. 40.0%, p: 0.009). The mortality and recurrence rates, as well as the occurrence of ileus and toxic megacolon, were similar in both groups. (54)

Systemic inflammation and clinical deterioration under maximal antibiotic treatment may manifest as toxic megacolon, acute abdomen and severe ileus. Risk factors for mortality after colectomy include increased levels of serum lactate (> 5mM), changes of the mental status, end-organ failure, renal failure, the need for preoperative intubation and ventilation and the development of shock (the need for vasopressors). The more risk factors a patient has already developed, the sooner a surgical intervention should be taken into account. It is recommended to operate before the patient develops severe colitis or before lactate levels exceed 5.0mM. (34)

The established technique for a surgical intervention had been a subtotal colectomy with end-ileostomy. Recently, a surgical alternative that perseveres the colon has been developed: At first, a diverting loop ileostomy is performed laparoscopically. Through the ileostomy, the colon is then lavaged with polyethene glycol 3350 or a balanced electrolyte solution. The colon is flushed postoperatively with vancomycin through a catheter and the patient receives metronidazole intravenously. (34,55) This new technique has been proposed by Neal et al. (55) after they had treated 42 patients and compared the outcomes to former patient groups treated with colectomy. Significantly lower mortality rates (19% vs. 50%, p: 0.006) have been detected and 93% of the colons could be preserved with the new treatment, although the severity of illness was similar in both groups. (55) A randomized controlled clinical trial was conducted, but the results have never been published. (56) A retrospective single centre review at the University of Virginia compared the mortality rates of all patients with CDI having received surgical treatment between April 2011 and June 2015. While the one-year-mortality was similar between patients having had a colectomy and an ileostomy, the 30-day-mortality was even lower in the

colectomy-group. Due to the low number of included patients, the results were not statistically significant, but the study shows that the new technique needs to be thoroughly validated through follow-up data and larger, prospective studies. (57)

1.6.3. Recurrences

The treatment of recurrences differs depending on how often the patient already has had CDI. Recommended procedures are listed in *Table 5*.

Criteria	*Therapy*
First recurrence	Antibiotics: vancomycin 4x125mg for 10 days (raise up to 500mg possible) or fidaxomicin p.o. 2x200mg for 10 days or metronidazole p.o. 3x500mg for 10 days
First recurrence with severe symptoms	Antibiotics: **fidaxomicin** p.o. 2x200mg for 10 days
More than one recurrence	Antibiotics: vancomycin 4x125mg for 10 days (pulsed/tapered) or fidaxomicin p.o. 2x200mg for 10 days faecal microbiota transplantation

Table 5: Therapeutic options for recurrences of CDI. (34)

Vancomycin should be administered pulsed or tapered. Pulsed administration means 125-500mg per day are given only every second or third day for at least 3 weeks. Tapered administration means the dose should be gradually decreased to 125mg per day.

Another highly effective treatment option for multiple recurrences is called faecal microbiota transplantation (FMT) following an antibiotic treatment with a glycopeptide. (34) The basic idea is to incorporate the intestinal microbiota of a healthy subject into a patient's colon. (9,38) Different routes of application are possible: via nasogastric or nasojejunal tube, during an upper endoscopy, colonoscopy or retention enema. (38) The donors have to be examined closely concerning disease and medication history, recent personal history, and illnesses that can be transmitted via blood or stool. (9)

Recently, two studies have been conducted to assess the efficacy of FMT versus the standard vancomycin regimen in patients with single or multiple recurrences of CDI. (38,39) Van Nood et al. (39) randomly divided 43 patients into 3 groups that received

different treatments: Group 1 was given a shorter regimen of vancomycin (500mg p.o. 4x daily for 4-5 days), a bowel lavage and an infusion of a donor's faecal microbiota. Group 2 received the standard treatment of vancomycin with the maximum dosage (500mg p.o. 4x daily for 14 days) followed by a bowel lavage, whereas group 3 was treated only with the standard vancomycin regimen. Faecal samples were donated by volunteers. The transplantation was applied via a nasoduodenal tube the day after the bowel lavage. All in all, 94% of the FMT-group, but only 31% of group 3 and 23% of group 2 were cured. Three patients had to receive a second infusion of faeces and two of them were cured thereafter. The therapy with FMT was superior to both vancomycin variations ($p < 0.01$ after the first infusion, $p < 0.001$ after the second). Being cured was defined as the absence of a relapse 10 weeks after the beginning of the treatment. Patients who had not been cured by the antibiotic therapy were offered FMT off protocol. The study also found out that the intestinal bacterial diversity increased within 2 weeks after the infusion and that the diversity level then equalled the donor's. The study was stopped after 43 patients had enrolled and the high benefit of the FMT had become evident. (39) In 2015, a similar Italian study was published. 39 patients were randomly assigned to the FMT-group or the vancomycin-group. The faecal infusion was conducted during a colonoscopy on the second or last day of an abbreviated regime of vancomycin (125mg p.o. 4x daily for 3 days). The other group received 125mg vancomycin p.o. four times daily for 10 days followed by a pulsed regimen with 125-500mg per days every 2-3 days for at least 3 weeks. Faeces were collected mostly from patient's relatives or intimates. After one year, 90% of the patients treated with FMT reported a resolution of diarrhoea, but only 26% of the vancomycin-group did so, too ($p < 0.0001$). (38)

After the FMT via nasogastric tube, the majority of patients (94%) had diarrhoea; cramping (31%) and belching (19%) were also reported. The symptoms disappeared within three hours. In the longer term, 19% of the patients complained about constipation. (39) After the instillation via colonoscopy, 94% suffered from diarrhoea, too. 60% reported bloating and abdominal cramping. The symptoms resolved within 12 hours. (38) However, in 2015, a patient died of aspiration pneumonia after the installation of FMT via the biopsy channel of an enteroscope. The author of the case report proposes to treat patients with an anti-emetic before the instillation to avoid aspiration. (58)

Advantages of the application via colonoscopy might be that the severity of intestinal inflammation and the presence of pseudomembranous colitis can be assessed

simultaneously. Moreover, large quantities of suspensions can be brought directly to the site of inflammation. A slightly better efficacy of instillation in the lower gastrointestinal tract has been noted. (9,38)

Both studies excluded important risk groups for CDI such as immunodeficient or critically ill patients or patients with additional antibiotic therapies. (9,38,39) Furthermore, both studies report a very low response rate to vancomycin, presumably because most patients had already been treated with vancomycin in earlier episodes and might have been pre-selected. (9,38,39) Further studies are necessary to assess the long-term safety of FMTs. The exact definition of a healthy microbiota is also still a field of ongoing investigations. (9) In 2014, an Austrian protocol concerning the screening of donors and the different methods of administration has been published by Kump et al. (59). The authors prefer the lower GI tract as route of administration due to the larger amount of volume that can be administered at once and due to the lower rate of severe complications. In 2017, 28 European experts published a general guideline offering protocols for the selection of donors, the preparation of material, indications, administration, clinical management and requirements for a FMT centre. (60) CDI, especially multiple recurrences, is listed as the major indication for FMT in both named guidelines. (59,60)

1.6.4. Adjunct therapeutic options

In 2010 a large, randomised, double-blind study examined the use of two monoclonal antibodies against the toxins A and B compared to a placebo. The recurrence rates could be decreased compared to a placebo (7% vs. 25%, $p < 0.001$), but the duration of clinical symptoms, the severity of infection or the duration of hospitalisation stayed the same. (15,61)

A vaccine for the prevention of recurrent CDI was successfully tested in a small study with only three patients who had had multiple recurrences of CDI in the past. The vaccine contains inactivated toxoids A and B. After the immunisation, all three patients went without further recurrences, although they had stopped their treatment with vancomycin. (62)

In 2013, a Cochrane review with regard to the prophylaxis of CDI with probiotics (63) has been conducted. Probiotics are living bacteria or yeast that might be able to restore the balance of the intestinal microbiome. Adverse effects include abdominal cramping, nausea,

fever, soft stools, flatulence and taste disturbance. The review indicates that probiotics reduce the rate of CDI cases by 60% on average. There was a subgroup effect concerning the baseline risk of developing CDI which showed that a baseline risk of > 5% leads to a higher reduction rate. At a baseline risk ≤ 5%, they do not seem to be effective. Even in immunocompromised or strongly debilitated patients, a short-term use of probiotics together with antibiotics seems to be safe. According to this Cochrane review, patients should therefore be informed about probiotics, especially if they belong to high-risk groups of CDI. (63) However, some studies (34) reported serious side effects in debilitated patients: Although it is still rare, the incidence of Saccharomyces invasive infections has risen since 1990. One of the risk factors might be the intake of probiotics containing *Saccharomyces boulardii*. In a case review of 92 cases, 37 infections were caused by *S. boulardii* and 32 of the affected patients had taken a probiotic with *S. boulardii* before. The most common risk factors are intravenous catheter use and previous antibiotic therapy. (64) As the evidence for a safe administration is not sufficient, the ESCMID-guideline does not recommend the use of probiotics. (34)

1.7. Prevention

As most cases of CDI are of nosocomial origin and iatrogenic, prevention measures in hospitals are very important. (4) In 2008, Vonberg et al. (13) published a guideline of infection control measures to limit the spread of *C. difficile* on behalf of the European Centre for Disease Control and Prevention (ECDC). Together they were able to identify ten crucial areas to prevent further transmission: 1. early diagnosis of CDI, 2. surveillance of CDI cases, 3. education of staff, 4. appropriate use of isolation precautions, 5. hand hygiene, 6. protective clothing, 7. cleaning of medical equipment, 8. environmental cleaning, 9. good antibiotic stewardship and 10. specific measures during outbreaks. The document can be used to adapt existing national guidelines or to create new ones. It evaluates the evidence level of each study according to the Oxford Centre for Evidence-Based Medicine ranging from Level 1a (systematic review (with homogeneity) of randomised controlled trials) to 5 (expert opinion without explicit critical appraisal, or based on physiology, bench research or ‘first principles’). The strength of recommendations is also assessed, ranging from IA (strongly recommended) to II (suggested for implementation) and unresolved issue (insufficient evidence). In the

following, the evidence levels and strength of recommendations are given in square brackets. (13)

1. Early diagnosis is only possible if tests for *C. difficile* toxins are performed in faecal samples of patients with nosocomial diarrhoea as well as patients that acquired it outside the hospital. If *C. difficile* was found, repeating testing should be refrained from unless a recurrence is suspected after a symptom-free period [IB/3b, 4]. That also means a test of cure should not be performed [IA/1a].]. Furthermore, it is only recommended to test symptomatic patients with diarrhoea, unless ileus is present. [IB/2b, 3b, 4] After CDI is diagnosed, the samples should be stored, especially if the patients show severe symptoms or in an outbreak situation [IB/1b, 3b, 4]. Hereby, retrospective typing is possible. (13)

2. If a patient is diagnosed with CDI, the case should be included in a surveillance system [IB/2b, 3b, 4, 5]. To correctly survey CDI cases in general, it is necessary to obtain a baseline incidence, so that you have something to compare new data to [IB/2c]. According to the baseline incidence, a threshold should be implemented that then triggers additional measures [IB/2b]. If changes in the incidence, complication rate or severity occur, new strains may be responsible [Unresolved/No data]. Fitting to the first point, early diagnosis, it is also important to ensure diagnostic testing of patients with acute diarrhoea not otherwise explained [IB/3b, 4]. (13)

3. The condition for the implementation of all the other measures is the education of the staff. Everyone that enters a patient's environment should know about clinical features, transmission and epidemiology of *C. difficile*. That includes not only healthcare workers but also cleaning personnel and visitors [1A/1a, 2b, 4, 5]. People with acute diarrhoea should not visit patients in a hospital. (13)

4. The staff should also be informed about the isolation precautions that have to be taken if a patient is diagnosed with CDI. As active infections represent a source of transmission, patients should ideally be isolated in single rooms with protective clothes for the staff [IB/1b, 2b]. If that is not possible, it is recommended to isolate special cohorts [IB/1b, 4], maybe in a designated unit for cohort isolation with designated staff [IB/1b, 4]. The benefits of this solution are numerous: Designated staff may be more experienced in caring for contagious patients and cleaning protocols may be more easily implemented. Furthermore, there are fewer people unnecessarily entering a cohort ward who might spread spores. If environmental contamination occurs, it may be tracked down to a single

focus, namely the cohort ward. The main transmission route is faecal-oral and the toilet or sluice rooms floors are an important reservoir for spores. (17) A special toilet or transportable toilet should be provided in case of CDI [IB/1b]. If the symptoms have ceased and the bowel movements have returned to normal, isolation precautions may be stopped after 48h [II/4]. (13)

5. As regular, alcohol-based hand disinfectants are not sporicidal and alcohol is even used to select for *C. difficile* spores in laboratories, other measures of hand hygiene have to be taken on when dealing with CDI patients [IB/2b, 2c]. It is important to wash the hands with soap and water because the action of rubbing and rinsing has been proven to be the best way to remove spores from the hands [IB/2a, 2b, 2c, 4]. Washing of hands is also recommended after taking off special aprons or gloves. The soap does not have to be antiseptic [Unresolved/2c]. Patients should be encouraged to wash their hands after using the toilet and before having a meal. (13)

6. When dealing with a patient who has diarrhoea, two things have to be considered: Firstly, one should always wear gloves when having contact with a CDI patient, his body fluids or potentially contaminated environment [IB/1b, 2b]. A prospective controlled trial in the US which instructed hospital workers in the correct use of vinyl gloves resulted in a decrease of CDI cases from 7.7 to 1.5 per 1000 patients within 6 months. (13) Secondly, wearing a special gown or an apron is recommended when caring for patients who have diarrhoea [IB/1a, 1b, 4]. (13)

7. Not only the hands or the clothes of contact persons but also medical equipment used for CDI patients may be contaminated by spores. Ideally, medical devices such as blood pressure cuffs or stethoscopes are only assigned to one patient [IB/1b, 4]. The same is true for thermometers [IA/1b, 2b]. Electronic thermometers with disposable sheaths should be avoided [IA/1b, 2b]. The use of tympanic thermometers is recommended. Transmission of CDI through endoscopes is not reported. After use, endoscopes have to be cleaned and disinfected with alkaline glutaraldehyde solution 2% or with peracetic acid for 5-10 minutes in order to kill off all remaining *C. difficile* spores. After contact with a CDI patient, the equipment should be cleaned using a sporicidal agent [IB/1b, 2c, 4]. Disposable material should be used, whenever possible [IB/1b]. (13)

8. Hospital wards, especially frequently touched surfaces, should be cleaned at least once a day [IB, 1b, 2a, 4]. Sporicidal agents are recommended for environmental cleaning and

cleaning of medical equipment, the best choice being a chlorine-containing agent with at least 1000 ppm available chlorine [IB, 2b, 2c, 4]. The most crucial areas are commodes and bedpans, which should be cleaned carefully and stored under dry conditions [IB/ 1b, 2a]. If faecal contamination of any surface has occurred, the cleaning staff should be informed and the cleaning process should start as soon as possible [IB/1b, 2a]. After a patient with CDI has been discharged, his room and toilet have to be cleaned and disinfected carefully [IB, 2b, 2c, 5]. (13)

9. The most important risk factor for the development of CDI is precedent treatment with antibiotics, that is why a good antibiotic stewardship is a fundamental part of prevention measures concerning CDI [IA/1a]. When a patient develops symptoms of CDI, any antimicrobial treatment not fighting the bacteria should be ceased as soon as possible. (13) Substances that are unlikely to cause CDI are ureidopenicillins with or without β-lactamase inhibitors and doxycycline. (13,25)

A good example for the beneficial impact of such measures is the implementation of an antibiotic stewardship in the Viennese Wilhelminenspital in 2013, a 1000-bed tertiary care hospital. (25) They closely monitored the numbers of CDI cases per year. In 2012, a slight increase from less than 200 to 313 was noticeable. When the cases did not cease to rise in the first quarter of 2013, an antibiotic stewardship team was established. As similar developments were not present in any other hospital in Vienna, hospital-specific causes were identified. In comparison with seven other Viennese hospitals, the moxifloxacin use in Wilhelminenspital was twice as high. Together with the fact that PCR-ribotype 027 has been more common in Austria since 2010 and it presents with intrinsic moxifloxacin resistance, the hospital decided to restrict the use of moxifloxacin. The increasing rate of 027-strains was also linked to an increase in recurrence and mortality rates in said hospital.

The intervention consisted of two components: a bundle of information and the antibiotic stewardship itself. Information included lectures about pathogenesis, epidemiology, prevention, diagnostics and treatment of CDI, which were also available via the hospital's intranet. Before doctors could prescribe moxifloxacin, they had to fill in a form describing the diagnosis, the dose, the route of administration, combined antibiotic treatments and pretreatment. If possible, a consultation between the physician and the pharmacist about indication, contraindications and possible alternatives to moxifloxacin then took place. It was thereby possible to reduce the defined daily doses (DDD) of moxifloxacin from 5189

in the preintervention period (period 1) to 291 in the intervention period (period 2). Simultaneously, the mean numbers of CDI dropped from 59±3 in period 1 to 32±3 in period 2, which equals a reduction of 46% (p=0.0044). It is to say that general hygiene measures such as protective clothing, hand hygiene or strict isolation of CDI patients have been established before and did not change during the intervention. Although the correlation between high moxifloxacin use and a large number of CDI cases does not prove a causal relationship, the study was able to reach lower CDI levels which can be seen as a success. (25)

10. In case of an outbreak, infection control staff should be informed [IB/1b] and all measures mentioned above should be reinforced [IB/1b, 4]. Reviewing the standard of environmental cleaning and antimicrobial prescribing is recommended [II/4]. Especially high-risk agents like broad-spectrum cephalosporins (especially second- and third-generation cephalosporins), fluoroquinolones and clindamycin are to be avoided [IB/1a, 2b, 3b, 4]. Policies for patient admission, placement and staffing should be implemented [IB/1b] and faecal samples of CDI cases should be stored, so that they can be cultured and typed [II/2b]. Ideally, isolates are compared to find out whether an outbreak is always caused by the same strain. If transmission continues despite all measures, the unit should be closed to new admission [IB/1b]. After that, the only option left is to vacate the unit for intensive environmental cleaning [II/2a]. (13)

In 2014, the Prevention of Hospital Infection by Intervention and Training (PROHIBIT) consortium conducted a study to estimate the scope of the ECDC guidelines and to get an overview of existing guidelines across Europe. They also wanted to compare some of the most important recommendations concerning their evidence and strength of recommendation. (65) The Austrian guideline published by the AGES in 2007 did include almost all highly recommended measures with high evidence. (3,65) The only IA-measure that was not explicitly mentioned was the avoidance of electronic thermometers with disposable sheaths, although the recommendation had good evidence levels [1b, 2b]. (65) The ECDC guideline advises educating visitors about *C. difficile*, whereas the Austrian guideline includes education for visitors only in outbreak situations. (3,13,65) Curiously, many different opinions about whether first washing or first disinfecting the hands were included throughout the guidelines. The Austrian and the German guidelines recommend washing the hands after disinfection. (65) As opposed to many other national guidelines, the AGES-guideline also provides the reader with information about the strength of

recommendation (IA-unresolved issue) and quality of evidence [1a-5]. (65) The PROHIBIT-consortium generally criticises that scheduled revisions of the guideline documents are missing and that they often lack implementation tools.

Hübner et al. (66) included long-term care facilities (LTCFs) as well as rehabilitation clinics, home care services and acute care hospitals in their study about the prevalence of certain pathogens and infection control measures. They stated that the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), which was the most prevalent pathogen, in home care services and LTCFs is similar to those in acute care hospitals. Many patients from LTCFs or home care services are admitted to acute care hospitals and vice versa. Unfortunately, infection control measures are often only reinforced in acute care hospitals and not in other care facilities, although the prevalence of certain pathogens is comparable. Moreover, existing studies on infection control often concentrate on acute care settings, the LTCFs and other facilities are left out. (66)

1.8. Objectives

Infection with *Clostridium difficile* is a widespread cause of diarrhoea in LTCF residents. (4,15) One of the ten crucial areas to prevent further transmission of CDI identified by the ECDC is a good surveillance system for CDI cases. Usually, it includes a baseline incidence, so that outbreaks can be recognised early. (13) However, the incidence of asymptomatic carriage should also be taken into account for two reasons: Firstly, carriers of *C. difficile* may act as a reservoir for the bacteria (41), as they are not treated. (34) It has been suggested that asymptomatic carriers contribute significantly to the transmission of CDI in LTCFs. (44) Secondly, the asymptomatic carriage is a risk factor for the development of symptomatic CDI. (43) Among hospitalised patients, the carriage rate might be up to 25%, whereas it is <5% in otherwise healthy adults. (13)

In order to detect risk factors for colonization and to decide whether further steps (e.g. screening for carriers in risk populations at admission (43)) are to be taken, it is necessary to assess the colonization rates first. Monitoring the *C. difficile* carriage rate among residents in nursing homes is therefore important for infection-control purposes. Existing studies about the incidence of CDI often concentrate on acute care hospitals, although many patients from acute care hospitals are admitted to other facilities like LTCFs and vice

versa. (66) No data is currently available concerning the colonization by *C. difficile* in long-term care facility residents in Austria.

The primary objective of this study is to obtain information about the prevalence of colonization by *C. difficile* in long-term care facility residents. Secondary objectives include risk factors for colonization by *C. difficile* in long-term care facility residents.

2. Methods

The point-prevalence study was carried out as cooperative effort between four LTCFs within the *Geriatrische Gesundheitszentren der Stadt Graz* (GGZ) and the Medical University of Graz. All full-time residents who did not show any clinical signs of an infection with *C. difficile* and who were willing to give written informed consent were included in the study. Residents who did not want to give written informed consent or showed clinical signs of *C. difficile* were excluded. There were no age restrictions. Ethical approval was obtained from the ethics committee of the Medical University of Graz.

Asymptomatic carriage was defined as the detection of *C. difficile* species but not of toxins A or B in the patient's faecal sample. At the time of stool collection, the patient must not show any signs of diarrhoea. Similar studies from Germany, Switzerland and Italy showed a carriage rate ranging from 2 to 5.1%. (40–42) To obtain at least 10 different strains, we calculated that we would need at least 200 samples (200*0.05 = 10). At our laboratory, a maximum of 200 samples can be analysed at the same time, so the targeted sample size was 200.

The samples were collected between 12th and 23rd March 2018 by the nursing staff of the GGZ and analysed at the Institute of Hygiene and Microbiology, Medical University of Graz. All samples were numbered consecutively and categorized according to the Bristol Stool Chart. The ESCMID suggests combining two tests in an algorithm in order to decrease the percentage of false-positive results. (45) Therefore, the highly sensitive glutamate-dehydrogenase-enzyme immunoassay (RIDA®QUICK) was applied first. All samples with a positive first test result were then tested with the highly specific Toxin A/B-enzyme immunoassay (RIDA®QUICK). (45) In addition, the samples were plated onto selective chromID *C. difficile* agar (bioMerieux). They were plated both manually and automatically (BD Kiestra) using 10µl of suspended faeces. All plates were incubated in an anaerobic chamber (Bartelt) at 35°C for 24 hours. Colonies suspicious of *C. difficile* were confirmed using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF, VitekMS) and their toxin production was tested again with Toxin A/B (Szabo-Scandic). Finally, the antimicrobial susceptibility was determined using the agar dilution method. (67)

After the evaluation of the results was finished, environmental swabs were taken from the affected residential units to detect a possible contamination with *C. difficile* spores on the

18th April 2018. The most frequent sites for contamination in a hospital environment are the floor, the bed frame, the toilet and the wet room. (3,14) The swabs were taken from the following sites: the toilet seat and flush, the toilet floor, the bed frame, the floor of the carrier's room, their personal nightstand, their table and chair in the common room, the shared kitchen, the corresponding medical trolley, and, if available, the participant's wheeled walker.

To evaluate possible risk factors for carriage of *C. difficile*, it was necessary to collect demographic and clinical details of the patients. These included age, sex, length of stay in the long-term care facility, accommodation, level of care, diabetes, haemodialysis, mobility, faecal and urinary incontinence, dementia or cognitive impairment, previous stay in acute care hospitals or surgery in the last 3 months, infection with *C. difficile* or diarrhoea in the last 3 months, administration of antibiotics in the last 3 months, urinary catheter and gastrostomy.

One month after the initial examination, participants that were identified as carriers were retested to see if they were still colonised by *C. difficile*.

SPSS was used for the statistical evaluation of the results. With regard to descriptive statistics, frequencies, mean values, standard deviations and ranges were calculated. Different methods were used to determine the correlations and the direction of the effect: Fisher's exact test with asymptotic or exact p-values or the likelihood-ratio and Phi or Cramer-V, depending on the type of the variable.

3. Results

3.1. General description of the study population

144 faecal samples were collected between 12th and 23rd March 2018. The four LTCFs included in the study were *Pflegewohnheim Aigner-Rollett am Rosenhain* (PWH AR), *Pflegewohnheim Peter Rosegger* (PWH PR), *Pflegewohnheim Erika Horn* (PWH EH) and *SeniorInnenresidenz Robert Stolz* (SRRS). 119 of the 144 participants were female (82.64%), 25 were male (17.36%). The mean age was 84.4 years (range: 60-103). On average, they had already spent 30.61 months in LTCFs (range: 0-241). Further general information about the study population can be found in *Table 6*.

Category	Mean value and standard deviation	Range
Age in years	84.40 ± 7.9	60-103
Length of stay in months	30.5 ± 38.7	0-241
Level of care	4.03 ± 1.3	0-7
Sex	Male: 25/144 (17.36%) Female: 119/144 (82.64%)	
Mobility	Bedridden: 4/144 (2.78%) Mobile with help: 112/144 (77.78%) Mobile without help: 28/144 (19.44%)	
Frequency		
	Affected	
Diabetes	31/144 (21.53%)	
Haemodialysis	0/144 (0%)	
Faecal incontinence	43/144 (29.86%)	
Urinary incontinence	108/144 (75%)	
Cognitive impairment/dementia	93/144 (64.58%)	
Stay in acute care hospital in the last 3 months	16/144 (11.11%)	
CDI or diarrhoea in the last 3 months	4/144 (2.78%)	
Antibiotics in the last 3 months	18/144 (12.5%)	
Urinary catheter	5/144 (3.47%)	
Gastrostomy	3/144 (2.08%)	

Table 6: Participants' characteristics.

3.2. Carriage rates

4/144 participants were tested positively for carrying *C. difficile* which results in a prevalence of 2.78%. They all had positive results in the GDH-enzyme immunoassay and negative results in the Toxin A/B-enzyme immunoassay when tested directly from stool.

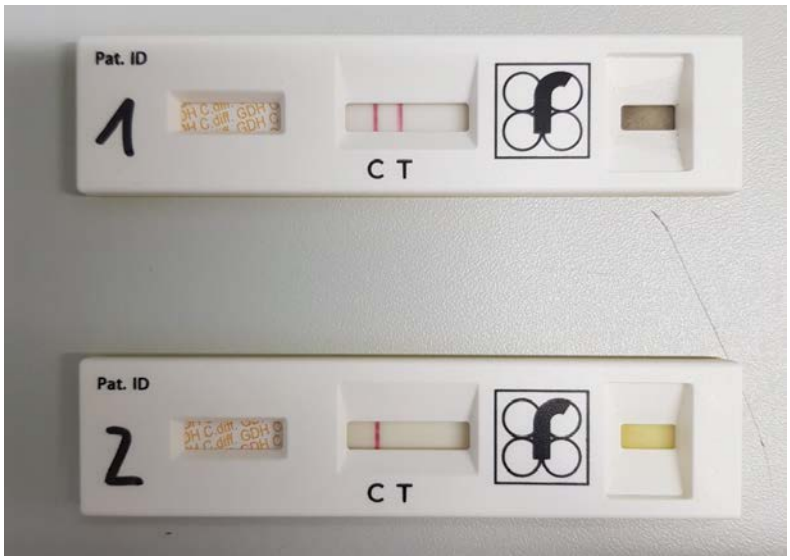


Figure 7: Comparison of positive and negative GDH-enzyme immunoassays. Photographer: Maria Neuhold.

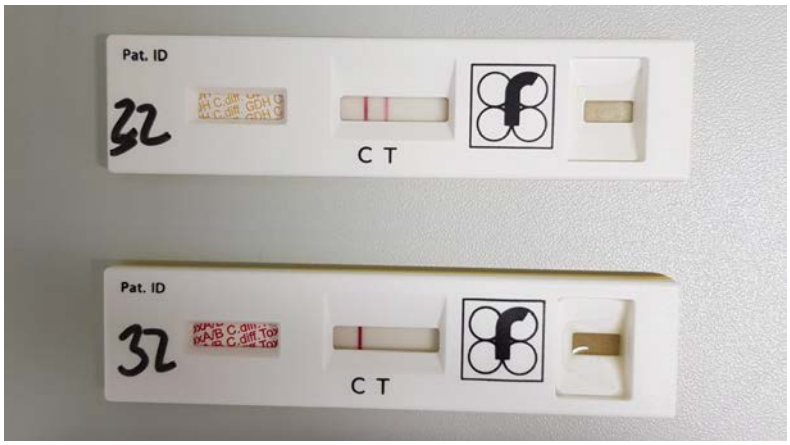


Figure 8: Comparison of positive GDH-enzyme-immunoassay and a negative Toxin A/B-enzyme immunoassay of the same participant. Photographer: Maria Neuhold.

The cultured isolates were identified as *C. difficile* in all four cases which were confirmed by MALDI-TOF (*Figure 9*). In three of the four cases, the Toxin A/B-test taken from the culture was positive which means that the bacterium is able to produce toxins.

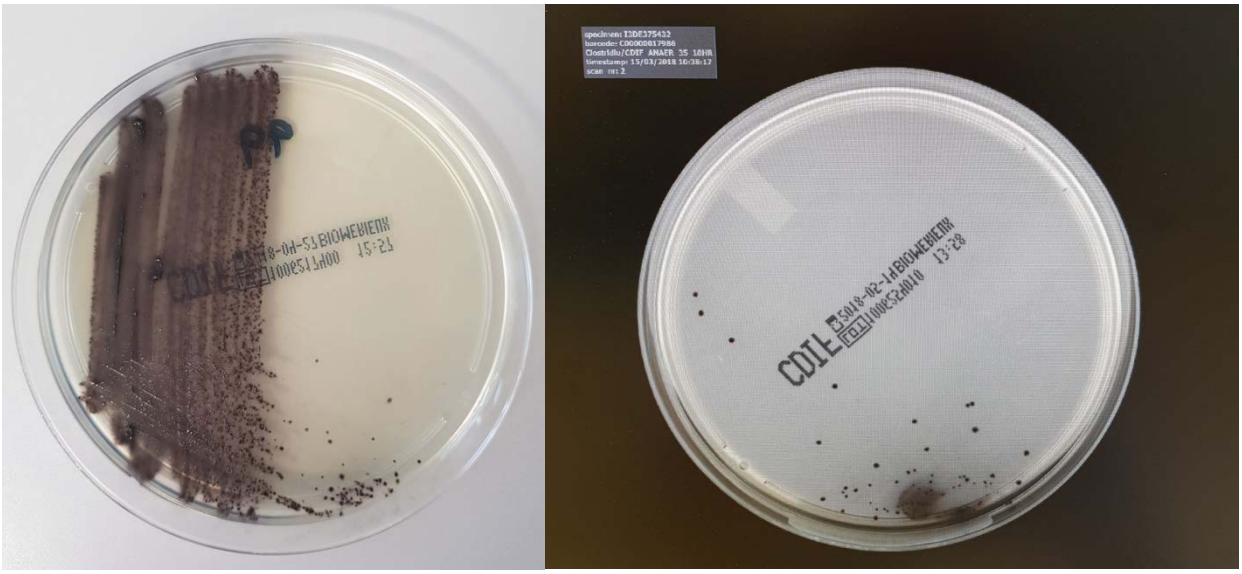


Figure 9: Comparison of manually (left) and automatically (right) plated isolates on selective chromID C. difficile *agar (bioMerieux). Photographer: Maria Neuhold.*

As the four carriers were found only in two of the four included LTCFs, we also calculated separate carriage rates for every single facility. The carriage rates were 4.65% and 7.69%, respectively, in the affected institutions. Two carriers lived not only in the same LTCF but also in the same residential unit. The absolute frequencies and the percentages of *C. difficile* carriers are listed in *Table 7*.

	In total	***C. difficile*-carriers**	***C. difficile*-carriage rate**
PWH AR	43 (29.86%)	2	4.65%
PWH PR	44 (30.56%)	0	0%
PWH EH	31 (21.53%)	0	0%
SRRS	26 (18.06%)	2	7.69%
In total	144	4	2.78%

Table 7: Comparison of the four participating LTCFs with respect to the carriage rates.

Environmental swabs were taken from different sites related to the carriers in PWH AR and SRRS to detect a possible contamination with *C. difficile* spores. None of the swabs showed a positive result for *C. difficile*. Detected microorganisms include *C. perfringens*, *Enterococci* and *Staphylococcus aureus*.

One month after the initial examination, we wanted to retest the four carriers to see if they were still colonised by *C. difficile*. Unfortunately, one of them died shortly after the first samples were taken. Two of the remaining three participants showed a positive result in the GDH-enzyme immunoassay and a negative result in the Toxin A/B-enzyme immunoassay

again. Consequently, they were still asymptomatic carriers of the bacterium one month after the first examination. In one of the two positive GDH-cases, the Toxin A/B-test taken from the culture was positive; the other one was negative (as it had been in the first round).

3.3. Antimicrobial susceptibility

The antimicrobial susceptibility of the *C. difficile* isolates was determined using the agar dilution method. In total, six bacterial strains were tested, four in the first sample collection and two in the second one. The minimal inhibitory concentrations (MIC) for metronidazole and vancomycin, the two most commonly used antibiotics for treating CDI, were tested according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST).

None of our strains showed a MIC higher than the cut-off value which is 2.0 µg/ml for both vancomycin and metronidazole. (68) Therefore, all of them were susceptible. The results are listed in *Table 8*. Two of the carriers were still colonised after one month. Compared to the first bacterial strain, the second showed higher MICs for metronidazole in both cases (0.25 vs. 0.125 µg/ml and 0.125 vs. 0.032 µg/ml) and for vancomycin in one case (0.5 vs. 0.25 µg/ml). None of the strains proved to be resistant to metronidazole or vancomycin.

	MIC metronidazole [µg/ml]		**MIC vancomycin [µg/ml]**	
Carrier 1	**0.125** **repeated: 0.25**	**S**	**0.5** **repeated: 0.5**	**S**
Carrier 2	**0.25**	**S**	**0.5**	**S**
Carrier 3	**0.032** **repeated: 0.125**	**S**	**0.25** **repeated: 0.5**	**S**
Carrier 4	**0.125**	**S**	**0.5**	**S**

Table 8: Minimal inhibitory concentrations (MICs) of the bacterial strains with regard to metronidazole and vancomycin; S: susceptible.

3.4. Evaluation of the risk factors

For the next calculations, the study population was split into two groups: the carriers, and the non-carriers. The general characteristics of the two groups regarding the 12 risk factors can be found in *Table 9*. The carrier who took antibiotics was given *Augmentin* which consists of amoxicillin and clavulanic acid. He developed an infection of which he died shortly after the samples were taken. Only one carrier had diarrhoea in the last 3 months, but two of the carriers are known to have had episodes of CDI in the past. One of them was still identified as a carrier after one month. It is not known if the other two carriers were

also formerly affected by CDI. As no one was on haemodialysis, it was not taken into account for the evaluation of the risk factors.

The carrier-status is a nominal variable and 10 out of 12 risk factors are also nominal. Firstly, a chi-squared test was performed using SPSS to compare them, but the results are not valid because our data did not meet all the conditions (69). Instead, we then used Fisher's exact test with an asymptotic p-value to measure the significance level for dichotomous nominal variables (69–71). If variables had more than two possible values, we conducted two different tests, on the one hand, Fisher's exact test with an exact p-value and on the other hand the likelihood ratio. Both tests do not require a certain expected frequency of an event in the study population and can be used for unordered r x c tables larger than 2 x 2 (67) The results are listed in *Table 9*.

The phi coefficient can be used to measure the strength of the correlation in nominal, dichotomous variables. Cramér's V has the same purpose as the phi coefficient, but can also be used for variables that are not dichotomous (72). Phi or Cramér's V are only listed in *Table 9* if the p-value showed a significant result.

Two categories were significantly associated with the carrier status: diabetes (p: 0.031) and urinary incontinence (p: 0.003). The phi-coefficient of diabetes with the carrier status was 0.22 which signifies a positive correlation. The phi coefficient for urinary incontinence and the carrier status was -0.293 which signifies a negative correlation. The participants' stay in an acute care hospital in the last 3 months also showed a trend towards significance when compared to the carrier status (p: 0.061). In the other cases, we had to accept the null hypothesis that the risk factor is independent of the carrier status.

Two of the twelve risk factors are not nominal variables: Level of care is an ordinal variable while length of stay is a metric variable with a ratio scale. In search of the best suited statistical method to compare them to the nominal carrier-status, we found the Kruskal-Wallis-test for nominal and ordinal variables (73) and the Eta-squared (η^2) for nominal and metric variables. (74) However, the nominal variable has to be the independent variable in both cases. That is why we decided to take the lowest scale level which is nominal as a reference and use the same statistical methods that are described for two nominal variables, although the results are less accurate. The results can also be found in *Table 9*.

	***C. difficile*-carriers** (mean ± standard deviation)	**Non-carriers** (mean ± standard deviation)	**p-value**
Mean length of stay	37.5 ± 22.4128	30.3 ± 39.075	$1.0^{L}/0.146^{Fe}$
Mean level of care	4.5 ± 0.577	4.01 ± 1.297	$0.8^{L}/0.773^{Fe}$
Diabetes	3/4 (75%)	28/140 (20%)	**0.031^{Fa}* (Phi: 0.220, p: 0.008)**
Mobility	Bedridden: 1/4 (25%) Mobile with help: 2/4 (50%) Mobile alone: 1/4 (25%)	Bedridden: 3/140 (2.14%) Mobile with help: 110/140 (78.57%) Mobile alone: 27/140 (19.29%)	$0.186^{L}/0.130^{Fe}$
Faecal incontinence	1/4 (25%)	42/140 (30%)	0.655^{Fa}
Urinary incontinence	0/4 (0%)	108/140 (77.14%)	**0.003^{Fa} ** (Phi: -0.293, p: 0.000)**
Dementia/cognitive impairment	1/4 (25%)	92/140 (65.71%)	0.127^{Fa}
Stay in acute care hospital in the last 3 months	2/4 (50%)	14/140 (10%)	0.061^{Fa}
CDI or diarrhoea in the last 3 months	1/4 (25%)	3/140 (2.14%)	0.108^{Fa}
Antibiotics in the last 3 months	1/4 (25%)	17/140 (12.14%)	0.417^{Fa}
Urinary catheter	0/4 (0%)	5/140 (3.57%)	0.867^{Fa}
Gastrostomy	1/4 (25%)	2/140 (1.42%)	0.082^{Fa}

*Table 9: Comparison between asymptomatic carriers of C. difficile and non-carriers (^{Fa}: Fisher's exact test with asymptotic p-value, ^{Fe}: Fisher's exact test with exact p-value, ^{L}: likelihood ratio; **: $p < 0.01$, *: $p < 0.05$).*

4. Discussion

The primary objective of this study was to obtain data about the prevalence of colonization with *C. difficile* in long-term care facility residents. In our study, we found an overall carriage rate of 2.78% (4/144). However, the rates were different in the four included LTCFs: 0% in PWH PR and PWH EH, 4.65% in PWH AR and 7.69% in SRRS. It was interesting to note that two institutions were not affected at all and that in one affected institution, two carriers lived in the same residential unit. This might suggest an indirect way of transmission through a vehicle in the patient's surroundings.

Similar studies in Austria's neighbouring countries Germany, Switzerland and Italy showed a carriage rate ranging from 2 to 5.1% (40–42). Our result of 2.78% lies within that range. The studies in Italy and Germany reported similar ranges in different LTCFs with some facilities that do not have any asymptomatic carriers while others have a relatively high prevalence. (40,42) The Swiss study was conducted in one single acute care hospital that is why we cannot compare the distributions. (41) In total, 406 people are living in the four participating LTCFs (PWH AR: 97 residents (75), SRRS: 100 residents (76), PWH EH: 105 residents (77), PWH PR: 104 residents (78)). With an average carriage rate of 2.78%, asymptomatic carriage of *C. difficile* might affect between eleven and twelve people in those LTCFs (406*0.0278 = 11.29). The carriage rates in our study are comparatively low.

Two categories correlated significantly with the carrier status: diabetes (p: 0.031) and urinary incontinence (p: 0.003). 75% of the carriers (3/4), but only 20% of the non-carriers (28/140) were affected by diabetes mellitus. With regard to urinary incontinence, the distribution was different: None of the carriers, but 77.14% (108/140) of the non-carriers were incontinent (p: 0.000). Theoretically, these results would suggest that people without urinary incontinence might be more likely to be colonised by *C. difficile*. However, that is not transferable to the general population, but seems to be an artefact. This assumption is backed by the results of similar studies. (40) Nevertheless, it should be reassessed in a greater number of patients. Another factor showed a trend towards significance which means that the p-value is almost 0.05: 50% of the carriers (2/4) stayed in an acute care hospital during the last three months while only 10% of the non-carriers (14/140) did so, too (p: 0.061). This trend should also be re-evaluated in a greater study population.

The Italian study conducted by Giufrè et al. (40) included 489 participants of 12 LTCFs. Their evaluation of risk factors showed that having recently been to an acute care hospital (p: 0.002) is an independent risk factors for colonisation with *C. difficile*. Our study showed a trend towards significance for the same factor. Another independent risk factor was being bedridden (p: 0.002). At the univariate analysis, a few other risk factors were found: pressure sore (p: 0.0179), PEG (p: 0.0055), urinary catheter (p: 0.0039), faecal incontinence (p: 0.0357) and autonomous mobility (p: 0.0266). Arvand et al. (42) included 240 nursing home residents. Admission to an acute care hospital was again significantly associated with asymptomatic colonisation (p: < 0.01). Other factors were: previous CDI (p: < 0.01) and antibiotic therapy in the last three months (p: 0.01). Another German study with a setting that differed a little from ours was conducted by Nissle et al. (43) They also examined the risk factors for asymptomatic carriage and again, the number of previous hospital stays showed a significant association with the carrier status. Other risk factors included an episode of CDI in the past, treatment with antibiotics in the past six months and the diagnosis "post-surgery hospital stay". Diabetes was not included as a possible risk factor by any of the three studies. (40,42,43) The Swiss study did not examine any risk factors statistically. (41)

Altogether, six bacterial isolates were tested for antimicrobial susceptibility to metronidazole and vancomycin in our study. All of them remained susceptible to both antibiotics. That was also the case in the Italian study by Giufrè et al. (40), although they tested for more substances such as fluoroquinolones and found multiple resistances there. The other three comparable studies from our neighbouring countries did not examine the antimicrobial susceptibility. (41–43)

Our study has a few limitations. Unfortunately, it was not possible to reach the targeted sample size of 200 due to difficulties obtaining the informed consent and acquiring the faecal samples. In the end, there were approximately 10 people from whom, despite signing the consent form and being willing to participate, no faecal samples were sent in. At the same time, the carriage rate was only 2.79% which indicated that the absolute number of carriers was relatively low. Hübner et al. (66) suggested that studies should concentrate less on the acute care setting and include different kinds of facilities. Although we did not focus on acute care hospitals, we did not include other settings like homecare services or rehabilitation clinics. Moreover, it was surprising that none of the locations showed a contamination with spores. A possible explanation might be the method that was

used to acquire the environmental swabs. In the future, the swabs will be taken using sponges. However, as *C. perfringens* was detected in the environmental screening, we assume that our method was suitable to detect spore-forming anaerobic bacteria.

The residents who were identified as asymptomatic carriers in our study can now be monitored with regard to the development of CDI in the future. If they develop clinical signs of CDI such as an abrupt onset of watery diarrhoea, fever, nausea or dehydration, an empirical therapy to treat *C. difficile* might be started on suspicion. To get a better idea of the general prevalence and other possible risk factors, bigger studies with more participating facilities are necessary.

To sum up, our carriage rate of 2.78% is comparable to a similar prevalence in Austria's neighbouring countries but is still low. Diabetes mellitus was associated significantly with the carrier status (p: 0.031) in our study, but it has not been included in the compared papers. The stay in an acute care hospital during the last three months showed a trend towards significance (p: 0.061). In three similar studies, it was significantly associated with the asymptomatic carrier status. Resistance to commonly used antibiotics against CDI was not detected in the examined strains.

5. Reference list

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